

UNIVERSITÀ DEGLI STUDI DI FERRARA

DOCTORAL COURSE IN "CHEMICAL SCIENCE"



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**Synthesis of small molecules with antitumoral activity  
that are able to interfere with the mitotic spindle  
formation**

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*Dedicata ai miei genitori*

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# CHAPTER 1

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## INTRODUCTION

### 1.1 General remarks

The last World Health Organization update, corresponding to the year 2016, put on the top 3 causes of disease mortality ischemic heart disease (9.43 million deaths worldwide), followed by cancer (8.97 million deaths) and stroke (5.78 million deaths). Overall, 18.08 million new cases of cancer have been diagnosed in 2018.

In the next four decades, cancer deaths are expected to overcome those for ischemic heart disease, with a 2.08-fold increase (1.76-fold for increase in ischemic heart disease) by the year 2060. Therefore, malignancies will become the leading causes of mortality around the world immediately after the year 2030 [31].

With these numbers, it is necessary undertake efforts for establishing or reinforcing the current strategies for cancer prevention, screening, diagnosis, and management.

#### Classification of diagnosed tumors and treatments

Overall, diagnosed tumors can be classified in three main classes [53]:

1. Indolent tumors that have low invasive and metastatic potential and will remain in such a state during the lifetime of the patient: these are not worthy of treatment (including surgery) and should be left undisturbed. Pancreatic neuroendocrine tumors and papillary thyroid carcinomas are some examples.
2. Highly aggressive tumors with a propensity to metastasize that have, with high probability, disseminated by the time that the primary tumor has been diagnosed: truly effective treatments for most kind of metastases are not available at present and drugs can only be palliative for treating correlated pain.
3. Tumors of intermediate grade that have the potential to disseminate but can be excised or treated with cytotoxic therapies before dissemination occurs and lifethreatening metastases are formed.

Even if surgery and radiotherapy (ionizing, thermic or photodynamic) are the best treatment for localised and well isolated tumors and chemotherapy and immunotherapy are used for systemic or disseminated malignancies [14], in many countries tumors of all classes are treated, often aggressively, with drugs and often incur numerous side effects and sometimes may actually increase the incidence of second-site cancers arising years later [53].

For these reasons is very important to get drugs that specifically act on tumoral cells, working on pathways that characterize these biologically different cells.

Amongst diverse cancer therapeutic targets, drugs targeting microtubules represent one of the most effective classes of cancer chemotherapeutic compounds available to date [12]: these act as disruptors of mitotic spindle assembly, and for this reason are called anti-mitotics agent. As result, the fate of tumoral cells is death, without completing the mitotic process [15].

## 1.2 Tubulin and microtubules

### 1.2.1 Composition and organization

Microtubules are highly dynamic structure that, together with microfilaments and intermediate filaments, form the cell cytoskeleton. The microtubule network is recognized for its role in regulating cell growth and movement as well as key signaling events, which modulate fundamental cellular processes.

As reported in fig 1.1, microtubules are composed of  $\alpha$ - and  $\beta$ -tubulin heterodimers that associate to form hollow cylindrical structures .

They are highly dynamic, and are constantly lengthening and shortening throughout all phases of the cell cycle. During interphase, microtubules are nucleated at the centrosome (minus end) and radiate toward the cell periphery (plus end). Interphase microtubules are involved in the maintenance of cell shape and in the trafficking of proteins and organelles.

Motor proteins translocate cell components on microtubule tracks, and protein–protein interactions with other adaptor proteins co-ordinate this process. Tubulin heterodimers also exist in soluble form in cells, and protein interactions with this tubulin population regulate microtubule behavior.

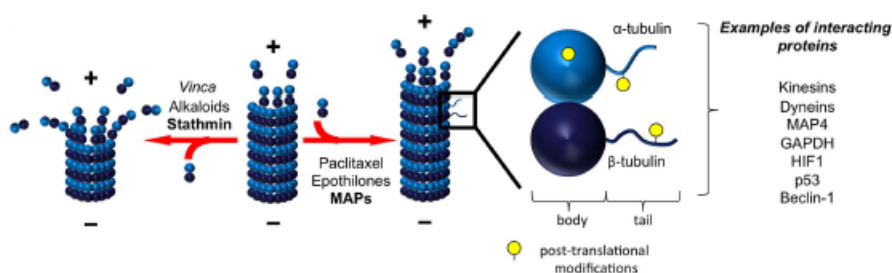


Figure 1.1: Microtubules are dynamic structures that interact with diverse proteins [35]

In humans, microtubules are composed of combinations of eight  $\alpha$ -tubulin isotypes and seven  $\beta$ -tubulin isotypes, with the different tubulin isotypes possessing specific tissue and developmental distributions. The members of the tubulin family share a high degree of structural homology and are distinguished from one another by highly divergent sequences at their carboxy-terminal (C-terminal) tail [21]; these tails are also thought to mediate protein-protein interactions and act as sites of post-translational modifications to confer unique functionality to each isotype [25].

The addition and removal of soluble tubulin heterodimers to dynamic microtubule ends is a highly regulated process. Tubulin dimers are nucleotide binding proteins, with  $\beta$ -tubulin also possessing GTPase activity. The manner in which tubulin heterodimers are orientated in microtubules gives rise to a polar molecule that differs in both structure and kinetics at each end of the microtubule. The dynamics of tubulin addition and release are much slower at the minus end of the microtubule, which terminates with  $\alpha$ -tubulin proteins, compared with the plus end of the microtubule, which terminates with  $\beta$ -tubulin proteins. The addition of a tubulin heterodimer to a microtubule activates the GTPase activity of  $\beta$ -tubulin, locking the  $\beta$ -tubulins in the microtubule in a GDP-bound state. The  $\beta$ -tubulins exposed to the solvent at the end of the microtubule form a GTP cap that is important in preventing microtubule depolymerization. Therefore, the binding of GTP at the microtubule plus end imparts structural and kinetic polarity to microtubules and is an important regulator of microtubule stability [35].

1.2.2 Tubulin alterations in cancer

Altered tubulin isotype expression, that results from increased gene transcription and enhanced mRNA stability [30], is the most widely characterized microtubule alteration reported in cancer and has been observed in both solid and hematological tumors. In particular, elevated  $\beta$ III-tubulin levels are found in different malignancies, suggesting that it may be act as a survival factor in cancer.

Post-translational modification are able to affect signaling events within the cell; for example, acetylated tubulin is implicated in intracellular trafficking, endoplasmic reticulum(ER) localization, and ER–mitochondria interactions, as well as the regulation of microtubule dynamics. But an increased acetylation of  $\alpha$ -tubulin on Lys40 (due to an increment of HDAC6 expression) has been observed in tumor cells, as well as an increment of Sirtuin-2 expression, responsible for tubulin deacetylation, has been linked with the regulation of autophagy in response to stress, allowing survival to cancer cells.

Tumoral cells are subjected to different kinds of stress: as reported in fig 1.2, microtubules influence homeostatic mechanisms and cell stress responses by regulating intracellular trafficking, acting as a scaffold for the co-localization and sequestration of stress response proteins, transmitting stress signals through cytoskeletal remodeling and modulating the induction of cell death pathways.

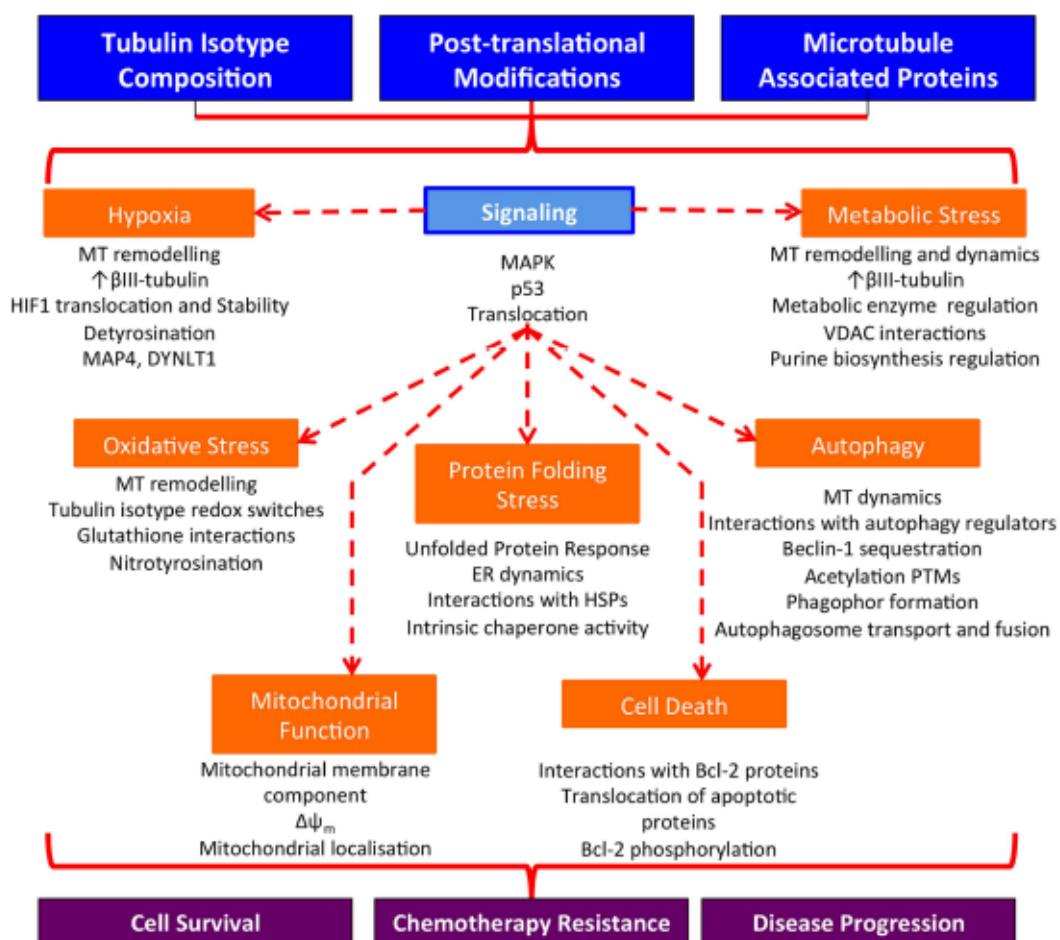


Figure 1.2: Microtubules regulate and co-ordinate diverse cellular stress responses in cancer [35]

Tumoral cells have to change and adapt their metabolism: they must survive (at the beginning) inside a microenvironment characterised by poor oxygen levels, massive presence of ROS and metabolic stress [35]. As reported in fig 1.2, microtubules modifications play crucial roles in this:

- *Hypoxia*, inside solid tumors, is the result of a rapid cell proliferation and poor

vascular development. An increased microtubule polymerization has been observed in physiological hypoxia (3% O<sub>2</sub>) [54]; its remodeling is dramatic and to maintain bioenergetics and organelle function there must be also an altered MAP signaling.

- *Oxydative stress* is very spread inside cancer cells; high levels of ROS are able to induce cell death, but tubulins interact with mediators of the oxidative stress response. Moreover, specific tubulin isotypes that are able to act as redox switchces are overexpressed, reducing the ROS total concentration [23].
- *Metabolic stress* occurs in cancer as a result of uncontrolled cell proliferation in the absence of adequate nutrients, and microtubules may act as a sensor of the energy state of the cell with ATP depletion causing instability of deetyrosinated microtubule plus ends. Moreover, metabolic modulation of microtubule dynamics and tubulin post-translational modifications may allow for rapid and widespread stress responses: for example, nutrient starvation induces hyperacetylation of tubulin, which may act in concert with AMPK to induce autophagy in response to decreased ATP levels, thereby engaging multiple stress response pathways through microtubule-related signaling [16].
- *Cell death signaling* is highly impacted by microtubules remodeling: for example, their dynamics are able to regulate p53 levels and translocation, with an overall pro-survival effect, as a result of reduction of the apoptotic potential of cancel cells [17].

As result of these cytoskeletal remodeling, tumoral cells are able to elude the activation of pathways that finally keep to cell death, promoting cell survival, chemotherapy resistance and disease progression.

### 1.2.3 Anti-mitotic drugs in cancer treatment

Drugs that disrupt mitotic progression, which are commonly referred to as ‘anti-mitotics’, are used extensively for the treatment of cancer. Currently, all such drugs that have been approved for clinical use target microtubules, with the taxanes and vinca alkaloids showing much success against a number of cancers [15].

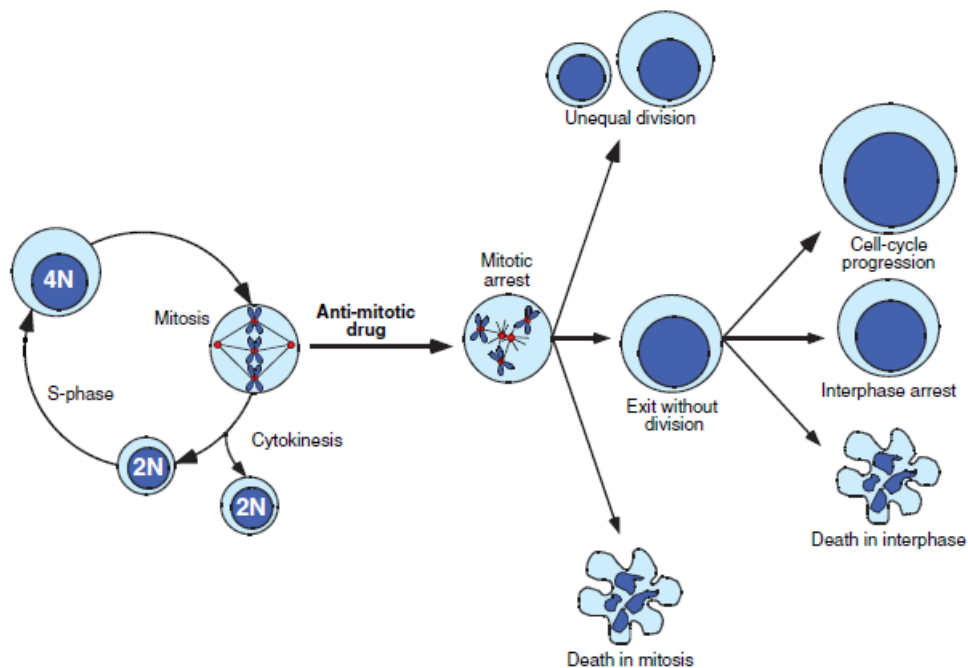


Figure 1.3: Cell fate in response to annti-mitotic drug treatment [15]

As showed in fig 1.3, during an unperturbed mitosis, mitotic exit is triggered by the rapid degradation of cyclin B1, that is prevented by the activity of the SAC (spindle-assembly



checkpoint) until chromosomes are correctly aligned. When cells are exposed to an anti-mitotic agent, they arrest in mitosis due to chronic activation of the SAC. As consequence, cells might die directly in mitosis, or divide unequally to produce aneuploid daughter cells. Alternatively, cells might exit mitosis without undergoing division: in this case, cells might then die in interphase, arrest in interphase indefinitely or enter additional cell cycles in the absence of division. Thus, it is likely that the fate of the cell in response to drug treatment is determined not only by events occurring during a mitotic arrest, but also the by the consequences of these events after mitotic exit, as well as additional signaling pathways that are active during interphase.

Even if the clinical success of the microtubule toxins suggests that mitotic disruption is an effective anti-cancer strategy, it must be consider that all somatic cells undergo to mitosis and two main factors must be evaluated: **resistance** and **toxicity** [32]. Resistance is the ability of tumoral cell to elude induced death mechanisms, while toxicity is related to the activity of this toxins also on somatic cells and in non-dividing cells, such as peripheral neurons. In the last case, these agents act disrupting the microtubules dynamic, inducing permanent neuropathies [42]. New chemical entities in vitro tests are very important in toxicity prediction: the activity of each compound must to be tested in different tumoral cells, but also in few somatic cells (like fibroblasts): a good compound should have no activity -or a very low- on non-tumoral cells.

### 1.3 Tubulin Binding Agents

Tubulin binding agents (TBAs or MTAs, microtubules-targeted agents) are molecules that are able to interact with tubulin. They disrupt microtubule dynamics in distinct ways and they are classified into two main groups, as shown in fig 1.4: microtubule destabilizing agents (MDAs) and microtubule stabilizing agents (MSAs).

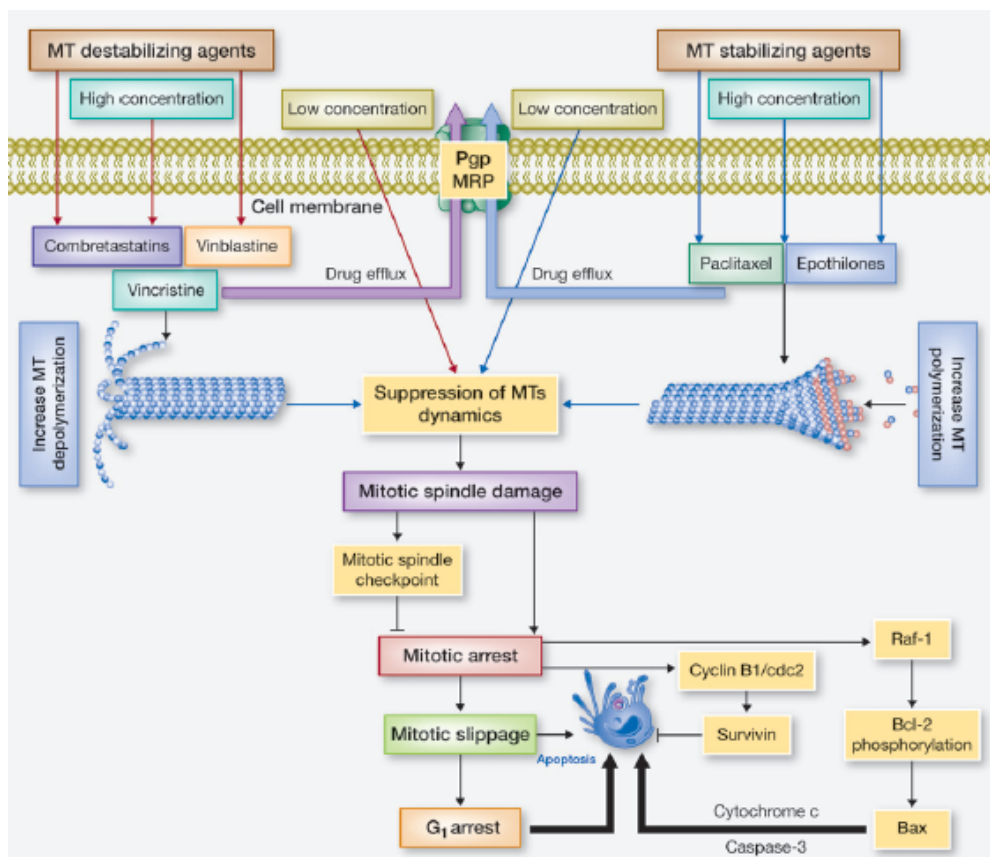


Figure 1.4: Schematic diagram of putative events involved in MTAs-induced apoptosis [33]

The most important action of the two classes of drugs is the suppression of spindle-microtubule dynamics.

At relatively high concentrations, MTAs either inhibit microtubule polymerization, destabilizing microtubules and decreasing microtubule polymer mass, or promote microtubule polymerization, stabilizing microtubules and increasing the polymer mass [24]. So, the treatment of human cancer cells with *low concentrations* of MSAs may lead to mitotic slippage, which is multipolar spindles formation, and subsequent cell-cycle arrest in G1. Thus, cells arrested in (aneuploidic) G1 state and subsequent undergo apoptosis. On the other hand, *higher drug concentrations* lead to a protracted mitotic block from which the cells eventually exit without division, thus forming tetraploid G1 cells [8], which will then undergo apoptosis. In fig 1.4 is also shown that a fundamental prerequisite for cell killing by MSAs is that cells must entry into mitosis; moreover, apoptosis does not (necessarily) occur from a G2–M arrested state.

There are lots of natural compounds that act as MTAs: among these, the most potent identified have been classified on the base of the site of their interaction with tubulin [32], as shown in fig 1.5. Currently there are three well-established drug-binding sites on  $\beta$ -tubulin:

1. The **vinca domain** is located adjacent to the exchangeable GTP-binding site in  $\beta$ -tubulin at the plus end interface.
2. The **taxane site** resides in a deep hydrophobic pocket at the lateral interface between adjacent protofilaments, within the lumen of the microtubule.
3. The **colchicine site** is located at the intradimer interface between  $\beta$ -tubulin and  $\alpha$ -tubulin.

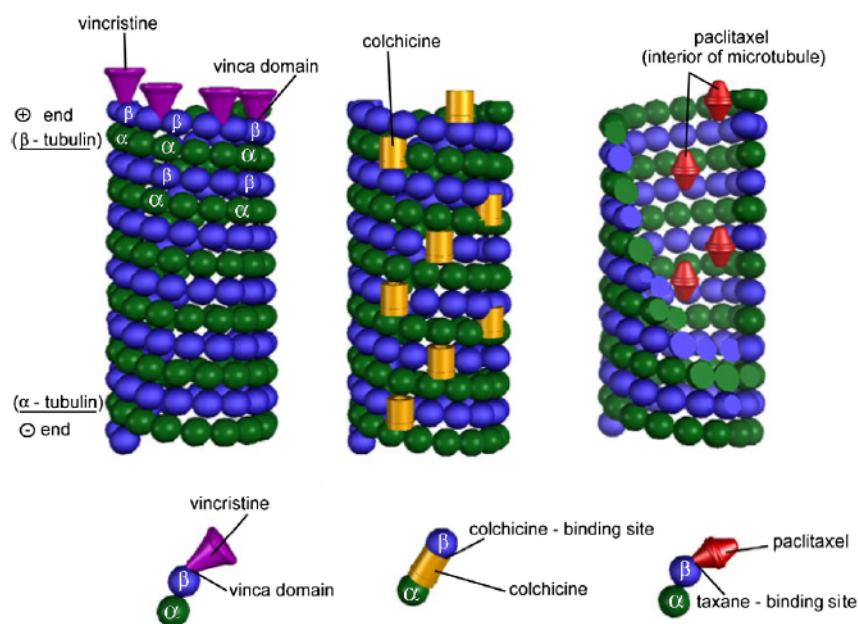


Figure 1.5: Antimitotic drugs bind to different sites on the tubulin heterodimers [32]

In the next pages is possible to find a brief description of these natural agents.

### 1.3.1 Taxane site binding agents

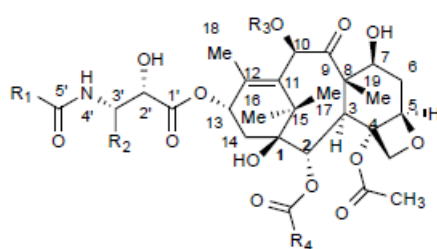
The first compound isolated from the stem bark of the western yew, *Taxus brevifolia* Nutt, is paclitaxel (structure reported in fig 1.6), a very complex chemical compound, characterised by a diterpene structure with a tetracyclic 17 carbon frame and 11 stereocenters [7].

## 1. INTRODUCTION

Paclitaxel can inhibit microtubule de-polymerization by binding to  $\beta$ -tubulin, resulting in mitotic arrest and subsequent activation of caspase-dependent apoptosis by Bcl-2 proteins [50]. The unique pharmacological effect of paclitaxel makes it be on a fast development in its clinical research: FDA approved it as antitumoral agent in 1992.

The fraction of surviving cells fell sharply after exposure for 24 h to paclitaxel concentrations ranging from 2 to 20 nM; the paclitaxel IC<sub>50</sub> was found to range between 2.5 and 7.5 nM. Increasing the paclitaxel concentration above 50 nM, however, resulted in no additional cytotoxicity after a 24 h drug exposure [28].

The main issue related to this natural compound is its low availability: the original plants not only grow slowly, but its highest content is of 0.069%, and it can be collected only by destroying plants (due to the complex molecular structure, the synthetic route only recently found cannot be applied to produce it in industrialization). Moreover, paclitaxel has poor solubility in water and drug resistance and side effects have been gradually appeared accompanied by the clinical application. For these reasons, it was necessary found new semi-synthetic products of paclitaxel, as reported in fig 1.6.



Paclitaxel (R<sub>1</sub> = R<sub>2</sub> = R<sub>4</sub> = Ph, R<sub>3</sub> = Ac)  
Docetaxel (R<sub>1</sub> = R<sub>4</sub> = Ph, R<sub>2</sub> = *t*-BuO, R<sub>3</sub> = H)  
Cabazitaxel (R<sub>1</sub> = *t*-BuO, R<sub>2</sub> = R<sub>4</sub> = Ph, R<sub>3</sub> = CH<sub>3</sub>)  
Milataxel (R<sub>1</sub> = *t*-BuO, R<sub>2</sub> = 2-furan, R<sub>3</sub> = H, R<sub>4</sub> = Ph)  
BMS-275183 (R<sub>1</sub> = *t*-BuO, R<sub>2</sub> = *t*-Bu, R<sub>3</sub> = Ac, R<sub>4</sub> = COOCH<sub>3</sub>)

Figure 1.6: Paclitaxel and its semi-synthetic products

Current researches about the SAR of paclitaxel indicate that the oxygen and the C-13 side chain are the necessary activity structure, and the benzoyl group at C-2 also play an important role in anticancer activity [9].

### 1.3.2 Vinca alkaloid site binding agents

Vinca alkaloids, which belong to a class of cell cycle phase M specific anti-tubulin agents, were one of the first plant alkaloids to be developed for use as anti-cancer agents in humans. Their anticancer mechanism is completely opposite to paclitaxel [7]. Among these, vincristina is one of the most common compounds (reported in fig 1.7) and FDA approved it as anticancer drug in 1962.

Vinca alkaloids function as microtubule inhibitors, inhibiting the polymerization of tubulin, which is necessary for spindle formation during the M-phase of the cell cycle. While they disrupt microtubular function at low doses, cell cycle arrest and apoptosis are seen at higher doses [3].

The concentrations of vincristine and vinblastine, respectively, that inhibited growth rates by 50% were: mouse leukemia L1210 cells, 4.4 and 4.0 nM; mouse lymphoma S49 cells, 5 and 3.5 nM; mouse neuroblastoma cells, 33 and 15 nM; HeLa cells, 1.4 and 2.6 nM; and human leukemia HL-60 cells, 4.1 and 5.3 nM [13].

These compounds are highly lipophilic and attain very high intracellular concentrations. Overproduction of P-glycoprotein, which is an efflux transporter protein, is a plausible mechanism of development of acquired drug resistance. Mutations in  $\beta$ -tubulin, with overexpression of different isotypes, may also correlate with the production of altered effects, leading to the development of drug resistance [3].

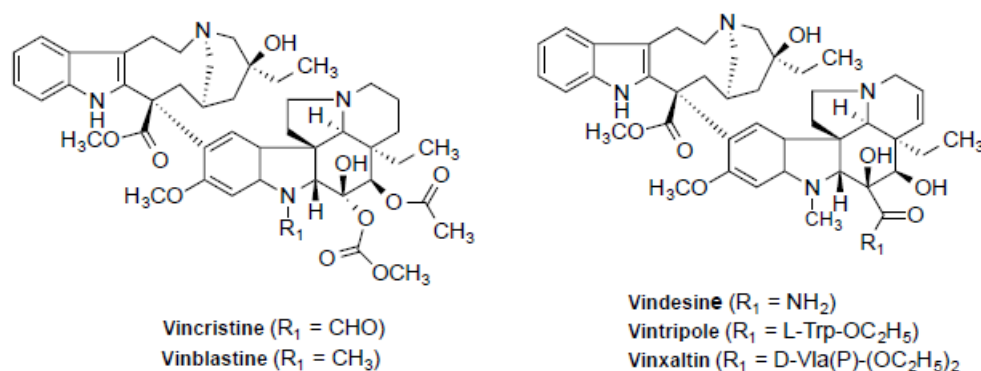


Figure 1.7: Most used vinca alkaloids

As taxanes, vinca alkaloids have extremely complex structures: it is important to synthesise new compounds with an easier chemical structure.

### 1.3.3 Colchicine alkaloid site binding agents

Colchicine is an alkaloid derived from the plant of the Lily family *Colchicum autumnale*. It was first extracted and isolated in 1883 and it has been used to treat gout and familial Mediterranean fever [47]. At therapeutic concentrations, colchicine has  $\text{IC}_{50}$  of 300 nM [11].

Colchicine binding site of tubulin is one of the most important pockets that have been focused on to design tubulin-destabilizing agents. The colchicine domain is divided in three zones: *zone 1*, located at the  $\alpha$  subunit interface; *zone 2*, an accessory hydrophobic pocket located in the  $\beta$  subunit and *zone 3*, buried deeper in the  $\beta$  subunit [27].

Its binding results in the formation of a tubulin-colchicine complex that prevents the formation of microtubules due to induced conformational change in tubulin dimer, making it incompetent for microtubule assembly [19]: this physically prevents the elongation of microtubule polymer [47].

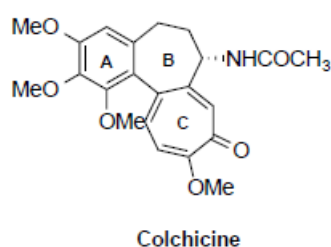


Figure 1.8

The simple structure of colchicine is reported in fig 1.8 can be broken up in three sections:

- **Ring A:** the trimethoxyphenyl moiety is fundamental; it plays a crucial role in interacting with tubulin: the insertion of a bulky group in ring A or the replacement of trimethoxyphenyl caused loss of activity [5].

To improve the compounds solubility, that ring can be replaced by urea moiety: N-phenyl-N'-(2-chloroethyl)urea is present in a very good derivative; that moiety is also responsible for the covalent interaction with the tubulin [52]. Another important structure used as covalent inhibitor is pentafluorophenyl [51].

- **Ring B:** the seven-membered ring and the C-7 side chain (that can be replaced with the bulky groups tert-butyl carbamate or N-phenylurea [19]) are not believed to be crucial for tubulin binding, even if they can affect the conformation of colchicinoids and their other tubulin binding properties [36].

That fused ring can be replaced by a bridge that is able to give the correct orientation to ring A and C to fit in the cavities of zone 1 and zone 2, respectively. That bridge can be a single atom, a cis-olefin or some heterocycles [27], as described in the next subsection.

- **Ring C:** it can accommodate into zone 1 on colchicine domain and usually forms a hydrogen bond with the residues around. Only two proper groups can accommodate in zone 1: 4-methoxyphenyl (such as in CA-4), that is a similar tropolone motif, and indoles; in both cases, the presence of a paramethoxy group is required for the biological activity [27].

Lots of compounds that bind tubulin at the colchicine binding site have been identified (both natural and synthetic); one of the most important is combretastatin A-4 (CA-4): since compounds that have been synthesised in this work are its derivatives, the next subsection has been dedicated to CA-4.

The colchicine site binders can also exhibit antiangiogenesis and vascular disruption activities, which are not found in other site binders [27] (topic detailed in the next section).

### 1.3.4 Combretastatins

The combretastatins are isolated from the South African tree *combretum caffrum kuntze*. The lead compound CA-4 (fig 1.9) has displayed remarkable cytotoxic effect in a wide variety of preclinical tumor models and inhibits tubulin polymerization by interacting at the colchicine binding site of microtubules [6]. CA-4 inhibited cell growth by 50% at 7nM and suppressed tubulin polymerization by 50% at 2.5  $\mu$ M in human bladder cancer cells [6].

Combretastatins are Z-stilbenoid products, consist of two substituted aromatic (aryl) rings linked by an olefinic bridge. The SAR of CA-4, that is one of the most powerful inhibitors of tubulin polymerization [48], is reported in fig 1.9:

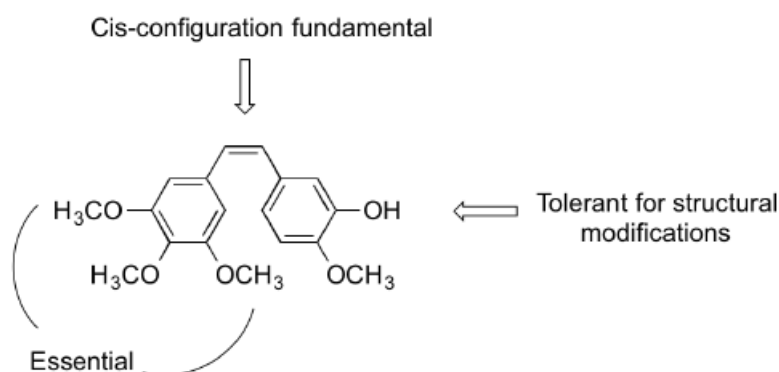


Figure 1.9: Combretastatin A-4

- The presence of a cis-configuration double bond in the C-2 bridge is fundamental to achieve high cytotoxicity as well as antitubulin activity [6].

Hydrogenation of the double bond leads to erianin, that retains activity but is less potent. Studies extending the bridge via methylene, ethylene, propylene and butylene revealed that two carbon linkers were the most active and other linkers were less active both in cytotoxicity and tubulin assays [27].

- The trimethoxyphenyl moiety is considered essential to obtain considerable efficient binding to tubulin.
- 3,4,5-trimethoxysubstituted A ring and the 4-methoxy substituted B ring are also very important for the substantial cytotoxic activity and efficiently binding at colchicine binding site of microtubule; however, B ring structural modification were tolerated by the target.

Although CA-4 is a promising clinical candidate, with very low side effects associated, it bears some very important issues: it has a very low solubility, a short biological half-life and undergoes to cis-trans isomerization in heat, light and protic media, forming the totally inactive trans form from the active cis form [20].

To improve the solubility of CA-4, its phosphate salt (CA-4P) has been synthesised, with very good results: this compound is still in clinical development for the treatment of ovarian and other cancers [18]. As mentioned before, compounds that bind to colchicine binding site also act as vascular disrupting agents (VDAs): it has been proved that CA-4P has this dual activity. The antivascular effect of this molecule derives from the role tubulin and microtubules play in determining the elongated shape of vascular endothelial cells. Since the cellular microtubule networks of the cytoskeleton plays a major role in maintaining cell shape, particularly in the case of the neovasculature, the result of the tubulin depolymerization is that endothelial cells round up: the blood flow is blocked very quickly through the vascular network [6]. As other VDAs, CA-4P is ineffective as monotherapy, since a thin peripheral rim of cells (thought to receive nutrients from the host vasculature) survives, even after destruction of the tumor vasculature. While the tumor center may necrose, the rim often repopulates rapidly. As such, several VDAs have been tested in combination with additional therapies, including radiotherapy, antiangiogenic agents (such as bevacizumab), traditional cytotoxic chemotherapy (like carboplatin, paclitaxel) and recently immunotherapy [29].

Even if CA-4P studies are very promising, the main issue is not resolved: in vivo, it isomerize to the inactive trans form. So, it is necessary to fix the conformational orientation of rings A and B: one approach, followed also in this work, is the rigidification of the double bond through the insertion of an appropriate heterocycle ring.

### Mechanisms of multidrug resistance

One of the major hurdles that continue to limit current anticancer agents is the development of drug resistance. Multidrug resistance (MDR) is the simultaneous resistance to a number of structurally and functionally unrelated chemotherapeutic drugs and is a substantial obstacle impeding the success of anticancer agents. Many cancers initially respond well to chemotherapy early on during treatment, but subsequently develop acquired resistance: more than 90% of patient with metastatic cancer fail to respond or to relapse from chemotherapeutics [2].

Two major mechanisms of MDR have been proposed:

- **Noncellular drug resistance:** is the inherent capacity of tumor cells to survive chemotherapy. It is always mediated by tumor microenvironment and is typically associated with solid tumors having unique properties, such as heterogeneous tumor vasculature, high interstitial fluid pressure, increased presence of noncycling tumors caused by insufficient supply of nutrients and oxygen as well as acidic environment.
- **Cellular drug resistance:** arise inside solid tumors; some examples are: elevated DNA repair, increased drug metabolism, altered apoptotic pathways to bypass drug targets, loss or change of drug target proteins and increased efflux of anticancer drugs.

To limit the MDR is thus very important to get new compounds that bear more than one activity. In this thesis two main classes of compound with this characteristic have been synthesised: compounds targeting tubulin and that are able to act as VDAs or as HDAC inhibitors.

### 1.4 Vascular disrupting agents

Solid tumor growth beyond about 1-3 mm in diameter depends extensively on angiogenesis initiating neovasculature for the supply of nutrient and oxygen to the fast growing tumor cells [29] and to remove toxic metabolites from the tumor site [10].

## 1. INTRODUCTION

Tumor vasculature presents abnormal and disorganized structures, lacking of the conventional blood vessel hierarchy. Arteries, capillaries and venules are not recognizable and they are tortuous, hyperpermeable and immature [37]. Furthermore, the high density of abnormal intratumoral blood vessels allows cancer cells to easily gain access to the blood thus triggering metastasis process [10].

Vascular-targeted anticancer therapy include two broad categories of agents with complementary mechanisms of action [18]:

1. **AAs, antiangiogenic agents:** they prevent tumor neovascularization by inhibiting vascular endothelial growth factor and other pro-angiogenic factors.
2. **VDAs, vascular disrupting agents:** they destroy established tumor vasculature. They are not effective alone, and this is attributable to a remaining viable rim of tumor cells that are supported by oxygen and nutrients from the surrounding normal vasculature.

As reported in fig 1.10, VDA activity results from microtubule disruption in activated endothelial cells, which initiates a signaling pathway characterized by profound cytoskeletal and morphological changes. Consequently, endothelial cells round up, leading to enhanced vascular leakage, and detachment from each other and from the underlying substratum to clog the tumor blood vessels. Direct vascular disruption is predicted to cause massive downstream starvation and hypoxiation, thereby potentiating the local effect and generating extensive necrosis. Thus, local damage generates massive amplification and tumor destruction [29].

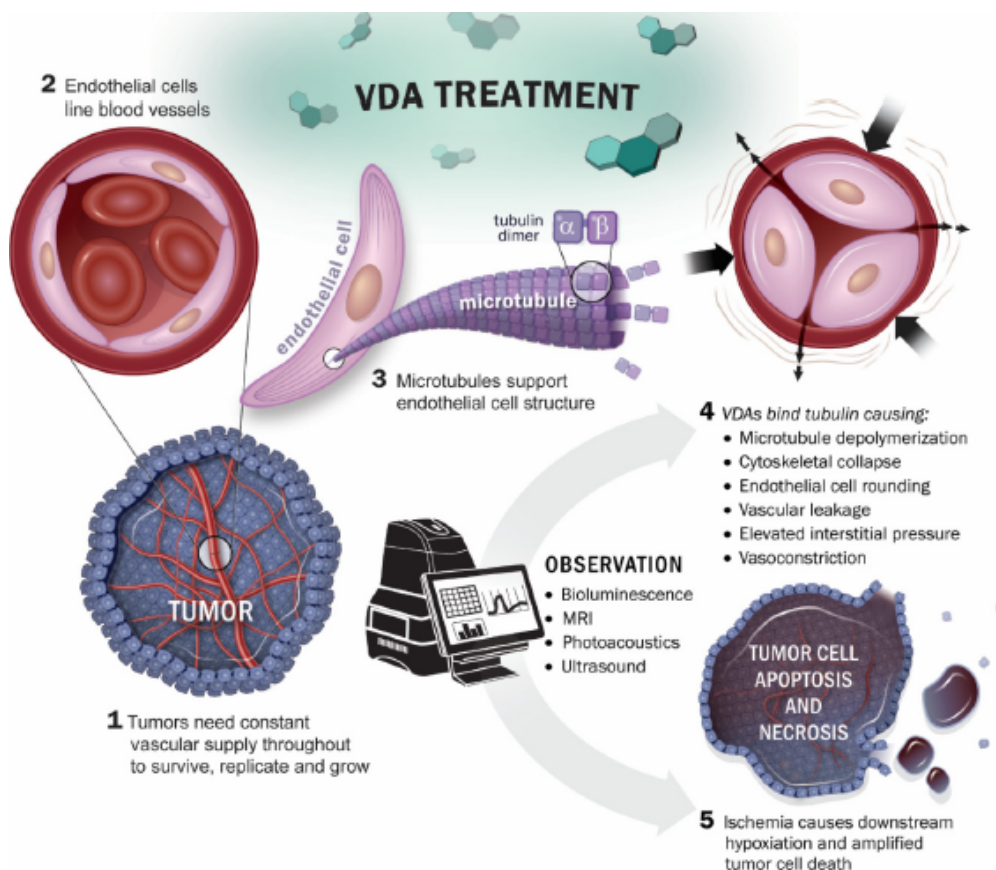


Figure 1.10: Imaging the action of VDAs on tumor-associated blood vessel [29]

### 1.5 Dual activity

Despite the continuous pharmacological and clinical progress of chemotherapeutic agents in treatment of cancer, most of the currently approved drugs based on the “single-target

single drug” design are becoming less and less effective in treatment of the complex, heterogeneous, multigenic cancer disease. As previously reported, this may be attributed to drug-resistance as well as to lack of selectivity.

It is now recognized that cancer is a multi-factorial disease caused by genetic and/or epigenetic alterations leading to the dysregulation of several and different pathways through diverse molecular mechanisms [4].

Actually there are three different approaches in use either in preclinical and/or clinical settings:

- The concomitant or simultaneous combination of two or more drugs acting on different targets; nevertheless, drug-combination therapies are often negated by adverse drug - drug interactions, unpredictable pharmacokinetic (PK) and safety profiles, and poor patient compliance [45].
- Multi-targeting or promiscuous drugs showing a wide variety of biological activities with probable adverse reactions [1].
- The smart hybridization of at least two different pharmacophore entities in a single drug; such so called ‘chimeras’ are able to simultaneously inhibit multiple cancer targets [26]. An hybrid compound guarantees the simultaneous transport of the two pharmacologically active entities to the tumor site in the required optimal ratio [4].

In recent years, multitarget therapy has been considered an effective strategy to achieve higher therapeutic efficacy, in particular dual-target drugs. As reported in fig 1.11, in terms of the synergetic effect of tubulin and other antitumor agents such as receptor tyrosine kinases inhibitors, histone deacetylases inhibitors, DNA-damaging agents, and topoisomerase inhibitors in combination therapy, designing dual-target tubulin inhibitors is regarded as a promising approach to overcome the limitations reported above and improve therapeutic efficacy.

Dual-target drugs not only retain the advantages of combination therapy but also circumvent the limitations of combination therapy. For example, dual-target drugs should have no or lower risks of drugdrug interactions due to one integrated molecule, compared to multiple drugs of combination therapy. The reduction of polypharmacy leads to lower adverse reactions and simplifies dosage regimens, that optimizes patient compliance. Another advantage for dual-target drugs is that they may possess more easily predictable PK profiles compared to multiple drugs used in combination therapy. Additionally, the development of target-based resistance of dual-target drugs is statistically lower than that of single-target drugs. Dual-target drugs also can overcome compensatory mechanisms which may limit the efficacy of single-target drugs. Moreover, the development of a dual-target drug may require fewer clinical trials than the combination therapy and have a similar cost and risk to those of a single-target agent [46].

### Design approaches for dual-target drugs

Since the design of dual-target drugs is more complicated than that of single-target agents, increased efforts have been made to develop them via diverse strategies. There are generally three design approaches, as reported in fig 1.11:

1. **Drug repurposing** is the application of known drugs to new therapeutic areas. Compared to the traditional drug discovery approaches, it is less expensive and has shorter development processes.
2. **Pharmacophore-based** can integrate the activities of multiple selective inhibitors into a single molecule. This strategy combines main pharmacophores of selected parent inhibitors via *linking* (where molecules often possess a large molecular weight (MW) -with potential unfavorable bioavailability and physicochemical properties- and



sometimes linkers may interfere with the interactions between targets and ligands) or *merging* (where it is maximized the overlapping level of pharmacophores; it is similar to a hybrid design -it requires active molecules to share similar pharmacophores-).

3. **Computational approach** facilitates the development of dual-target tubulin inhibitors with novel scaffold, through ligand-based and structure-based screening, and enlarges structural diversity.

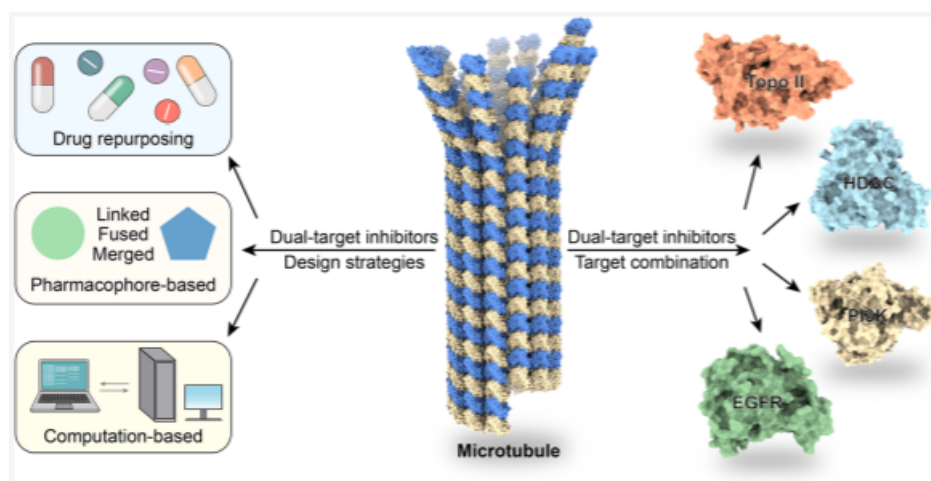


Figure 1.11: Design approaches for dual-target drugs [46]

### 1.5.1 HDAC inhibitors

There is a powerful machinery that drive malignancies without affecting the DNA structure itself: **epigenetic alterations** are post-translational histone modifications, such as the acetylation of histone lysine residue, that are determined by the inverse action of histone acetyl-transferases (HATs) and histone deacetylases (HDACs: include four families with 18 different HDACs, [21]). In non-tumoral cells, HATs and HDACs work properly, but during cancer progressions their alterations may occur at any time, altering the structure of the chromatin, the accessibility for transcription factors and thus the transcription of genes.

In cancer cells, HDACs are often aberrantly overexpressed predominantly leading to the transcriptional repression of tumor suppressor genes, for examples genes encoding for  $\alpha$ -tubulin, p53 or Hsp90 [46]. Thus, histone deacetylase inhibitors (HDAC-is) can be a good class of new anti-cancer drugs [22].

Among all the isoforms of HDACs, the one that targets tubulin is **HDAC6**: it contributes to the degradation of aggregasomal proteins, since it is able to bind to both poly-ubiquitinated and dynein motor proteins as an adaptor protein to transport misfolded proteins along microtubules into aggregasomes, which are finally degraded by the lysosomes (aggregasome pathway). As result, an inhibition of HDAC6 results in a lower microtubule stability and consequently in an increased cell stress and cell death [43].

#### Structural features of HDAC inhibitors

Available crystallographic data have confirmed a highly conserved nature for the HDAC isoforms: they all feature a variably sized cavity on the surface and a narrow tunnel of mutable length and width, leading to a Zinc ion located in the active site [49].

Thus, a reliable pharmacophore for HDAC inhibitors has been established, as reported in fig 1.12: SAHA is an FDA approved drug, and it bears a **capping group** occupying the entrance area to the active site, a **zinc binding group (ZBG)** interacting with the zinc ion in the catalytic center and a **hydrophobic linker** connecting the two units through the enzyme's channel rime [22].

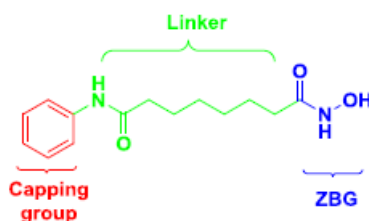


Figure 1.12: SAHA structure [55]

HDAC6 is the only isoform possessing two functional catalytic domains and an additional zinc finger, serving as an ubiquitin-binding domain (HDAC6 UBD). For the second catalytic domain (CD2) of HDAC6, the tunnel appears to be slightly more spacious but shorter, while the entrance area on the enzyme's surface is larger compared to other isoforms. HDAC6 is located predominantly in the cytoplasm, while HDAC1 is found in the nucleus of cells, where it is responsible for the eponymous deacetylation of histones [44]. Selectivity for HDAC6 can therefore be achieved by incorporating large cap groups and benzyl linkers instead of aliphatic chains [21].

### Molecular mechanisms of HDACis-promoted anticancer effects

HDAC are involved in a lot of cellular mechanisms responsible for cancer initiation and propagation. Thus, HDACis play more than one role in treating cancer.

The *induction of apoptotic cell death* represent one of the main anticancer effects promoted by HDAC inhibitors, since they can increase the expression or activity of proapoptotic proteins of the intrinsic pathway, such as Bax or Bak, or decrease the expression of antiapoptotic Bcl proteins such as Bcl-2 or Bcl-xL [21].

They are able to *induce autophagy*, that at the beginning serves as a survival mechanism upon cellular stress, but for a longer time it carries to cell death.

They are able to *induce senescence*, so a stable cell cycle arrest.

HDAC inhibitors are able to *induce DNA damage* through the induction of oxydative stress and the inhibition of the DNA repair machinery, with the subsequent accumulation of DNA lesions evoked by exogenous or endogenous mutagenes [22].

Finally, in hormone-dependent cancers, there are evidence suggesting that HDACis *affect the expression of hormone receptors* and can *influence the immune system* in many ways, often depending on the cellular context and the tumor microenvironment: they can affect dendritic cell activation and the antigen presenting machinery, as well as T-cell activation or the presence of regulatory T-cells and myeloid derived suppressor cells in the tumor microenvironment [22].

### 1.5.2 Dual inhibitors

Currently there are a lot of dual inhibitors at different stages of the clinical and preclinical research. Some examples are: HDAC/phosphoinositide-3-kinase $\alpha$  inhibitors, EGFR/HDAC inhibitors, HDACis/alkilating agents [22] and some that act as HDACis and TBAs.

One example of the last category is reported in fig 1.13: Compound 1 becomes from the merging of colchicine and the HDAC inhibitor SAHA. From the potent in vitro antiproliferative activity, is possible to state that the colchicine moiety is an appropriate capping group for HDAC inhibitors [55].

For HDAC-involved dual inhibitors, ZBG might contribute to the binding activities of both targets [55]; the best ZBG is represented from hydroxamic acid moiety, that is also able to facilitate water solubility of different compounds [46].

Docking analysis performed by Schmitt et al showed that the molecules with longer linker length tend to have a higher affinity to HDAC1 and HDAC6 [44].

Noteworthy are studies reported indicating the selective targeting of HDAC1 causes apoptosis while inhibition of HDAC6 causes angiogenesis and antimetastasis [56]. Thus, in

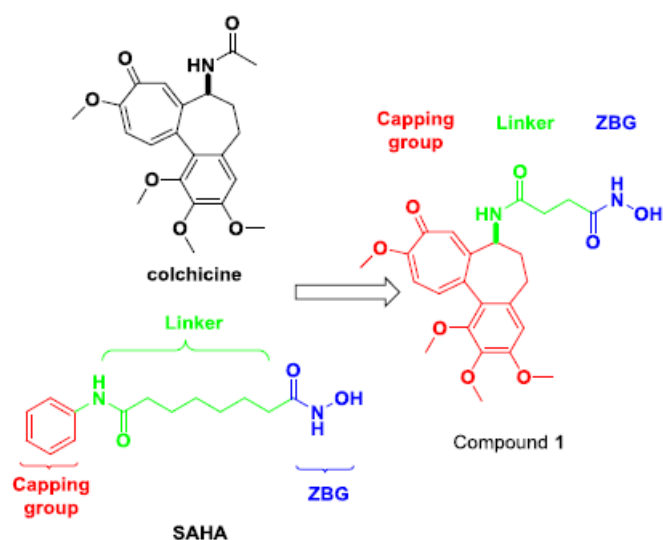


Figure 1.13: Design of novel tubulin-HDAC inhibitor [55]

this work one of the target is to synthesise compounds that are able to act as TBAs and also as HDAC1 or HDAC6 inhibitors.

## 1.6 Scopes of the thesis

As mentioned before, for a compound targeting only the dynamic of microtubules is not enough to reach the anti-tumoral activity: in this work, the aim is to synthesise compounds bearing more than one biological activity.

All designed and synthesised compounds are rigid analogues of CA-4: thus, these compounds should target tubulin at the colchicine binding site. With this purpose, the stilbene linker had been replaced by different five member rings (ring C): many works have been published, as reported in fig 1.14, and in all cases compounds had comparable or better activity than CA-4 against different tumor cell lines.

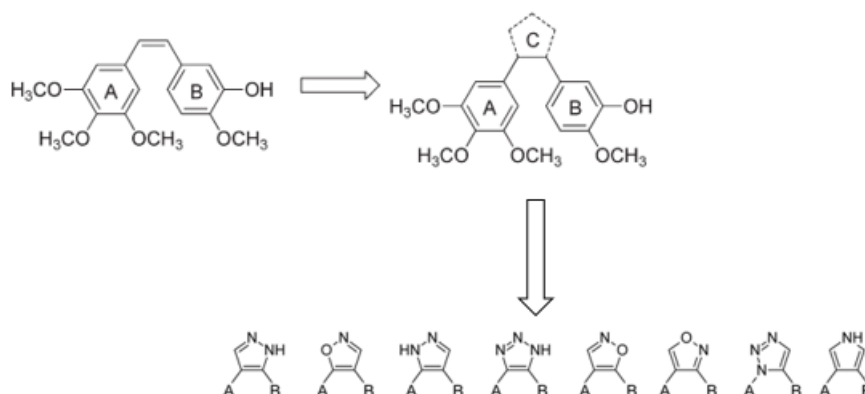


Figure 1.14: Rational for CA-4 constraintment

As mentioned above, a wide number of five member rings had been explored: in this work, **3,4-disubstituted pyrazole** had been the first heterocycle investigated. Both regioisomers had been taken in consideration, because in previous works there were significant differences in biological activities: 3',4',5'-trimethoxyphenyl moiety (ring A, that plays a crucial role in interacting with tubulin) had been placed first in position 3-, then in position 4-. In this case, structure-activity relationship (SAR) information was obtained by adding

## 1. INTRODUCTION

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electron-withdrawing ( $\text{OCF}_3$  and  $\text{CF}_3$ ) or electron-releasing (alkyl and alkoxy) groups on the second aryl ring, corresponding to ring B of CA-4, either in at the 4- or 3- position of the pyrazole nucleus. Ring B had also been replaced with a benzo[b]thien-2-yl moiety.

The second heterocycle investigated was **3,4-disubstituted pyrrole**. As for pyrazole analogues, ring A (3',4',5'-trimethoxyphenyl moiety) was maintained and ring B was chosen with the same consideration previously described.

With the purpose to get dual inhibitors, analogues esters of the best compounds were synthesised (**3,4,5-trimethoxyphenylpyrrol-1-yl esters**) just to check if the biological activity was maintained. Esters are easier to synthesise than hydroxamic acids (the ZBG of HDAC inhibitors), and for this reason esters had been synthesised first.

Another class synthesised was **1H-1,2,4-triazole-3,5-diamines**: compared to previous classes, the replacement of stilbene linker was bigger. Ring A was maintained, while ring C was constituted from a benzylamine, mainly decorated with small electron-withdrawing groups (the only exception was chlorine). In this class, the 3',4',5'-trimethoxyphenyl moiety had the possibility to assume different position: the insertion of the carbonyl group in the [1,2,4]triazolo [1,5-a]pyrimidine blocked its conformation, and generated the class of **7-N-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo [1,5-a]pyrimidine-2,7-diamines**.

Moving to a completely different class, the **hydroxamic acids derivatives** had been designed with the purpose of get compounds with dual activity: they should be both tubulin-inhibitors and HDAC-inhibitors.

Each compound synthesised was characterised with UPLC/MS and NMR analyses; biologist had the primary tests and other scientists performed specific experiments and computational work: the synthetic work will be described in the next chapter, while the job of other scientists will be presented in the "results" chapter.

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# CHAPTER 2

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## EXPERIMENTAL PART

In this chapter are reported the successful reactions performed to get the final compounds. For each series are reported a short description of the scheme of the general route and then the synthesis of each target compound, showed in the picture. Each synthesis begins from the first step, until the description of a common intermediate; other synthesis are described starting from that intermediate. In some examples, to get a large amount of one or more intermediates, reactions were performed more than once: for this reason, in these cases are reported different batches.

### 2.1 Synthesis of (3,4,5-trimethoxyphenyl)-1H-pyrazoles

Compounds belonging to this series came from the same synthetic route: each target is characterised by a pyrazole as core, a 3,4,5-trimethoxyphenyl ring at position 3 or 4 of the core and another decorated ring at position 4 or 3.

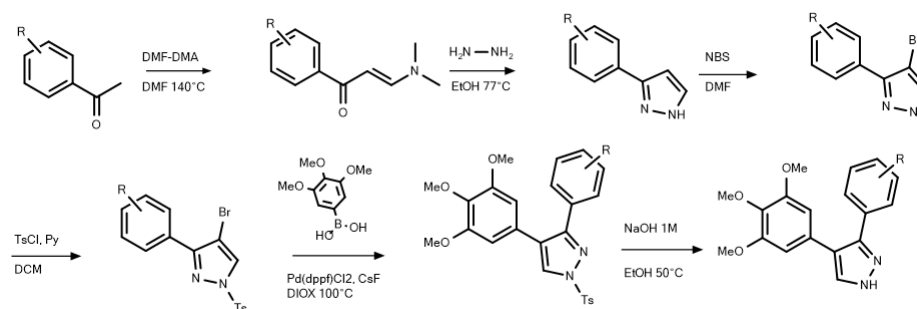


Figure 2.1: general route for 4-(3,4,5-trimethoxyphenyl)-1H-pyrazoles

The general route for the synthesis of 4-(3,4,5-trimethoxyphenyl)-1H-pyrazoles (fig 2.1) started from the reaction between DMF-DMA and the substituted acetophenone; the cyclization to form the pyrazole occurred with hydrazine. The core was first brominated and then protected on the N-H. The Suzuki's coupling with (3,4,5-trimethoxyphenyl)boronic acid gave the intermediate that, deprotected under basic conditions, afforded target compounds.

For other compounds of this series, the general route for the synthesis of 3-(3,4,5-trimethoxyphenyl)-1H-pyrazoles (fig 2.2) followed the same kind of reaction of the previous route and carried to a common intermediate (4-bromo-3-(3,4,5-trimethoxyphenyl)-1H-pyrazole), starting from 1-(3,4,5-trimethoxyphenyl)ethanone. At this point occurred the Suzuki's coupling with

## 2. EXPERIMENTAL PART

the appropriate boronic acid, giving the intermediates that, deprotected under basic conditions, afforded target compounds.

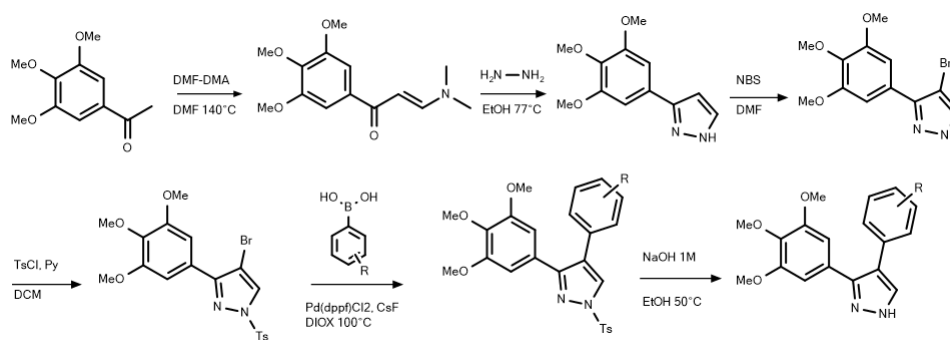


Figure 2.2: general route for 3-(3,4,5-trimethoxyphenyl)-1H-pyrazoles

### N0237-16 (TR998) 3-(4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrazole

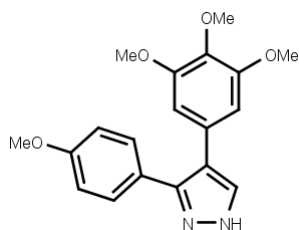


Figure 2.3: TR998

1,1-dimethoxy-*N,N*-dimethylmethanamine (10.64 mL, 79.91 mmol) was added to a stirred solution of 1-(4-methoxyphenyl)ethanone (3.0 g, 19.98 mmol) in DMF (8 mL). The reaction mixture was refluxed (145 °C) for 16h, then it was left to reach RT and concentrated. Et<sub>2</sub>O was added, the mixture stirred for 15 min and filtrated. Cake was washed with Et<sub>2</sub>O; it was left under vacuo for 2h, giving (*E*)-3-(dimethylamino)-1-(4-methoxyphenyl)prop-2-en-1-one (N0237-01-1: 3.076 g, 14.99 mmol, 75.02% yield) as yellow solid.

LCMS acidic r.t. 0.71 min, MS (ESI) *m/z*= 206.2 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ ppm 2.91 (3 H, br. s.) 3.12 (3 H, br. s.) 3.79 - 3.83 (3 H, m) 5.80 (1 H, d, *J*=12.32 Hz) 6.94 - 6.98 (2 H, m) 7.66 (1 H, d, *J*=12.10 Hz) 7.86 - 7.90 (2 H, m).

Hydrazine hydrate (0.33 mL, 4.38 mmol) was added to a stirred solution of (*E*)-3-(dimethylamino)-1-(4-methoxyphenyl)prop-2-en-1-one (0.75 g, 3.65 mmol) in Ethanol (15 mL). The reaction mixture was stirred at 77 °C for 4h, then solvent was evaporated. DCM and water were added: phases were separated, the organic one was washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 25g, Cy/EtOAc from 9:1 to 1:1), affording 3-(4-methoxyphenyl)-1H-pyrazole (N0237-03-1: 436 mg, 2.503 mmol, 68.5% yield) as pale yellow solid.

LCMS acidic r.t. 0.75 min, MS (ESI) *m/z*= 174.9 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ ppm 3.75 - 3.81 (3 H, m) 6.54 - 6.66 (1 H, m) 6.90 - 7.06 (2 H, m) 7.65 - 7.80 (3 H, m) 12.70 - 13.17 (1 H, m).

A solution of 1-bromopyrrolidine-2,5-dione (445.48 mg, 2.5 mmol) in DMF (1 mL) was added dropwise to a stirred solution of 3-(4-methoxyphenyl)-1H-pyrazole (436.0 mg, 2.5 mmol) in DMF (5 mL). The reaction mixture was stirred at RT for 2h, then a solution of NBS (0.1 eq) in DMF (0.4 mL) was added again. The reaction mixture was stirred at RT for 1h, then it was concentrated. Crude was dissolved in DCM and a saturated aq solution of NaHCO<sub>3</sub> were added: phases were separated, the organic one washed with brine, dried over sodium sulfate,

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filtered and concentrated. Crude was purified by flash chromatography (silica 25g, Cy/EtOAc from 9:1 to 6:4) affording *4-bromo-3-(4-methoxyphenyl)-1H-pyrazole* (N0237-06-1: 409 mg, 1.616 mmol, 64.56% yield) as white solid.

LCMS acidic r.t. 0.92 min, MS (ESI)  $m/z$  = 253.3 – 256.3 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 3.79 - 3.83 (3 H, m) 7.00 - 7.09 (2 H, m) 7.65 - 7.78 (3 H, m) 13.33 (1 H, br. s.).

4-methylbenzenesulfonyl chloride (369.7 mg, 1.94 mmol) was added to a stirred solution of *4-bromo-3-(4-methoxyphenyl)-1H-pyrazole* (409.0 mg, 1.62 mmol) and Pyridine (0.39 mL, 4.85 mmol) in DCM (5 mL). The reaction mixture was stirred at RT for 4.5h, then DCM and HCl 0.1 M were added: phases were separated, the organic one was washed with aq NaHCO<sub>3</sub> and brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 25g, Cy/EtOAc from 95:5 to 6:4) affording *4-bromo-3-(4-methoxyphenyl)-1-(4-methylphenyl)sulfonylpyrazole* (N0237-10-1: 370 mg, 0.908 mmol, 56.22% yield) as colourless oil.

LCMS acidic r.t. 1.31 min, MS (ESI)  $m/z$  = 407.5 – 410.4 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 2.39 - 2.42 (3 H, m) 3.79 - 3.82 (3 H, m) 7.03 - 7.07 (2 H, m) 7.49 - 7.53 (2 H, m) 7.68 - 7.73 (2 H, m) 7.92 - 7.97 (2 H, m) 8.87 (1 H, s).

A mixture of *4-bromo-3-(4-methoxyphenyl)-1-(4-methylphenyl)sulfonylpyrazole* (370.0 mg, 0.910 mmol), (3,4,5-trimethoxyphenyl)boronic acid (288.91 mg, 1.36 mmol) and CsF (344.99 mg, 2.27 mmol) was degassed for 10 min. [1,1-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) (66.66 mg, 0.090 mmol) was added and the mixture degassed again for 10 min. The reaction mixture was stirred at 100 °C for 2.5h, then it was left to reach RT; it was diluted with EtOAc and filtered, then concentrated. Crude was purified by flash chromatography (silica 25g + 25g, Cy/EtOAc from 95:5 to 6:4) affording *3-(4-methoxyphenyl)-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrazole* (N0237-14-1: 325 mg, 0.657 mmol, 72.34% yield) as yellowish solid.

LCMS acidic r.t. 1.31 min, MS (ESI)  $m/z$  = 495.1 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 2.40 - 2.43 (3 H, m) 3.63 (6 H, s) 3.66 (3 H, s) 3.75 - 3.77 (3 H, m) 6.59 (2 H, s) 6.94 - 6.99 (2 H, m) 7.30 - 7.38 (2 H, m) 7.50 - 7.55 (2 H, m) 7.94 - 8.00 (2 H, m) 8.72 - 8.75 (1 H, m).

A mixture of *3-(4-methoxyphenyl)-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrazole* (325.0 mg, 0.660 mmol) and sodium hydroxide (14.79 mL, 14.79 mmol) in Ethanol (45 mL) was stirred ON at 50 °C. The mixture was left to reach RT, then cooled in an ice bath and HCl 1M (14.79 mL) was added dropwise. Solvent was partially eliminated on vacuum, then a saturated aq solution of NaHCO<sub>3</sub> was added, followed by DCM: phases were separated, the organic one washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 25g, Cy/EtOAc from 8:2 to 2:8) affording *3-(4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrazole* (N0237-16-1: 88 mg, 0.259 mmol, 39.34% yield) as white foam.

LCMS acidic r.t. 0.91 min, MS (ESI)  $m/z$  = 341.1 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-d)  $\delta$  ppm 3.73 - 3.76 (6 H, m) 3.82 - 3.86 (3 H, m) 3.86 - 3.90 (3 H, m) 6.52 - 6.56 (2 H, m) 6.88 - 6.93 (2 H, m) 7.40 - 7.46 (2 H, m) 7.67 - 7.71 (1 H, m).

### N0237-17 (TRR12) *3-(4-methylphenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrazole*

1,1-dimethoxy-N,N-dimethylmethanamine (5.97 mL, 44.72 mmol) was added to a stirred solution of 1-(4-methylphenyl)ethanone (1.49 mL, 11.18 mmol) in DMF (4 mL). The reaction mixture was refluxed (145 °C) for 16h, then it was left to reach RT and concentrated. Et<sub>2</sub>O was added, the mixture stirred for 15 min and filtrated. Cake was washed with Et<sub>2</sub>O; it was left under vacuo on, giving (*E*)-*3-(dimethylamino)-1-(4-methylphenyl)prop-2-en-1-one* (N0237-02-1: 1.001 g, 5.289 mmol, 47.31% yield) as yellow solid.

LCMS acidic r.t. 0.79 min, MS (ESI)  $m/z$  = 190 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 2.35 (3 H, s) 2.91 (3 H, br. s.) 3.08 - 3.18 (3 H, m)

## 2. EXPERIMENTAL PART

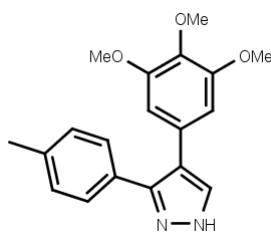


Figure 2.4: TRR12

5.81 (1 H, d, J=12.32 Hz) 7.21 - 7.26 (2 H, m) 7.68 (1 H, d, J=12.32 Hz) 7.77 - 7.81 (2 H, m).

Hydrazine hydrate (0.36 mL, 4.76 mmol) was added to a stirred solution of (E)-3-(dimethylamino)-1-(4-methylphenyl)prop-2-en-1-one (0.75 g, 3.96 mmol) in Ethanol (15 mL). The reaction mixture was stirred at 77 °C for 4h, then solvent was evaporated. DCM and water were added: phases were separated, the organic one washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 25g, Cy/EtOAc from 9:1 to 1:1) affording 3-(4-methylphenyl)-1H-pyrazole (N0237-04-1: 519 mg, 3.281 mmol, 82.78% yield) as yellowish oil.

LCMS acidic r.t. 0.84 min, MS (ESI) m/z= 158.9 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 2.29 - 2.36 (3 H, m) 6.61 - 6.69 (1 H, m) 7.16 - 7.31 (2 H, m) 7.47 - 7.67 (1 H, m) 7.67 - 7.79 (2 H, m) 12.73 - 13.25 (1 H, m).

A solution of 1-bromopyrrolidine-2,5-dione (583.89 mg, 3.28 mmol) in DMF (1.19 mL) was added dropwise to a stirred solution of 3-(4-methylphenyl)-1H-pyrazole (519.0 mg, 3.28 mmol) in DMF (5.952 mL). The reaction mixture was stirred at RT for 2h, then a solution of NBS (0.1 eq) in DMF (0.6 mL) was added dropwise. The reaction mixture was stirred at RT for 1h, then it was concentrated. DCM and a saturated aq solution of NaHCO<sub>3</sub> were added: phases were separated, the organic one was washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 25g + 25g, Cy/EtOAc from 9:1 to 1:1) affording 4-bromo-3-(4-methylphenyl)-1H-pyrazole (N0237-07-1: 390 mg, 1.645 mmol, 50.14% yield) as white solid.

LCMS acidic r.t. 1.00 min, MS (ESI) m/z= 237.3-240.3 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 2.34 - 2.38 (3 H, m) 7.26 - 7.34 (2 H, m) 7.68 (2 H, d, J=7.92 Hz) 7.78 - 7.97 (1 H, m) 13.38 (1 H, br. s.).

4-methylbenzenesulfonyl chloride (376.31 mg, 1.97 mmol) was added to a stirred solution of 4-bromo-3-(4-methylphenyl)-1H-pyrazole (390.0 mg, 1.64 mmol) and Pyridine (0.4 mL, 4.93 mmol) in DCM (4.768 mL). The reaction mixture was stirred at RT for 4.5h, then DCM and HCl 0.1 M were added: phases were separated, the organic one washed with saturated aq solution of NaHCO<sub>3</sub>, then brine. It was dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 25g, Cy/EtOAc from 95:5 to 8:2) affording 4-bromo-3-(4-methylphenyl)-1-(4-methylphenyl)sulfonylpyrazole (N0237-11-1: 244 mg, 0.624 mmol, 37.91% yield) as colourless oil.

LCMS acidic r.t. 1.39 min, MS (ESI) m/z= 393.4 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 2.35 (3 H, s) 2.40 (3 H, s) 7.28 - 7.33 (2 H, m) 7.49 - 7.54 (2 H, m) 7.63 - 7.67 (2 H, m) 7.93 - 7.98 (2 H, m) 8.88 - 8.91 (1 H, m).

A mixture of 4-bromo-3-(4-methylphenyl)-1-(4-methylphenyl)sulfonylpyrazole (240.0 mg, 0.610 mmol), (3,4,5-trimethoxyphenyl)boronic acid (195.06 mg, 0.920 mmol) and CsF (232.93 mg, 1.53 mmol) in 1,4-Dioxane (5.838 mL) was degassed for 10 min. [1,1-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) (45.0 mg, 0.060 mmol) was added: the mixture was degassed and then stirred at 100 °C for 2.5h. The reaction mixture was left to reach RT, diluted with EtOAc, filtered and concentrated. Crude was purified by flash chromatography (silica 25g+25g, Cy/EtOAc from 95:5 to 6:4) affording 3-(4-methylphenyl)-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrazole (N0237-15-1: 220 mg, 0.460 mmol, 74.95% yield) as white foam.

LCMS acidic r.t. 1.37 min, MS (ESI) m/z= 479.1 [M+H]<sup>+</sup>.



## 2. EXPERIMENTAL PART

$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  ppm 2.30 - 2.32 (3 H, m) 2.40 - 2.43 (3 H, m) 3.60 - 3.62 (6 H, m) 3.65 - 3.67 (3 H, m) 6.55 - 6.59 (2 H, m) 7.18 - 7.23 (2 H, m) 7.27 - 7.31 (2 H, m) 7.50 - 7.55 (2 H, m) 7.95 - 8.00 (2 H, m) 8.73 - 8.77 (1 H, m).

A mixture of 3-(4-methylphenyl)-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrazole (217.8 mg, 0.460 mmol) and sodium hydroxide (10.24 mL, 10.24 mmol) in Ethanol (29.91 mL) was stirred ON at 50 °C. The reaction mixture was left to reach RT, then it was cooled with an ice bath and HCl 1M (10.24 mL) was added dropwise. Solvent was partially eliminated under vacuum, then a saturated aq solution of  $\text{NaHCO}_3$  was added, followed by DCM: phases were separated, the organic one washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 25g, Cy/EtOAc from 8:2 to 3:7) affording 3-(4-methylphenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrazole (N0237-17-1: 77.6 mg, 0.239 mmol, 52.57% yield) as white solid.

LCMS acidic r.t. 0.99 min, MS (ESI)  $m/z$ = 325.2  $[\text{M}+\text{H}]^+$ .

$^1\text{H}$  NMR (400 MHz, chloroform- $d$ )  $\delta$  ppm 2.39 (3 H, s) 3.72 - 3.75 (6 H, m) 3.87 - 3.90 (3 H, m) 6.53 - 6.55 (2 H, m) 7.16 - 7.21 (2 H, m) 7.37 - 7.42 (2 H, m) 7.69 - 7.71 (1 H, m).

$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  ppm 2.28 - 2.37 (3 H, s) 3.63 (6 H, s) 3.65 - 3.66 (3 H, m) 6.50 - 6.59 (2 H, m) 7.14 - 7.30 (2 H, m) 7.32 - 7.39 (2 H, m) 7.70 - 8.01 (1 H, m) 12.91 - 13.15 (1 H, m).

### N0237-25 (TRR6) 4-[4-(trifluoromethyl)phenyl]-3-(3,4,5-trimethoxyphenyl)-1H-pyrazole

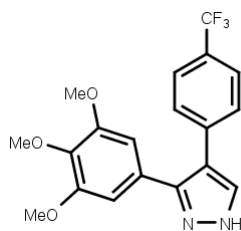


Figure 2.5: TRR6

*Batch 1* : 1,1-dimethoxy- $N,N$ -dimethylmethanamine (7.62 mL, 57.08 mmol) was added to a stirred solution of 1-(3,4,5-trimethoxyphenyl)ethanone (3.0 g, 14.27 mmol) in DMF (8 mL). The reaction mixture was refluxed (141 °C) for 24h, then it was left to reach RT and concentrated.  $\text{Et}_2\text{O}$  was added, the mixture stirred for 15 min and filtrated. Cake was washed with  $\text{Et}_2\text{O}$ ; it was left under vacuo for 2 h, giving (*E*)-3-(dimethylamino)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (N0237-05-1: 3.496 g, 13.18 mmol, 92.34% yield) as yellow solid.

LCMS acidic r.t. 0.69 min, MS (ESI)  $m/z$ = 266.1  $[\text{M}+\text{H}]^+$ .

$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  ppm 2.88 - 3.02 (3 H, m) 3.06 - 3.19 (3 H, m) 3.70 - 3.73 (3 H, m) 3.82 - 3.86 (6 H, m) 5.82 (1 H, d,  $J=12.32$  Hz) 7.14 - 7.19 (2 H, m) 7.66 - 7.72 (1 H, m).

*Batch 2*: 1,1-dimethoxy- $N,N$ -dimethylmethanamine (17.77 mL, 133.19 mmol) was added to a stirred solution of 1-(3,4,5-trimethoxyphenyl)ethanone (7.0 g, 33.3 mmol) in DMF (18.67 mL). The reaction mixture was refluxed (141 °C) for 24h, then it was left to reach RT and concentrated.  $\text{Et}_2\text{O}$  was added, the mixture stirred for 15 min and filtrated. Cake was washed with  $\text{Et}_2\text{O}$ ; it was left under vacuo for 2 h, giving (*E*)-3-(dimethylamino)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (N0237-13-1: 8.383 g, 31.6 mmol, 94.9% yield) as yellow solid.

LCMS acidic r.t. 0.72 min, MS (ESI)  $m/z$ = 266.2  $[\text{M}+\text{H}]^+$ .

$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  ppm 2.89 - 2.99 (3 H, m) 3.08 - 3.20 (3 H, m) 3.69 - 3.72 (3 H, m) 3.82 - 3.87 (6 H, m) 5.82 (1 H, d,  $J=12.10$  Hz) 7.15 - 7.19 (2 H, m) 7.66 - 7.72 (1 H, m).

*Batch 1*: Hydrazine hydrate (1.01 mL, 13.19 mmol) was added to a stirred solution of (*E*)-3-(dimethylamino)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (3.5 g, 13.19 mmol) in Ethanol (50 mL). The reaction mixture was stirred at 77 °C for 4h, then solvent

## 2. EXPERIMENTAL PART

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was evaporated. DCM and water were added: phases were separated, the organic one was washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 100g, Cy/EtOAc from 9:1 to 1:1), affording *3-(3,4,5-trimethoxyphenyl)-1H-pyrazole* (N0237-08-1: 2.141 g, 9.14 mmol, 69.28% yield) as yellowish foam.

LCMS acidic r.t. 0.70 min, MS (ESI)  $m/z=235.4$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 3.64 - 3.70 (3 H, m) 3.82 - 3.87 (6 H, m) 6.69 - 6.76 (1 H, m) 7.06 - 7.14 (2 H, m) 7.48 - 7.78 (1 H, m) 12.78 - 13.24 (1 H, m).

*Batch 2:* Hydrazine hydrate (2.32 mL, 30.32 mmol) was added to a stirred solution of (E)-3-(dimethylamino)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (8.38 g, 30.32 mmol) in Ethanol (114.93 mL). The reaction mixture was stirred at 77 °C for 4h, then solvent was evaporated. DCM and water were added: phases were separated, the organic one was washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 100g, Cy/EtOAc from 9:1 to 0:10), affording *3-(3,4,5-trimethoxyphenyl)-1H-pyrazole* (N0237-19-1: 7.877 g, 33.63 mmol, 110.89% yield) as yellowish oil.

LCMS acidic r.t. 0.71 min, MS (ESI)  $m/z=235.1$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, CHLOROFORM-d)  $\delta$  ppm 3.89 - 3.92 (3 H, m) 3.94 - 3.97 (6 H, m) 6.60 - 6.62 (1 H, m) 7.03 (2 H, s) 7.63 - 7.65 (1 H, m).

*Batch 1:* A solution of 1-bromopyrrolidine-2,5-dione (1.71 g, 9.59 mmol) in DMF (3 mL) was added dropwise to a stirred solution of 3-(3,4,5-trimethoxyphenyl)-1H-pyrazole (2.14 g, 9.14 mmol) in DMF (12 mL). The reaction mixture was stirred at RT for 2h, then a solution of NBS (0.1 eq) in DMF (0.4 mL) was added again. The reaction mixture was stirred at RT for 1h, then it was concentrated. Crude was dissolved in DCM and a saturated aq solution of NaHCO<sub>3</sub> were added: phases were separated, the organic one washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 100g, Cy/EtOAc from 9:1 to 6:4) affording *4-bromo-3-(3,4,5-trimethoxyphenyl)-1H-pyrazole* (N0237-09-1: 1.8 g, 5.748 mmol, 62.92% yield) as yellowish solid.

LCMS acidic r.t. 0.87 min, MS (ESI)  $m/z=315.3$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 3.71 (3 H, s) 3.82 - 3.85 (6 H, m) 6.99 - 7.18 (2 H, m) 7.91 (1 H, br. s.) 13.42 (1 H, br. s.).

*Batch 2:* A solution of 1-bromopyrrolidine-2,5-dione (6.28 g, 35.28 mmol) in DMF (11.03 mL) was added dropwise to a stirred solution of 3-(3,4,5-trimethoxyphenyl)-1H-pyrazole (7.87 g, 33.6 mmol) in DMF (44.13 mL). The reaction mixture was stirred at RT for 2h, then it was concentrated. Crude was dissolved in DCM and a saturated aq solution of NaHCO<sub>3</sub> were added: phases were separated, the organic one washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 340g, Cy/EtOAc from 9:1 to 6:4) affording *4-bromo-3-(3,4,5-trimethoxyphenyl)-1H-pyrazole* (N0237-20-1: 3.654 g, 11.67 mmol, 34.73% yield) as yellowish solid.

LCMS acidic r.t. 0.89 min, MS (ESI)  $m/z=315.0$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-d)  $\delta$  ppm 3.92 (3 H, s) 3.93 - 3.95 (6 H, m) 7.03 - 7.08 (2 H, m) 7.68 (1 H, s).

*Batch 1:* 4-methylbenzenesulfonyl chloride (1.315 g, 6.9 mmol) was added to a stirred solution of 4-bromo-3-(3,4,5-trimethoxyphenyl)-1H-pyrazole (1.8 g, 5.75 mmol) and Pyridine (1.39 mL, 17.24 mmol) in DCM (22 mL). The reaction mixture was stirred at RT for 4.5h, then DCM and HCl 0.1 M were added: phases were separated, the organic one was washed with aq NaHCO<sub>3</sub> and brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 50g, Cy/EtOAc from 95:5 to 6:4) affording *4-bromo-1-(4-methylphenyl)sulfonyl-3-(3,4,5-trimethoxyphenyl)pyrazole* (N0237-12-1: 1.74 g, 3.723 mmol, 64.77% yield) as white foam.

LCMS acidic r.t. 1.28 min, MS (ESI)  $m/z=469$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 2.39 - 2.42 (3 H, m) 3.71 (3 H, s) 3.81 - 3.83 (6 H, m)

## 2. EXPERIMENTAL PART

6.98 - 7.01 (2 H, m) 7.51 (2 H, dd, J=8.80, 0.66 Hz) 7.94 - 7.98 (2 H, m) 8.91 (1 H, s).

*Batch 2:* 4-methylbenzenesulfonyl chloride (2.53 g, 13.29 mmol) was added to a stirred solution of 4-bromo-3-(3,4,5-trimethoxyphenyl)-1H-pyrazole (3.65 g, 11.07 mmol) and Pyridine (2.67 mL, 33.22 mmol) in DCM (42.39 mL). The reaction mixture was stirred at RT on, then DCM and HCl 0.1 M were added: phases were separated, the organic one was washed with aq NaHCO<sub>3</sub> and brine, dried over sodium sulfate, filtered and concentrated. crude was purified by flash chromatography (silica 340g, Cy/EtOAc from 95:5 to 6:4) affording 4-bromo-1-(4-methylphenyl)sulfonyl-3-(3,4,5-trimethoxyphenyl)pyrazole (N0237-30-1: 4.03 g, 8.623 mmol, 77.88% yield) as white foam.

LCMS acidic r.t. 1.28 min, MS (ESI) m/z= 467.1 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, CHLOROFORM-d)  $\delta$  ppm 2.44 - 2.47 (3 H, m) 3.90 - 3.90 (3 H, m) 3.92 - 3.93 (6 H, m) 7.10 - 7.13 (2 H, m) 7.37 (2 H, d, J=7.92 Hz) 7.94 - 7.98 (2 H, m) 8.19 - 8.21 (1 H, m).

A mixture of 4-bromo-1-(4-methylphenyl)sulfonyl-3-(3,4,5-trimethoxyphenyl)pyrazole (270.0 mg, 0.580 mmol), [4-(trifluoromethyl)phenyl]boronic acid (164.6 mg, 0.870 mmol) and was degassed for 10 min. [1,1-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) (42.39 mg, 0.060 mmol) was added and the mixture degassed again for 10 min. The reaction mixture was stirred previously at 45 degrees for 20 min, then at 100 °C for 1.5h. It was left to reach RT, diluted with EtOAc and filtered, then concentrated. Crude was purified by flash chromatography (silica 25g + 25g, Cy/EtOAc from 95:5 to 6:4) affording 1-(4-methylphenyl)sulfonyl-4-[4-(trifluoromethyl)phenyl]-3-(3,4,5-trimethoxyphenyl)pyrazole (N0237-18-1: 253 mg, 0.475 mmol, 82.23% yield) as white foam.

LCMS acidic r.t. 1.40 min, MS (ESI) m/z= 533.1 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-d)  $\delta$  ppm 2.47 (3 H, s) 3.66 - 3.70 (6 H, m) 3.85 - 3.88 (3 H, m) 6.61 - 6.64 (2 H, m) 7.38 - 7.44 (4 H, m) 7.62 (2 H, d, J=7.92 Hz) 8.00 - 8.06 (2 H, m) 8.22 - 8.24 (1 H, m).

An aqueous solution 1N of sodium hydroxide (10.69 mL, 10.69 mmol) was added to a mixture of 1-(4-methylphenyl)sulfonyl-4-[4-(trifluoromethyl)phenyl]-3-(3,4,5-trimethoxyphenyl)pyrazole (253.0 mg, 0.480 mmol) in Ethanol (31.67 mL). The reaction mixture was stirred on at 50 °C, then it was left to reach RT and cooled at 0°C. HCl 1N (10.69 mL) was added and solvents were evaporated. A saturated aq solution of NaHCO<sub>3</sub> was added, followed by DCM: phases were separated, the organic one washed with water and brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 25g, Cy/EtOAc from 95:5 to 1:9) affording 4-[4-(trifluoromethyl)phenyl]-3-(3,4,5-trimethoxyphenyl)-1H-pyrazole (N0237-25-1: 96 mg, 0.254 mmol, 53.41% yield) as white foam.

LCMS acidic r.t. 1.11 min, MS (ESI) m/z= 379.1 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-d)  $\delta$  ppm 3.72 (6 H, s) 3.90 - 3.91 (3 H, m) 6.64 - 6.67 (2 H, m) 7.45 - 7.50 (2 H, m) 7.58 - 7.63 (2 H, m) 7.75 - 7.78 (1 H, m).

### N0237-26 (TR992) 4-naphthalen-2-yl-3-(3,4,5-trimethoxyphenyl)-1H-pyrazole

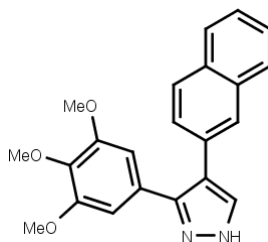


Figure 2.6: TR992

A mixture of 4-bromo-1-(4-methylphenyl)sulfonyl-3-(3,4,5-trimethoxyphenyl)pyrazole (257.03 mg, 0.550 mmol), 2-naphthalenylboronic acid (141.89 mg, 0.830 mmol) and CsF

## 2. EXPERIMENTAL PART

(208.86 mg, 1.38 mmol) in 1,4-Dioxane (7 mL) and few drops of water was degassed for 10 min. [1,1-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) (40.35 mg, 0.060 mmol) was added: the mixture was degassed again for 10 min, then stirred at 45 degrees for 20min and at 100 °C for 2h. The reaction mixture was left to reach RT, then it was diluted with EtOAc and filtered. Solvents were evaporated and crude purified by flash chromatography (silica 25g + 25g, Cy/EtOAc from 95:5 to 6:4) affording *1-(4-methylphenyl)sulfonyl-4-naphthalen-2-yl-3-(3,4,5-trimethoxyphenyl)pyrazole* (N0237-21-1: 239 mg, 0.464 mmol, 84.44% yield) as yellowish foam.

LCMS acidic r.t. 1.42 min, MS (ESI)  $m/z = 515.2$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-d)  $\delta$  ppm 2.47 (3 H, s) 3.57 - 3.60 (6 H, m) 3.84 - 3.86 (3 H, m) 6.73 - 6.75 (2 H, m) 7.35 (1 H, dd,  $J=8.58, 1.76$  Hz) 7.40 (2 H, d,  $J=7.92$  Hz) 7.49 - 7.53 (2 H, m) 7.77 - 7.87 (4 H, m) 8.02 - 8.07 (2 H, m) 8.27 (1 H, s).

An aqueous solution 1N of sodium hydroxide (10.45 mL, 10.45 mmol) was added to a stirred solution of *1-(4-methylphenyl)sulfonyl-4-naphthalen-2-yl-3-(3,4,5-trimethoxyphenyl)pyrazole* (239.0 mg, 0.460 mmol) in Ethanol (30.96 mL). The reaction mixture was stirred on at 50 °C, then it was left to reach RT and cooled at 0°C. HCl 1N was added (10.45 mL) and solvents evaporated. A saturated aq solution of NaHCO<sub>3</sub> was added, followed by DCM: phases were separated, the organic one washed with water and brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 25g, Cy/EtOAc from 8:2 to 0:10) affording *4-naphthalen-2-yl-3-(3,4,5-trimethoxyphenyl)-1H-pyrazole* (N0237-26-1: 87 mg, 0.241 mmol, 51.97% yield) as white foam.

LCMS acidic r.t. 1.11 min, MS (ESI)  $m/z = 361.2$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-d)  $\delta$  ppm 3.59 - 3.63 (6 H, m) 3.88 - 3.91 (3 H, m) 6.75 - 6.79 (2 H, m) 7.43 - 7.51 (3 H, m) 7.75 - 7.85 (4 H, m) 7.86 - 7.89 (1 H, m).

### N0237-27 (TRR13) 4-(3-chloro-4-methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)-1H-pyrazole

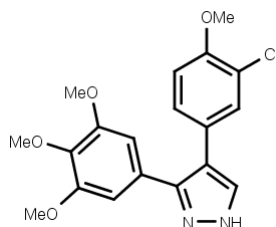


Figure 2.7: TRR13

A mixture of 4-bromo-1-(4-methylphenyl)sulfonyl-3-(3,4,5-trimethoxyphenyl)pyrazole (0.26 g, 0.550 mmol), (3-chloro-4-methoxyphenyl)boronic acid (153.78 mg, 0.830 mmol) and CsF (208.86 mg, 1.38 mmol) in 1,4-Dioxane (7 mL) and few drops of water was degassed for 10 min. [1,1-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) (40.35 mg, 0.060 mmol) was added and the mixture was degassed for other 10 min, then it was stirred at 45 degrees for 20 min and at 100 °C for 2h. The reaction mixture was left to reach RT: EtOAc was added and it was filtered. Solvents were evaporated and crude purified by flash chromatography (silica 25g + 25g, Cy/EtOAc from 95:5 to 1:1) affording *4-(3-chloro-4-methoxyphenyl)-1-(4-methylphenyl)sulfonyl-3-(3,4,5-trimethoxyphenyl)pyrazole* (N0237-22-1: 250 mg, 0.473 mmol, 85.93% yield) as colourless oil.

LCMS acidic r.t. 1.35 min, MS (ESI)  $m/z = 529.2$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-d)  $\delta$  ppm 3.72 (6 H, s) 3.87 (3 H, s) 3.92 - 3.94 (3 H, m) 6.71 (2 H, s) 6.88 - 6.91 (1 H, m) 7.12 (1 H, dd,  $J=8.47, 2.09$  Hz) 7.36 (1 H, d,  $J=2.20$  Hz) 7.37 - 7.40 (2 H, m) 8.00 (2 H, d,  $J=8.36$  Hz) 8.13 (1 H, s).

An aq solution 1N of sodium hydroxide (10.63 mL, 10.63 mmol) was added

## 2. EXPERIMENTAL PART

to a stirred mixture of 4-(3-chloro-4-methoxyphenyl)-1-(4-methylphenyl)sulfonyl-3-(3,4,5-trimethoxyphenyl)pyrazole (250.0 mg, 0.470 mmol) in Ethanol (31.51 mL). The reaction mixture was stirred on at 50 °C. The reaction mixture was left to reach RT, then cooled at 0°C: HCl 1N was added (10.63 mL) and solvents evaporated. A saturated aq solution of NaHCO<sub>3</sub> was added, followed by DCM: phases were separated, the organic one washed with water and brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 25g, Cy/EtOAc from 8:2 to 0:10) affording 4-(3-chloro-4-methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)-1H-pyrazole (N0237-27-1: 32 mg, 0.085 mmol, 18.07% yield) as white foam.

LCMS acidic r.t. 1.01 min, MS (ESI) m/z= 375.1 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-d) δ ppm 3.72 - 3.74 (6 H, m) 3.89 - 3.90 (3 H, m) 3.92 - 3.93 (3 H, m) 6.69 - 6.74 (2 H, m) 6.91 (1 H, d, J=8.58 Hz) 7.19 (1 H, dd, J=8.36, 2.20 Hz) 7.40 - 7.43 (1 H, m) 7.65 - 7.68 (1 H, m).

### N0237-31 (TRR11) 4-(3,4-dimethylphenyl)-3-(3,4,5-trimethoxyphenyl)-1H-pyrazole

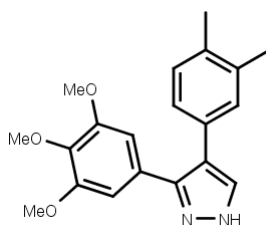


Figure 2.8: TRR11

A mixture of 4-bromo-1-(4-methylphenyl)sulfonyl-3-(3,4,5-trimethoxyphenyl)pyrazole (0.26 g, 0.550 mmol), 3,4-dimethylbenzeneboronic acid (123.73 mg, 0.830 mmol) and CsF (208.86 mg, 1.38 mmol) in 1,4-Dioxane (7 mL) and few drops of water was degassed for 10 min. [1,1-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) (40.35 mg, 0.060 mmol) was added: the mixture was degassed for 10 min, then stirred at 45°C for 20 min and at 100 °C for 80 min. The reaction mixture was left to reach RT, it was diluted with EtOAc and filtered: solvents were evaporated and crude purified by flash chromatography (silica 25g + 25g, Cy/EtOAc from 95:5 to 6:4) affording 4-(3,4-dimethylphenyl)-1-(4-methylphenyl)sulfonyl-3-(3,4,5-trimethoxyphenyl)pyrazole (N0237-23-1: 222 mg, 0.451 mmol, 81.94% yield) as yellowish foam.

LCMS acidic r.t. 1.43 min, MS (ESI) m/z= 493.1 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-d) δ ppm 2.24 (3 H, s) 2.28 (3 H, s) 2.43 - 2.47 (3 H, m) 3.66 - 3.70 (6 H, m) 3.83 - 3.88 (3 H, m) 6.71 - 6.76 (2 H, m) 7.00 (1 H, dd, J=7.70, 1.76 Hz) 7.08 - 7.10 (1 H, m) 7.11 - 7.12 (1 H, m) 7.34 - 7.40 (2 H, m) 7.98 - 8.02 (2 H, m) 8.10 - 8.13 (1 H, m).

An aq solution 1N of sodium hydroxide (10.14 mL, 10.14 mmol) was added to a stirred solution of 4-(3,4-dimethylphenyl)-1-(4-methylphenyl)sulfonyl-3-(3,4,5-trimethoxyphenyl)pyrazole (222.0 mg, 0.450 mmol) in Ethanol (30.05 mL). The reaction mixture was stirred on at 50 °C. The mixture was left to reach RT and cooled at 0°C: HCl 1N was added (10.14 mL) and solvents evaporated. A saturated aq solution of NaHCO<sub>3</sub> was added, followed by DCM: phases were separated, the organic one washed with water and brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 25g, Cy/EtOAc from 8:2 to 3:7) affording 4-(3,4-dimethylphenyl)-3-(3,4,5-trimethoxyphenyl)-1H-pyrazole (N0237-31-1: 61 mg, 0.180 mmol, 40% yield) as white foam.

LCMS acidic r.t. 1.10 min, MS (ESI) m/z= 339.2 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-d) δ ppm 2.24 - 2.26 (3 H, m) 2.28 (3 H, s) 3.67 - 3.70 (6 H, m) 3.87 - 3.90 (3 H, m) 6.73 - 6.77 (2 H, m) 7.07 - 7.12 (2 H, m) 7.14 - 7.18 (1 H, m) 7.65 -

## 2. EXPERIMENTAL PART

7.68 (1 H, m).

### N0237-32 (TRR4) 4-[4-(trifluoromethoxy)phenyl]-3-(3,4,5-trimethoxyphenyl)-1H-pyrazole

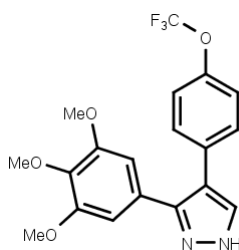


Figure 2.9: TRR4

A mixture of 4-bromo-1-(4-methylphenyl)sulfonyl-3-(3,4,5-trimethoxyphenyl)pyrazole (0.26 g, 0.550 mmol), [4-(trifluoromethoxy)phenyl]boronic acid (169.89 mg, 0.830 mmol) and CsF (208.86 mg, 1.38 mmol) in 1,4-Dioxane (7 mL) and few drops of water was degassed for 10 min. [1,1-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) (40.35 mg, 0.060 mmol) was added: the mixture was degassed for 10 min, then stirred at 45°C for 20 min and at 100 °C for 70 min. The mixture was left to reach RT, diluted with EtOAc, filtered and concentrated. Crude was purified by flash chromatography (silica 25g + 25g, Cy/EtOAc from 95:5 to 6:4) affording 1-(4-methylphenyl)sulfonyl-4-[4-(trifluoromethoxy)phenyl]-3-(3,4,5-trimethoxyphenyl)pyrazole (N0237-24-1: 236 mg, 0.430 mmol, 78.23% yield) as white foam.

LCMS acidic r.t. 1.42 min, MS (ESI)  $m/z = 549$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-d)  $\delta$  ppm 2.45 - 2.48 (3 H, m) 3.66 - 3.69 (6 H, m) 3.84 - 3.87 (3 H, m) 6.63 - 6.65 (2 H, m) 7.21 - 7.25 (2 H, m) 7.30 - 7.34 (2 H, m) 7.37 - 7.41 (2 H, m) 8.00 - 8.04 (2 H, m) 8.18 (1 H, s).

An aq solution 1N of sodium hydroxide (9.68 mL, 9.68 mmol) was added to a stirred mixture of 1-(4-methylphenyl)sulfonyl-4-[4-(trifluoromethoxy)phenyl]-3-(3,4,5-trimethoxyphenyl)pyrazole (236.0 mg, 0.430 mmol) in Ethanol (28.68 mL). The reaction mixture was stirred on at 50 °C, then it was left to reach RT and cooled st 0°C: HCl 1N (9.68 mL) was added and solvents evaporated. A saturated solution of NaHCO<sub>3</sub> was added, followed by DCM: phases were separated, the organic one washed with water and brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 25g, Cy/EtOAc from 8:2 to 3:7) affording 4-[4-(trifluoromethoxy)phenyl]-3-(3,4,5-trimethoxyphenyl)-1H-pyrazole (N0237-32-1: 90 mg, 0.228 mmol, 53.05% yield) as white solid.

LCMS acidic r.t. 1.14 min, MS (ESI)  $m/z = 395.1$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-d)  $\delta$  ppm 3.67 - 3.70 (6 H, m) 3.88 - 3.90 (3 H, m) 6.63 - 6.68 (2 H, m) 7.21 (2 H, ddt, J=7.73, 2.12, 1.13, 1.13 Hz) 7.36 - 7.40 (2 H, m) 7.69 - 7.71 (1 H, m).

### N0237-33 (TR87) 4-(4-methylphenyl)-3-(3,4,5-trimethoxyphenyl)-1H-pyrazole

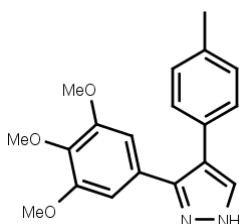


Figure 2.10: TR87

## 2. EXPERIMENTAL PART

A mixture of 4-bromo-1-(4-methylphenyl)sulfonyl-3-(3,4,5-trimethoxyphenyl)pyrazole (0.22 g, 0.470 mmol), (4-methylphenyl)boronic acid (96.01 mg, 0.710 mmol) and CsF (178.77 mg, 1.18 mmol) in 1,4-Dioxane (7 mL) and few drops of water was degassed for 10 min. [1,1-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) (34.54 mg, 0.050 mmol) was added: the mixture was degassed again for 10 min, then stirred at 45°C for 20 min and 100 °C for 2h. The reaction mixture was left to reach RT, then it was diluted with EtOAc, filtered and concentrated. Crude was purified by flash chromatography (silica 25g +25g, Cy/EtOAc from 95:5 to 6:4) affording 4-(4-methylphenyl)-1-(4-methylphenyl)sulfonyl-3-(3,4,5-trimethoxyphenyl)pyrazole (N0237-28-1: 208 mg, 0.435 mmol, 92.33% yield) as colourless oil.

LCMS acidic r.t. 1.39 min, MS (ESI)  $m/z = 479.1$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-d)  $\delta$  ppm 2.37 (3 H, s) 2.44 - 2.48 (3 H, m) 3.68 (6 H, s) 3.84 - 3.88 (3 H, m) 6.68 - 6.73 (2 H, m) 7.19 (4 H, s) 7.38 (2 H, d,  $J = 7.92$  Hz) 7.98 - 8.02 (2 H, m) 8.11 - 8.13 (1 H, m).

A solution 1M of sodium hydroxide (9.78 mL, 9.78 mmol) was added to a stirred mixture of 4-(4-methylphenyl)-1-(4-methylphenyl)sulfonyl-3-(3,4,5-trimethoxyphenyl)pyrazole (208.0 mg, 0.430 mmol) in Ethanol (28.98 mL). The reaction mixture was stirred at 50 °C on, then it was left to reach RT. The mixture was cooled at 0°C and HCl was added (9.78 mL): solvents were evaporated. A saturated aq solution of NaHCO<sub>3</sub> was added, followed by DCM: phases were separated, the organic one was washed with water and brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 25g, Cy/EtOAc from 8:2 to 3:7) affording 4-(4-methylphenyl)-3-(3,4,5-trimethoxyphenyl)-1H-pyrazole (N0237-33-1: 60 mg, 0.185 mmol, 42.56% yield) as white foam.

LCMS acidic r.t. 1.05 min, MS (ESI)  $m/z = 325.1$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-d)  $\delta$  ppm 2.34 - 2.39 (3 H, m) 3.67 - 3.71 (6 H, m) 3.87 - 3.91 (3 H, m) 6.69 - 6.75 (2 H, m) 7.14 - 7.19 (2 H, m) 7.23 - 7.27 (2 H, m) 7.65 - 7.69 (1 H, m).

### N0237-34 (TR989) 4-(4-methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)-1H-pyrazole

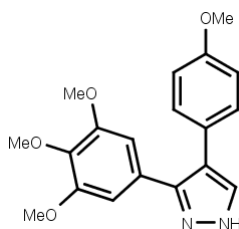


Figure 2.11: TR989

A mixture of 4-bromo-1-(4-methylphenyl)sulfonyl-3-(3,4,5-trimethoxyphenyl)pyrazole (0.22 g, 0.470 mmol), CsF (178.77 mg, 1.18 mmol) and (4-methoxyphenyl)boronic acid (107.3 mg, 0.710 mmol) in 1,4-Dioxane (7 mL) and few drops of water was degassed for 10 min. [1,1-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) (34.54 mg, 0.050 mmol) was added and the mixture was degassed for other 10 min, then it was stirred at 45 degrees for 20 min and at 100 °C for 2h. The reaction mixture was left to reach RT: EtOAc was added and it was filtered. Solvents were evaporated and crude purified by flash chromatography (silica 25g + 25g, Cy/EtOAc from 95:5 to 1:1) affording 4-(4-methoxyphenyl)-1-(4-methylphenyl)sulfonyl-3-(3,4,5-trimethoxyphenyl)pyrazole (N0237-29-1: 154 mg, 0.311 mmol, 66.15% yield) as colourless oil.

LCMS acidic r.t. 1.31 min, MS (ESI)  $m/z = 495.1$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-d)  $\delta$  ppm 2.44 - 2.47 (3 H, m) 3.69 (6 H, s) 3.81 - 3.84 (3 H, m) 3.84 - 3.87 (3 H, m) 6.71 (2 H, s) 6.86 - 6.92 (2 H, m) 7.18 - 7.23 (2 H, m) 7.38 (2 H, d,  $J = 7.92$  Hz) 7.98 - 8.03 (2 H, m) 8.09 - 8.12 (1 H, m).

## 2. EXPERIMENTAL PART

An aq solution 1M of sodium hydroxide (7.01 mL, 7.01 mmol) was added to a stirred mixture of 4-(4-methoxyphenyl)-1-(4-methylphenyl)sulfonyl-3-(3,4,5-trimethoxyphenyl)pyrazole (154.0 mg, 0.310 mmol) in Ethanol (20.76 mL). The reaction mixture was stirred on at 50 °C. It was left to reach RT, then cooled at 0°C and HCl 1N (7.01 mL) was added: solvents were evaporated. An aq saturated solution of NaHCO<sub>3</sub> was added, followed by DCM: phases were separated, the organic one washed with water and brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 25g, Cy/EtOAc from 8:2 to 4:6) affording 4-(4-methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)-1H-pyrazole (N0237-34-1: 47 mg, 0.138 mmol, 44.35% yield) as white solid.

LCMS acidic r.t. 0.95 min, MS (ESI) m/z= 341.1 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-d) δ ppm 3.67 - 3.71 (6 H, m) 3.83 (3 H, s) 3.87 - 3.89 (3 H, m) 6.71 - 6.74 (2 H, m) 6.87 - 6.92 (2 H, m) 7.25 - 7.29 (2 H, m) 7.64 (1 H, s).

### N0237-37 (TR996) 4-(1-benzothiophen-2-yl)-3-(3,4,5-trimethoxyphenyl)-1H-pyrazole

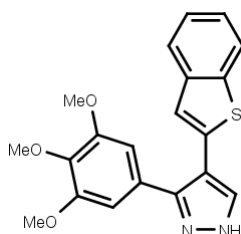


Figure 2.12: TR996

A mixture of 4-bromo-1-(4-methylphenyl)sulfonyl-3-(3,4,5-trimethoxyphenyl)pyrazole (0.27 g, 0.540 mmol), 1-benzothiophen-2-ylboronic acid (144.2 mg, 0.810 mmol) and CsF (205.07 mg, 1.35 mmol) in 1,4-Dioxane (8.178 mL) and few drops of water was degassed for 10 min. [1,1-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) (39.62 mg, 0.050 mmol) was added: the mixture was degassed again for 10 min, then stirred at 45°C for 20 min and 100 °C for 2h. The reaction mixture was left to reach RT, then it was diluted with EtOAc, filtered and concentrated. Crude was purified by flash chromatography (silica 25g +25g, Cy/EtOAc from 95:5 to 6:4) affording 4-(1-benzothiophen-2-yl)-1-(4-methylphenyl)sulfonyl-3-(3,4,5-trimethoxyphenyl)pyrazole (N0237-35-1: 286 mg, 0.549 mmol, 101.73% yield) as colourless oil.

LCMS acidic r.t. 1.43 min, MS (ESI) m/z= 521.1 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-d) δ ppm 2.46 - 2.48 (3 H, m) 3.69 - 3.72 (6 H, m) 3.88 (3 H, s) 6.81 - 6.84 (2 H, m) 7.19 (1 H, d, J=0.66 Hz) 7.33 - 7.37 (2 H, m) 7.39 - 7.42 (2 H, m) 7.67 - 7.71 (1 H, m) 7.78 - 7.82 (1 H, m) 8.00 - 8.05 (2 H, m) 8.30 - 8.32 (1 H, m).

An aqueous solution 1N of sodium hydroxide (12.36 mL, 12.36 mmol) was added to a mixture of 4-(1-benzothiophen-2-yl)-1-(4-methylphenyl)sulfonyl-3-(3,4,5-trimethoxyphenyl)pyrazole (286.0 mg, 0.550 mmol) in Ethanol (36.62 mL). The reaction mixture was stirred on at 50 °C, then it was left to reach RT and cooled at 0°C. HCl 1N (13.36 mL) was added: solvents were evaporated. A saturated aq solution of NaHCO<sub>3</sub> was added, followed by DCM: phases were separated, the organic one washed with water and brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 25g, Cy/EtOAc from 8:2 to 4:6) affording 4-(1-benzothiophen-2-yl)-3-(3,4,5-trimethoxyphenyl)-1H-pyrazole (N0237-37-1: 72 mg, 0.196 mmol, 35.77% yield) as white foam.

LCMS acidic r.t. 1.12 min, MS (ESI) m/z= 367.1 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-d) δ ppm 3.74 (6 H, s) 3.92 - 3.94 (3 H, m) 6.84 - 6.88 (2 H, m) 7.24 (1 H, d, J=0.66 Hz) 7.28 - 7.37 (2 H, m) 7.67 - 7.72 (1 H, m) 7.76 - 7.81 (1 H, m) 7.81 - 7.83 (1 H, m).



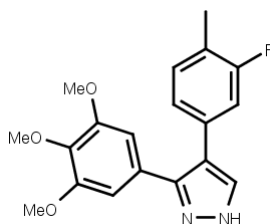
**N0237-38 (TRR5) 4-(3-fluoro-4-methylphenyl)-3-(3,4,5-trimethoxyphenyl)-1H-pyrazole**

Figure 2.13: TRR5

A mixture of 4-bromo-1-(4-methylphenyl)sulfonyl-3-(3,4,5-trimethoxyphenyl)pyrazole (266.0 mg, 0.540 mmol), (3-fluoro-4-methylphenyl)boronic acid (124.87 mg, 0.810 mmol) and CsF (205.34 mg, 1.35 mmol) in 1,4-Dioxane (8.04 mL) and few drops of water was degassed for 10 min. [1,1-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) (39.67 mg, 0.050 mmol) was added: the mixture was degassed again for 10 min, then stirred at 45°C for 20 min and 100 °C for 2h. The reaction mixture was left to reach RT, then it was diluted with EtOAc, filtered and concentrated. Crude was purified by flash chromatography (silica 25g +25g, Cy/EtOAc from 95:5 to 6:4) affording 4-(3-fluoro-4-methylphenyl)-1-(4-methylphenyl)sulfonyl-3-(3,4,5-trimethoxyphenyl)pyrazole (N0237-36-1: 267 mg, 0.538 mmol, 99.44% yield) as yellowish oil.

LCMS acidic r.t. 1.39 min, MS (ESI)  $m/z = 497.2$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-d)  $\delta$  ppm 2.27 - 2.31 (3 H, m) 3.68 - 3.71 (6 H, m) 3.84 - 3.87 (3 H, m) 6.67 - 6.71 (2 H, m) 6.96 (2 H, d,  $J=9.24$  Hz) 7.16 (1 H, t,  $J=7.81$  Hz) 7.36 - 7.41 (2 H, m) 7.98 - 8.03 (2 H, m) 8.13 - 8.15 (1 H, m).

An aqueous solution 1N of sodium hydroxide (12.1 mL, 12.1 mmol) was added to a stirred mixture of 4-(3-fluoro-4-methylphenyl)-1-(4-methylphenyl)sulfonyl-3-(3,4,5-trimethoxyphenyl)pyrazole (267.0 mg, 0.540 mmol) in Ethanol (35.85 mL). The reaction mixture was stirred on at 50 °C, then it was left to reach RT and cooled at 0°C. HCl 1N was added (12.1 mL) and solvents evaporated. A saturated aq solution of NaHCO<sub>3</sub> was added, followed by DCM: phases were separated, the organic one washed with water and brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 25g, Cy/EtOAc from 8:2 to 4:6) affording 4-(3-fluoro-4-methylphenyl)-3-(3,4,5-trimethoxyphenyl)-1H-pyrazole (N0237-38-1: 81.4 mg, 0.238 mmol, 44.22% yield) as white foam.

LCMS acidic r.t. 1.07 min, MS (ESI)  $m/z = 343.1$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-d)  $\delta$  ppm 2.29 (3 H, d,  $J=1.54$  Hz) 3.70 - 3.74 (6 H, m) 3.87 - 3.91 (3 H, m) 6.69 - 6.73 (2 H, m) 6.99 - 7.02 (1 H, m) 7.03 - 7.05 (1 H, m) 7.11 - 7.17 (1 H, m) 7.66 - 7.68 (1 H, m).

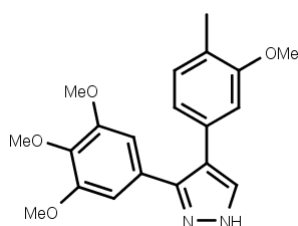
**N0237-41 (TRR3) 4-(3-methoxy-4-methylphenyl)-3-(3,4,5-trimethoxyphenyl)-1H-pyrazole**

Figure 2.14: TRR3

A mixture of 4-bromo-1-(4-methylphenyl)sulfonyl-3-(3,4,5-trimethoxyphenyl)pyrazole

## 2. EXPERIMENTAL PART

(267.0 mg, 0.570 mmol), 3-methoxy-4-methylbenzeneboronic acid(142.24 mg, 0.860 mmol) and CsF (216.96 mg, 1.43 mmol) in 1,4-Dioxane (8.653 mL) and few drops of water was degassed for 10 min. [1,1-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) (41.92 mg, 0.060 mmol) was added: the mixture was degassed again for 10 min, then stirred at 45°C for 20 min and 100 °C for 2h. The reaction mixture was left to reach RT, then it was diluted with EtOAc, filtered and concentrated. Crude was purified by flash chromatography (silica 25g +25g, Cy/EtOAc from 95:5 to 6:4) affording 4-(3-methoxy-4-methylphenyl)-1-(4-methylphenyl)sulfonyl-3-(3,4,5-trimethoxyphenyl)pyrazole (N0237-39-1: 234 mg, 0.460 mmol, 80.53% yield) as yellowish foam.

LCMS acidic r.t. 1.40 min, MS (ESI) m/z= 509.1 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-d) δ ppm 2.23 (3 H, s) 2.46 (3 H, t, J=0.77 Hz) 3.68 - 3.70 (6 H, m) 3.70 (3 H, s) 3.84 - 3.87 (3 H, m) 6.70 (1 H, d, J=1.54 Hz) 6.72 - 6.75 (2 H, m) 6.80 (1 H, dd, J=7.48, 1.54 Hz) 7.09 - 7.12 (1 H, m) 7.38 (2 H, dd, J=8.58, 0.66 Hz) 7.99 - 8.03 (2 H, m) 8.14 - 8.16 (1 H, m).

An aq solution 1N of sodium hydroxide (10.35 mL, 10.35 mmol) was added to a stirred solution of 4-(3-methoxy-4-methylphenyl)-1-(4-methylphenyl)sulfonyl-3-(3,4,5-trimethoxyphenyl)pyrazole (234.0 mg, 0.460 mmol) in Ethanol (31.67 mL). The reaction mixture was stirred on at 50 °C. The mixture was left to reach RT and cooled at 0°C: HCl 1N was added (10.35 mL) and solvents evaporated. A saturated aq solution of NaHCO<sub>3</sub> was added, followed by DCM: phases were separated, the organic one washed with water and brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 25g, Cy/EtOAc from 8:2 to 3:7) affording 4-(3-methoxy-4-methylphenyl)-3-(3,4,5-trimethoxyphenyl)-1H-pyrazole (N0237-41-1: 86 mg, 0.243 mmol, 52.74% yield) as white solid.

LCMS acidic r.t. 1.08 min, MS (ESI) m/z= 355.2 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-d) δ ppm 2.23 (3 H, d, J=0.66 Hz) 3.69 - 3.71 (6 H, m) 3.71 (3 H, s) 3.88 (3 H, s) 6.73 - 6.77 (2 H, m) 6.80 (1 H, d, J=1.32 Hz) 6.86 - 6.90 (1 H, m) 7.09 - 7.13 (1 H, m) 7.70 (1 H, s).

### N0237-42 (TRR7) 4-(3-fluoro-4-methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)-1H-pyrazole

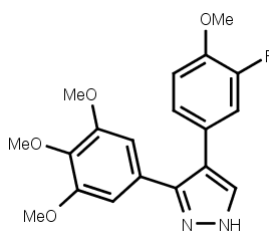


Figure 2.15: TRR7

A mixture of 4-bromo-1-(4-methylphenyl)sulfonyl-3-(3,4,5-trimethoxyphenyl)pyrazole (266.0 mg, 0.570 mmol), (3-fluoro-4-methoxyphenyl)boronic acid (145.1 mg, 0.850 mmol) and CsF (216.15 mg, 1.42 mmol) in 1,4-Dioxane (8.464 mL) and few drops of water was degassed for 10 min. [1,1-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) (41.76 mg, 0.060 mmol) was added: the mixture was degassed again for 10 min, then stirred at 45°C for 20 min and 100 °C for 2h. The reaction mixture was left to reach RT, then it was diluted with EtOAc, filtered and concentrated. Crude was purified by flash chromatography (silica 25g +25g, Cy/EtOAc from 95:5 to 6:4) affording 4-(3-fluoro-4-methoxyphenyl)-1-(4-methylphenyl)sulfonyl-3-(3,4,5-trimethoxyphenyl)pyrazole (N0237-40-1: 260 mg, 0.507 mmol, 89.12% yield) as yellowish oil.

LCMS acidic r.t. 1.32 min, MS (ESI) m/z= 513.1 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-d) δ ppm 2.46 (3 H, s) 3.71 (6 H, s) 3.87 (3 H, s) 3.92 (3 H, s) 6.68 - 6.71 (2 H, m) 6.90 - 6.96 (1 H, m) 6.97 - 7.01 (1 H, m) 7.02 - 7.07 (1 H, m) 7.38 (2

## 2. EXPERIMENTAL PART

H, d, J=7.92 Hz) 7.97 - 8.03 (2 H, m) 8.11 - 8.14 (1 H, m).

An aqueous solution 1N of sodium hydroxide (11.41 mL, 11.41 mmol) was added to a stirred solution of 4-(3-fluoro-4-methoxyphenyl)-1-(4-methylphenyl)sulfonyl-3-(3,4,5-trimethoxyphenyl)pyrazole (260.0 mg, 0.510 mmol) in Ethanol (35.19 mL). The reaction mixture was stirred on at 50 °C. The mixture was left to reach RT and cooled at 0°C: HCl 1N was added (10.35 mL) and solvents evaporated. A saturated aq solution of NaHCO<sub>3</sub> was added, followed by DCM: phases were separated, the organic one washed with water and brine, dried over sodium sulfate, filtered and concentrated. crude was purified by flash chromatography (silica 25g, Cy/EtOAc from 8:2 to 3:7) affording 4-(3-fluoro-4-methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)-1H-pyrazole (N0237-42-1: 111 mg, 0.310 mmol, 61.06% yield) as white foam.

LCMS acidic r.t. 0.98 min, MS (ESI) m/z= 513.1 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-d) δ ppm 3.71 - 3.74 (6 H, m) 3.89 - 3.90 (3 H, m) 3.91 - 3.92 (3 H, m) 6.68 - 6.72 (2 H, m) 6.91 - 6.97 (1 H, m) 7.04 - 7.08 (1 H, m) 7.11 (1 H, dd, J=12.21, 2.09 Hz) 7.64 - 7.67 (1 H, m).

### 2.2 Synthesis of 4-(3,4,5-trimethoxyphenyl)-1H-pyrroles

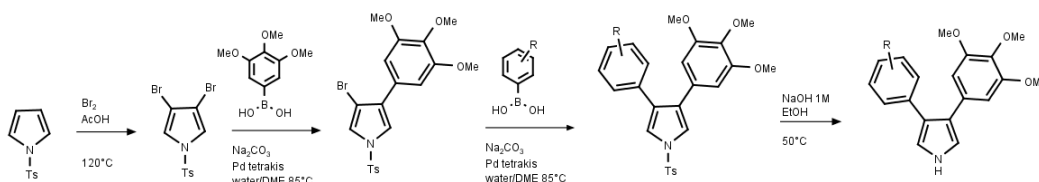


Figure 2.16: general route for 4-(3,4,5-trimethoxyphenyl)-1H-pyrrole

The general route reported in fig 2.16 was a good one: the first reaction is the double pyrrole's bromination, followed by two Suzuki's coupling (the first one gave the common intermediate *3-bromo-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole*) and the last step was the basic deprotection of pyrrole.

The worst step of this route was the first one: this reaction was performed at strong conditions (in acetic acid 0.25M at 120°C), it was very dirty and not selective. In fig 2.17 is reported the crude material's chromatogram: there are lots of peaks with unclear m/z.

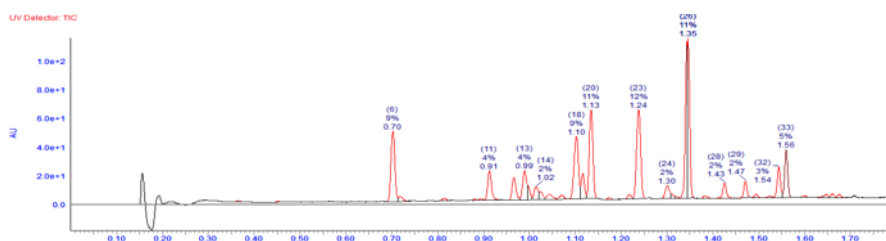


Figure 2.17: UPLC analysis of bromination -crude material-

Lots of attempts were performed to clean the crude material: best purification conditions came from reverse phase (silica c18 120g, water+0.1% formic acid / MeCN+0.1% formic acid, from 98:2 to 0:10): in fig 2.18 is reported the chromatogram after purification. Another issue of this first step is the reaction scale: because of the use of a large amount of acetic acid and the big size of columns used for purification, reaction had to be performed different times (maximum 4g of starting material).

The second step was the Suzuki's coupling. Three attempts were performed, exploring catalysts, bases, solvents, temperature and reactors (fig 2.19).

The first one (Pd tetrakis PPh<sub>3</sub> and sodium carbonate in 1,2-dimethoxyethane/water (1:0.15) stirred at 85°C for 24h), with 45% yield, was chosen for both the Suzuki's coupling.

## 2. EXPERIMENTAL PART

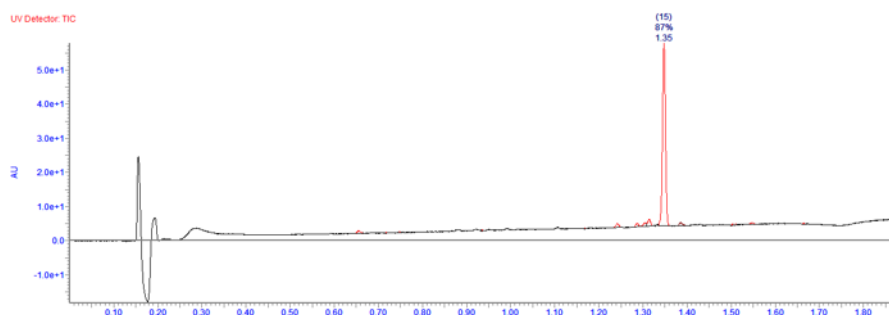


Figure 2.18: UPLC analysis of bromination after purification

O.I.	reaction condition	yield%
N0423-04-1	Pd tetrakis PPh <sub>3</sub> , Na <sub>2</sub> CO <sub>3</sub> in 1,2-dimethoxyethane/water (1:0.15), 85°C 24h	45%
N0423-05-1	Pd(dppf)Cl <sub>2</sub> , Na <sub>2</sub> CO <sub>3</sub> in MeCN/water (1:1), 110°C 4*25 min microwave reactor	0%
N0423-07-1	Pd(dppf)Cl <sub>2</sub> , CsF in 1,4-dioxane, 100°C 16h	14%

Figure 2.19: reaction conditions for Suzuki's coupling

### N0423-15 (CP001) 3-(4-methylphenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole

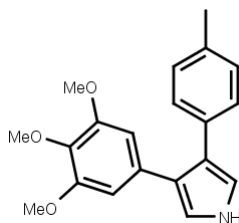


Figure 2.20: CP001

*Batch 1* : A solution of molecular bromine (2.13 mL, 41.58 mmol) in Acetic acid (24 mL) was added dropwise to a stirred solution of 1-(4-methylphenyl)sulfonylpyrrole (4.0 g, 18.08 mmol) in Acetic acid (56 mL), then the reaction mixture was stirred at 120 °C for 90 min. The black mixture was left to reach RT and volatiles removed under reduced pressure. The dark oil was dissolved in DCM and the mixture was filtered over a pad of celite that was washed with DCM. Volatiles were removed and crude purified by flash chromatography, reverse phase (silica c18 120g + 120g, water+0.1% formic acid / MeCN+0.1% formic acid, from 98:2 to 10:85), affording 3,4-dibromo-1-(4-methylphenyl)sulfonylpyrrole (N0423-06-1: 1.945 g, 5.131 mmol, 28.38% yield) as brownish solid.

LCMS acidic r.t. 1.34 min, MS (ESI) m/z= 380.3 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 2.40 - 2.42 (3 H, m) 7.48 - 7.52 (2 H, m) 7.75 - 7.77 (2 H, m) 7.92 - 7.97 (2 H, m)

*Batch 2* : A solution of molecular bromine (3.2 mL, 62.36 mmol) in Acetic acid (36 mL) was added dropwise to a stirred solution of 1-(4-methylphenyl)sulfonylpyrrole (6.0 g, 27.11 mmol) in Acetic acid (84 mL), then the reaction mixture was stirred at 120 °C for 90 min. The black mixture was left to reach RT and volatiles removed under reduced pressure. The dark oil was dissolved in DCM and the mixture was filtered over a pad of celite that was washed with DCM. Volatiles were removed and crude purified by flash chromatography, reverse phase (silica c18 120g, water+0.1% formic acid / MeCN+0.1% formic acid, from 98:2 to 0:10), affording 3,4-dibromo-1-(4-methylphenyl)sulfonylpyrrole (N0758-02-1: 2.242 g, 5.914 mmol,

## 2. EXPERIMENTAL PART

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21.81% yield) as brownish solid.

LCMS acidic r.t. 1.35 min, no mass detected.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 2.40 (3 H, t, J=0.77 Hz) 7.46 - 7.52 (2 H, m) 7.73 - 7.77 (2 H, m) 7.91 - 7.96 (2 H, m)

<sup>1</sup>H NMR (400 MHz, chloroform-d) δ ppm 2.42 - 2.49 (3 H, m) 7.18 - 7.22 (2 H, m) 7.33 - 7.40 (2 H, m) 7.75 - 7.82 (2 H, m)

*Batch 1* : A mixture of 3,4-dibromo-1-(4-methylphenyl)sulfonylpyrrole (2.63 g, 6.94 mmol) and 3,4,5-trimethoxyphenylboronic acid (1618.03 mg, 7.63 mmol) and sodium carbonate (2206.09 mg, 20.81 mmol) in 1,2-dimethoxyethane (52.6 mL) and Water (7.89 mL) was degassed for 10 min; palladium tetrakis triphenylphosphine (801.73 mg, 0.690 mmol) was added and the mixture degassed for 10min, then stirred at 85 °C for 24h. The reaction mixture was left to reach RT, then it was diluted with EtOAc and filtered. Volatiles were removed and crude purified by flash chromatography (silica 100g + 100g, Cy/EtOAc from 98:2 to 6:4) affording *3-bromo-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole* (N0423-20-1: 1.325 g, 2.841 mmol, 40.95% yield) as yellowish foam.

LCMS acidic r.t. 1.34 min, MS (ESI) m/z= 468.1-469.2 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 2.41 (3 H, d, J=0.66 Hz) 3.68 (3 H, s) 3.79 - 3.82 (6 H, m) 6.80 - 6.84 (2 H, m) 7.48 - 7.53 (2 H, m) 7.72 (2 H, q, J=2.64 Hz) 7.95 - 7.99 (2 H, m).

*Batch 2* : A mixture of 3,4-dibromo-1-(4-methylphenyl)sulfonylpyrrole (2.14 g, 5.65 mmol) and 3,4,5-trimethoxyphenylboronic acid (1.32 g, 6.21 mmol) and sodium carbonate (1.8 g, 16.94 mmol) in 1,2-dimethoxyethane (42.8 mL) and Water (6.42 mL) was degassed for 10 min; palladium tetrakis triphenylphosphine (652.36 mg, 0.560 mmol) was added and the mixture degassed for 10min, then stirred at 85 °C for 24h. The reaction mixture was left to reach RT, then it was diluted with EtOAc and filtered. Volatiles were removed and crude purified by flash chromatography (silica 100g + 100g, Cy/EtOAc from 98:2 to 6:4) affording *3-bromo-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole* (N0758-04-1: 968 mg, 2.076 mmol, 36.77% yield) as yellowish foam.

LCMS acidic r.t. 1.35 min, MS (ESI) m/z= 468-469.3 [M+H]<sup>+</sup>.

A mixture of 3-bromo-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (91.0 mg, 0.200 mmol), (4-methylphenyl)boronic acid (26.53 mg, 0.200 mmol) and sodium carbonate (62.05 mg, 0.590 mmol) in 1,2-dimethoxyethane (2 mL) and Water (0.300 mL) was degassed for 10 min. palladium tetrakis triphenylphosphine (22.55 mg, 0.020 mmol) was added and the mixture degassed again for 10 min, then stirred on at 85 °C. The reaction mixture was left to reach RT, EtOAc was added and it was filtered and concentrated. Crude was purified by flash chromatography (silica 10g + 10g, Cy/EtOAc from 98:2 to 7:3) giving *3-(4-methylphenyl)-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole* (N0423-14-1: 64 mg, 0.134 mmol, 68.68% yield) as yellowish oil.

LCMS acidic r.t. 1.43 min, MS (ESI) m/z= 478.3 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 2.26 - 2.30 (3 H, m) 2.40 (3 H, s) 3.53 - 3.58 (6 H, m) 3.63 (3 H, s) 6.44 (2 H, s) 7.08 - 7.16 (4 H, m) 7.47 - 7.49 (2 H, m) 7.49 - 7.51 (1 H, m) 7.63 (1 H, d, J=2.64 Hz) 7.98 - 8.03 (2 H, m).

Sodium hydroxide (3.02 mL, 3.02 mmol) 1M was added to a solution of 3-(4-methylphenyl)-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (64.0 mg, 0.130 mmol) in Ethanol (8.934 mL). The reaction mixture was stirred on at 50 °C. The mixture was cooled to RT, then HCl 1M (3.02 mL) was added at 0°C. Volatiles were removed: a saturated aq solution of NaHCO<sub>3</sub> was added, followed by DCM: phases were separated, the organic one washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 10g, Cy/EtOAc from 95:5 to 6:4) affording *3-(4-methylphenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole* (N0423-15-1: 29.5 mg, 0.091 mmol, 68.07% yield) as white solid.

LCMS acidic r.t. 1.15 min, MS (ESI) m/z= 324.3 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 1.51 (3 H, t, J=0.66 Hz) 2.83 - 2.85 (6 H, m) 2.94 - 2.96 (3 H, m) 5.69 - 5.72 (2 H, m) 6.01 (1 H, d, J=2.20 Hz) 6.11 (1 H, d, J=2.20 Hz) 6.25 -

## 2. EXPERIMENTAL PART

6.30 (2 H, m) 6.32 - 6.36 (2 H, m).

### N0423-18 (CP002) 3-naphthalen-2-yl-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole

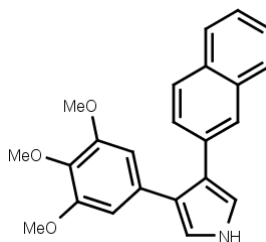


Figure 2.21: CP002

A mixture of 3-bromo-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (100.0 mg, 0.210 mmol), sodium carbonate (67.5 mg, 0.640 mmol) and palladium tetrakis triphenylphosphine (24.53 mg, 0.020 mmol) in 1,2-dimethoxyethane (2.176 mL) and Water (0.326 mL) was degassed for 10 min. 2-naphthalenylboronic acid (38.34 mg, 0.220 mmol) was added and the mixture degassed again for 10 min, then stirred on at 85 °C. The reaction mixture was left to reach RT, EtOAc was added and it was filtered and concentrated. Crude was purified by flash chromatography (silica 10g + 10g, Cy/EtOAc from 98:2 to 7:3) giving *1-(4-methylphenyl)sulfonyl-3-naphthalen-2-yl-4-(3,4,5-trimethoxyphenyl)pyrrole* (N0423-16-1: 65 mg, 0.127 mmol, 59.62% yield) as yellowish oil.

LCMS acidic r.t. 1.48 min, MS (ESI)  $m/z = 524.1$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 2.41 - 2.43 (3 H, m) 3.46 - 3.50 (6 H, m) 3.60 - 3.64 (3 H, m) 6.47 - 6.51 (2 H, m) 7.30 (1 H, dd,  $J = 8.58, 1.76$  Hz) 7.47 - 7.54 (4 H, m) 7.65 - 7.68 (1 H, m) 7.72 - 7.74 (1 H, m) 7.80 - 7.85 (2 H, m) 7.86 - 7.91 (2 H, m) 8.01 - 8.06 (2 H, m).

1-(4-methylphenyl)sulfonyl-3-naphthalen-2-yl-4-(3,4,5-trimethoxyphenyl)pyrrole (65.0 mg, 0.130 mmol) 1M was added to a solution of sodium hydroxide (2.85 mL, 2.85 mmol) in Ethanol (9.074 mL). The reaction mixture was stirred on at 50 °C. The mixture was cooled to RT, then HCl 1M (3.02 mL) was added at 0°C. Volatiles were removed: a saturated aq solution of NaHCO<sub>3</sub> was added, followed by DCM: phases were separated, the organic one washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 10g, Cy/EtOAc from 95:5 to 6:4) affording *3-naphthalen-2-yl-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole* (N0423-18-1: 5.8 mg, 0.016 mmol, 12.75% yield) as white solid.

LCMS acidic r.t. 1.21 min, MS (ESI)  $m/z = 360.3$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 3.51 (6 H, s) 3.63 - 3.65 (3 H, m) 6.50 (2 H, s) 7.08 (2 H, quin,  $J = 2.42$  Hz) 7.36 - 7.39 (1 H, m) 7.40 - 7.48 (2 H, m) 7.75 - 7.81 (3 H, m) 7.85 (1 H, d,  $J = 7.70$  Hz).

### N0423-19 (CP003) 3-(3-chloro-4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole

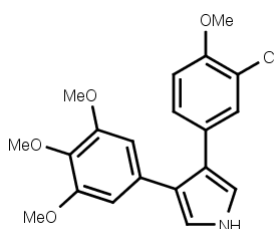


Figure 2.22: CP003

## 2. EXPERIMENTAL PART

A mixture of 3-bromo-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (100.0 mg, 0.210 mmol), sodium carbonate (68.18 mg, 0.640 mmol) and palladium tetrakis triphenylphosphine (24.78 mg, 0.020 mmol) in 1,2-dimethoxyethane (2.198 mL) and Water (0.330 mL) was degassed for 10 min. (3-chloro-4-methoxyphenyl)boronic acid (41.97 mg, 0.230 mmol) was added and the mixture degassed again for 10 min, then stirred on at 85 °C. The reaction mixture was left to reach RT, EtOAc was added and it was filtered and concentrated. Crude was purified by flash chromatography (silica 10g + 10g, Cy/EtOAc from 98:2 to 1:1) giving 3-(3-chloro-4-methoxyphenyl)-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (N0423-17-1: 72 mg, 0.136 mmol, 63.59% yield) as white foam.

LCMS acidic r.t. 1.41 min, MS (ESI)  $m/z$ = 528.4-531.2 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 2.39 - 2.43 (3 H, m) 3.59 - 3.62 (6 H, m) 3.63 - 3.66 (3 H, m) 3.83 - 3.85 (3 H, m) 6.45 - 6.48 (2 H, m) 7.08 - 7.15 (2 H, m) 7.33 (1 H, d, J=1.98 Hz) 7.47 - 7.51 (2 H, m) 7.60 (1 H, d, J=2.42 Hz) 7.62 - 7.64 (1 H, m) 7.98 - 8.03 (2 H, m).

3-(3-chloro-4-methoxyphenyl)-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (72.0 mg, 0.140 mmol) 1M was added to a solution of sodium hydroxide (3.06 mL, 3.06 mmol) in Ethanol (10.05 mL). The reaction mixture was stirred on at 50 °C. The mixture was cooled to RT, then HCl 1M (3.02 mL) was added at 0°C. Volatiles were removed: a saturated aq solution of NaHCO<sub>3</sub> was added, followed by DCM: phases were separated, the organic one washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 10g, Cy/EtOAc from 95:5 to 6:4) affording 3-(3-chloro-4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (N0423-19-1: 6.3 mg, 0.017 mmol, 12.36% yield) as white solid.

LCMS acidic r.t. 1.12 min, MS (ESI)  $m/z$ = 374.2 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 3.62 (6 H, s) 3.64 - 3.65 (3 H, m) 3.83 (3 H, s) 6.47 (2 H, s) 6.94 - 6.97 (1 H, m) 7.00 (1 H, t, J=2.20 Hz) 7.05 - 7.10 (2 H, m) 7.16 (1 H, dd, J=8.58, 2.20 Hz) 7.26 (1 H, d, J=2.20 Hz).

### N0423-23 (CP004) 3-[4-(methoxymethoxy)phenyl]-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole

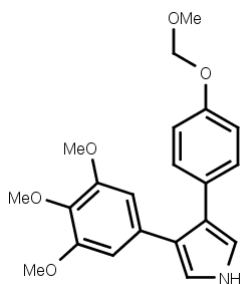


Figure 2.23: CP004

A mixture of 3-bromo-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (100.0 mg, 0.210 mmol), sodium carbonate (68.18 mg, 0.640 mmol) and [4-(methoxymethoxy)phenyl]boronic acid (40.97 mg, 0.230 mmol) in 1,2-dimethoxyethane (2.198 mL) and Water (0.330 mL) was degassed for 10 min. Palladium tetrakis triphenylphosphine (24.78 mg, 0.020 mmol) was added and the mixture degassed again for 10 min, then stirred on at 85 °C. The reaction mixture was left to reach RT, EtOAc was added and it was filtered and concentrated. Crude was purified by flash chromatography (silica 10g + 10g, Cy/EtOAc from 98:2 to 6:4) giving 3-[4-(methoxymethoxy)phenyl]-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (N0423-21-1: 62 mg, 0.118 mmol, 55.22% yield) as yellowish oil.

LCMS acidic r.t. 1.37 min, MS (ESI)  $m/z$ = 524.3 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 2.39 - 2.43 (3 H, m) 3.34 - 3.36 (3 H, m) 3.56 - 3.59 (6

## 2. EXPERIMENTAL PART

H, m) 3.62 - 3.64 (3 H, m) 5.18 (2 H, s) 6.41 - 6.46 (2 H, m) 6.96 - 7.01 (2 H, m) 7.11 - 7.17 (2 H, m) 7.46 - 7.47 (1 H, m) 7.48 - 7.52 (2 H, m) 7.64 (1 H, d, J=2.42 Hz) 7.97 - 8.02 (2 H, m).

3-[4-(methoxymethoxy)phenyl]-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl) pyrrole (65.0 mg, 0.120 mmol) 1M was added to a solution of sodium hydroxide (2.79 mL, 2.79 mmol) in Ethanol (9.074 mL). The reaction mixture was stirred on at 50 °C. The mixture was cooled to RT, then HCl 1M (3.02 mL) was added at 0°C. Volatiles were removed: a saturated aq solution of NaHCO<sub>3</sub> was added, followed by DCM: phases were separated, the organic one washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 10g, Cy/EtOAc from 95:5 to 6:4) affording 3-[4-(methoxymethoxy)phenyl]-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (N0423-23-1: 9.4 mg, 0.025 mmol, 20.5% yield) as white solid.

LCMS acidic r.t. 1.06 min, MS (ESI) m/z= 370.3 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 3.36 (3 H, s) 3.58 (6 H, s) 3.63 (3 H, s) 5.15 - 5.17 (2 H, m) 6.45 (2 H, s) 6.86 (1 H, t, J=2.31 Hz) 6.93 - 6.96 (2 H, m) 7.00 (1 H, t, J=2.42 Hz) 7.13 - 7.17 (2 H, m) 11.01 (1 H, br. s.).

### N0423-24 (CP005) 3-(1-benzothiophen-2-yl)-4-(3,4,5-trimethoxyphenyl)- 1H-pyrrole

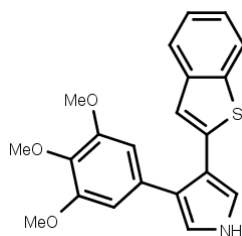


Figure 2.24: CP005

A mixture of 3-bromo-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (100.0 mg, 0.210 mmol), sodium carbonate (68.18 mg, 0.640 mmol) and 1-benzothiophen-2-ylboronic acid (38.17 mg, 0.210 mmol) in DMF (2.198 mL) and Water (0.330 mL) was degassed for 10 min. Palladium tetrakis triphenylphosphine (24.78 mg, 0.020 mmol) was added and the mixture degassed again for 10 min, then stirred on at 85 °C. The reaction mixture was left to reach RT, EtOAc was added and it was filtered and concentrated. Crude was purified by flash chromatography (silica 10g + 10g, Cy/EtOAc from 98:2 to 7:3) giving 3-(1-benzothiophen-2-yl)-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (N0423-22-1: 77 mg, 0.148 mmol, 69.1% yield) as yellowish oil.

LCMS acidic r.t. 1.47 min, MS (ESI) m/z= 520.3 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 2.41 (3 H, t, J=0.66 Hz) 3.68 (3 H, s) 3.80 - 3.82 (6 H, m) 6.80 - 6.84 (2 H, s) 7.30 - 7.36 (2 H, m) 7.47 - 7.54 (3 H, m) 7.68 (1 H, d, J=2.42 Hz) 7.71 - 7.74 (2 H, m) 7.75 - 7.77 (1 H, m) 7.95 - 7.99 (2 H, m).

Sodium hydroxide (3.33 mL, 3.33 mmol) 1M was added to a solution of 3-(1-benzothiophen-2-yl)-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (77.0 mg, 0.150 mmol) in Ethanol (10.75 mL). The reaction mixture was stirred on at 50 °C. The mixture was cooled to RT, then HCl 1M (3.33 mL) was added at 0°C. Volatiles were removed: a saturated aq solution of NaHCO<sub>3</sub> was added, followed by DCM: phases were separated, the organic one washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography, reverse phase (silica c18 12g, water+0.1%formic acid / MeCN+0.1%formic acid, from 98:2 to 4:6) affording 3-(1-benzothiophen-2-yl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (N0423-24-1: 12.9 mg, 0.035 mmol, 23.82% yield) as yellow solid.

LCMS acidic r.t. 1.21 min, MS (ESI) m/z= 366.3 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 3.63 (6 H, s) 3.67 (3 H, s) 6.64 (2 H, s) 7.02 - 7.04 (1



## 2. EXPERIMENTAL PART

H, m) 7.13 - 7.16 (2 H, m) 7.21 - 7.32 (2 H, m) 7.69 (1 H, d, J=7.26 Hz) 7.84 (1 H, d, J=7.70 Hz) 11.29 (1 H, br. s.).

### N0423-27 (CP006) 3-(4-ethoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole

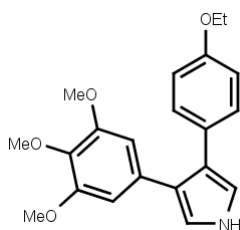


Figure 2.25: CP006

A mixture of 3-bromo-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (100.0 mg, 0.210 mmol), sodium carbonate (66.82 mg, 0.630 mmol) and (4-ethoxyphenyl)boronic acid (34.88 mg, 0.210 mmol) in 1,2-dimethoxyethane (2.154 mL) and Water (0.323 mL) was degassed for 10 min. Palladium tetrakis triphenylphosphine (24.28 mg, 0.020 mmol) was added and the mixture degassed again for 10 min, then stirred on at 85 °C. The reaction mixture was left to reach RT, EtOAc was added and it was filtered and concentrated. Crude was purified by flash chromatography (silica 10g + 10g, Cy/EtOAc from 98:2 to 6:4) giving 3-(4-ethoxyphenyl)-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (N0423-25-1: 88 mg, 0.173 mmol, 82.5% yield) as colourless oil.

LCMS acidic r.t. 1.42 min, MS (ESI) m/z= 508.4 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 1.27 - 1.34 (3 H, m) 2.40 (3 H, s) 3.58 (6 H, s) 3.62 - 3.65 (3 H, m) 3.97 - 4.03 (2 H, m) 6.45 (2 H, s) 6.85 - 6.91 (2 H, m) 7.10 - 7.14 (2 H, m) 7.45 (1 H, d, J=2.42 Hz) 7.49 (2 H, d, J=7.92 Hz) 7.62 (1 H, d, J=2.42 Hz) 7.96 - 8.02 (2 H, m).

A mixture of 3-(4-ethoxyphenyl)-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (88.0 mg, 0.170 mmol) in Ethanol (12.38 mL) and sodium hydroxide (3.9 mL, 3.9 mmol) 1M was stirred on at 50 °C. The solution was cooled to 0°C and HCl 1M (3.9 mL) was added. Volatiles were removed and a ss aq solution of NaHCO<sub>3</sub> was added, followed by DCM: phases were separated, the organic one washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 10g, Cy/EtOAc from 9:1 to 6:4) giving 3-(4-ethoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (N0423-27-1: 31 mg, 0.088 mmol, 50.6% yield) as whitish solid.

LCMS acidic r.t. 1.13 min, MS (ESI) m/z= 354.3 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 1.31 (3 H, t, J=7.04 Hz) 3.57 - 3.61 (6 H, m) 3.62 - 3.65 (3 H, m) 3.97 - 4.04 (2 H, m) 6.44 - 6.48 (2 H, m) 6.82 - 6.87 (3 H, m) 6.98 - 7.00 (1 H, m) 7.12 - 7.16 (2 H, m) 10.99 (1 H, br. s.).

### N0423-28 (CP007) 3-(4-ethoxy-3-fluorophenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole

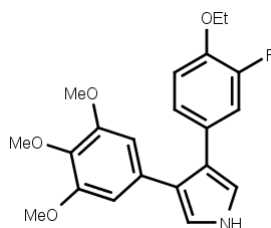


Figure 2.26: CP007

A mixture of 3-bromo-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (100.0 mg, 0.210 mmol), sodium carbonate (66.82 mg, 0.630 mmol) and

## 2. EXPERIMENTAL PART

(4-ethoxy-3-fluorophenyl)boronic acid (40.59 mg, 0.220 mmol) in 1,2-dimethoxyethane (2.154 mL) and Water (0.323 mL) was degassed for 10 min. Palladium tetrakis triphenylphosphine (24.28 mg, 0.020 mmol) was added and the mixture degassed again for 10 min, then stirred on at 85 °C. The reaction mixture was left to reach RT, EtOAc was added and it was filtered and concentrated. Crude was purified by flash chromatography (silica 10g + 10g, Cy/EtOAc from 98:2 to 6:4) giving *3-(4-ethoxy-3-fluorophenyl)-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole* (N0423-26-1: 64 mg, 0.122 mmol, 57.95% yield) as whitish oil.

LCMS acidic r.t. 1.41 min, MS (ESI)  $m/z = 526.4$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 1.29 - 1.35 (3 H, m) 2.41 (3 H, d,  $J=0.66$  Hz) 3.60 (6 H, s) 3.64 (3 H, s) 4.05 - 4.12 (2 H, m) 6.46 (2 H, s) 6.94 (1 H, dd,  $J=8.36, 1.10$  Hz) 7.06 - 7.13 (2 H, m) 7.47 - 7.52 (2 H, m) 7.56 (1 H, d,  $J=2.42$  Hz) 7.62 (1 H, d,  $J=2.64$  Hz) 7.98 - 8.03 (2 H, m).

A mixture of *3-(4-ethoxy-3-fluorophenyl)-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole* (64.0 mg, 0.120 mmol) in Ethanol (8.698 mL) and sodium hydroxide (2.74 mL, 2.74 mmol) 1M was stirred on at 50 °C. The mixture was left to reach RT and cooled at 0°C, then HCl 1M (2.74 mL) was added. Volatiles were removed and a ss aq solution of NaHCO<sub>3</sub> was added, followed by DCM: phases were separated, the organic one washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 10g, Cy/EtOAc from 9:1 to 6:4) affording *3-(4-ethoxy-3-fluorophenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole* (N0423-28-1: 42 mg, 0.113 mmol, 92.87% yield) as yellowish solid.

LCMS acidic r.t. 1.14 min, MS (ESI)  $m/z = 372.3$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 1.29 - 1.36 (3 H, m) 3.60 - 3.63 (6 H, m) 3.64 - 3.66 (3 H, m) 4.03 - 4.12 (2 H, m) 6.45 - 6.48 (2 H, m) 6.92 - 6.97 (2 H, m) 6.97 - 6.99 (1 H, m) 7.00 (1 H, d,  $J=1.98$  Hz) 7.04 - 7.09 (1 H, m) 11.07 (1 H, br. s.).

### N0423-33 (CP008) *3-(4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole*

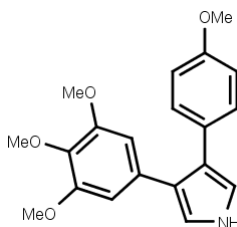


Figure 2.27: CP008

A mixture of 3-bromo-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (100.0 mg, 0.210 mmol), sodium carbonate (68.18 mg, 0.640 mmol) and (4-methoxyphenyl)boronic acid (34.21 mg, 0.230 mmol) in 1,2-dimethoxyethane (2.198 mL) and Water (0.330 mL) was degassed for 10 min. Palladium tetrakis triphenylphosphine (24.78 mg, 0.020 mmol) was added and the mixture degassed again for 10 min, then stirred on at 85 °C. The reaction mixture was left to reach RT, EtOAc was added and it was filtered and concentrated. Crude was purified by flash chromatography (silica 10g + 10g, Cy/EtOAc from 98:2 to 6:4) giving *3-(4-methoxyphenyl)-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole* (N0423-29-1: 84 mg, 0.170 mmol, 79.37% yield) as white foam.

LCMS acidic r.t. 1.37 min, MS (ESI)  $m/z = 494.4$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 2.39 - 2.42 (3 H, m) 3.58 (6 H, s) 3.63 - 3.65 (3 H, m) 3.72 - 3.75 (3 H, m) 6.45 (2 H, s) 6.87 - 6.93 (2 H, m) 7.11 - 7.17 (2 H, m) 7.45 (1 H, d,  $J=2.42$  Hz) 7.47 - 7.52 (2 H, m) 7.62 (1 H, d,  $J=2.42$  Hz) 7.97 - 8.02 (2 H, m).

A mixture of *3-(4-methoxyphenyl)-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxy*

## 2. EXPERIMENTAL PART

phenyl)pyrrole (84.0 mg, 0.170 mmol) in Ethanol (12.16 mL) and sodium hydroxide (3.83 mL, 3.83 mmol) 1M was stirred on at 50 °C. The mixture was left to reach RT, then cooled at 0°C and HCl 1M (3.83 mL) was added. Volatiles were removed; DCM and a ss aq solution of sodium bicarbonate were added. Phases were separated, the organic one washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 10g, Cy/EtOAc from 9:1 to 6:4) affording *3-(4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole* (N0423-33-1: 40 mg, 0.118 mmol, 69.25% yield) as yellowish solid.

LCMS acidic r.t. 1.06 min, MS (ESI)  $m/z = 340.3$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 3.59 (6 H, s) 3.63 - 3.64 (3 H, m) 3.73 - 3.74 (3 H, m) 6.46 (2 H, s) 6.83 - 6.85 (1 H, m) 6.85 - 6.89 (2 H, m) 6.98 - 7.00 (1 H, m) 7.13 - 7.18 (2 H, m) 10.99 (1 H, d, J=3.74 Hz).

### N0423-34 (CP009) 3-(3-fluoro-4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole

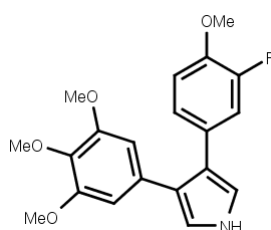


Figure 2.28: CP009

A mixture of 3-bromo-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (100.0 mg, 0.210 mmol), sodium carbonate (68.18 mg, 0.640 mmol) and (3-fluoro-4-methoxyphenyl)boronic acid (36.44 mg, 0.210 mmol) in 1,2-dimethoxyethane (2.198 mL) and Water (0.330 mL) was degassed for 10 min. Palladium tetrakis triphenylphosphine (24.78 mg, 0.020 mmol) was added and the mixture degassed again for 10 min, then stirred on at 85 °C. The reaction mixture was left to reach RT, EtOAc was added and it was filtered and concentrated. Crude was purified by flash chromatography (silica 10g + 10g, Cy/EtOAc from 98:2 to 6:4) giving *3-(3-fluoro-4-methoxyphenyl)-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole* (N0423-30-1: 75 mg, 0.147 mmol, 68.37% yield) as colourless oil.

LCMS acidic r.t. 1.36 min, MS (ESI)  $m/z = 512.4$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 2.39 - 2.42 (3 H, m) 3.58 - 3.62 (6 H, m) 3.64 (3 H, s) 3.80 - 3.84 (3 H, m) 6.44 - 6.49 (2 H, m) 6.96 (1 H, dt, J=8.42, 1.62 Hz) 7.07 - 7.15 (2 H, m) 7.47 - 7.52 (2 H, m) 7.56 (1 H, d, J=2.64 Hz) 7.62 (1 H, d, J=2.42 Hz) 7.98 - 8.04 (2 H, m).

A mixture of 3-(3-fluoro-4-methoxyphenyl)-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (75.0 mg, 0.150 mmol) in Ethanol (10.47 mL) and sodium hydroxide (3.3 mL, 3.3 mmol) 1M was stirred on at 50 °C. The reaction mixture was left to reach RT, then HCl 1M (3.3 mL) was added: volatile were removed. DCM and a ss aq solution of sodium bicarbonate were added: phases were separated, the organic one washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 10g, Cy/EtOAc from 9:1 to 6:4) affording *3-(3-fluoro-4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole* (N0423-34-1: 35 mg, 0.098 mmol, 66.8% yield) as yellowish solid.

LCMS acidic r.t. 1.07 min, MS (ESI)  $m/z = 358.3$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 3.62 (6 H, s) 3.65 (3 H, s) 3.81 (3 H, s) 6.45 - 6.48 (2 H, m) 6.94 (1 H, t, J=2.42 Hz) 6.96 - 7.00 (2 H, m) 7.00 - 7.05 (1 H, m) 7.05 - 7.11 (1 H, m) 11.07 (1 H, br. s.).

## 2. EXPERIMENTAL PART

### N0423-35 (CP010) 3-(3-chloro-4-ethoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole

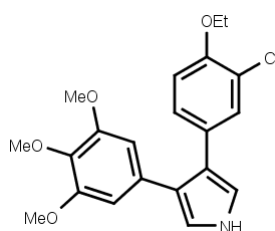


Figure 2.29: CP010

A mixture of 3-bromo-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (100.0 mg, 0.210 mmol), sodium carbonate (68.18 mg, 0.640 mmol) and (3-chloro-4-ethoxyphenyl)boronic acid (45.13 mg, 0.230 mmol) in 1,2-dimethoxyethane (2.198 mL) and Water (0.330 mL) was degassed for 10 min. Palladium tetrakis triphenylphosphine (24.78 mg, 0.020 mmol) was added and the mixture degassed again for 10 min, then stirred on at 85 °C. The reaction mixture was left to reach RT, EtOAc was added and it was filtered and concentrated. Crude was purified by flash chromatography (silica 10g + 10g, Cy/EtOAc from 98:2 to 6:4) giving 3-(3-chloro-4-ethoxyphenyl)-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (N0423-31-1: 93 mg, 0.172 mmol, 80.01% yield) as colourless oil.

LCMS acidic r.t. 1.46 min, MS (ESI)  $m/z$ = 542.3 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 1.33 (3 H, t, J=6.93 Hz) 2.38 - 2.45 (3 H, m) 3.58 - 3.62 (6 H, m) 3.63 - 3.68 (3 H, m) 4.06 - 4.14 (2 H, m) 6.45 - 6.49 (2 H, m) 7.06 - 7.12 (2 H, m) 7.33 (1 H, d, J=1.76 Hz) 7.49 (2 H, d, J=7.92 Hz) 7.58 - 7.60 (1 H, m) 7.62 (1 H, d, J=2.64 Hz) 7.98 - 8.02 (2 H, m).

A mixture of 3-(3-chloro-4-ethoxyphenyl)-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (3.86 mL, 0.170 mmol) in Ethanol (12.26 mL) and sodium hydroxide (3.86 mL, 3.86 mmol) was stirred on at 50 °C. The mixture was left to reach RT, then cooled at 0°C: HCl 1M (3.86 mL) was added and volatiles were removed. DCM and a ss aq solution of sodium bicarbonate were added: phases were separated, the organic one washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 10g, Cy/EtOAc from 9:1 to affording 3-(3-chloro-4-ethoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (N0423-35-1: 27 mg, 0.070 mmol, 40.57% yield)

LCMS acidic r.t. 1.20 min, MS (ESI)  $m/z$ = 388.3 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 1.34 (3 H, t, J=6.93 Hz) 3.60 - 3.63 (6 H, m) 3.64 - 3.67 (3 H, m) 4.09 (2 H, q, J=6.97 Hz) 6.45 - 6.49 (2 H, m) 6.95 (1 H, t, J=2.42 Hz) 7.00 (1 H, t, J=2.42 Hz) 7.04 - 7.08 (1 H, m) 7.11 - 7.15 (1 H, m) 7.26 (1 H, d, J=1.98 Hz) 11.08 (1 H, br. s.).

### N0423-36 (CP011) 3-(4-ethylphenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole

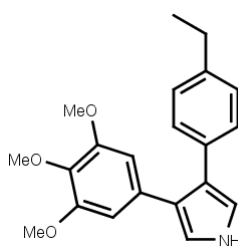


Figure 2.30: CP011

## 2. EXPERIMENTAL PART

A mixture of 3-bromo-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (100.0 mg, 0.210 mmol), sodium carbonate (68.18 mg, 0.640 mmol) and (4-ethylphenyl)boronic acid (33.77 mg, 0.230 mmol) in 1,2-dimethoxyethane (2.198 mL) and Water (0.330 mL) was degassed for 10 min. Palladium tetrakis triphenylphosphine (24.78 mg, 0.020 mmol) was added and the mixture degassed again for 10 min, then stirred on at 85 °C. The reaction mixture was left to reach RT, EtOAc was added and it was filtered and concentrated. Crude was purified by flash chromatography (silica 10g + 10g, Cy/EtOAc from 98:2 to 6:4) giving 3-(4-ethylphenyl)-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (N0423-32-1: 97 mg, 0.197 mmol, 92.02% yield) as colourless oil.

LCMS acidic r.t. 1.49 min, MS (ESI) m/z= 492.4 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 1.12 - 1.18 (3 H, m) 2.41 (3 H, s) 2.56 - 2.62 (2 H, m) 3.55 (6 H, s) 3.63 (3 H, s) 6.42 - 6.45 (2 H, m) 7.10 - 7.19 (4 H, m) 7.46 - 7.52 (3 H, m) 7.65 (1 H, d, J=2.42 Hz) 7.97 - 8.02 (2 H, m).

A mixture of 3-(4-ethylphenyl)-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (97.0 mg, 0.200 mmol) in Ethanol (14.09 mL) and sodium hydroxide (4.44 mL, 4.44 mmol) 1M was stirred on at 50 °C. The mixture was left to reach RT, then cooled at 0°C and HCl 1M (4.44 mL) was added: volatiles were removed. DCM and a ss aq solution of sodium bicarbonate was added: phases were separated, the organic one washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 10g, Cy/EtOAc from 9:1 to 6:4) affording 3-(4-ethylphenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (N0423-36-1: 34 mg, 0.101 mmol, 51.07% yield) as white solid.

LCMS acidic r.t. 1.22 min, MS (ESI) m/z= 338.3 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 1.16 (3 H, t, J=7.48 Hz) 2.54 - 2.61 (2 H, m) 3.55 - 3.59 (6 H, m) 3.61 - 3.65 (3 H, m) 6.44 (2 H, s) 6.88 (1 H, t, J=2.42 Hz) 7.00 (1 H, t, J=2.42 Hz) 7.10 - 7.16 (4 H, m) 11.02 (1 H, br. s.).

### N0423-39 (CP012) 3-(4-ethylsulfanylphenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole

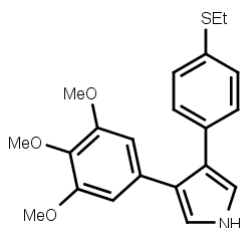


Figure 2.31: CP012

A mixture of 3-bromo-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (100.0 mg, 0.210 mmol), sodium carbonate (68.18 mg, 0.640 mmol) and (4-ethylsulfanylphenyl)boronic acid (40.99 mg, 0.230 mmol) in 1,2-dimethoxyethane (2.198 mL) and Water (0.330 mL) was degassed for 10 min. Palladium tetrakis triphenylphosphine (24.78 mg, 0.020 mmol) was added and the mixture degassed again for 10 min, then stirred on at 85 °C. The reaction mixture was left to reach RT, EtOAc was added and it was filtered and concentrated. Crude was purified by flash chromatography (silica 10g + 10g, Cy/EtOAc from 98:2 to 6:4) giving 3-(4-ethylsulfanylphenyl)-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (N0423-38-1: 79 mg, 0.151 mmol, 70.35% yield) as white foam.

LCMS acidic r.t. 1.48 min, MS (ESI) m/z= 524.4 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 1.16 - 1.23 (3 H, m) 2.96 (2 H, q, J=7.41 Hz) 3.56 - 3.59 (6 H, m) 3.61 - 3.65 (3 H, m) 6.42 - 6.46 (2 H, m) 7.14 - 7.19 (2 H, m) 7.25 - 7.30 (2 H, m) 7.47 - 7.52 (2 H, m) 7.54 - 7.56 (1 H, m) 7.64 - 7.66 (1 H, m) 7.98 - 8.03 (2 H, m).

A mixture of 3-(4-ethylsulfanylphenyl)-1-(4-methylphenyl)sulfonyl-4-

## 2. EXPERIMENTAL PART

(3,4,5-trimethoxyphenyl)pyrrole (79.0 mg, 0.150 mmol) in Ethanol (10.78 mL) and sodium hydroxide (3.39 mL, 3.39 mmol) 1M was stirred on at 50 °C. The mixture was left to reach RT, then cooled at 0°C and HCl 1M (3.39 mL) was added: volatiles were removed. DCM and a ss aq solution of sodium bicarbonate was added: phases were separated, the organic one washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 10g, Cy/EtOAc from 95:5 to 7:3) affording *3-(4-ethylsulfanylphenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole* (N0423-39-1: 33.5 mg, 0.091 mmol, 60.1% yield) as white solid.

LCMS acidic r.t. 1.22 min, MS (ESI)  $m/z=370.3$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 1.17 - 1.24 (3 H, m) 2.93 (2 H, q, J=7.26 Hz) 3.58 - 3.61 (6 H, m) 3.63 - 3.67 (3 H, m) 6.45 (2 H, s) 6.94 (1 H, t, J=2.42 Hz) 7.01 (1 H, t, J=2.42 Hz) 7.16 - 7.21 (2 H, m) 7.23 - 7.28 (2 H, m) 11.09 (1 H, br. s.).

### N0423-42 (CP013) 3-[4-(trifluoromethyl)phenyl]-4-(3,4,5-trimethoxy phenyl)-1H-pyrrole

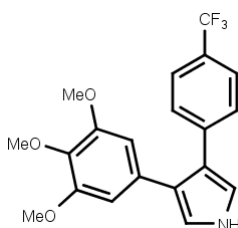


Figure 2.32: CP013

A mixture of 3-bromo-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (100.0 mg, 0.210 mmol), sodium carbonate (68.18 mg, 0.640 mmol) and [4-(trifluoromethyl)phenyl]boronic acid (42.76 mg, 0.230 mmol) in 1,2-dimethoxyethane (2.198 mL) and Water (0.330 mL) was degassed for 10 min. Palladium tetrakis triphenylphosphine (24.78 mg, 0.020 mmol) was added and the mixture degassed again for 10 min, then stirred on at 85 °C. The reaction mixture was left to reach RT, EtOAc was added and it was filtered and concentrated. Crude was purified by flash chromatography (silica 10g + 10g, Cy/EtOAc from 98:2 to 6:4) giving *1-(4-methylphenyl)sulfonyl-3-[4-(trifluoromethyl)phenyl]-4-(3,4,5-trimethoxyphenyl)pyrrole* (N0423-40-1: 84 mg, 0.158 mmol, 73.7% yield) as white foam.

LCMS acidic r.t. 1.45 min, MS (ESI)  $m/z=532.4$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 2.41 (3 H, s) 3.56 - 3.58 (6 H, m) 3.63 - 3.65 (3 H, m) 6.41 - 6.43 (2 H, m) 7.42 - 7.46 (2 H, m) 7.48 - 7.53 (2 H, m) 7.66 - 7.73 (4 H, m) 7.99 - 8.05 (2 H, m).

A mixture of 1-(4-methylphenyl)sulfonyl-3-[4-(trifluoromethyl)phenyl]-4-(3,4,5-trimethoxyphenyl)pyrrole (84.0 mg, 0.160 mmol) in Ethanol (11.29 mL) and sodium hydroxide (3.56 mL, 3.56 mmol) 1M was stirred on at 50 °C. The mixture was left to reach RT, then it was cooled at 0°C and HCl 1 M (3.56 mL) was added: volatiles were removed, then DCM and a ss aq solution of sodium bicarbonate were added. Phases were separated, the organic one washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography, reverse phase (silica 12g, water+0.1% formic acid / MeCN+0.1% formic acid, from 98:2 to 1:1) affording *3-[4-(trifluoromethyl)phenyl]-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole* (N0423-42-1: 34.5 mg, 0.091 mmol, 57.85% yield) as white solid.

LCMS acidic r.t. 1.21 min, MS (ESI)  $m/z=378.3$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 3.60 (6 H, s) 3.65 (3 H, s) 6.44 (2 H, s) 7.04 (1 H, t, J=2.53 Hz) 7.11 (1 H, t, J=2.42 Hz) 7.44 (2 H, d, J=7.92 Hz) 7.62 (2 H, d, J=8.14 Hz) 11.25 (1 H, br. s.).

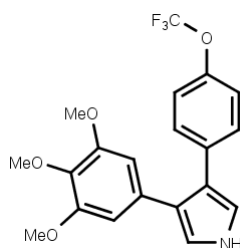
**N0423-43 (CP014) 3-[4-(trifluoromethoxy)phenyl]-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole**

Figure 2.33: CP014

A mixture of 3-bromo-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (100.0 mg, 0.210 mmol), sodium carbonate (68.18 mg, 0.640 mmol) and [4-(trifluoromethoxy)phenyl]boronic acid (44.16 mg, 0.210 mmol) in 1,2-dimethoxyethane (2.198 mL) and Water (0.330 mL) was degassed for 10 min. Palladium tetrakis triphenylphosphine (24.78 mg, 0.020 mmol) was added and the mixture degassed again for 10 min, then stirred on at 85 °C. The reaction mixture was left to reach RT, EtOAc was added and it was filtered and concentrated. Crude was purified by flash chromatography (silica 10g + 10g, Cy/EtOAc from 98:2 to 6:4) giving *1-(4-methylphenyl)sulfonyl-3-[4-(trifluoromethoxy)phenyl]-4-(3,4,5-trimethoxyphenyl)pyrrole* (N0423-41-1: 85 mg, 0.155 mmol, 72.4% yield) as white foam.

LCMS acidic r.t. 1.47 min, MS (ESI)  $m/z$ = 548.4 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 2.39 - 2.42 (3 H, m) 3.54 - 3.58 (6 H, m) 3.63 (3 H, s) 6.41 (2 H, s) 7.32 - 7.36 (4 H, m) 7.47 - 7.52 (2 H, m) 7.63 (1 H, d,  $J$ =2.42 Hz) 7.69 (1 H, d,  $J$ =2.42 Hz) 7.99 - 8.03 (2 H, m).

A mixture of 1-(4-methylphenyl)sulfonyl-3-[4-(trifluoromethoxy)phenyl]-4-(3,4,5-trimethoxyphenyl)pyrrole (85.0 mg, 0.160 mmol) in sodium hydroxide (3.49 mL, 3.49 mmol) 1M and Ethanol (11.09 mL) was stirred on at 50 °C. The mixture was left to reach RT, then it was cooled at 0°C and HCl 1M (3.49 mL) was added: volatiles were removed. DCM and a ss aq solution of sodium bicarbonate were added: phases were separated, the organic one washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography, reverse phase (silica c18 12g, water+0.1% formic acid / MeCN+0.1% formic acid, from 98:2 to 1:1) affording *3-[4-(trifluoromethoxy)phenyl]-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole* (N0423-43-1: 34.4 mg, 0.087 mmol, 56.33% yield) as white solid.

LCMS acidic r.t. 1.24 min, MS (ESI)  $m/z$ = 394.3 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 3.58 (6 H, s) 3.64 (3 H, s) 6.42 (2 H, s) 7.00 (1 H, t,  $J$ =2.42 Hz) 7.04 (1 H, t,  $J$ =2.42 Hz) 7.25 - 7.30 (2 H, m) 7.31 - 7.35 (2 H, m) 11.16 (1 H, br. s.).

**N0423-44 (CP015) 3-(4-propoxyphenyl)-4-(3,4,5-trimethoxyphenyl)- 1H-pyrrole**

A mixture of 3-bromo-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (100.0 mg, 0.210 mmol), sodium carbonate (68.18 mg, 0.640 mmol) and (4-propoxyphenyl)boronic acid (40.53 mg, 0.230 mmol) in 1,2-dimethoxyethane (2.198 mL) and Water (0.330 mL) was degassed for 10 min. Palladium tetrakis triphenylphosphine (24.78 mg, 0.020 mmol) was added and the mixture degassed again for 10 min, then stirred on at 85 °C. The reaction mixture was left to reach RT, EtOAc was added and it was filtered and concentrated. Crude was purified by flash chromatography (silica 10g + 10g, Cy/EtOAc from 98:2 to 6:4) giving *1-(4-methylphenyl)sulfonyl-3-(4-propoxyphenyl)-4-(3,4,5-trimethoxyphenyl)pyrrole* (N0423-37-1: 28 mg, 0.054 mmol, 25.03% yield) as colourless oil.

LCMS acidic r.t. 1.48 min, MS (ESI)  $m/z$ = 522.5 [M+H]<sup>+</sup>.

## 2. EXPERIMENTAL PART

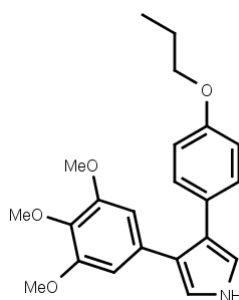


Figure 2.34: CP015

$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  ppm 0.93 - 0.99 (3 H, m) 1.67 - 1.74 (2 H, m) 2.40 (3 H, s) 3.58 (6 H, s) 3.62 - 3.65 (3 H, m) 3.91 (2 H, t,  $J=6.60$  Hz) 6.45 (2 H, s) 6.88 (2 H, d,  $J=9.02$  Hz) 7.11 - 7.15 (2 H, m) 7.45 (1 H, d,  $J=2.64$  Hz) 7.49 (2 H, d,  $J=7.92$  Hz) 7.62 (1 H, d,  $J=2.64$  Hz) 7.97 - 8.02 (2 H, m).

A mixture of 1-(4-methylphenyl)sulfonyl-3-(4-propoxyphenyl)-4-(3,4,5-trimethoxyphenyl)pyrrole (28.0 mg, 0.050 mmol) in Ethanol (3.834 mL) and sodium hydroxide (1.21 mL, 1.21 mmol) 1M was stirred on at 50 °C. The reaction mixture was left to reach RT, then cooled at 0°C and HCl 1M (1.21 mL) was added. Volatiles were removed, then DCM and a ss aq solution of sodium bicarbonate were added: phases were separated, the organic one washed with brine, dried over sodium sulfate, filtered and concentrated. crude was purified by flash chromatography, reverse phase (silica c18 12g, water+0.1% formic acid / MeCN+0.1% formic acid, from 98:2 to 4:6) affording 3-(4-propoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (N0423-44-1: 16 mg, 0.044 mmol, 81.12% yield) as white solid.

LCMS acidic r.t. 1.22 min, MS (ESI)  $m/z$ = 368.3  $[\text{M}+\text{H}]^+$ .

$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  ppm 0.97 (3 H, t,  $J=7.37$  Hz) 1.66 - 1.76 (2 H, m) 3.57 - 3.61 (6 H, m) 3.63 (3 H, s) 3.90 (2 H, t,  $J=6.49$  Hz) 6.44 - 6.48 (2 H, m) 6.82 - 6.88 (3 H, m) 6.99 (1 H, t,  $J=2.42$  Hz) 7.11 - 7.16 (2 H, m) 10.99 (1 H, br. s.).

### 2.3 Synthesis of (3,4,5-trimethoxyphenyl)pyrrol-1-yl esters

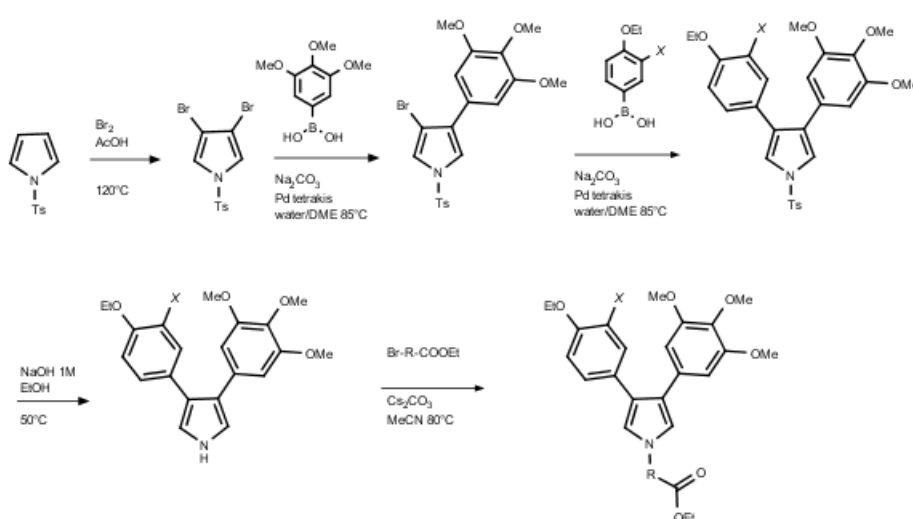


Figure 2.35: general route for 4-(3,4,5-trimethoxyphenyl)pyrrol-1-yl]esters

Two of the best compound of previous series (CP007 -fig 2.26- and CP010 -fig 2.29-) were chosen to generate few derivatives: if they will maintain the activity, hydroxamic acids



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will be synthesized instead of these esters (that have a shorter synthesis). The synthetic route, reported in fig 2.35, compared to the one of the previous series, has a further step: the N-pyrrole's alkylation (with the appropriate bromo-alkyl ester).

### N0758-07 (CP022) ethyl 3-[3-(4-ethoxy-3-fluorophenyl)-4-(3,4,5-trimethoxyphenyl)pyrrol-1-yl]propanoate

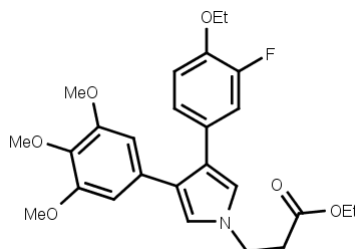


Figure 2.36: CP022

A mixture of 3-bromo-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (500.0 mg, 1.06 mmol), sodium carbonate (337.5 mg, 3.18 mmol) and (4-ethoxy-3-fluorophenyl)boronic acid (205.04 mg, 1.11 mmol) in 1,2-dimethoxyethane (8.703 mL) and Water (1.305 mL) was degassed for 10 min. Palladium tetrakis triphenylphosphine (122.66 mg, 0.110 mmol) was added and the mixture degassed again for 10 min, then stirred on at 85 °C. The reaction mixture was left to reach RT, EtOAc was added and it was filtered over a pad of celite and concentrated. Crude was purified by flash chromatography (silica 25g + 25g, DCM/EtOAc from 10:0 to 8:2) giving 3-(4-ethoxy-3-fluorophenyl)-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (N0758-05-1: 520 mg, 0.989 mmol, 93.21% yield) as yellowish oil.

LCMS acidic r.t. 1.40 min, MS (ESI) m/z= 526.17 [M+H]<sup>+</sup>.

A mixture of 3-(4-ethoxy-3-fluorophenyl)-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (520.0 mg, 0.970 mmol) in Ethanol (69.26 mL) and sodium hydroxide (21.82 mL, 21.82 mmol) 1M was stirred on at 50 °C. The mixture was left to reach RT and cooled at 0°C, then HCl 1M (21.82 mL) was added. Volatiles were removed and a ss aq solution of NaHCO<sub>3</sub> was added, followed by DCM: phases were separated, the organic one washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 25g + 25g, Cy/EtOAc from 9:1 to 6:4) affording 3-(4-ethoxy-3-fluorophenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (N0758-06-1: 120 mg, 0.323 mmol, 33.32% yield) as a yellow solid.

LCMS acidic r.t. 1.13 min, MS (ESI) m/z= 372.2 [M+H]<sup>+</sup>.

3-bromopropanoic acid ethyl ester (0.01 mL, 0.080 mmol) was added to a mixture of 3-(4-ethoxy-3-fluorophenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (20.0 mg, 0.050 mmol) and 3-bromopropanoic acid ethyl ester (0.01 mL, 0.080 mmol) in MeCN (1 mL). The reaction mixture was stirred at 90 °C for 2hours, then it was left to reach RT. Water and EtOAc were added: phases were separated, the organic one dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (SFAR D 10g, Cy/EtOAc from 10:0 to 6:4) affording ethyl 3-[3-(4-ethoxy-3-fluorophenyl)-4-(3,4,5-trimethoxyphenyl)pyrrol-1-yl]propanoate (N0758-07-1: 15.5 mg, 0.033 mmol, 61.04% yield) as yellowish oil.

LCMS acidic r.t. 1.27 min, MS (ESI) m/z= 472.2 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 1.15 - 1.25 (3 H, m) 1.28 - 1.38 (3 H, m) 2.88 (2 H, t, J=6.82 Hz) 3.58 - 3.63 (6 H, m) 3.64 - 3.67 (3 H, m) 4.03 - 4.13 (4 H, m) 4.16 (2 H, t, J=6.82 Hz) 6.39 - 6.50 (2 H, m) 6.90 - 7.11 (5 H, m).

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### N0758-08 (CP023) ethyl 4-[3-(4-ethoxy-3-fluorophenyl)-4-(3,4,5-trimethoxyphenyl)pyrrol-1-yl]butanoate

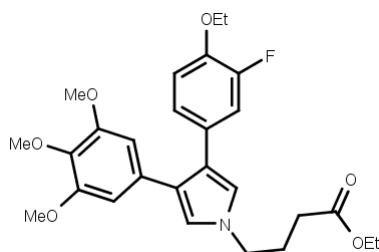


Figure 2.37: CP023

4-bromobutanoic acid ethyl ester (0.01 mL, 0.080 mmol) was added to a mixture of 3-(4-ethoxy-3-fluorophenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (20.0 mg, 0.050 mmol) and dicesium carbonate (52.64 mg, 0.160 mmol) in MeCN (1 mL). The reaction mixture was stirred at 90 °C on, then it was left to reach RT. Water and EtOAc were added: phases were separated, the organic one dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (SFAR D 10g, Cy/EtOAc from 10:0 to 6:4) affording *ethyl 4-[3-(4-ethoxy-3-fluorophenyl)-4-(3,4,5-trimethoxyphenyl)pyrrol-1-yl]butanoate* (N0758-08-1: 9.5 mg, 0.020 mmol, 36.33% yield) as a yellow glassy solid.

LCMS acidic r.t. 1.31 min, MS (ESI)  $m/z = 486.17$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 1.15 - 1.20 (3 H, m) 1.29 - 1.36 (3 H, m) 1.99 - 2.07 (2 H, m) 2.30 - 2.38 (2 H, m) 3.61 - 3.63 (6 H, m) 3.64 - 3.66 (3 H, m) 3.92 (2 H, t, J=6.93 Hz) 4.03 - 4.11 (4 H, m) 6.44 - 6.46 (2 H, m) 6.93 - 6.97 (2 H, m) 6.98 - 7.03 (2 H, m) 7.05 - 7.10 (1 H, m).

### N0758-09 (CP024) ethyl 5-[3-(4-ethoxy-3-fluorophenyl)-4-(3,4,5-trimethoxyphenyl)pyrrol-1-yl]pentanoate

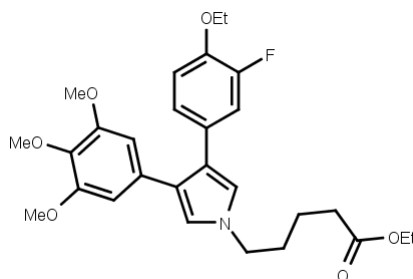


Figure 2.38: CP024

5-bromopentanoic acid ethyl ester (0.01 mL, 0.080 mmol) was added to a mixture of 3-(4-ethoxy-3-fluorophenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (20.0 mg, 0.050 mmol) and dicesium carbonate (52.64 mg, 0.160 mmol) in MeCN (1 mL). The reaction mixture was stirred at 90 °C for 2 hours, then it was left to reach RT. Water and EtOAc were added: phases were separated, the organic one dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (SFAR D 10g, Cy/EtOAc from 10:0 to 6:4) affording *ethyl 5-[3-(4-ethoxy-3-fluorophenyl)-4-(3,4,5-trimethoxyphenyl)pyrrol-1-yl]pentanoate* (N0758-09-1: 19.3 mg, 0.039 mmol, 71.74% yield) as yellow solid.

LCMS acidic r.t. 1.35 min, MS (ESI)  $m/z = 500.2$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 1.13 - 1.20 (3 H, m) 1.30 - 1.36 (3 H, m) 1.52 - 1.59 (2 H, m) 1.73 - 1.83 (2 H, m) 2.31 - 2.38 (2 H, m) 3.61 - 3.63 (6 H, m) 3.64 - 3.66 (3 H, m)

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3.87 - 3.93 (2 H, m) 4.01 - 4.12 (4 H, m) 6.44 - 6.46 (2 H, m) 6.92 - 6.97 (2 H, m) 6.97 - 7.02 (2 H, m) 7.04 - 7.09 (1 H, m).

### N0758-12 (CP025) ethyl 3-[3-(3-chloro-4-ethoxyphenyl)-4-(3,4,5-trimethoxyphenyl)pyrrol-1-yl]propanoate

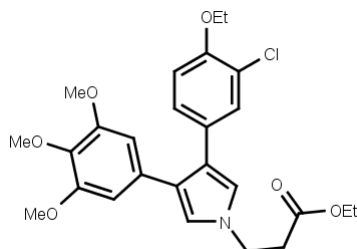


Figure 2.39: CP025

A mixture of 3-bromo-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (500.0 mg, 1.07 mmol), sodium carbonate (340.91 mg, 3.22 mmol) and (3-chloro-4-ethoxyphenyl)boronic acid (225.64 mg, 1.13 mmol) in 1,2-dimethoxyethane (7.033 mL) and Water (1.055 mL) was degassed for 10 min. Palladium tetrakis triphenylphosphine (123.89 mg, 0.110 mmol) was added and the mixture degassed again for 10 min, then stirred on at 85 °C. The reaction mixture was left to reach RT, EtOAc was added and it was filtered over a pad of celite and concentrated. Crude was purified by flash chromatography (silica 25g + 25g, Cy/EtOAc from 10:0 to 6:4) giving 3-(3-chloro-4-ethoxyphenyl)-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (N0758-10-1: 529 mg, 0.976 mmol, 91.03% yield) as a yellow foam.

LCMS acidic r.t. 1.46 min, MS (ESI)  $m/z$  = 542.16 [M+H]<sup>+</sup>.

A mixture of 3-(3-chloro-4-ethoxyphenyl)-1-(4-methylphenyl)sulfonyl-4-(3,4,5-trimethoxyphenyl)pyrrole (529.0 mg, 0.940 mmol) in Ethanol (69.02 mL) and sodium hydroxide (21.08 mL, 21.08 mmol) 1M was stirred on at 50 °C. The mixture was left to reach RT and cooled at 0°C, then HCl 1M (21.82 mL) was added. Volatiles were removed and a ss aq solution of NaHCO<sub>3</sub> was added, followed by DCM: phases were separated, the organic one washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 25g + 25g, Cy/EtOAc from 9:1 to 6:4) affording 3-(3-chloro-4-ethoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (N0758-11-1: 184 mg, 0.474 mmol, 50.63% yield) as a yellow solid.

LCMS acidic r.t. 1.19 min, MS (ESI)  $m/z$  = 388.21 [M+H]<sup>+</sup>.

3-bromopropanoic acid ethyl ester (0.01 mL, 0.070 mmol) was added to a mixture of 3-(3-chloro-4-ethoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (20.0 mg, 0.050 mmol) and dicesium carbonate (50.4 mg, 0.150 mmol) in MeCN (1 mL). The reaction mixture was stirred at 90 °C overnight, then it was left to reach RT. Water and EtOAc were added: phases were separated, the organic one dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (SFAR D 10g, Cy/EtOAc from 10:0 to 6:4) affording ethyl 3-[3-(3-chloro-4-ethoxyphenyl)-4-(3,4,5-trimethoxyphenyl)pyrrol-1-yl]propanoate (N0758-12-1: 17.3 mg, 0.035 mmol, 68.75% yield) as yellowish oil.

LCMS acidic r.t. 1.33min, MS (ESI)  $m/z$  = 488.16 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 1.16 - 1.25 (3 H, m) 1.30 - 1.39 (3 H, m) 2.88 (2 H, t, J=6.93 Hz) 3.59 - 3.63 (6 H, m) 3.63 - 3.66 (3 H, m) 4.05 - 4.13 (4 H, m) 4.15 - 4.23 (2 H, m) 6.42 - 6.46 (2 H, m) 6.95 - 7.04 (2 H, m) 7.04 - 7.12 (2 H, m) 7.20 - 7.25 (1 H, m).

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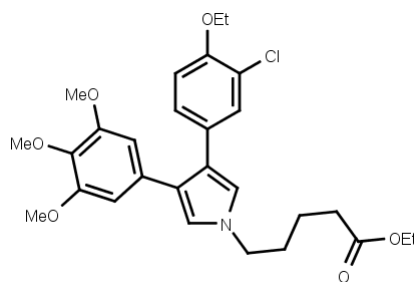


Figure 2.40: CP026

### **N0758-14 (CP026) ethyl 5-[3-(3-chloro-4-ethoxyphenyl)-4-(3,4,5-trimethoxyphenyl)pyrrol-1-yl]pentanoate**

5-bromopentanoic acid ethyl ester (0.01 mL, 0.070 mmol) was added to a mixture of 3-(3-chloro-4-ethoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (20.0 mg, 0.050 mmol) and dicesium carbonate (50.4 mg, 0.150 mmol) in MeCN (5 mL). The reaction mixture was stirred at 90 °C overnight, then it was left to reach RT. Water and EtOAc were added: phases were separated, the organic one dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (SFAR D 10g, Cy/EtOAc from 10:0 to 6:4) affording *ethyl 5-[3-(3-chloro-4-ethoxyphenyl)-4-(3,4,5-trimethoxyphenyl)pyrrol-1-yl]pentanoate* (N0758-14-1: 15 mg, 0.029 mmol, 56.37% yield) as yellow solid.

LCMS acidic r.t. 1.40 min, MS (ESI)  $m/z = 516.2$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 1.13 - 1.23 (3 H, m) 1.30 - 1.37 (3 H, m) 1.48 - 1.59 (2 H, m) 1.75 - 1.84 (2 H, m) 2.35 (2 H, t, J=7.37 Hz) 3.59 - 3.63 (6 H, m) 3.64 - 3.68 (3 H, m) 3.87 - 3.95 (2 H, m) 4.02 - 4.15 (4 H, m) 6.44 - 6.51 (2 H, m) 6.95 - 6.99 (1 H, m) 7.02 (1 H, d, J=2.20 Hz) 7.04 - 7.08 (1 H, m) 7.09 - 7.13 (1 H, m) 7.23 - 7.30 (1 H, m).

### **N0758-15 (CP027) ethyl 4-[3-(3-chloro-4-ethoxyphenyl)-4-(3,4,5-trimethoxyphenyl)pyrrol-1-yl]butanoate**

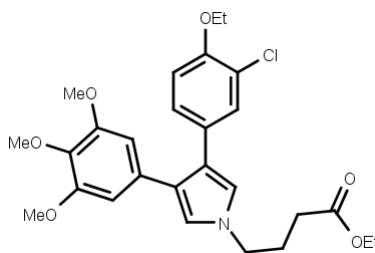


Figure 2.41: CP027

4-bromobutanoic acid ethyl ester (0.01 mL, 0.070 mmol) was added to a mixture of 3-(3-chloro-4-ethoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole (20.0 mg, 0.050 mmol) and dicesium carbonate (50.4 mg, 0.150 mmol) in MeCN (2.5 mL). The reaction mixture was stirred at 90 °C on, then it was left to reach RT. Water and EtOAc were added: phases were separated, the organic one dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (SFAR D 10g, Cy/EtOAc from 10:0 to 6:4) affording 25 mg of a mixture named N0758-15-1 as a yellow glassy solid. Compound was impure, so it was purified again by flash chromatography, reverse phase (silica c18 12g, water + 0.1% formic acid / MeCN + 0.1% formic acid, from 98:2 to 2:8) giving *ethyl 4-[3-(3-chloro-4-ethoxyphenyl)-4-(3,4,5-trimethoxyphenyl)pyrrol-1-yl]butanoate* (N0758-15-2: 9.5 mg, 0.019 mmol, 36.7% yield) as yellow solid.

LCMS acidic r.t. 1.37 min, MS (ESI)  $m/z = 502.18$  [M+H]<sup>+</sup>.

## 2. EXPERIMENTAL PART

$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  ppm 1.15 - 1.21 (3 H, m) 1.30 - 1.35 (3 H, m) 1.99 - 2.05 (2 H, m) 2.28 - 2.32 (2 H, m) 3.61 (6 H, s) 3.64 (3 H, s) 3.90 - 3.95 (2 H, m) 4.07 (4 H, dq,  $J=9.46, 7.04$  Hz) 6.45 (2 H, s) 6.95 - 7.02 (2 H, m) 7.04 - 7.07 (1 H, m) 7.09 - 7.12 (1 H, m) 7.25 (1 H, d,  $J=1.98$  Hz).

### 2.4 Synthesis of 1H-1,2,4-triazole-3,5-diamines

The aim of this synthesis was to obtain 1,2,4-triazole-3,5-diamines acylated with 3,4,5-trimethoxybenzoyl chloride in N1 position of the triazole: for each compounds synthesis started from the appropriate benzylamine, followed by the cyclization with hydrazine hydrate to give the 1H-1,2,4-triazole-3,5-diamines.

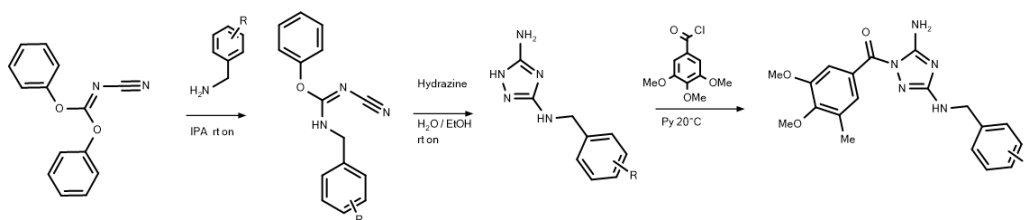


Figure 2.42: general route for 1H-1,2,4-triazole-3,5-diamines

In my hand, it was not possible to acylate selectively the desired position: for each intermediate, there were 3 possible sites that could be acylated and, even changing reaction conditions (solvents, temperature and bases), for most of compounds was not possible to get the correct one, maybe due to the batch of the reagent 3,4,5-trimethoxybenzoyl chloride used. For this reason, my synthetic job ended with the 1H-1,2,4-triazole-3,5-diamines, that were shipped at the University of Ferrara, where it was possible to acylate the correct position.

#### N0237-47-1 3-N-[(4-fluorophenyl)methyl]-1H-1,2,4-triazole-3,5-diamine

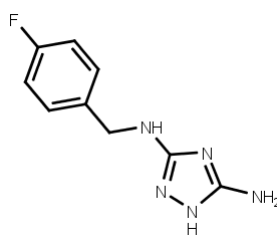


Figure 2.43: N0237-47-1

A mixture of diphenoxymethylidene cyanamide (500.0 mg, 2.1 mmol) and (4-fluorophenyl)methanamine (0.24 mL, 2.1 mmol) in IPA (7 mL) was stirred at RT for 3.5h. The mixture was filtered, cake was washed with diethyl ether, giving *phenyl N'-cyano-N-[(4-fluorophenyl)methyl]carbamimidate* (N0237-45-1: 315 mg, 1.17 mmol, 55.74% yield) as white solid.

LCMS acidic r.t. 0.99 min, MS (ESI)  $m/z=270.1$   $[\text{M}+\text{H}]^+$ .

$^1\text{H}$  NMR (400 MHz, chloroform- $d_6$ )  $\delta$  ppm 4.49 - 4.65 (2 H, m) 6.86 (1 H, br. s.) 7.00 - 7.19 (4 H, m) 7.30 - 7.54 (5 H, m).

Phenyl *N'*-cyano-*N*-[(4-fluorophenyl)methyl]carbamimidate (314.0 mg, 1.17 mmol) was added to a stirred mixture of hydrazine hydrate (0.18 mL, 2.33 mmol) in Ethanol (4.971 mL). The reaction mixture was stirred at RT on, then it was concentrated. DCM was added and the mixture filtered, giving *3-N-[(4-fluorophenyl)methyl]-1H-1,2,4-triazole-3,5-diamine* (N0237-47-1: 186 mg, 0.898 mmol, 76.98% yield) as yellowish solid.

## 2. EXPERIMENTAL PART

LCMS acidic r.t. 0.38 min, MS (ESI)  $m/z=208$   $[M+H]^+$ .

$^1\text{H}$  NMR (400 MHz, methanol- $d_6$ )  $\delta$  ppm 4.31 - 4.36 (2 H, m) 7.01 - 7.08 (2 H, m) 7.34 - 7.39 (2 H, m).

### N0237-48-1 3-N-[(3,5-dimethylphenyl)methyl]-1H-1,2,4-triazole-3,5-diamine

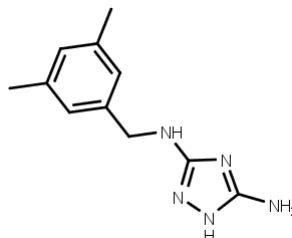


Figure 2.44: N0237-48-1

A mixture of diphenoxymethylidencyanamide (500.0 mg, 2.1 mmol) and 3,5-dimethylbenzylamine (0.3 mL, 2.1 mmol) in IPA (7 mL) was stirred at RT for 3.5h. The mixture was filtered, cake was washed with diethyl ether, giving *phenyl N'-cyano-N-[(3,5-dimethylphenyl)methyl]carbamimidate* (N0237-46-1: 314 mg, 1.124 mmol, 53.56% yield) as white solid.

LCMS acidic r.t. 1.13 min, MS (ESI)  $m/z=280.2$   $[M+H]^+$ .

$^1\text{H}$  NMR (400 MHz, chloroform- $d_6$ )  $\delta$  ppm 2.27 - 2.45 (6 H, m) 4.55 - 4.63 (2 H, m) 6.16 (1 H, br. s.) 6.91 - 7.05 (3 H, m) 7.10 (2 H, d,  $J=7.48$  Hz) 7.33 (1 H, d,  $J=7.48$  Hz) 7.41 - 7.50 (2 H, m).

Phenyl *N'-cyano-N-[(3,5-dimethylphenyl)methyl]carbamimidate* (314.0 mg, 1.12 mmol) was added to a stirred mixture of hydrazine hydrate (0.17 mL, 2.25 mmol) in Ethanol (4.971 mL). The reaction mixture was stirred at RT on, then it was concentrated. DCM was added and the mixture filtered, giving *3-N-[(3,5-dimethylphenyl)methyl]-1H-1,2,4-triazole-3,5-diamine* (N0237-48-1: 153 mg, 0.704 mmol, 62.65% yield) as yellowish solid.

LCMS acidic r.t. 0.51 min, MS (ESI)  $m/z=218.1$   $[M+H]^+$ .

$^1\text{H}$  NMR (400 MHz, chloroform- $d_6$ )  $\delta$  ppm 2.31 - 2.34 (6 H, m) 4.04 (2 H, br. s.) 4.33 - 4.39 (2 H, m) 4.61 (1 H, br. s.) 6.95 (1 H, s) 6.97 - 7.00 (2 H, m).

### N0237-52-1 3-N-[(3-fluorophenyl)methyl]-1H-1,2,4-triazole-3,5-diamine

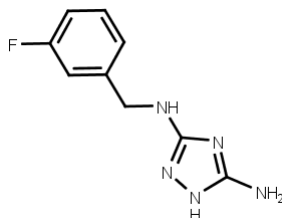


Figure 2.45: N0237-52-1

A mixture of diphenoxymethylidencyanamide (500.0 mg, 2.1 mmol) and (3-fluorophenyl)methanamine (0.24 mL, 2.1 mmol) in IPA (7 mL) was stirred at RT for 4.5h. The mixture was filtered, cake was washed with diethyl ether, giving *phenyl N'-cyano-N-[(3-fluorophenyl)methyl]carbamimidate* (N0237-49-1: 360 mg, 1.337 mmol, 63.7% yield) as white solid.

LCMS acidic r.t. 0.99 min, MS (ESI)  $m/z=270.1$   $[M+H]^+$ .

## 2. EXPERIMENTAL PART

$^1\text{H}$  NMR (400 MHz, chloroform- $d_6$ )  $\delta$  ppm 4.54 - 4.65 (2 H, m) 7.00 - 7.24 (5 H, m) 7.30 - 7.51 (4 H, m).

Phenyl  $N'$ -cyano- $N$ -[(3-fluorophenyl)methyl]carbamimidate (360.0 mg, 1.34 mmol) was added to a stirred mixture of hydrazine hydrate (0.2 mL, 2.67 mmol) in Ethanol (5.699 mL). The reaction mixture was stirred at RT on, then it was concentrated. DCM was added and the mixture filtered, giving *3-N-[(3-fluorophenyl)methyl]-1H-1,2,4-triazole-3,5-diamine* (N0237-52-1: 54 mg, 0.261 mmol, 19.49% yield) as white solid.

LCMS acidic r.t. 0.38 min, MS (ESI)  $m/z$  = 208 [M+H] $^+$ .

$^1\text{H}$  NMR (400 MHz, chloroform- $d_6$ )  $\delta$  ppm 4.14 (2 H, br. s.) 4.43 - 4.48 (2 H, m) 4.56 (1 H, br. s.) 6.99 (1 H, td,  $J$ =8.47, 2.86 Hz) 7.10 (1 H, dt,  $J$ =9.68, 2.42 Hz) 7.15 (1 H, dd,  $J$ =7.48, 0.88 Hz) 7.30 - 7.36 (1 H, m).

### N0237-53-1 3-N-[(3-methoxyphenyl)methyl]-1H-1,2,4-triazole-3,5-diamine

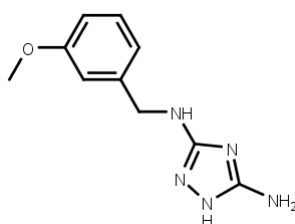


Figure 2.46: N0237-53-1

A mixture of diphenoxymethylidene cyanamide (500.0 mg, 2.1 mmol) and (3-methoxyphenyl)methanamine (0.27 mL, 2.1 mmol) in IPA (7 mL) was stirred at RT for 4.5h. The mixture was filtered, cake was washed with diethyl ether, giving *phenyl N'-cyano-N-[(3-methoxyphenyl)methyl]carbamimidate* (N0237-50-1: 347 mg, 1.234 mmol, 58.7% yield) as white solid.

LCMS acidic r.t. 0.98 min, MS (ESI)  $m/z$  = 282.1 [M+H] $^+$ .

$^1\text{H}$  NMR (400 MHz, chloroform- $d_6$ )  $\delta$  ppm 4.55 - 4.65 (2 H, m) 6.78 (1 H, d,  $J$ =6.60 Hz) 6.87 - 7.00 (3 H, m) 7.03 - 7.13 (2 H, m) 7.30 - 7.49 (4 H, m).

Phenyl  $N'$ -cyano- $N$ -[(3-methoxyphenyl)methyl]carbamimidate (347.0 mg, 1.23 mmol) was added to a stirred mixture of hydrazine hydrate (0.19 mL, 2.47 mmol) in Ethanol (5.493 mL). The reaction mixture was stirred at RT on, then it was concentrated. DCM was added and the mixture filtered, giving *3-N-[(3-methoxyphenyl)methyl]-1H-1,2,4-triazole-3,5-diamine* (N0237-53-1: 214 mg, 0.976 mmol, 79.13% yield) as white solid.

LCMS acidic r.t. 0.38 min, MS (ESI)  $m/z$  = 220 [M+H] $^+$ .

$^1\text{H}$  NMR (400 MHz, chloroform- $d_6$ )  $\delta$  ppm 3.80 - 3.84 (3 H, m) 4.08 (2 H, br. s.) 4.42 (2 H, d,  $J$ =5.72 Hz) 4.60 (1 H, br. s.) 6.85 (1 H, dd,  $J$ =8.36, 2.42 Hz) 6.93 (1 H, s) 6.96 (1 H, d,  $J$ =7.92 Hz) 7.27 (1 H, br. s.).

### N0237-54-1 3-N-[(4-propan-2-ylphenyl)methyl]-1H-1,2,4-triazole-3,5-diamine

A mixture of diphenoxymethylidene cyanamide (500.0 mg, 2.1 mmol) and 4-isopropylbenzylamine (0.34 mL, 2.1 mmol) in IPA (7 mL) was stirred at RT for 4.5h. The mixture was filtered, cake was washed with diethyl ether, giving *phenyl N'-cyano-N-[(4-propan-2-ylphenyl)methyl]carbamimidate* (N0237-51-1: 227 mg, 0.774 mmol, 36.87% yield) as white solid.

LCMS acidic r.t. 1.19 min, MS (ESI)  $m/z$  = 294.1 [M+H] $^+$ .

$^1\text{H}$  NMR (400 MHz, chloroform- $d_6$ )  $\delta$  ppm 1.25 - 1.34 (6 H, m) 2.91 - 3.01 (1 H, m) 4.56 - 4.64 (2 H, m) 6.53 (1 H, br. s.) 7.09 (2 H, d,  $J$ =7.48 Hz) 7.29 - 7.36 (4 H, m) 7.39 - 7.48 (2 H, m).

Phenyl  $N'$ -cyano- $N$ -[(4-propan-2-ylphenyl)methyl]carbamimidate (227.0 mg, 0.770 mmol) was added to a stirred mixture of hydrazine hydrate (0.12 mL,

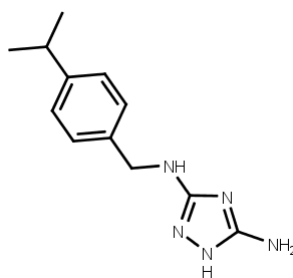


Figure 2.47: N0237-54-1

1.55 mmol) in Ethanol (3.594 mL). The reaction mixture was stirred at RT on, then it was concentrated. DCM was added and the mixture filtered, giving *3-N-[(4-isobutylphenyl)methyl]-1H-1,2,4-triazole-3,5-diamine* (N0237-54-1: 140 mg, 0.605 mmol, 78.22% yield) as white solid.

LCMS acidic r.t. 0.59 min, MS (ESI)  $m/z = 232.1$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-d<sub>6</sub>)  $\delta$  ppm 1.23 - 1.29 (6 H, m) 2.86 - 2.97 (1 H, m) 4.11 (2 H, br. s.) 4.35 - 4.41 (2 H, m) 4.78 (1 H, br. s.) 7.21 - 7.25 (2 H, m) 7.28 - 7.32 (2 H, m).

#### N0237-59-1 3-N-[(3-chlorophenyl)methyl]-1H-1,2,4-triazole-3,5-diamine

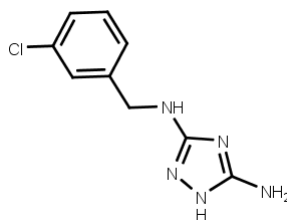


Figure 2.48: N0237-59-1

A mixture of diphenoxymethylidene cyanamide (500.0 mg, 2.1 mmol) and (3-chlorophenyl)methanamine (0.26 mL, 2.1 mmol) in IPA (7 mL) was stirred at RT for 4.5h. The mixture was filtered, cake was washed with diethyl ether, giving *phenyl N-[(3-chlorophenyl)methyl]-N'-cyanocarbamimidate* (N0237-56-1: 281 mg, 0.983 mmol, 46.86% yield) as white solid.

LCMS acidic r.t. 1.06 min, MS (ESI)  $m/z = 286.1$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-d<sub>6</sub>)  $\delta$  ppm 4.53 - 4.62 (2 H, m) 7.00 - 7.08 (2 H, m) 7.24 - 7.28 (1 H, m) 7.30 - 7.48 (7 H, m).

Phenyl N-[(3-chlorophenyl)methyl]-N'-cyanocarbamimidate (281.0 mg, 0.980 mmol) was added to a stirred mixture of hydrazine hydrate (0.15 mL, 1.97 mmol) in Ethanol (4.449 mL). The reaction mixture was stirred at RT on, then it was concentrated. DCM was added and the mixture filtered, giving *3-N-[(3-chlorophenyl)methyl]-1H-1,2,4-triazole-3,5-diamine* (N0237-59-1: 199 mg, 0.890 mmol, 90.47% yield) as yellowish solid.

LCMS acidic r.t. 0.45 min, MS (ESI)  $m/z = 224$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, methanol-d<sub>6</sub>)  $\delta$  ppm 4.33 - 4.39 (2 H, m) 7.21 - 7.33 (3 H, m) 7.36 - 7.39 (1 H, m).

<sup>1</sup>H NMR (400 MHz, chloroform-d<sub>6</sub>)  $\delta$  ppm 4.13 (2 H, br. s.) 4.42 - 4.46 (2 H, m) 4.51 (1 H, s) 7.26 - 7.31 (3 H, m) 7.39 (1 H, s).

#### N0237-60-1 3-N-[(3,4-dimethoxyphenyl)methyl]-1H-1,2,4-triazole-3,5-diamine

A mixture of diphenoxymethylidene cyanamide (500.0 mg, 2.1 mmol) and (3,4-dimethoxyphenyl)methanamine (0.32 mL, 2.1 mmol) in IPA (7 mL) was stirred at



## 2. EXPERIMENTAL PART

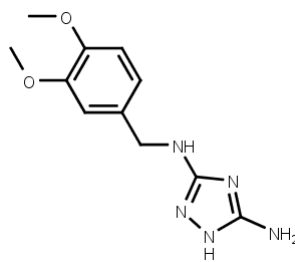


Figure 2.49: N0237-60-1

RT for 4.5h. The mixture was filtered, cake was washed with diethyl ether, giving *phenyl N'-cyano-N-[(3,4-dimethoxyphenyl)methyl]carbamiimidate* (N0237-57-1: 468 mg, 1.503 mmol, 71.62% yield) as white solid.

LCMS acidic r.t. 0.91 min, MS (ESI)  $m/z = 312.1$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-d<sub>6</sub>)  $\delta$  ppm 3.90 - 3.93 (6 H, m) 4.51 - 4.59 (2 H, m) 6.78 - 6.86 (1 H, m) 6.86 - 6.97 (3 H, m) 7.04 - 7.15 (2 H, m) 7.29 - 7.37 (1 H, m) 7.43 (2 H, t, J=7.59 Hz).

Phenyl *N'-cyano-N-[(3,4-dimethoxyphenyl)methyl]carbamiimidate* (468.0 mg, 1.5 mmol) was added to a stirred mixture of hydrazine hydrate (0.23 mL, 3.01 mmol) in Ethanol (7.409 mL). The reaction mixture was stirred at RT on, then it was concentrated. DCM was added and the mixture filtered, giving *3-N-[(3,4-dimethoxyphenyl)methyl]-1H-1,2,4-triazole-3,5-diamine* (N0237-60-1: 347 mg, 1.392 mmol, 92.61% yield) as yellowish solid.

LCMS acidic r.t. 0.35 min, MS (ESI)  $m/z = 250.1$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, methanol-d<sub>6</sub>)  $\delta$  ppm 3.82 - 3.83 (3 H, m) 3.83 (3 H, s) 4.27 - 4.31 (2 H, m) 6.89 - 6.92 (2 H, m) 6.99 (1 H, t, J=1.43 Hz).

<sup>1</sup>H NMR (400 MHz, chloroform-d<sub>6</sub>)  $\delta$  ppm 3.90 (6 H, d, J=0.88 Hz) 4.06 (2 H, d, J=3.96 Hz) 4.37 (2 H, d, J=5.72 Hz) 4.52 - 4.56 (1 H, m) 6.90 - 6.95 (2 H, m) 7.27 (1 H, s).

### N0237-65-1 3-N-[(4-chlorophenyl)methyl]-1H-1,2,4-triazole-3,5-diamine

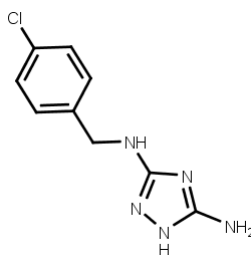


Figure 2.50: N0237-65-1

A mixture of diphenoxymethylidencyanamide (500.0 mg, 2.1 mmol) and (4-chlorophenyl)methanamine (0.26 mL, 2.1 mmol) in IPA (7 mL) was stirred at RT for 4.5h. The mixture was filtered, cake was washed with diethyl ether, giving *phenyl N-[(4-chlorophenyl)methyl]-N'-cyanocarbamimidate* (N0237-62-1: 375 mg, 1.312 mmol, 62.53% yield) as white solid.

LCMS acidic r.t. 1.07 min, MS (ESI)  $m/z = 286$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-d<sub>6</sub>)  $\delta$  ppm 4.51 - 4.60 (2 H, m) 6.97 - 7.09 (2 H, m) 7.30 - 7.35 (2 H, m) 7.36 - 7.48 (5 H, m).

Phenyl *N-[(4-chlorophenyl)methyl]-N'-cyanocarbamimidate* (375.0 mg, 1.31 mmol) was added to a stirred mixture of hydrazine hydrate (0.2 mL, 2.62 mmol) in Ethanol (5.937 mL). The reaction mixture was stirred at RT on, then it was concentrated. DCM was added and the mixture filtered, giving *3-N-[(4-chlorophenyl)methyl]-1H-1,2,4-triazole-3,5-diamine*

## 2. EXPERIMENTAL PART

(N0237-65-1: 264 mg, 1.18 mmol, 89.94% yield) as white solid.

LCMS acidic r.t. 0.41 min, MS (ESI)  $m/z = 224$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-d<sub>6</sub>)  $\delta$  ppm 4.12 (2 H, br. s.) 4.41 - 4.44 (2 H, m) 4.47 - 4.52 (1 H, m) 7.26 - 7.30 (2 H, m) 7.33 (2 H, s).

<sup>1</sup>H NMR (400 MHz, methanol-d<sub>6</sub>)  $\delta$  ppm 4.34 (2 H, s) 7.29 - 7.37 (4 H, m).

### N0237-66-1 3-N-(pyridin-4-ylmethyl)-1H-1,2,4-triazole-3,5-diamine

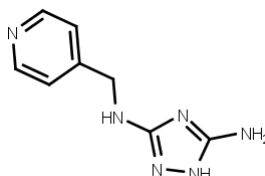


Figure 2.51: N0237-66-1

A mixture of diphenoxymethylidene cyanamide (500.0 mg, 2.1 mmol) and pyridin-4-ylmethanamine (0.21 mL, 2.1 mmol) in IPA (7 mL) was stirred at RT for 4.5h. The mixture was concentrated and crude purified by flash chromatography (silica 25g + 25g, EtOAc/MeOH from 10:0 to 9:1) affording *phenyl N'-cyano-N-(pyridin-4-ylmethyl)carbamimidate* (N0237-63-1: 257 mg, 1.019 mmol, 48.54% yield) as white foam.

LCMS acidic r.t. 0.38 min, MS (ESI)  $m/z = 253$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-d<sub>6</sub>)  $\delta$  ppm 4.51 - 4.65 (2 H, m) 6.94 - 7.07 (2 H, m) 7.24 - 7.31 (2 H, m) 7.36 - 7.47 (2 H, m) 8.11 (1 H, br. s.) 8.59 - 8.68 (2 H, m).

Phenyl *N'*-cyano-*N*-(pyridin-4-ylmethyl)carbamimidate (257.0 mg, 1.02 mmol) was added to a stirred mixture of hydrazine hydrate (0.16 mL, 2.04 mmol) in Ethanol (4.069 mL). The reaction mixture was stirred at RT on, then it was concentrated. DCM was added and the mixture filtered, giving *3-N-(pyridin-4-ylmethyl)-1H-1,2,4-triazole-3,5-diamine* (N0237-66-1: 180 mg, 0.946 mmol, 92.89% yield) as white solid.

LCMS acidic r.t. 0.25 min, MS (ESI)  $m/z = 191$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, methanol-d<sub>6</sub>)  $\delta$  ppm 4.43 (2 H, s) 7.42 (2 H, ddt, J=4.62, 1.65, 0.94, 0.94 Hz) 8.43 - 8.49 (2 H, m).

### N0237-94-2 [5-amino-3-[(3,5-dichlorophenyl)methylamino]-1,2,4-triazol-1-yl]-(3,4,5-trimethoxyphenyl)methanone

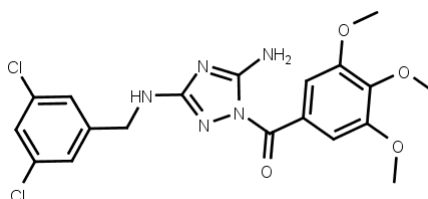


Figure 2.52: N0237-94-2

A mixture of diphenoxymethylidene cyanamide (500.0 mg, 2.1 mmol) and 3,5-dichlorobenzenemethanamine (0.28 mL, 2.1 mmol) in IPA (7 mL) was stirred at RT for 3.5h. The mixture was filtered, cake was washed with diethyl ether, giving *phenyl N'-cyano-N-[(3,5-dichlorophenyl)methyl]carbamimidate* (N0237-82-1: 416 mg, 1.299 mmol, 61.91% yield) as white solid.

LCMS acidic r.t. 1.17 min, MS (ESI)  $m/z = 321.2$ - $324.2$  [M+H]<sup>+</sup>.

## 2. EXPERIMENTAL PART

$^1\text{H}$  NMR (400 MHz, chloroform- $d_6$ )  $\delta$  ppm 4.45 - 4.58 (2 H, m) 6.97 - 7.09 (2 H, m) 7.29 - 7.34 (1 H, m) 7.36 (1 H, td,  $J=2.09, 0.66$  Hz) 7.38 - 7.50 (2 H, m) 7.82 - 7.98 (1 H, m).

Hydrazine hydrate (0.2 mL, 2.6 mmol) was added to a mixture of phenyl  $N'$ -cyano- $N$ -[(3,5-dichlorophenyl)methyl]carbamiimidate (416.0 mg, 1.3 mmol) in Ethanol (5.459 mL). The reaction mixture was stirred on at RT, then volatiles were eliminated. DCM was added and the mixture was sonicated, then filtered. Cake was washed with DCM, giving *3-N-[(3,5-dichlorophenyl)methyl]-1H-1,2,4-triazole-3,5-diamine* (N0237-91-1: 299 mg, 1.158 mmol, 89.16% yield) as white solid.

LCMS acidic r.t. 0.57 min, MS (ESI)  $m/z=258.1$  [M+H] $^+$ .

$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  ppm 4.11 - 4.32 (2 H, m) 5.49 - 5.73 (2 H, m) 6.02 (1 H, br. s.) 7.32 - 7.36 (2 H, m) 7.42 (1 H, d,  $J=1.98$  Hz) 10.69 (1 H, br. s.).

3,4,5-trimethoxybenzoyl chloride (253.7 mg, 1.1 mmol) was added portionwise to a cold mixture ( $-20$  °C) of *3-N-[(3,5-dichlorophenyl)methyl]-1H-1,2,4-triazole-3,5-diamine* (258.11 mg, 1 mmol) in Pyridine (10 mL). The reaction mixture was slowly left to reach RT and stirred on. Volatiles were removed; DCM (75 mL) was added, followed by a saturated aq solution of  $\text{NaHCO}_3$ : phases were separated, the organic one was washed with water and then brine, dried over sodium sulfate, filtered and concentrated.  $\text{Et}_2\text{O}$  was added: the mixture was first sonicated and then triturated and filtered, giving 70 mg of N0237-94-1, that was purified by flash chromatography (silica NH 11g + 11g, EtOAc/MeOH from 100:0 to 97:3) affording *[5-amino-3-[(3,5-dichlorophenyl)methylamino]-1,2,4-triazol-1-yl]-(3,4,5-trimethoxyphenyl) methanone* (N0237-94-2: 12 mg, 0.027 mmol, 2.65% yield) as white solid.

LCMS acidic r.t. 1.89 min, MS (ESI)  $m/z=453.2-455.2$  [M+H] $^+$ .

$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  ppm 3.71 - 3.73 (6 H, m) 3.75 (3 H, s) 4.30 - 4.35 (2 H, m) 7.02 - 7.07 (1 H, m) 7.33 - 7.36 (2 H, m) 7.45 (1 H, t,  $J=1.87$  Hz) 7.55 - 7.58 (2 H, m) 7.72 (2 H, br. s.).

### N0237-88-1 *3-N-[(4-methoxyphenyl)methyl]-1H-1,2,4-triazole-3,5-diamine*

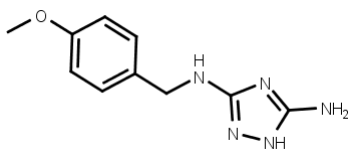


Figure 2.53: N0237-88-1

A mixture of diphenoxymethylidene cyanamide (500.0 mg, 2.1 mmol) and (4-methoxyphenyl)methanamine (0.27 mL, 2.1 mmol) in IPA (7 mL) was stirred at RT on. The mixture was filtered, cake was washed with diethyl ether, giving *phenyl N'*-cyano- $N$ -[(4-methoxyphenyl)methyl]carbamiimidate (N0237-79-1: 303 mg, 1.077 mmol, 51.32% yield) as yellowish solid.

LCMS acidic r.t. 1.00 min, MS (ESI)  $m/z=282.2$  [M+H] $^+$ .

$^1\text{H}$  NMR (400 MHz, chloroform- $d_6$ )  $\delta$  ppm 3.81 - 3.89 (3 H, m) 4.56 (2 H, d,  $J=4.84$  Hz) 6.75 (1 H, br. s.) 6.89 - 6.99 (2 H, m) 7.04 - 7.14 (2 H, m) 7.29 - 7.36 (2 H, m) 7.39 - 7.49 (2 H, m).

Hydrazine hydrate (0.16 mL, 2.15 mmol) was added to a mixture of phenyl  $N'$ -cyano- $N$ -[(4-methoxyphenyl)methyl]carbamiimidate (303.0 mg, 1.08 mmol) in Ethanol (4.526 mL). The reaction was stirred on at RT, then volatiles were removed. DCM was added and the mixture first sonicated, then filtered. Cake was washed with DCM, giving *3-N-[(4-methoxyphenyl)methyl]-1H-1,2,4-triazole-3,5-diamine* (N0237-88-1: 194 mg, 0.885 mmol, 82.15% yield) as white solid.

LCMS acidic r.t. 0.37 min, MS (ESI)  $m/z=220.1$  [M+H] $^+$ .

$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  ppm 3.68 - 3.75 (3 H, m) 4.14 (2 H, d,  $J=5.50$  Hz) 5.58 (2 H, br. s.) 6.80 - 6.90 (2 H, m) 7.18 - 7.28 (2 H, m) 10.64 (1 H, br. s.).

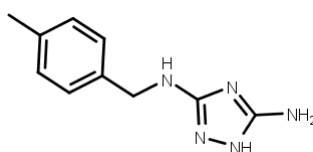
**N0237-89-1 3-N-[(4-methylphenyl)methyl]-1H-1,2,4-triazole-3,5-diamine**

Figure 2.54: N0237-89-1

A mixture of diphenoxymethylidencyanamide (500.0 mg, 2.1 mmol) and (4-methylphenyl)methanamine (0.27 mL, 2.1 mmol) in IPA (7 mL) was stirred at RT on. The mixture was filtered, cake was washed with diethyl ether, giving *phenyl N'-cyano-N-[(4-methylphenyl)methyl]carbamimidate* (N0237-80-1: 322 mg, 1.214 mmol, 57.83% yield) as white solid.

LCMS acidic r.t. 1.07 min, MS (ESI)  $m/z = 266.2$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-d<sub>6</sub>)  $\delta$  ppm 2.36 - 2.44 (3 H, m) 4.55 - 4.64 (2 H, m) 6.57 (1 H, br. s.) 7.04 - 7.26 (6 H, m) 7.31 - 7.51 (3 H, m).

Hydrazine (0.186 mL) was added to a mixture of *phenyl N'-cyano-N-[(4-methylphenyl)methyl]carbamimidate* (322 mg) in EtOH (5 mL). The reaction mixture was stirred at RT on, then volatiles were removed. DCM was added, the mixture sonicated and then filtered, giving *3-N-[(4-methylphenyl)methyl]-1H-1,2,4-triazole-3,5-diamine* N0237-89-1 (207 mg) as white solid.

LCMS acidic r.t. 0.43 min, MS (ESI)  $m/z = 204.1$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 2.22 - 2.29 (3 H, m) 4.17 (2 H, dd, J=6.38, 1.32 Hz) 4.83 (1 H, br. s.) 5.24 - 5.75 (2 H, m) 7.03 - 7.12 (2 H, m) 7.14 - 7.22 (2 H, m) 10.67 (1 H, br. s.).

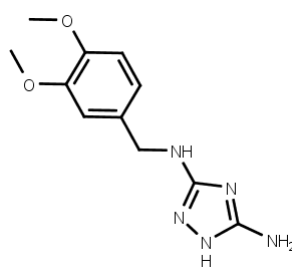
**N0237-90-1 3-N-[(3,4-dimethoxyphenyl)methyl]-1H-1,2,4-triazole-3,5-diamine**

Figure 2.55: N0237-90-1

A mixture of diphenoxymethylidencyanamide (500.0 mg, 2.1 mmol) and (3,4-dimethoxyphenyl)methanamine (350.93 mg, 2.1 mmol) in IPA (7 mL) was stirred at RT on. The mixture was filtered, cake was washed with diethyl ether, giving *phenyl N'-cyano-N-[(3,4-dimethoxyphenyl)methyl]carbamimidate* (N0237-81-1: 443 mg, 1.423 mmol, 67.8% yield) as white solid.

LCMS acidic r.t. 0.93 min, MS (ESI)  $m/z = 312.2$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-d<sub>6</sub>)  $\delta$  ppm 3.89 - 3.97 (6 H, m) 4.52 - 4.61 (2 H, m) 6.61 (1 H, d, J=16.95 Hz) 6.82 - 6.97 (3 H, m) 7.04 - 7.17 (2 H, m) 7.30 - 7.36 (1 H, m) 7.39 - 7.51 (2 H, m).

Hydrazine hydrate (0.22 mL, 2.85 mmol) was added to a mixture of *phenyl N'-cyano-N-[(3,4-dimethoxyphenyl)methyl]carbamimidate* (443.0 mg, 1.42 mmol) in Ethanol (5.978 mL). The reaction mixture was stirred at RT on, then volatiles were removed. DCM was added and the mixture sonicated, then it was filtered and cake washed with DCM,

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giving 3-N-[(3,4-dimethoxyphenyl)methyl]-1H-1,2,4-triazole-3,5-diamine (N0237-90-1: 315 mg, 1.264 mmol, 88.81% yield) as white solid.

LCMS acidic r.t. 0.35 min, MS (ESI)  $m/z = 250.2$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 3.70 - 3.74 (6 H, m) 4.11 - 4.18 (2 H, m) 4.89 - 5.56 (2 H, m) 5.62 - 5.91 (1 H, m) 6.80 - 6.88 (2 H, m) 6.95 (1 H, d,  $J = 1.76$  Hz) 10.69 (1 H, br. s.).

### N0237-91-1 3-N-[(3,5-dichlorophenyl)methyl]-1H-1,2,4-triazole-3,5-diamine

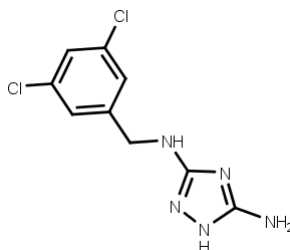


Figure 2.56: N0237-91-1

A mixture of diphenoxymethylidene cyanamide (500.0 mg, 2.1 mmol) and 3,5-dichlorobenzene methanamine (0.28 mL, 2.1 mmol) in IPA (7 mL) was stirred at RT for 3.5h. The mixture was filtered, cake was washed with diethyl ether, giving *phenyl N'-cyano-N-[(3,5-dichlorophenyl)methyl]carbamimidate* (N0237-82-1: 416 mg, 1.299 mmol, 61.91% yield) as white solid.

LCMS acidic r.t. 1.17 min, MS (ESI)  $m/z = 320.2-324.2$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-d<sub>6</sub>)  $\delta$  ppm 4.48 - 4.55 (2 H, m) 6.98 - 7.10 (2 H, m) 7.30 - 7.37 (3 H, m) 7.39 - 7.50 (2 H, m) 7.91 (1 H, d,  $J = 8.14$  Hz).

Hydrazine hydrate (0.2 mL, 2.6 mmol) was added to a mixture of *phenyl N'-cyano-N-[(3,5-dichlorophenyl)methyl]carbamimidate* (416.0 mg, 1.3 mmol) in Ethanol (5.459 mL). The reaction mixture was stirred on at RT, then volatiles were eliminated. DCM was added and the mixture was sonicated, then filtered. Cake was washed with DCM, giving *3-N-[(3,5-dichlorophenyl)methyl]-1H-1,2,4-triazole-3,5-diamine* (N0237-91-1: 299 mg, 1.158 mmol, 89.16% yield) as white solid.

LCMS acidic r.t. 0.57 min, MS (ESI)  $m/z = 258.2-260$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 4.21 (2 H, d,  $J = 5.72$  Hz) 5.64 (2 H, d,  $J = 2.86$  Hz) 6.02 (1 H, br. s.) 7.29 - 7.37 (2 H, m) 7.42 (1 H, t,  $J = 2.20$  Hz) 10.69 (1 H, br. s.).

### N0237-92-1 3-N-(1,3-benzodioxol-5-ylmethyl)-1H-1,2,4-triazole-3,5-diamine

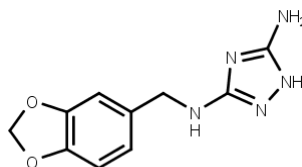


Figure 2.57: N0237-92-1

A mixture of diphenoxymethylidene cyanamide (500.0 mg, 2.1 mmol) and 1,3-benzodioxol-5-ylmethanamine (0.26 mL, 2.1 mmol) in IPA (7 mL) was stirred at RT for 3.5h. The mixture was filtered, cake was washed with diethyl ether, giving *phenyl N-(1,3-benzodioxol-5-ylmethyl)-N'-cyanocarbamimidate* (N0237-83-1: 329 mg, 1.114 mmol, 53.09% yield) as white solid.

LCMS acidic r.t. 0.97 min, MS (ESI)  $m/z = 296.2$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-d<sub>6</sub>)  $\delta$  ppm 4.48 - 4.57 (2 H, m) 6.00 - 6.04 (2 H, m) 6.62 (1

## 2. EXPERIMENTAL PART

H, br. s.) 6.79 - 6.89 (3 H, m) 7.09 (2 H, d, J=7.26 Hz) 7.30 - 7.37 (1 H, m) 7.39 - 7.50 (2 H, m).

Hydrazine hydrate (0.17 mL, 2.23 mmol) was added to a mixture of phenyl N-(1,3-benzodioxol-5-ylmethyl)-N'-cyanocarbamimidate (329.0 mg, 1.11 mmol) in Ethanol (4.681 mL). The reaction mixture was stirred at RT on. Volatiles were removed and cake washed with DCM, giving 3-N-(1,3-benzodioxol-5-ylmethyl)-1H-1,2,4-triazole-3,5-diamine (N0237-92-1: 282 mg, 1.209 mmol, quantitative yield) as white solid.

LCMS acidic r.t. 0.36 min, MS (ESI) m/z= 234.1 [M+H]+.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 4.09 - 4.15 (2 H, m) 5.40 (2 H, br. s.) 5.93 - 5.98 (2 H, m) 6.74 - 6.79 (1 H, m) 6.79 - 6.83 (1 H, m) 6.88 (1 H, d, J=1.32 Hz) 10.69 (1 H, br. s.).

### N0237-97-1 3-N-[[4-(trifluoromethyl)phenyl]methyl]-1H-1,2,4-triazole-3,5-diamine

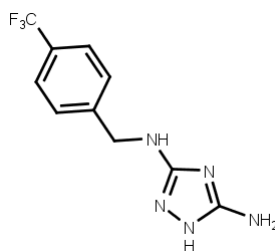


Figure 2.58: N0237-97-1

*Batch 1* : A mixture of diphenoxymethylidene cyanamide (500.0 mg, 2.1 mmol) and [4-(trifluoromethyl)phenyl]methanamine (0.3 mL, 2.1 mmol) in IPA (7 mL) was stirred at RT for 3.5h. The mixture was filtered, cake was washed with diethyl ether, giving phenyl N'-cyano-N-[[4-(trifluoromethyl)phenyl]methyl]carbamide (N0237-85-1: 44 mg, 0.138 mmol, 6.56% yield) as white solid.

LCMS acidic r.t. 1.11 min, MS (ESI) m/z= 320.2 [M+H]+.

<sup>1</sup>H NMR (400 MHz, chloroform-d<sub>6</sub>) δ ppm 4.61 - 4.73 (2 H, m) 7.00 - 7.14 (2 H, m) 7.31 - 7.55 (5 H, m) 7.69 (2 H, d, J=7.26 Hz).

*Batch 2* : A mixture of diphenoxymethylidene cyanamide (500.0 mg, 2.1 mmol) and [4-(trifluoromethyl)phenyl]methanamine (0.3 mL, 2.1 mmol) in IPA (7 mL) was stirred at RT overnight. The mixture was filtered, cake was washed with diethyl ether, giving phenyl N'-cyano-N-[[4-(trifluoromethyl)phenyl]methyl]carbamide (N0237-95-1: 216 mg, 0.677 mmol, 32.23% yield) as white solid.

LCMS acidic r.t. 1.11 min, MS (ESI) m/z= 320.2 [M+H]+.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 4.45 - 4.64 (2 H, m) 7.07 (1 H, d, J=7.92 Hz) 7.23 - 7.44 (3 H, m) 7.46 - 7.56 (2 H, m) 7.61 (1 H, d, J=7.70 Hz) 7.70 - 7.82 (2 H, m).

Hydrazine hydrate (0.12 mL, 1.63 mmol) was added to a mixture of phenyl N'-cyano-N-[[4-(trifluoromethyl)phenyl]methyl]carbamide (260.0 mg, 0.810 mmol) in Ethanol (3.422 mL). The reaction mixture was stirred on at RT, then volatiles were removed. DCM was added and the mixture sonicated, then filtered and washed with DCM, affording 3-N-[[4-(trifluoromethyl)phenyl]methyl]-1H-1,2,4-triazole-3,5-diamine (N0237-97-1: 158 mg, 0.614 mmol, 75.43% yield) as white solid.

LCMS acidic r.t. 0.54 min, MS (ESI) m/z= 258.2 [M+H]+.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 4.25 - 4.36 (2 H, m) 5.59 (2 H, br. s.) 5.99 (1 H, br. s.) 7.47 - 7.55 (2 H, m) 7.62 - 7.69 (2 H, m) 10.55 - 10.79 (1 H, m).

### N0237-98-1 3-N-[(3-methylphenyl)methyl]-1H-1,2,4-triazole-3,5-diamine

*Batch 1* : A mixture of diphenoxymethylidene cyanamide (500.0 mg, 2.1 mmol) and (3-methylphenyl)methanamine (0.27 mL, 2.1 mmol) in IPA (7 mL) was stirred at RT on. The mixture was filtered, cake was washed with diethyl ether, giving phenyl

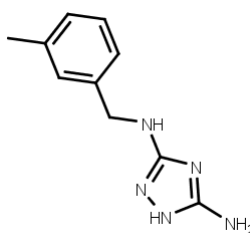


Figure 2.59: N0237-98-1

*N'*-cyano-*N*-[(3-methylphenyl)methyl]carbamimidate (N0237-78-1: 65 mg, 0.245 mmol, 11.67% yield) as white solid.

LCMS acidic r.t. 1.07 min, MS (ESI)  $m/z$ = 266.2 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, chloroform-*d*<sub>6</sub>)  $\delta$  ppm 2.40 (3 H, t,  $J$ =1.98 Hz) 4.59 (2 H, dd,  $J$ =4.40, 2.86 Hz) 6.71 (1 H, d,  $J$ =6.82 Hz) 7.08 (2 H, d,  $J$ =7.48 Hz) 7.14 - 7.22 (3 H, m) 7.32 (2 H, d,  $J$ =7.70 Hz) 7.39 - 7.52 (2 H, m).

*Batch 2* : A mixture of diphenoxymethylidene cyanamide (500.0 mg, 2.1 mmol) and (3-methylphenyl)methanamine (0.27 mL, 2.1 mmol) in IPA (7 mL) was stirred at RT on. The mixture was filtered, cake was washed with diethyl ether, giving *phenyl N'*-cyano-*N*-[(3-methylphenyl)methyl]carbamimidate (N0237-96-1: 171 mg, 0.645 mmol, 30.71% yield) as white solid.

LCMS acidic r.t. 1.07 min, MS (ESI)  $m/z$ = 266.2 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 2.29 - 2.36 (3 H, m) 4.33 - 4.50 (2 H, m) 7.01 - 7.21 (4 H, m) 7.21 - 7.36 (3 H, m) 7.38 - 7.54 (2 H, m) 8.78 - 9.50 (1 H, m).

Hydrazine hydrate (0.14 mL, 1.78 mmol) was added to a stirred mixture of *phenyl N'*-cyano-*N*-[(3-methylphenyl)methyl]carbamimidate (236.0 mg, 0.890 mmol) in Ethanol (3.738 mL). The reaction mixture was stirred on at RT, then concentrated. DCM was added: the mixture was sonicated, then filtered, giving *3-N*-[(3-methylphenyl)methyl]-1*H*-1,2,4-triazole-3,5-diamine (N0237-98-1: 145 mg, 0.713 mmol, 80.2% yield) as white solid.

LCMS acidic r.t. 0.43 min, MS (ESI)  $m/z$ = 204.1 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 2.25 - 2.29 (3 H, m) 4.19 (2 H, d,  $J$ =5.50 Hz) 4.71 (1 H, br. s.) 5.42 - 5.76 (2 H, m) 6.97 - 7.04 (1 H, m) 7.07 - 7.11 (1 H, m) 7.12 (1 H, tt,  $J$ =1.65, 0.77 Hz) 7.14 - 7.21 (1 H, m) 10.64 (1 H, br. s.).

## 2.5 Synthesis of 7-*N*-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo[1,5-*a*]pyrimidine-2,7-diamines

First and second steps of this route (fig 2.60) were common to the previous one: after the cyclization with hydrazine, the condensation reaction gave the core were, once got the chlorinated intermediate, with a S<sub>N</sub>Ar with 3,4,5-trimethoxyaniline was possible to get all the target compounds.

### N0423-80 (CP017) 2-*N*-[(4-fluorophenyl)methyl]-5-methyl-7-*N*-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo[1,5-*a*]pyrimidine-2,7-diamine

A mixture of diphenoxymethylidene cyanamide (1.12 g, 4.7 mmol) and (4-fluorophenyl)methanamine (0.54 mL, 4.7 mmol) in IPA (15 mL) was stirred on at RT. The mixture was filtered, cake was washed with diethyl ether, giving *phenyl N'*-cyano-*N*-[(4-fluorophenyl)methyl]carbamimidate (N0423-76-1: 222 mg, 0.824 mmol, 17.54% yield) as white solid.

LCMS acidic r.t. 1.02 min, MS (ESI)  $m/z$ = 270.1 [M+H]<sup>+</sup>.

## 2. EXPERIMENTAL PART

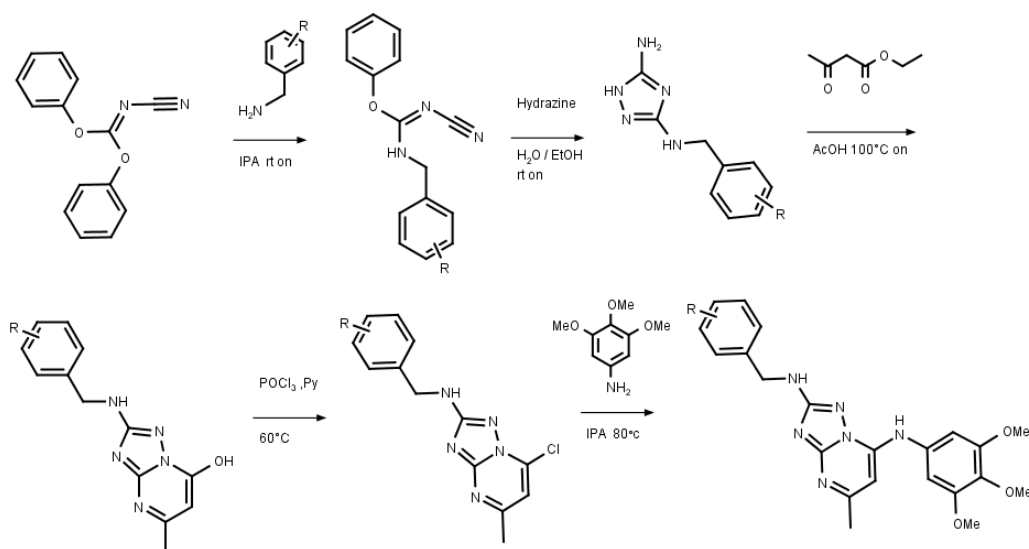


Figure 2.60: general route for 7-N-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-2,7-diamines

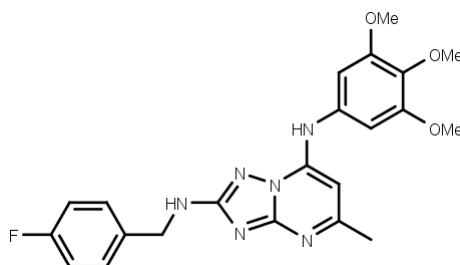


Figure 2.61: CP017

$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  ppm 4.34 - 4.52 (2 H, m) 7.00 - 7.12 (1 H, m) 7.15 - 7.55 (7 H, m) 8.78 - 9.45 (1 H, m).

Hydrazine hydrate (0.12 mL, 1.57 mmol) was added to a stirred mixture of phenyl  $N$ -cyano- $N$ -[(4-fluorophenyl)methyl]carbamimidate (222.0 mg, 0.780 mmol) in Ethanol (3.34 mL). The reaction mixture was stirred on at RT, then concentrated. DCM was added: the mixture was sonicated, then filtered, giving 3- $N$ -[(4-fluorophenyl)methyl]-1 $H$ -1,2,4-triazole-3,5-diamine (N0423-77-1: 141 mg, 0.680 mmol, 86.88% yield) as white solid.

LCMS acidic r.t. 0.38 min, MS (ESI)  $m/z$ = 208.1 [M+H] $^+$ .

$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  ppm 4.20 (2 H, d,  $J$ =6.38 Hz) 5.23 - 6.12 (2 H, m) 7.05 - 7.16 (2 H, m) 7.30 - 7.37 (2 H, m) 10.35 - 10.96 (1 H, m).

A mixture of 3- $N$ -[(4-fluorophenyl)methyl]-1 $H$ -1,2,4-triazole-3,5-diamine (141.0 mg, 0.680 mmol) and 3-oxobutanoic acid ethyl ester (0.17 mL, 1.36 mmol) in Acetic acid (3.7 mL) was stirred on at 100°C. Volatiles were removed and crude suspended in EtOAc and filtered, giving 2-[(4-fluorophenyl)methylamino]-5-methyl-[1,2,4]triazolo[1,5- $a$ ]pyrimidin-7-ol (N0423-78-1: 150 mg, 0.549 mmol, 80.67% yield) as pink solid.

LCMS acidic r.t. 0.64 min, MS (ESI)  $m/z$ = 274.08 [M+H] $^+$

$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  ppm 2.22 (3 H, d,  $J$ =0.66 Hz) 4.36 (2 H, d,  $J$ =6.38 Hz) 5.63 (1 H, d,  $J$ =0.88 Hz) 7.08 - 7.18 (3 H, m) 7.34 - 7.41 (2 H, m) 12.68 (1 H, br. s.).

A mixture of 2-[(4-fluorophenyl)methylamino]-5-methyl-[1,2,4]triazolo[1,5- $a$ ]pyrimidin-7-ol (150.0 mg, 0.550 mmol), Pyridine (22.2  $\mu\text{L}$ , 0.270 mmol) and Phosphorus(V) oxychloride (500.0  $\mu\text{L}$ , 5.35 mmol) was stirred at 60 °C for 4h, then volatiles were removed. DCM was added, followed by a 10% aq solution of sodium carbonate: phases were separated, the organic one washed with brine, dried over sodium sulfate, filtered and concentrated, giving



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7-chloro-N-[(4-fluorophenyl)methyl]-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-2-amine (N0423-79-1: 115 mg, 0.394 mmol, 71.82% yield) as brown solid.

LCMS acidic r.t. 0.90 min, MS (ESI)  $m/z$ = 292.05 - 294.05 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 2.50 (3 H, s) 4.46 (2 H, d, J=6.38 Hz) 7.11 - 7.18 (2 H, m) 7.26 (1 H, s) 7.38 - 7.43 (2 H, m) 7.68 (1 H, t, J=6.49 Hz).

A mixture of 7-chloro-N-[(4-fluorophenyl)methyl]-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-2-amine (115.0 mg, 0.390 mmol) and 3,4,5-trimethoxyaniline (144.44 mg, 0.790 mmol) in IPA (8 mL) was stirred at 80 °C for 3h. Volatiles were removed; DCM and water were added: phases were separated, the organic one was washed with brine, dried over sodium sulfate, filtered and concentrated. crude was purified by flash chromatography (silica NH 28g, Cy/EtOAc from 6:4 to 0:10) affording 2-N-[(4-fluorophenyl)methyl]-5-methyl-7-N-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-2,7-diamine (N0423-80-1: 102 mg, 0.233 mmol, 59.01% yield) as brownish foam. LCMS acidic r.t. 0.86 min, MS (ESI)  $m/z$ = 439.2 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 2.32 (3 H, s) 3.69 (3 H, s) 3.78 (6 H, s) 4.50 (2 H, d, J=6.38 Hz) 6.22 (1 H, s) 6.73 (2 H, s) 7.06 - 7.19 (3 H, m) 7.40 - 7.49 (2 H, m) 9.25 (1 H, br. s.).

### N0423-97(CP018) 2-N-[(3-fluorophenyl)methyl]-5-methyl-7-N-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-2,7-diamine

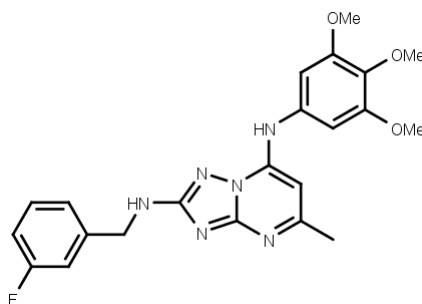


Figure 2.62: CP018

A mixture of diphenoxymethylidencyanamide (1.25 g, 5.25 mmol) and (3-fluorophenyl)methanamine (0.6 mL, 5.25 mmol) in IPA (16 mL) was stirred on at RT. The mixture was filtered, cake was washed with diethyl ether, giving *phenyl N'-cyano-N-[(3-fluorophenyl)methyl]carbamimidate* (N0423-81-1: 558 mg, 2.072 mmol, 39.5% yield) as white solid.

LCMS acidic r.t. 1.01 min, MS (ESI)  $m/z$ = 270.1 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 4.33 - 4.62 (2 H, m) 6.96 - 7.60 (9 H, m) 8.69 - 9.49 (1 H, m).

Hydrazine hydrate (0.31 mL, 4.06 mmol) was added to a stirred mixture of *phenyl N'-cyano-N-[(3-fluorophenyl)methyl]carbamimidate* (558.0 mg, 2.03 mmol) in Ethanol (8.66 mL). The reaction mixture was stirred for 7.5 h at RT, then concentrated. DCM was added: the mixture was sonicated, then filtered, giving *3-N-[(3-fluorophenyl)methyl]-1H-1,2,4-triazole-3,5-diamine* (N0423-85-1: 68 mg, 0.328 mmol, 16.16% yield) as white solid.

LCMS acidic r.t. 0.38 min, MS (ESI)  $m/z$ = 208.1 - 209.2 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 4.23 (2 H, d, J=6.38 Hz) 5.48 (2 H, br. s.) 5.87 - 6.11 (1 H, m) 7.01 (1 H, td, J=8.64, 2.09 Hz) 7.08 - 7.16 (2 H, m) 7.29 - 7.36 (1 H, m) 10.66 (1 H, br. s.).

A mixture of 3-N-[(3-fluorophenyl)methyl]-1H-1,2,4-triazole-3,5-diamine (68.0 mg, 0.330 mmol) and 3-oxobutanoic acid ethyl ester (0.08 mL, 0.660 mmol) in Acetic acid (1.784 mL) was stirred on at 100°C.

## 2. EXPERIMENTAL PART

Volatiles were removed and crude suspended in EtOAc and filtered, giving 2-[(3-fluorophenyl)methylamino]-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-7-ol (N0423-89-1: 55 mg, 0.201 mmol, 61.33% yield) as pink solid.

LCMS acidic r.t. 0.64 min, MS (ESI)  $m/z$ = 274.13 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 2.23 (3 H, d, J=0.66 Hz) 4.37 - 4.44 (2 H, m) 7.04 (1 H, td, J=8.47, 2.86 Hz) 7.11 - 7.22 (3 H, m) 7.36 (1 H, td, J=7.98, 6.05 Hz) 12.69 (1 H, br. s.).

A mixture of 2-[(3-fluorophenyl)methylamino]-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-7-ol (55.0 mg, 0.200 mmol), Pyridine (8.14  $\mu$ L, 0.100 mmol) and Phosphorus(V) oxychloride (183.33  $\mu$ L, 1.96 mmol) was stirred at 60 °C for 2h, then volatiles were removed. DCM was added, followed by a 10% aq solution of sodium carbonate: phases were separated, the organic one washed with brine, dried over sodium sulfate, filtered and concentrated, giving 7-chloro-N-[(3-fluorophenyl)methyl]-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-2-amine (N0423-93-1: 34 mg, 0.117 mmol, 57.91% yield) as brown solid.

LCMS acidic r.t. 0.90 min, MS (ESI)  $m/z$ = 292.1 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 2.49 (3 H, br. s.) 4.48 - 4.52 (2 H, m) 7.05 (1 H, td, J=8.47, 2.42 Hz) 7.14 - 7.23 (2 H, m) 7.27 (1 H, s) 7.32 - 7.41 (1 H, m) 7.73 (1 H, t, J=6.49 Hz).

A mixture of 7-chloro-N-[(3-fluorophenyl)methyl]-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-2-amine (45.0 mg, 0.150 mmol) and 3,4,5-trimethoxyaniline (56.52 mg, 0.310 mmol) in IPA (3.13 mL) was stirred at 80 °C for 45 min. Volatiles were removed; DCM and water were added: phases were separated, the organic one was washed with brine, dried over sodium sulfate, filtered and concentrated. crude was purified by flash chromatography (silica NH 11g, Cy/EtOAc from 6:4 to 0:10) affording 2-N-[(3-fluorophenyl)methyl]-5-methyl-7-N-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-2,7-diamine (N0423-97-1: 23.4 mg, 0.053 mmol, 34.6% yield) as whitish foam.

LCMS acidic r.t. 0.87 min, MS (ESI)  $m/z$ = 439.25 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 2.31 (3 H, s) 3.69 (3 H, s) 3.78 (6 H, s) 4.53 (2 H, d, J=6.16 Hz) 6.21 (1 H, br. s.) 6.72 (2 H, br. s.) 7.05 (1 H, t, J=9.79 Hz) 7.15 (1 H, br. s.) 7.19 - 7.27 (2 H, m) 7.34 - 7.40 (1 H, m) 9.25 (1 H, br. s.).

### N0423-98(CP019) 2-N-[(3-chlorophenyl)methyl]-5-methyl-7-N-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-2,7-diamine

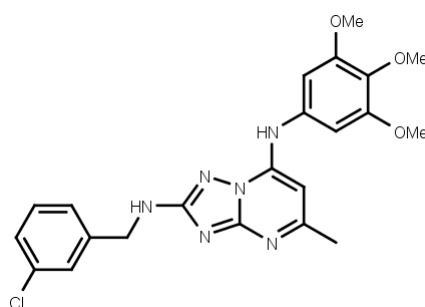


Figure 2.63: CP019

A mixture of diphenoxymethylidene cyanamide (1.25 g, 5.25 mmol) and (3-chlorophenyl)methanamine (0.64 mL, 5.25 mmol) in IPA (16 mL) was stirred on at RT. The mixture was filtered, cake was washed with diethyl ether, giving *phenyl N-[(3-chlorophenyl)methyl]-N'-cyanocarbamimidate* (N0423-82-1: 284 mg, 0.994 mmol, 18.% yield) as white solid.

LCMS acidic r.t. 1.08 min, MS (ESI)  $m/z$ = 286.1 - 288.1 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 4.37 - 4.55 (2 H, m) 7.06 (1 H, d, J=7.92 Hz) 7.21 - 7.55 (8 H, m) 8.79 - 9.44 (1 H, m).

Hydrazine hydrate (0.15 mL, 1.97 mmol) was added to a stirred mixture

## 2. EXPERIMENTAL PART

of phenyl N-[(3-chlorophenyl)methyl]-N'-cyanocarbamimidate (284.0 mg, 0.980 mmol) in Ethanol (4.453 mL). The reaction mixture was stirred for 7.5 h at RT, then concentrated. DCM was added: the mixture was sonicated, then filtered, giving 3-N-[(3-chlorophenyl)methyl]-1H-1,2,4-triazole-3,5-diamine (N0423-86-1: 204 mg, 0.912 mmol, 92.69% yield) as white solid.

LCMS acidic r.t. 0.46 min, MS (ESI) m/z= 224.06 - 226.03 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 4.22 (2 H, d, J=6.60 Hz) 5.27 - 5.68 (2 H, m) 7.22 - 7.28 (2 H, m) 7.31 (1 H, d, J=7.48 Hz) 7.36 (1 H, s) 10.47 - 10.89 (1 H, m).

A mixture of 3-N-[(3-chlorophenyl)methyl]-1H-1,2,4-triazole-3,5-diamine (202.0 mg, 0.890 mmol) and 3-oxobutanoic acid ethyl ester (0.23 mL, 1.79 mmol) in Acetic acid (5.248 mL) was stirred on at 100°C. Volatiles were removed and crude suspended in EtOAc and filtered, giving 2-[(3-chlorophenyl)methylamino]-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-7-ol (N0423-90-1: 182 mg, 0.628 mmol, 70.26% yield) as pink solid.

LCMS acidic r.t. 0.72 min, MS (ESI) m/z= 290.08 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 2.22 (3 H, s) 4.39 (2 H, d, J=6.38 Hz) 5.64 (1 H, s) 7.17 (1 H, br. s.) 7.26 - 7.40 (4 H, m) 12.70 (1 H, br. s.).

A mixture of 2-[(3-chlorophenyl)methylamino]-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-7-ol (182.0 mg, 0.630 mmol), Pyridine (25.4 μL, 0.310 mmol) and Phosphorus(V) oxychloride (572.22 μL, 6.12 mmol) was stirred at 60 °C for 2 h, then volatiles were removed. DCM was added, followed by a 10% aq solution of sodium carbonate: phases were separated, the organic one washed with brine, dried over sodium sulfate, filtered and concentrated, giving 7-chloro-N-[(3-chlorophenyl)methyl]-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-2-amine (N0423-94-1: 95 mg, 0.308 mmol, 49.07% yield) as brown solid.

LCMS acidic r.t. 0.98 min, MS (ESI) m/z= 308.03 - 310 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 2.49 (3 H, br. s.) 4.47 - 4.51 (2 H, m) 7.27 (1 H, s) 7.28 - 7.38 (3 H, m) 7.42 (1 H, t, J=1.98 Hz) 7.74 (1 H, t, J=6.38 Hz).

A mixture of 7-chloro-N-[(3-chlorophenyl)methyl]-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-2-amine (95.0 mg, 0.280 mmol) and 3,4,5-trimethoxyaniline (101.66 mg, 0.550 mmol) in IPA (5.948 mL) was stirred at 80 °C for 45 min. Volatiles were removed; DCM and water were added: phases were separated, the organic one was washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica NH 11g, Cy/EtOAc from 6:4 to 0:10) affording 2-N-[(3-chlorophenyl)methyl]-5-methyl-7-N-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-2,7-diamine (N0423-98-1: 55.7 mg, 0.122 mmol, 44.13% yield) as yellowish foam.

LCMS acidic r.t. 0.93 min, MS (ESI) m/z= 455.24 - 457.19 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 2.31 (3 H, s) 3.69 (3 H, s) 3.78 (6 H, s) 4.53 (2 H, d, J=6.38 Hz) 6.22 (1 H, br. s.) 6.72 (2 H, s) 7.17 (1 H, br. s.) 7.27 - 7.32 (1 H, m) 7.36 (2 H, d, J=5.28 Hz) 7.44 (1 H, s) 9.27 (1 H, br. s.).

### **N0423-99(CP020) 5-methyl-2-N-(pyridin-3-ylmethyl)-7-N-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-2,7-diamine**

A mixture of diphenoxymethylidene cyanamide (1.25 g, 5.25 mmol) and 3-pyridinylmethanamine (0.53 mL, 5.25 mmol) in IPA (16 mL) was stirred on at RT. The mixture was filtered, cake was washed with diethyl ether, giving phenyl N'-cyano-N-(pyridin-3-ylmethyl)carbamide (N0423-84-1: 371 mg, 1.471 mmol, 28.03% yield) as white solid.

LCMS acidic r.t. 0.46 min, MS (ESI) m/z= 253.1 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 4.39 - 4.58 (2 H, m) 7.08 (1 H, d, J=7.04 Hz) 7.23 - 7.54 (5 H, m) 7.67 - 7.85 (1 H, m) 8.48 - 8.63 (2 H, m) 8.76 - 9.45 (1 H, m).

Hydrazine hydrate (0.22 mL, 2.94 mmol) was added to a stirred mixture of phenyl N'-cyano-N-(pyridin-3-ylmethyl)carbamide (371.0 mg, 1.47 mmol) in

## 2. EXPERIMENTAL PART

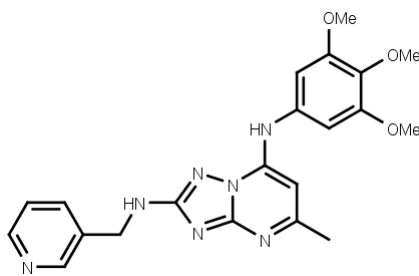


Figure 2.64: CP020

Ethanol (5.875 mL). The reaction mixture was stirred for 7.5 h at RT, then concentrated. DCM was added: the mixture was sonicated, then filtered, giving 3-*N*-(pyridin-3-ylmethyl)-1*H*-1,2,4-triazole-3,5-diamine (N0423-87-1: 237 mg, 1.246 mmol, 84.72% yield) as white solid.

LCMS basic r.t. 0.29 min, MS (ESI)  $m/z$ = 189.11 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 4.19 - 4.28 (2 H, m) 5.40 - 5.72 (2 H, m) 5.84 - 6.07 (1 H, m) 7.31 (1 H, ddd,  $J$ =7.76, 4.79, 0.66 Hz) 7.67 - 7.73 (1 H, m) 8.41 (1 H, dd,  $J$ =4.73, 1.65 Hz) 8.52 (1 H, dd,  $J$ =2.53, 0.77 Hz) 10.64 (1 H, br. s.).

A mixture of 3-*N*-(pyridin-3-ylmethyl)-1*H*-1,2,4-triazole-3,5-diamine (235.0 mg, 1.24 mmol) and 3-oxobutanoic acid ethyl ester (0.31 mL, 2.47 mmol) in Acetic acid (6.167 mL) was stirred on at 100°C. Volatiles were removed and crude suspended in EtOAc and filtered, giving 5-methyl-2-(pyridin-3-ylmethylamino)-[1,2,4]triazolo[1,5-*a*]pyrimidin-7-ol (N0423-91-1: 250 mg, 0.976 mmol, 78.96% yield) as pink solid.

LCMS acidic r.t. 0.15 min, MS (ESI)  $m/z$ = 257.11 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 2.23 (3 H, s) 4.41 (2 H, d,  $J$ =6.16 Hz) 5.64 (1 H, s) 7.17 (1 H, t,  $J$ =6.16 Hz) 7.34 (1 H, dd,  $J$ =7.26, 4.84 Hz) 7.74 (1 H, d,  $J$ =7.70 Hz) 8.44 (1 H, dd,  $J$ =4.73, 1.65 Hz) 8.56 (1 H, d,  $J$ =1.54 Hz) 12.70 (1 H, br. s.).

A mixture of 5-methyl-2-(pyridin-3-ylmethylamino)-[1,2,4]triazolo[1,5-*a*]pyrimidin-7-ol (250.0 mg, 0.980 mmol), Pyridine (39.45  $\mu$ L, 0.490 mmol) and Phosphorus(V) oxychloride (888.65  $\mu$ L, 9.5 mmol) was stirred at 90 °C for 2h, then volatiles were removed. DCM was added, followed by a 10% aq solution of sodium carbonate: phases were separated, the organic one washed with brine, dried over sodium sulfate, filtered and concentrated, giving 7-chloro-5-methyl-*N*-(pyridin-3-ylmethyl)-[1,2,4]triazolo[1,5-*a*]pyrimidin-2-amine (N0423-95-1: 154 mg, 0.561 mmol, 57.46% yield) as brown solid.

LCMS acidic r.t. 0.35 min, MS (ESI)  $m/z$ = 275.08 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 2.62 - 2.69 (3 H, m) 4.53 (2 H, d,  $J$ =5.94 Hz) 7.28 (1 H, s) 7.45 - 7.52 (1 H, m) 7.72 - 7.81 (2 H, m) 7.88 - 7.96 (2 H, m) 8.50 - 8.55 (1H, m) 8.62 - 8.67 (1 H, m).

A mixture of 7-chloro-5-methyl-*N*-(pyridin-3-ylmethyl)-[1,2,4]triazolo[1,5-*a*]pyrimidin-2-amine (154.0 mg, 0.560 mmol) and 3,4,5-trimethoxyaniline (205.4 mg, 1.12 mmol) in IPA (10.71 mL) was stirred at 80 °C for 1.5 h. Volatiles were removed; DCM and water were added: phases were separated, the organic one was washed with brine, dried over sodium sulfate, filtered and concentrated. crude was purified by flash chromatography (silica NH 28g, Cy/EtOAc from 6:4 to 0:10 and then EtOAc/MeOH 9:1) affording 5-methyl-2-*N*-(pyridin-3-ylmethyl)-7-*N*-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo[1,5-*a*]pyrimidine-2,7-diamine (N0423-99-1: 12 mg, 0.028 mmol, 5.079% yield) as brownish foam.

LCMS acidic r.t. 0.49 min, MS (ESI)  $m/z$ = 422.26 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 2.31 (3 H, s) 3.66 - 3.71 (3 H, m) 3.74 - 3.81 (6 H, m) 4.52 (2 H, d,  $J$ =6.16 Hz) 6.20 (1 H, br. s.) 6.72 (2 H, br. s.) 7.18 (1 H, br. s.) 7.35 (1 H, dd,  $J$ =7.48, 4.62 Hz) 7.81 (1 H, d,  $J$ =7.70 Hz) 8.44 (1 H, dd,  $J$ =5.06, 1.98 Hz) 8.64 (1 H, d,  $J$ =2.42 Hz) 9.26 (1 H, br. s.).

## 2. EXPERIMENTAL PART

### N0758-01(CP021) 5-methyl-2-N-(pyridin-4-ylmethyl)-7-N-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-2,7-diamine

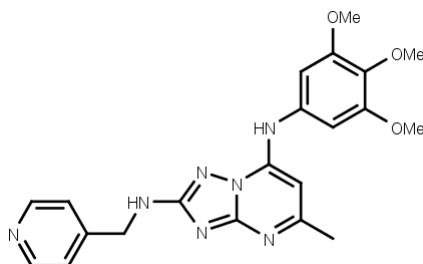


Figure 2.65: CP021

A mixture of diphenoxymethylidene cyanamide (1.25 g, 5.25 mmol) and pyridin-4-ylmethanamine (0.53 mL, 5.25 mmol) in IPA (16 mL) was stirred on at RT. The mixture was concentrated and crude purified by flash chromatography, reverse phase (silica c18 30g + 30g, water + 0.1% formic acid / MeCN + 0.1% formic acid, from 98:2 to 6:4) affording *formic acid; phenyl N'-cyano-N-(pyridin-4-ylmethyl)carbamimidate* (N0423-83-1: 476 mg, 1.596 mmol, 30.41% yield) as yellow oil.

LCMS acidic r.t. 0.42 min, MS (ESI)  $m/z = 253.1$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 4.40 - 4.60 (2 H, m) 7.02 - 7.10 (1 H, m) 7.26 - 7.45 (5 H, m) 7.51 (1 H, d,  $J = 5.94$  Hz) 8.14 (0.5 H, s) 8.57 (2 H, d,  $J = 7.92$  Hz).

Hydrazine hydrate (0.24 mL, 3.19 mmol) was added to a stirred mixture of *formic acid; phenyl N'-cyano-N-(pyridin-4-ylmethyl)carbamimidate* (476.0 mg, 1.6 mmol) in Ethanol (7.538 mL). The reaction mixture was stirred for 7.5 h at RT, then concentrated. DCM was added: the mixture was sonicated, then filtered, giving *3-N-(pyridin-4-ylmethyl)-1H-1,2,4-triazole-3,5-diamine* (N0423-88-1: 292 mg, 1.535 mmol, 96.2% yield) as white solid.

LCMS acidic r.t. 0.10 min, MS (ESI)  $m/z = 191.08$  [M+H]<sup>+</sup>

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 4.24 (2 H, d,  $J = 6.38$  Hz) 5.39 (2 H, br. s.) 6.19 (1 H, br. s.) 7.25 - 7.32 (2 H, m) 8.42 - 8.49 (2 H, m).

A mixture of *3-N-(pyridin-4-ylmethyl)-1H-1,2,4-triazole-3,5-diamine* (290.0 mg, 1.52 mmol) and 3-oxobutanoic acid ethyl ester (0.39 mL, 3.05 mmol) in Acetic acid (7.61 mL) was stirred on at 100°C. Volatiles were removed and crude suspended in EtOAc and filtered, giving *5-methyl-2-(pyridin-4-ylmethylamino)-[1,2,4]triazolo[1,5-a]pyrimidin-7-ol* (N0423-92-1: 282 mg, 1.1 mmol, 72.18% yield) as pink solid.

LCMS acidic r.t. 0.15 min, MS (ESI)  $m/z = 257.14$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 2.22 (3 H, s) 4.41 (2 H, d,  $J = 6.38$  Hz) 5.64 (1 H, s) 7.22 (1 H, br. s.) 7.32 (2 H, d,  $J = 6.16$  Hz) 8.45 - 8.51 (2 H, m) 12.70 (1 H, br. s.).

A mixture of *5-methyl-2-(pyridin-4-ylmethylamino)-[1,2,4]triazolo[1,5-a]pyrimidin-7-ol* (282.0 mg, 1.1 mmol), Pyridine (44.5  $\mu$ L, 0.550 mmol) and Phosphorus(V) oxychloride (1002.4  $\mu$ L, 10.72 mmol) was stirred at 100 °C for 4h, then volatiles were removed. DCM was added, followed by a 10% aq solution of sodium carbonate: phases were separated, the organic one washed with brine, dried over sodium sulfate, filtered and concentrated, giving *7-chloro-5-methyl-N-(pyridin-4-ylmethyl)-[1,2,4]triazolo[1,5-a]pyrimidin-2-amine* (N0423-96-1: 70 mg, 0.255 mmol, 23.16% yield) as brown solid.

LCMS acidic r.t. 0.33 min, MS (ESI)  $m/z = 275.1$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 3.18 (3 H, d,  $J = 5.28$  Hz) 4.51 (2 H, d,  $J = 6.38$  Hz) 7.26 - 7.29 (1 H, m) 7.35 (2 H, d,  $J = 5.94$  Hz) 7.79 (1 H, t,  $J = 6.60$  Hz) 8.48 - 8.52 (2 H, m).

A mixture of *7-chloro-5-methyl-N-(pyridin-4-ylmethyl)-[1,2,4]triazolo[1,5-a]pyrimidin-2-amine* (70.0 mg, 0.250 mmol) and 3,4,5-trimethoxyaniline (93.36 mg, 0.510 mmol) in IPA (4.87 mL) was stirred at 80 °C for 1.5 h. Volatiles were removed; DCM and water were added: phases were separated, the organic one was washed with brine, dried

## 2. EXPERIMENTAL PART

over sodium sulfate, filtered and concentrated. crude was purified by flash chromatography (silica NH 11g, Cy/EtOAc from 6:4 to 0:10 and then EtOAc/MeOH 9:1) affording *5-methyl-2-N-(pyridin-4-ylmethyl)-7-N-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine-2,7-diamine* (N0758-01-1: 14.1 mg, 0.033 mmol, 13.13% yield) as brownish foam. LCMS acidic r.t. 0.48 min, MS (ESI)  $m/z = 422.26$   $[M+H]^+$ .

$^1\text{H NMR}$  (400 MHz, DMSO- $d_6$ )  $\delta$  ppm 2.31 (3 H, s) 3.68 (3 H, s) 3.78 (6 H, s) 4.55 (2 H, d,  $J=6.38$  Hz) 6.22 (1 H, s) 6.72 (2 H, s) 7.23 (1 H, br. s.) 7.37 (2 H, d,  $J=5.94$  Hz) 8.48 - 8.54 (2 H, m) 9.25 (1 H, s).

### 2.6 Synthesis of hydroxamic acids

These compounds, as reported in fig 2.66, were obtained starting from the bromination of the methyl of the appropriate methyl-phenyl keton (bearing 0-3 methoxy group) and the bromination of the aromatic ring that will be part of the central core. These two intermediates were then combined, giving the cyclized compound that took part in the Sonogashira's coupling with 2-propyonic acid.

To get the hydroxamic acid, the product of the Sonogashira was first combined with hydroxylamine and carbonyldiimidazole: no reaction occurred, both with hydroxylamine hydrochloride and free hydroxylamine. For this reason, it was necessary add another step: first, the coupling with the protected hydroxylamine, as *O*-(2-oxanyl)hydroxylamine, and then its deprotection with para toluene sulfonic acid, to give target compounds.

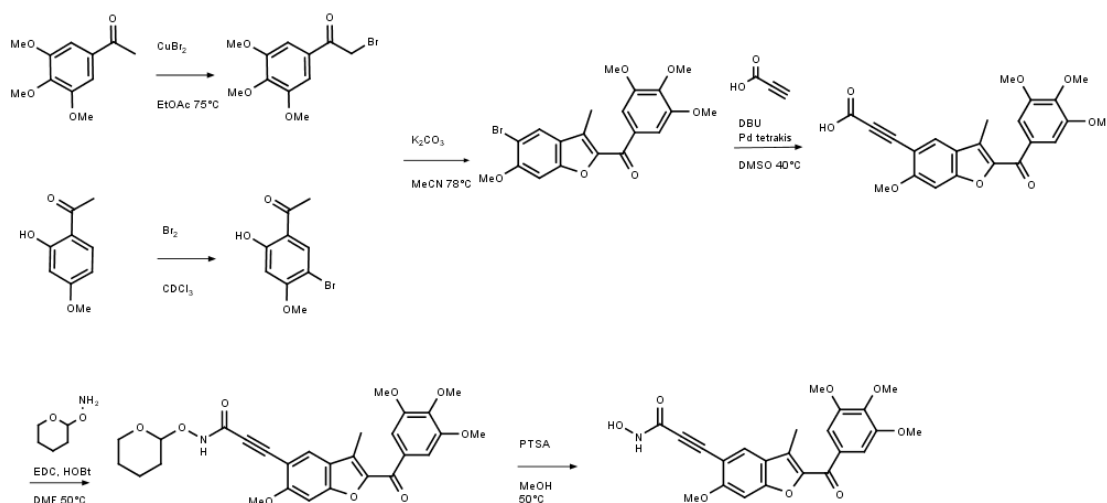


Figure 2.66: general route for hydroxamic acid

#### N0758-28 (CP016) N-hydroxy-3-[6-methoxy-3-methyl-2-(3,4,5-trimethoxybenzoyl)-1-benzofuran-5-yl]prop-2-ynamide

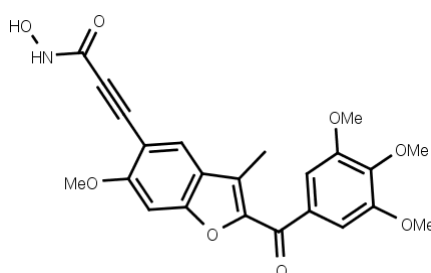


Figure 2.67: CP016

## 2. EXPERIMENTAL PART

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Copper (II) bromide (9.03 g, 40.43 mmol) was added to a stirred solution of 1-(3,4,5-trimethoxyphenyl)ethanone (5.0 g, 23.78 mmol) in Ethyl acetate (100 mL). The reaction mixture was stirred at 75 °C for 7h, then it was left to reach RT. Ground charcoal was added and the mixture stirred for 10 min, then filtered and washed with EtOAc. Volatiles were removed and crude purified by flash chromatography, reverse phase (silica c18 120g, water+0.1% formic acid/ MeCN+0.1% formic acid, from 98:2 to 4:6) giving 2-bromo-1-(3,4,5-trimethoxyphenyl)ethanone (N0423-51-1: 3.84 g, 13.28 mmol, 55.84% yield) as brownish solid.

LCMS acidic r.t. 0.94 min, MS (ESI)  $m/z$ = 289.03 - 291.06 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 3.75 - 3.78 (3 H, m) 3.85 - 3.88 (6 H, m) 4.97 (2 H, s) 7.31 (2 H, s).

*Batch 1* : A solution of molecular bromine (0.15 mL, 3.01 mmol) in Chloroform (3 mL) was added dropwise to a cold solution (0 °C) of 1-(2-hydroxy-4-methoxyphenyl)ethanone (0.5 g, 3.01 mmol) in Chloroform (27 mL). The reaction mixture was stirred at RT for 1h, then a ss aq solution of sodium bisulfite was added, followed by DCM: phases were separated, the organic one was washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 25g +25g, Cy/EtOAc from 10:0 to 85:15) affording 1-(5-bromo-2-hydroxy-4-methoxyphenyl)ethanone (N0423-60-1: 496 mg, 2.024 mmol, 67.26% yield) as white solid.

LCMS acidic r.t. 1.05 min, MS (ESI)  $m/z$ =244.88 - 246.92 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 2.60 (3 H, s) 3.92 (3 H, s) 6.68 (1 H, s) 8.06 - 8.10 (1 H, m) 12.54 (1 H, s).

*Batch 2* : A solution of molecular bromine (0.62 mL, 12.04 mmol) in Chloroform (12 mL) was added dropwise to a cold solution (0 °C) of 1-(2-hydroxy-4-methoxyphenyl)ethanone (2.0 g, 12.04 mmol) in Chloroform (108 mL). The reaction mixture was stirred at RT for 1h, then a ss aq solution of sodium bisulfite was added, followed by DCM: phases were separated, the organic one was washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 100g, Cy/EtOAc from 10:0 to 85:15) affording 1-(5-bromo-2-hydroxy-4-methoxyphenyl)ethanone (N0423-63-1: 2.014 g, 8.218 mmol, 68.28% yield) as white solid.

LCMS acidic r.t. 1.05 min, MS (ESI)  $m/z$ = 244.98 - 247.03 [M+H]<sup>+</sup>.

A mixture of 1-(5-bromo-2-hydroxy-4-methoxyphenyl)ethanone (2.18 g, 8.9 mmol), 2-bromo-1-(3,4,5-trimethoxyphenyl)ethanone (2.571 g, 8.9 mmol) and potassium carbonate (1.229 g, 8.9 mmol) in MeCN (47.25 mL) was stirred at 78 °C for 5h, then it was left to reach RT. Water was added (1.8 mL) and the mixture filtered; cake was dried under reduced pressure and purified by flash chromatography (silica 100g, Cy/EtOAc from 10:0 to 2:8) affording two batches: (5-bromo-6-methoxy-3-methyl-1-benzofuran-2-yl)-(3,4,5-trimethoxyphenyl)methanone (N0423-65-1: 2.04 g, 4.687 mmol, 52.69% yield) and (5-bromo-6-methoxy-3-methyl-1-benzofuran-2-yl)-(3,4,5-trimethoxyphenyl)methanone (N0423-65-2: 327 mg, 0.751 mmol, 8.445% yield) as whitish foams.

LCMS acidic N0423-65-1: r.t. 1.36 min, MS (ESI)  $m/z$ = 435.03 - 437.07 [M+H]<sup>+</sup>.

LCMS acidic N0423-65-2: r.t. 1.36 min, MS (ESI)  $m/z$ = 435.04 - 437.00 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 2.53 (3 H, s) 3.80 (3 H, s) 3.88 (6 H, s) 3.96 (3 H, s) 7.33 (2 H, s) 7.56 (1 H, s) 8.15 (1 H, s).

A solution of 2-propyonic acid (38.89  $\mu$ L, 0.630 mmol) in DMSO (0.333 mL) was added to a mixture of (5-bromo-6-methoxy-3-methyl-1-benzofuran-2-yl)-(3,4,5-trimethoxyphenyl)methanone (250.0 mg, 0.570 mmol), palladium tetrakis triphenylphosphine (46.46 mg, 0.040 mmol) and 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-a]azepine (257.43  $\mu$ L, 1.72 mmol) in DMSO (1.667 mL). The reaction mixture was stirred on at 40°C, then at room temperature for 2 days. The reaction mixture was poured into EtOAc, then a ss aq solution of NaHCO<sub>3</sub> was added: phases were separated, to the aq one were added cold HCl 1M and then DCM: phases

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were separated, and the organic one dried over sodium sulfate, filtered and concentrated, giving 3-[6-methoxy-3-methyl-2-(3,4,5-trimethoxybenzoyl)-1-benzofuran-5-yl]prop-2-ynoic acid (N0758-25-1: 225 mg, 0.530 mmol, 92.31% yield) as yellow solid.

LCMS acidic r.t. 1.16 min, MS (ESI)  $m/z=425.10$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 2.54 - 2.55 (3 H, m) 3.80 (3 H, s) 3.88 (6 H, s) 3.96 (3 H, s) 7.33 (2 H, s) 7.53 (1 H, s) 8.13 (1 H, br. s.).

A mixture of 3-[6-methoxy-3-methyl-2-(3,4,5-trimethoxybenzoyl)-1-benzofuran-5-yl]prop-2-ynoic acid (225.0 mg, 0.530 mmol), 1-hydroxybenzotriazole hydrate (121.78 mg, 0.800 mmol) and 3-(ethyliminomethylideneamino)-N,N-dimethyl-1-propanamine;hydrochloride (121.96 mg, 0.640 mmol) in DMF (8 mL) was stirred at RT for 30 min, then O-(2-oxanyl)hydroxylamine (93.16 mg, 0.800 mmol) was added and stirred at 50 °C for 2.5h. Volatiles were removed and crude dissolved in DCM: a ss aq solution of NaHCO<sub>3</sub> was added and phases separated; the organic one was washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 25g, Cy/EtOAc from 8:2 to 1:1) affording 3-[6-methoxy-3-methyl-2-(3,4,5-trimethoxybenzoyl)-1-benzofuran-5-yl]-N-(oxan-2-yloxy)prop-2-ynamide (N0758-27-1: 62 mg, 0.118 mmol, 22.34% yield) as orange solid.

LCMS acidic r.t. 1.16 min, MS (ESI)  $m/z=524.2$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 2.54 (3 H, s) 2.74 (4 H, d, J=0.66 Hz) 2.88 - 2.91 (4 H, m) 3.80 (3 H, s) 3.88 (6 H, s) 3.95 - 3.98 (3 H, m) 4.95 (1 H, br. s.) 5.97 (1 H, br. s.) 7.33 (2 H, s) 7.54 (1 H, s) 7.96 (1 H, s).

4-methylbenzenesulfonic acid hydrate (4.37 mg, 0.020 mmol) was added to a mixture of 3-[6-methoxy-3-methyl-2-(3,4,5-trimethoxybenzoyl)-1-benzofuran-5-yl]-N-(oxan-2-yloxy)prop-2-ynamide (62.0 mg, 0.110 mmol) in Methanol (2 mL): the reaction mixture was stirred on at RT, then at 50°C for 2h. Volatiles were removed and crude purified by flash chromatography, reverse phase (silica c18 6g, water + 0.1% formic acid / MeCN + 0.1% formic acid, from 98:2 to 1:1) affording N-hydroxy-3-[6-methoxy-3-methyl-2-(3,4,5-trimethoxybenzoyl)-1-benzofuran-5-yl]prop-2-ynamide (N0758-28-1: 24 mg, 0.055 mmol, 47.55% yield) as a yellowish solid.

LCMS acidic r.t. 0.94 min, MS (ESI)  $m/z=440.17$  [M+H]<sup>+</sup>

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 2.55 (3 H, s) 3.80 (3 H, s) 3.88 (6 H, s) 3.96 (3 H, m) 7.32 - 7.35 (2 H, m) 7.51 - 7.55 (1 H, m) 8.03 - 8.05 (1 H, m) 9.22 (1 H, br. s.) 11.27 (1 H, s).

### N0758-45 (CP028) N-hydroxy-3-[6-methoxy-2-(4-methoxybenzoyl)-3-methyl-1-benzofuran-5-yl]prop-2-ynamide

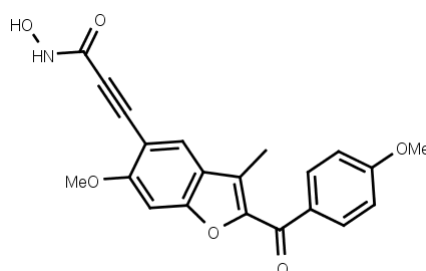


Figure 2.68: CP028

*Batch 1* : A solution of 2-propyonic acid (18.05  $\mu$ L, 0.290 mmol) in DMSO (0.133 mL) was added to a mixture of (5-bromo-6-methoxy-3-methyl-1-benzofuran-2-yl)-(4-methoxyphenyl)methanone (100.0 mg, 0.270 mmol), palladium tetrakis triphenylphosphine (21.56 mg, 0.020 mmol) and 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-a]zepine (119.45  $\mu$ L, 0.800 mmol) in DMSO (0.667 mL). The reaction mixture was stirred on at 40°C, then at room temperature for 2 days. The reaction mixture was poured into EtOAc, then a ss aq solution of NaHCO<sub>3</sub> was added:



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phases were separated, to the aq one were added cold HCl 1M and then DCM: phases were separated, and the organic one dried over sodium sulfate, filtered and concentrated, giving 3-[6-methoxy-2-(4-methoxybenzoyl)-3-methyl-1-benzofuran-5-yl]prop-2-ynoic acid (N0758-29-1: 64 mg, 0.176 mmol, 65.91% yield) as yellow solid. Compound was impure, so it was purified by flash chromatography (silica 10g, Cy/EtOAc from 8:2 to 0:10 and then EtOAc/MeOH from 95:5 to 75:25) affording 3-[6-methoxy-2-(4-methoxybenzoyl)-3-methyl-1-benzofuran-5-yl]prop-2-ynoic acid (N0758-29-2: 14 mg, 0.038 mmol, 14.42% yield) as yellow solid.

LCMS acidic r.t. 1.21 min, MS (ESI)  $m/z$  = 365.1 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 2.54 (3 H, s) 3.89 (3 H, s) 3.92 (3 H, s) 7.10 - 7.15 (2 H, m) 7.36 (1 H, s) 7.83 - 7.85 (1 H, m) 8.03 - 8.07 (2 H, m).

*Batch 2* : A solution of 2-propyonic acid (38.8  $\mu$ L, 0.630 mmol) in DMSO (0.287 mL) was added to a mixture of (5-bromo-6-methoxy-3-methyl-1-benzofuran-2-yl)-(4-methoxyphenyl)methanone (215.0 mg, 0.570 mmol), palladium tetrakis triphenylphosphine (46.35 mg, 0.040 mmol) and 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-a]azepine (256.83  $\mu$ L, 1.72 mmol) in DMSO (1.433 mL). The reaction mixture was stirred on at 40°C, then at room temperature for 2 days. The reaction mixture was poured into EtOAc, then a ss aq solution of NaHCO<sub>3</sub> was added: phases were separated, to the aq one were added cold HCl 6M and then DCM: phases were separated, and the organic one dried over sodium sulfate, filtered and concentrated, giving 3-[6-methoxy-2-(4-methoxybenzoyl)-3-methyl-1-benzofuran-5-yl]prop-2-ynoic acid (N0758-33-1: 160 mg, 0.439 mmol, 76.64% yield) as yellow solid.

LCMS acidic r.t. 1.22 min, MS (ESI)  $m/z$  = 365.16 [M+H]<sup>+</sup>.

A mixture of 3-[6-methoxy-2-(4-methoxybenzoyl)-3-methyl-1-benzofuran-5-yl]prop-2-ynoic acid (174.0 mg, 0.480 mmol), 3-(ethyliminomethylideneamino)-N,N-dimethyl-1-propanamine hydrochloride (109.86 mg, 0.570 mmol) and 1-hydroxybenzotriazole;hydrate (109.7 mg, 0.720 mmol) in DMF (6 mL) was stirred at RT for 30 min, then O-(2-oxanyl)hydroxylamine (83.92 mg, 0.720 mmol) was added and the reaction mixture stirred at 50 °C for 90 min. Volatiles were removed; DCM and ss aq NaHCO<sub>3</sub> were added: phases were separated, the organic one washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 10g, Cy/EtOAc from 9:1 to 3:7) affording 3-[6-methoxy-2-(4-methoxybenzoyl)-3-methyl-1-benzofuran-5-yl]-N-(oxan-2-yloxy)prop-2-ynamide (N0758-40-1: 90 mg, 0.194 mmol, 40.66% yield) as yellowish foam.

LCMS acidic r.t. 1.19 min, MS (ESI)  $m/z$  = 464.17 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 1.54 (3 H, br. s.) 1.69 (3 H, br. s.) 2.53 - 2.57 (3 H, m) 3.53 (2 H, d, J=13.20 Hz) 3.87 - 3.91 (3 H, m) 3.94 - 3.97 (3 H, m) 4.94 (1 H, br. s.) 7.10 - 7.15 (2 H, m) 7.48 - 7.52 (1 H, m) 8.04 - 8.09 (3 H, m) 11.82 (1 H, br. s.).

A mixture of 3-[6-methoxy-2-(4-methoxybenzoyl)-3-methyl-1-benzofuran-5-yl]-N-(oxan-2-yloxy)prop-2-ynamide (90.0 mg, 0.190 mmol) and 4-methylbenzenesulfonic acid hydrate (7.39 mg, 0.040 mmol) in Methanol (3.407 mL) was stirred at 50 °C for 90min, then volatiles were removed. Crude was purified by flash chromatography, reverse phase (silica c18 12g + 12g, water + 0.1% formic acid / MeCN + 0.1% formic acid, from 98:2 to 45:55) affording N-hydroxy-3-[6-methoxy-2-(4-methoxybenzoyl)-3-methyl-1-benzofuran-5-yl]prop-2-ynamide (N0758-45-1: 38.7 mg, 0.102 mmol, 52.53% yield).

LCMS acidic r.t. 0.98 min, MS (ESI)  $m/z$  = 380.17 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 2.54 - 2.56 (3 H, m) 3.89 (3 H, s) 3.94 - 3.97 (3 H, m) 7.10 - 7.16 (2 H, m) 7.47 - 7.51 (1 H, m) 8.02 - 8.09 (3 H, m) 9.27 (1 H, br. s.) 11.25 (1 H, br. s.).

## 2. EXPERIMENTAL PART

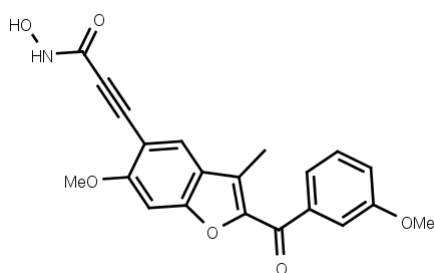


Figure 2.69: CP029

### **N0758-46 (CP029) N-hydroxy-3-[6-methoxy-2-(3-methoxybenzoyl)-3-methyl-1-benzofuran-5-yl]prop-2-ynamide**

A solution of 2-propyonic acid (44.75  $\mu$ L, 0.730 mmol) in DMSO (0.331 mL) was added to a mixture of (5-bromo-6-methoxy-3-methyl-1-benzofuran-2-yl)-(3-methoxyphenyl)methanone (248.0 mg, 0.660 mmol), palladium tetrakis triphenylphosphine (53.46 mg, 0.050 mmol) and 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-a]zazepine (296.25  $\mu$ L, 1.98 mmol) in DMSO (1.653 mL). The reaction mixture was stirred on at 40°C, then at room temperature for 2 days. The reaction mixture was poured into EtOAc, then a ss aq solution of NaHCO<sub>3</sub> was added: phases were separated, to the aq one were added cold HCl 6M and then DCM: phases were separated, and the organic one dried over sodium sulfate, filtered and concentrated, giving 3-[6-methoxy-2-(3-methoxybenzoyl)-3-methyl-1-benzofuran-5-yl]prop-2-ynoic acid (N0758-34-1: 187 mg, 0.513 mmol, 77.65% yield) as yellow solid.

LCMS acidic r.t. 1.23 min, MS (ESI) m/z= 365.11 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 2.54 (3 H, s) 3.84 - 3.88 (3 H, m) 3.94 - 3.97 (3 H, m) 7.24 - 7.29 (1 H, m) 7.46 - 7.54 (3 H, m) 7.56 - 7.60 (1 H, m) 8.14 (1 H, s) 13.53 - 13.83 (1 H, m).

A mixture of 3-[6-methoxy-2-(3-methoxybenzoyl)-3-methyl-1-benzofuran-5-yl]prop-2-ynoic acid (187.0 mg, 0.510 mmol), 3-(ethyliminomethylideneamino)-N,N-dimethyl-1-propanamine hydrochloride (118.07 mg, 0.620 mmol) and 1-hydroxybenzotriazole hydrate (117.9 mg, 0.770 mmol) in DMF (6 mL) was stirred at RT for 30 min, then O-(2-oxanyl)hydroxylamine (90.19 mg, 0.770 mmol) was added and the reaction mixture stirred at 50 °C for 90 min. Volatiles were removed; DCM and ss aq NaHCO<sub>3</sub> were added: phases were separated, the organic one washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 10g, Cy/EtOAc from 9:1 to 3:7) affording 3-[6-methoxy-2-(3-methoxybenzoyl)-3-methyl-1-benzofuran-5-yl]-N-(oxan-2-yloxy)prop-2-ynamide (N0758-41-1: 84 mg, 0.181 mmol, 35.31% yield) as brownish oil.

LCMS acidic r.t. 1.21 min, MS (ESI) m/z= 494.25 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 1.55 (3 H, br. s.) 1.65 - 1.73 (3 H, m) 2.54 - 2.58 (3 H, m) 3.54 (1 H, d, J=10.12 Hz) 3.85 (3 H, s) 3.93 - 3.97 (3 H, m) 4.94 (1 H, br. s.) 7.27 (1 H, ddd, J=8.14, 2.64, 1.10 Hz) 7.48 (1 H, dd, J=2.53, 1.43 Hz) 7.49 - 7.54 (2 H, m) 7.56 - 7.59 (1 H, m) 8.10 (1 H, s) 11.82 (1 H, br. s.).

A mixture of 3-[6-methoxy-2-(3-methoxybenzoyl)-3-methyl-1-benzofuran-5-yl]-N-(oxan-2-yloxy)prop-2-ynamide (84.0 mg, 0.180 mmol) and 4-methylbenzenesulfonic acid hydrate (6.9 mg, 0.040 mmol) in Methanol (3.18 mL) was stirred at 50 °C for 90min. Volatiles were removed and crude purified by flash chromatography, reverse phase (silica c18 12g + 12g, water + 0.1% formic acid / MeCN + 0.1% formic acid, from 98:2 to 4:6) affording N-hydroxy-3-[6-methoxy-2-(3-methoxybenzoyl)-3-methyl-1-benzofuran-5-yl]prop-2-ynamide (N0758-46-1: 34 mg, 0.090 mmol, 49.45% yield) as yellowish solid.

LCMS acidic r.t. 0.99 min, MS (ESI) m/z= 380.11 [M+H]<sup>+</sup>

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 2.52 - 2.56 (3 H, m) 3.84 (3 H, s) 3.92 - 3.96 (3 H, m)

## 2. EXPERIMENTAL PART

7.22 - 7.28 (1 H, m) 7.45 - 7.53 (3 H, m) 7.55 - 7.60 (1 H, m) 8.02 - 8.07 (1 H, m) 9.26 (1 H, br. s.) 11.25 (1 H, br. s.).

### N0758-47 (CP030) 3-[2-(3,4-dimethoxybenzoyl)-6-methoxy-3-methyl-1-benzofuran-5-yl]-N-hydroxyprop-2-ynamide

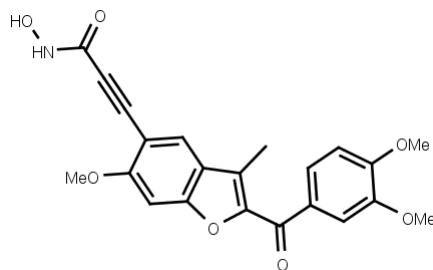


Figure 2.70: CP030

*Batch 1* : A solution of 2-propyonic acid (15.04 uL, 0.240 mmol) in DMSO (0.120 mL) was added to a mixture of (5-bromo-6-methoxy-3-methyl-1-benzofuran-2-yl)-(3,4-dimethoxyphenyl)methanone (90.0 mg, 0.220 mmol), palladium tetrakis triphenylphosphine (17.96 mg, 0.020 mmol) and 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-a]azepine (99.54 uL, 0.670 mmol) in DMSO (0.600 mL). The reaction mixture was stirred on at 40°C, then at room temperature for 2 days. The reaction mixture was poured into EtOAc, then a ss aq solution of NaHCO<sub>3</sub> was added: phases were separated, to the aq one were added cold HCl 1M and then DCM: phases were separated, and the organic one dried over sodium sulfate, filtered and concentrated, giving 3-[2-(3,4-dimethoxybenzoyl)-6-methoxy-3-methyl-1-benzofuran-5-yl]prop-2-ynoic acid (N0758-31-1: 26 mg, 0.066 mmol, 29.69% yield) as yellow solid.

LCMS acidic r.t. 1.15 min, MS (ESI) m/z= 395.17 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 2.53 - 2.55 (3 H, m) 3.85 - 3.91 (6 H, m) 3.94 - 3.99 (3 H, m) 7.12 - 7.17 (1 H, m) 7.46 - 7.51 (1 H, m) 7.55 - 7.59 (1 H, m) 7.74 - 7.79 (1 H, m) 7.99 - 8.14 (1 H, m).

*Batch 2* : A solution of 2-propyonic acid (14.54 uL, 0.240 mmol) in DMSO (0.116 mL) was added to a mixture of (5-bromo-6-methoxy-3-methyl-1-benzofuran-2-yl)-(3,4-dimethoxyphenyl)methanone (87.0 mg, 0.210 mmol), palladium tetrakis triphenylphosphine (17.37 mg, 0.020 mmol) and 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-a]azepine (96.22 uL, 0.640 mmol) in DMSO (0.580 mL). The reaction mixture was stirred on at 40°C, then at room temperature for 2 days. The reaction mixture was poured into EtOAc, then a ss aq solution of NaHCO<sub>3</sub> was added: phases were separated, to the aq one were added cold HCl 6M and then DCM: phases were separated, and the organic one dried over sodium sulfate, filtered and concentrated, giving 3-[2-(3,4-dimethoxybenzoyl)-6-methoxy-3-methyl-1-benzofuran-5-yl]prop-2-ynoic acid (N0758-35-1: 49 mg, 0.124 mmol, 57.87% yield) as yellow solid.

LCMS acidic r.t. 1.15 min, MS (ESI) m/z= 395.13 [M+H]<sup>+</sup>.

A mixture of 3-[2-(3,4-dimethoxybenzoyl)-6-methoxy-3-methyl-1-benzofuran-5-yl]prop-2-ynoic acid (75.0 mg, 0.190 mmol), 1-hydroxybenzotriazole hydrate (43.69 mg, 0.290 mmol) and 3-(ethyliminomethylideneamino)-N,N-dimethyl-1-propanamine hydrochloride (43.75 mg, 0.230 mmol) in DMF (2.6 mL) was stirred at RT for 30 min, then O-(2-oxanyl)hydroxylamine (33.42 mg, 0.290 mmol) was added and the reaction mixture stirred at 50 °C for 90 min. Volatiles were removed; DCM and ss aq NaHCO<sub>3</sub> were added: phases were separated, the organic one washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 10g, Cy/EtOAc from 9:1 to 2:8) affording 3-[2-(3,4-dimethoxybenzoyl)-6-methoxy-3-methyl-1-benzofuran-5-yl]-N-(oxan-2-yloxy)prop-

## 2. EXPERIMENTAL PART

2-ynamide (N0758-42-1: 29 mg, 0.059 mmol, 30.9% yield) as yellowish foam.

LCMS acidic r.t. 1.13 min, MS (ESI)  $m/z$ = 494.25 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 1.55 (3 H, br. s.) 1.70 (3 H, d, J=1.98 Hz) 2.52 - 2.55 (3 H, m) 3.54 (1 H, d, J=11.00 Hz) 3.83 - 3.87 (3 H, m) 3.88 - 3.92 (3 H, m) 3.94 - 3.98 (3 H, m) 4.95 (1 H, t, J=2.75 Hz) 7.13 - 7.17 (1 H, m) 7.50 - 7.52 (1 H, m) 7.57 (1 H, d, J=1.98 Hz) 7.74 - 7.78 (1 H, m) 8.08 (1 H, s) 11.82 (1 H, br. s.).

A mixture of 3-[2-(3,4-dimethoxybenzoyl)-6-methoxy-3-methyl-1-benzofuran-5-yl]-N-(oxan-2-yloxy)prop-2-ynamide (29.0 mg, 0.060 mmol) and 4-methylbenzenesulfonic acid hydrate (2.24 mg, 0.010 mmol) in Methanol (1.031 mL) was stirred at 50 °C for 90 min, then volatiles were removed. Crude was purified by flash chromatography, reverse phase (silica c18 12g + 12g, water + 0.1% formic acid / MeCN + 0.1% formic acid, from 98:2 to 45:55) affording 3-[2-(3,4-dimethoxybenzoyl)-6-methoxy-3-methyl-1-benzofuran-5-yl]-N-hydroxyprop-2-ynamide (N0758-47-1: 15.6 mg, 0.038 mmol, 64.85% yield) as yellowish solid.

LCMS acidic r.t. 0.91 min, MS (ESI)  $m/z$ = 410.19 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 2.52 - 2.54 (3 H, m) 3.85 (3 H, s) 3.88 (3 H, s) 3.93 - 3.98 (3 H, m) 7.11 - 7.16 (1 H, m) 7.48 - 7.51 (1 H, m) 7.54 - 7.58 (1 H, m) 7.72 - 7.79 (1 H, m) 8.00 - 8.05 (1 H, m) 9.25 (1 H, br. s.) 11.25 (1 H, br. s.).

### N0758-48 (CP031) N-hydroxy-3-[6-methoxy-2-(2-methoxybenzoyl)-3-methyl-1-benzofuran-5-yl]prop-2-ynamide

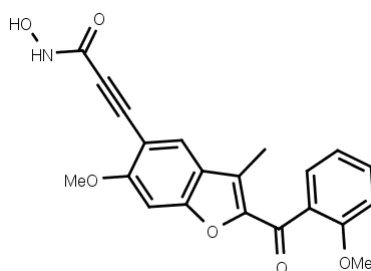


Figure 2.71: CP031

*Batch 1* : A solution of 2-propyonic acid (18.05  $\mu$ L, 0.290 mmol) in DMSO (0.133 mL) was added to a mixture of (5-bromo-6-methoxy-3-methyl-1-benzofuran-2-yl)-(2-methoxyphenyl)methanone (100.0 mg, 0.270 mmol), palladium tetrakis triphenylphosphine (21.56 mg, 0.020 mmol) and 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-a]azepine (119.45  $\mu$ L, 0.800 mmol) in DMSO (0.667 mL). The reaction mixture was stirred on at 40°C, then at room temperature for 2 days. The reaction mixture was poured into EtOAc, then a ss aq solution of NaHCO<sub>3</sub> was added: phases were separated, to the aq one were added cold HCl 1M and then DCM: phases were separated, and the organic one dried over sodium sulfate, filtered and concentrated, giving 3-[6-methoxy-2-(2-methoxybenzoyl)-3-methyl-1-benzofuran-5-yl]prop-2-ynoic acid (N0758-30-1: 24 mg, 0.066 mmol, 24.72% yield) as yellow solid.

LCMS acidic r.t. 1.14 min, MS (ESI)  $m/z$ = 365.11 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 2.38 (3 H, s) 3.74 (3 H, s) 3.90 - 3.93 (3 H, m) 7.07 - 7.12 (1 H, m) 7.21 (1 H, dd, J=8.25, 0.77 Hz) 7.37 - 7.40 (1 H, m) 7.41 (1 H, s) 7.53 - 7.59 (1 H, m) 8.08 (1 H, s).

*Batch 2* : A solution of 2-propyonic acid (26.71  $\mu$ L, 0.430 mmol) in DMSO (0.197 mL) was added to a mixture of (5-bromo-6-methoxy-3-methyl-1-benzofuran-2-yl)-(2-methoxyphenyl)methanone (148.0 mg, 0.390 mmol), palladium tetrakis triphenylphosphine (31.91 mg, 0.030 mmol) and 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-a]azepine (176.79  $\mu$ L, 1.18 mmol) in DMSO (0.987 mL). The reaction mixture was stirred on at 40°C, then at room temperature for 2 days.

## 2. EXPERIMENTAL PART

The reaction mixture was poured into EtOAc, then a ss aq solution of NaHCO<sub>3</sub> was added: phases were separated, to the aq one were added cold HCl 6M and then DCM: phases were separated, and the organic one dried over sodium sulfate, filtered and concentrated, giving 3-[6-methoxy-2-(2-methoxybenzoyl)-3-methyl-1-benzofuran-5-yl]prop-2-ynoic acid (N0758-36-1: 134 mg, 0.368 mmol, 93.24% yield) as yellow solid.

LCMS acidic r.t. 1.14 min, MS (ESI) m/z= 365.14 [M+H]<sup>+</sup>.

A mixture of 3-[6-methoxy-2-(2-methoxybenzoyl)-3-methyl-1-benzofuran-5-yl]prop-2-ynoic acid (158.0 mg, 0.430 mmol), 1-hydroxybenzotriazole;hydrate (99.61 mg, 0.650 mmol) and 3-(ethyliminomethylideneamino)-N,N-dimethyl-1-propanamine;hydrochloride (99.76 mg, 0.520 mmol) in DMF (5.6 mL) as stirred at RT for 30 min, then O-(2-oxanyl)hydroxylamine (76.2 mg, 0.650 mmol) was added and the reaction mixture stirred at 50 °C for 90 min. Volatiles were removed; DCM and ss aq NaHCO<sub>3</sub> were added: phases were separated, the organic one washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 10g, Cy/EtOAc from 9:1 to 2:8) affording 3-[6-methoxy-2-(2-methoxybenzoyl)-3-methyl-1-benzofuran-5-yl]-N-(oxan-2-yloxy)prop-2-ynamide (N0758-43-1: 41 mg, 0.088 mmol, 20.4% yield) as yellowish oil.

LCMS acidic r.t. 1.13 min, MS (ESI) m/z= 464.16 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 1.54 (3 H, br. s.) 1.69 (3 H, br. s.) 2.38 (3 H, s) 3.74 (3 H, s) 3.89 - 3.93 (3 H, m) 7.07 - 7.12 (1 H, m) 7.21 (1 H, dd, J=8.36, 0.66 Hz) 7.38 (1 H, dd, J=7.48, 1.76 Hz) 7.42 (1 H, s) 7.56 (1 H, td, J=7.92, 1.76 Hz) 8.02 - 8.08 (1 H, m) 11.80 (1 H, br. s.).

A mixture of 3-[6-methoxy-2-(2-methoxybenzoyl)-3-methyl-1-benzofuran-5-yl]-N-(oxan-2-yloxy)prop-2-ynamide (41.0 mg, 0.090 mmol) and 4-methylbenzenesulfonic acid hydrate (3.37 mg, 0.020 mmol) in Methanol (1.552 mL) was stirred at 50 °C for 90 min, then volatiles were removed. Crude was purified by flash chromatography, reverse phase (silica c18 12g+ 12g, water + 0.1% formic acid / MeCN + 0.1% formic acid, from 98:2 to 45:55) affording N-hydroxy-3-[6-methoxy-2-(2-methoxybenzoyl)-3-methyl-1-benzofuran-5-yl]prop-2-ynamide (N0758-48-1: 6.3 mg, 0.017 mmol, 18.77% yield) as a yellowish foam.

LCMS acidic r.t. 0.92 min, MS (ESI) m/z= 380.15 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 2.34 - 2.40 (3 H, m) 3.71 - 3.75 (3 H, m) 3.87 - 3.94 (3 H, m) 7.06 - 7.12 (1 H, m) 7.20 (1 H, d, J=8.14 Hz) 7.37 (1 H, dd, J=7.48, 1.76 Hz) 7.41 (1 H, s) 7.53 - 7.58 (1 H, m) 8.00 (1 H, s) 9.20 - 9.33 (1 H, m) 11.23 (1 H, br. s.).

### N0758-49 (CP032) 3-[2-(3,5-dimethoxybenzoyl)-6-methoxy-3-methyl-1-benzofuran-5-yl]-N-hydroxyprop-2-ynamide

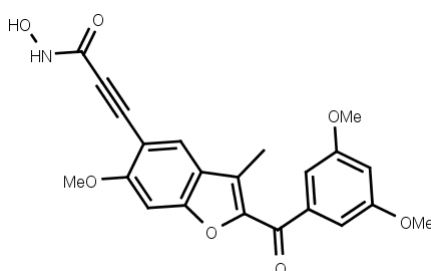


Figure 2.72: CP032

A solution of 2-propyonic acid (42.61 uL, 0.690 mmol) in DMSO (0.340 mL) was added to a mixture of (5-bromo-6-methoxy-3-methyl-1-benzofuran-2-yl)-(3,5-dimethoxyphenyl)methanone (255.0 mg, 0.630 mmol), palladium tetrakis triphenylphosphine (50.9 mg, 0.040 mmol) and 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-a]zepine (282.04 uL, 1.89 mmol) in DMSO (1.7 mL). The reaction mixture was stirred on at 40°C, then at room temperature for 2 days. The reaction mixture was poured into EtOAc, then a ss aq solution of NaHCO<sub>3</sub> was added:

## 2. EXPERIMENTAL PART

phases were separated, to the aq one were added cold HCl 6M and then DCM: phases were separated, and the organic one dried over sodium sulfate, filtered and concentrated, giving *3-[2-(3,5-dimethoxybenzoyl)-6-methoxy-3-methyl-1-benzofuran-5-yl]prop-2-ynoic acid* (N0758-37-1: 380 mg, 0.964 mmol, 153.13% yield) as brownish solid.

LCMS acidic r.t. 1.24 min, MS (ESI)  $m/z=295.2$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 2.54 (3 H, br. s.) 3.82 - 3.84 (6 H, m) 3.96 (3 H, s) 6.82 (1 H, t,  $J=2.31$  Hz) 7.10 (2 H, d,  $J=2.20$  Hz) 7.52 (1 H, s) 8.12 - 8.15 (1 H, m) 8.13 (1 H, s).

A mixture of *3-[2-(3,5-dimethoxybenzoyl)-6-methoxy-3-methyl-1-benzofuran-5-yl]prop-2-ynoic acid* (404.26 mg, 0.960 mmol), 1-hydroxybenzotriazole;hydrate (221.34 mg, 1.45 mmol) and 3-(ethyliminomethylideneamino)-N,N-dimethyl-1-propanamine hydrochloride (221.66 mg, 1.16 mmol) in DMF (9 mL) was stirred at RT for 30 min, then O-(2-oxanyl)hydroxylamine (169.32 mg, 1.45 mmol) was added and the reaction mixture stirred at 50 °C for 90 min. Volatiles were removed; DCM and ss aq NaHCO<sub>3</sub> were added: phases were separated, the organic one washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 10g, Cy/EtOAc from 9:1 to 2:8) affording *3-[2-(3,5-dimethoxybenzoyl)-6-methoxy-3-methyl-1-benzofuran-5-yl]-N-(oxan-2-yloxy)prop-2-ynamide* (N0758-44-1: 60 mg, 0.122 mmol, 12.62% yield) was yellowish foam.

LCMS acidic r.t. 1.22 min, MS (ESI)  $m/z=494.11$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 1.50 - 1.61 (3 H, m) 1.69 (3 H, br. s.) 2.53 - 2.55 (3 H, m) 3.54 (1 H, d,  $J=11.44$  Hz) 3.81 - 3.86 (6 H, m) 3.92 - 3.98 (3 H, m) 4.95 (1 H, br. s.) 5.98 (1 H, br. s.) 6.82 (1 H, t,  $J=2.31$  Hz) 7.08 - 7.12 (2 H, m) 7.53 (1 H, s) 8.10 (1 H, s) 11.82 (1 H, br. s.).

A mixture of *3-[2-(3,5-dimethoxybenzoyl)-6-methoxy-3-methyl-1-benzofuran-5-yl]-N-(oxan-2-yloxy)prop-2-ynamide* (60.0 mg, 0.120 mmol) and 4-methylbenzenesulfonic acid hydrate (4.63 mg, 0.020 mmol) in Methanol (2.133 mL) was stirred at 50 °C for 4h, then volatiles were removed. Crude was purified by flash chromatography, reverse phase (silica c18 12g + 12g, water + 0.1% formic acid / MeCN + 0.1% formic acid, from 98:2 to 1:1) affording *3-[2-(3,5-dimethoxybenzoyl)-6-methoxy-3-methyl-1-benzofuran-5-yl]-N-hydroxyprop-2-ynamide* (N0758-49-1: 16.9 mg, 0.041 mmol, 33.95% yield) as yellowish solid.

LCMS acidic r.t. 1.01 min, MS (ESI)  $m/z=410.16$  [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 2.52 - 2.54 (3 H, m) 3.83 (6 H, s) 3.93 - 3.96 (3 H, m) 6.81 (1 H, t,  $J=2.31$  Hz) 7.09 (2 H, d,  $J=2.20$  Hz) 7.51 (1 H, s) 8.03 - 8.06 (1 H, m) 9.26 (1 H, br. s.) 11.25 (1 H, br. s.).

### N0758-59 (CP034) 3-(2-benzoyl-6-methoxy-3-methyl-benzofuran-5-yl)prop-2-ynehydroxamic acid

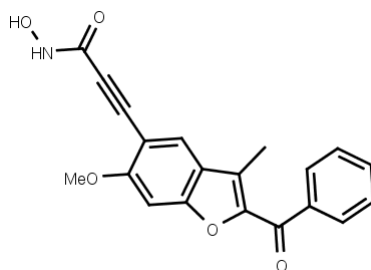


Figure 2.73: CP034

A solution of 2-propyonic acid (33.35  $\mu$ L, 0.540 mmol) in DMSO (0.227 mL) was added to a mixture of (5-bromo-6-methoxy-3-methyl-benzofuran-2-yl)-phenyl-methanone (170.0 mg, 0.490 mmol), palladium tetrakis triphenylphosphine (39.84 mg, 0.030 mmol) and 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-a]azepine (220.73  $\mu$ L, 1.48 mmol) in DMSO

## 2. EXPERIMENTAL PART

(1.133 mL). The reaction mixture was stirred on at 45°C. 2-propyonic acid (33.35 uL, 0.540 mmol) in DMSO (0.227 mL), 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-a]azepine (220.73 uL, 1.48 mmol) and palladium tetrakis triphenylphosphine (39.84 mg, 0.030 mmol) were added again: the mixture was degassed, then stirred at 45 °C on. The reaction mixture was poured into EtOAc, then a ss aq solution of NaHCO<sub>3</sub> was added: phases were separated, to the aq one were added cold HCl 6M and then DCM: phases were separated, and the organic one dried over sodium sulfate, filtered and concentrated, giving 3-(2-benzoyl-6-methoxy-3-methyl-benzofuran-5-yl)prop-2-ynoic acid (N0758-55-1: 186 mg, 0.556 mmol, 112.97% yield) as brownish solid.

LCMS acidic r.t. 1.23 min, MS (ESI) m/z= 335.09 [M+H]<sup>+</sup>.

A mixture of 3-(2-benzoyl-6-methoxy-3-methyl-benzofuran-5-yl)prop-2-ynoic acid (180.0 mg, 0.540 mmol), 3-(ethyliminomethylideneamino)-N,N-dimethyl-1-propanamine hydrochloride (123.85 mg, 0.650 mmol) and 1-hydroxybenzotriazole;hydrate (123.68 mg, 0.810 mmol) in DMF (6.207 mL) was stirred at RT for 30 min, then O-(2-oxanyl)hydroxylamine (94.61 mg, 0.810 mmol) was added and the reaction mixture stirred at 50 °C for 3.5h. Volatiles were removed; DCM and ss aq NaHCO<sub>3</sub> were added: phases were separated, the organic one washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 10g, Cy/EtOAc from 9:1 to 3:7) affording 3-(2-benzoyl-6-methoxy-3-methyl-benzofuran-5-yl)-N-tetrahydropyran-2-yloxy-prop-2-ynamide (N0758-57-1: 34 mg, 0.078 mmol, 14.57% yield) as yellowish foam.

LCMS acidic r.t. 1.20 min, MS (ESI) m/z= 434.27 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 1.55 (3 H, br. s.) 1.69 (3 H, br. s.) 2.55 - 2.59 (3 H, m) 3.54 (1 H, d, J=11.66 Hz) 3.96 (3 H, s) 4.95 (1 H, t, J=2.64 Hz) 7.49 - 7.52 (1 H, m) 7.57 - 7.63 (2 H, m) 7.67 - 7.72 (1 H, m) 7.95 - 8.02 (2 H, m) 8.09 - 8.13 (1 H, m) 11.77 - 11.86 (1 H, m).

A mixture of 3-(2-benzoyl-6-methoxy-3-methyl-benzofuran-5-yl)-N-tetrahydropyran-2-yloxy-prop-2-ynamide (29.86 mg, 0.070 mmol) and 4-methylbenzenesulfonic acid hydrate (2.62 mg, 0.010 mmol) in Methanol (1.209 mL) was stirred at 50 °C for 4h, then volatiles were removed. Crude was purified by flash chromatography, reverse phase (silica c18 6g + 6g, water + 0.1% formic acid / MeCN + 0.1% formic acid, from 98:2 to 1:1) affording 3-(2-benzoyl-6-methoxy-3-methyl-benzofuran-5-yl)prop-2-ynehydroxamic acid (N0758-59-1: 10.48 mg, 0.030 mmol, 43.54% yield) as yellowish solid.

LCMS acidic r.t. 0.98 min, MS (ESI) m/z= 350.17 [M+H]<sup>+</sup>

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 2.54 - 2.57 (3 H, m) 3.94 (3 H, s) 7.48 (1 H, s) 7.57 - 7.61 (2 H, m) 7.66 - 7.71 (1 H, m) 7.96 - 8.00 (2 H, m) 8.05 - 8.07 (1 H, m) 9.24 (1 H, br. s.) 11.17 - 11.37 (1 H, m).

### N0758-60 (CP035) 3-[3-methyl-2-(3,4,5-trimethoxybenzoyl)benzofuran-5-yl]prop-2-ynehydroxamic acid

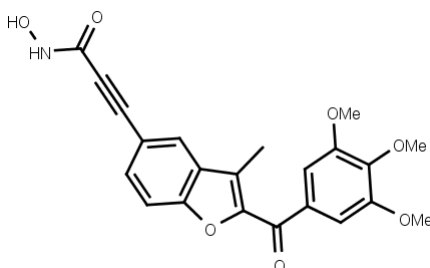


Figure 2.74: CP035

A solution of 2-propyonic acid (50.13 uL, 0.810 mmol) in DMSO (0.400 mL) was added to a mixture of (5-bromo-3-methyl-benzofuran-2-yl)-(3,4,5-trimethoxyphenyl)methanone

## 2. EXPERIMENTAL PART

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(300.0 mg, 0.740 mmol), palladium tetrakis triphenylphosphine (59.88 mg, 0.050 mmol) and 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-a]azepine (331.81  $\mu$ L, 2.22 mmol) in DMSO (2 mL). The reaction mixture was stirred on at 45°C, then 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-a]azepine (331.81  $\mu$ L, 2.22 mmol), palladium tetrakis triphenylphosphine (59.88 mg, 0.050 mmol) and 2-propyonic acid (50.13  $\mu$ L, 0.810 mmol) in DMSO (0.400 mL) were added: the mixture was degassed, then stirred on at 45°C. The reaction mixture was poured into EtOAc, then a ss aq solution of NaHCO<sub>3</sub> was added: phases were separated, to the aq one were added cold HCl 6M and then DCM: phases were separated, and the organic one dried over sodium sulfate, filtered and concentrated, giving 3-[3-methyl-2-(3,4,5-trimethoxybenzoyl)benzofuran-5-yl]prop-2-ynoic acid (N0758-56-1: 194 mg, 0.492 mmol, 66.45% yield) as brown oil.

LCMS acidic r.t. 1.22min, MS (ESI) m/z= 395.17 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 2.56 - 2.58 (3 H, m) 3.80 (3 H, s) 3.86 - 3.88 (6 H, m) 7.34 - 7.36 (2 H, m) 7.76 - 7.81 (1 H, m) 7.83 - 7.87 (1 H, m) 8.25 (1 H, dd, J=1.87, 0.77 Hz) 13.83 (1 H, br. s.).

A mixture of 3-[3-methyl-2-(3,4,5-trimethoxybenzoyl)benzofuran-5-yl]prop-2-ynoic acid (185.0 mg, 0.470 mmol), 3-(ethyliminomethylideneamino)-N,N-dimethyl-1-propanamine hydrochloride (107.91 mg, 0.560 mmol) and 1-hydroxybenzotriazole hydrate (107.76 mg, 0.700 mmol) in DMF (6.379 mL) was stirred at RT for 30 min, then O-(2-oxanyl)hydroxylamine (82.43 mg, 0.700 mmol) was added and the reaction mixture stirred at 50 °C for 4.5h. Volatiles were removed; DCM and ss aq NaHCO<sub>3</sub> were added: phases were separated, the organic one washed with brine, dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 25g, Cy/EtOAc from 9:1 to 3:7) affording 3-[3-methyl-2-(3,4,5-trimethoxybenzoyl)benzofuran-5-yl]-N-tetrahydropyran-2-yloxy-prop-2-ynamide (N0758-58-1: 14 mg, 0.028 mmol, 6.047% yield) as yellowish foam.

LCMS acidic r.t. 1.17 min, MS (ESI) m/z= 494.27 [M+H]<sup>+</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 1.51 - 1.59 (3 H, m) 1.67 - 1.73 (3 H, m) 2.56 - 2.58 (3 H, m) 3.55 (1 H, d, J=11.00 Hz) 3.79 - 3.82 (3 H, m) 3.85 - 3.90 (6 H, m) 4.95 (1 H, br. s.) 7.35 (2 H, s) 7.73 - 7.78 (1 H, m) 7.83 - 7.88 (1 H, m) 8.18 - 8.22 (1 H, m) 11.85 (1 H, br. s.).

A mixture of 3-[3-methyl-2-(3,4,5-trimethoxybenzoyl)benzofuran-5-yl]-N-tetrahydropyran-2-yloxy-prop-2-ynamide (14.0 mg, 0.030 mmol) and 4-methylbenzenesulfonic acid hydrate (1.08 mg, 0.010 mmol) in Methanol (0.498 mL) was stirred at 50 °C for 90 min, then volatiles were removed. Crude was purified by flash chromatography, reverse phase (silica c18 12g + 12g, water + 0.1% formic acid / MeCN + 0.1% formic acid, from 98:2 to 45:55) affording 3-[3-methyl-2-(3,4,5-trimethoxybenzoyl)benzofuran-5-yl]prop-2-ynehydroxamic acid (N0758-60-1: 4.87 mg, 0.012 mmol, 41.93% yield) as yellowish solid.

LCMS acidic r.t. 0.95 min, MS (ESI) m/z= 410.25 [M+H]<sup>+</sup>

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 2.54 - 2.57 (3 H, m) 3.79 (3 H, s) 3.84 - 3.88 (6 H, m) 7.29 - 7.38 (2 H, m) 7.69 - 7.75 (1 H, m) 7.80 - 7.86 (1 H, m) 8.12 - 8.19 (1 H, m) 9.32 (1 H, br. s.) 11.30 (1 H, br. s.).

### **N0758-54 (CP033) (5-ethynyl-6-methoxy-3-methyl-benzofuran-2-yl)-(3,4,5-trimethoxy phenyl)methanone**

A mixture of (5-bromo-6-methoxy-3-methyl-1-benzofuran-2-yl)-(3,4,5-trimethoxy phenyl)methanone (200.0 mg, 0.460 mmol), ethynyl(trimethyl)silane (0.1 mL, 0.690 mmol), triethylamine (0.19 mL, 1.38 mmol), palladium(2+) triphenylphosphine dichloride (32.25 mg, 0.050 mmol) and copper (I) iodide (8.75 mg, 0.050 mmol) in THF (4 mL) was degassed, then submitted to microwave reactor (5\*30 min at 100 °C). Ethynyl(trimethyl)silane (0.02 mL, 0.170 mmol), triethylamine (0.05 mL, 0.340 mmol), palladium(2+) triphenylphosphine dichloride (8.06 mg, 0.010 mmol) and copper (I) iodide (2.19 mg, 0.010 mmol) were added again: the reaction mixture was degassed, then submitted to microwave reactor (5\*30 min at 100 °C). The mixture was diluted with



## 2. EXPERIMENTAL PART

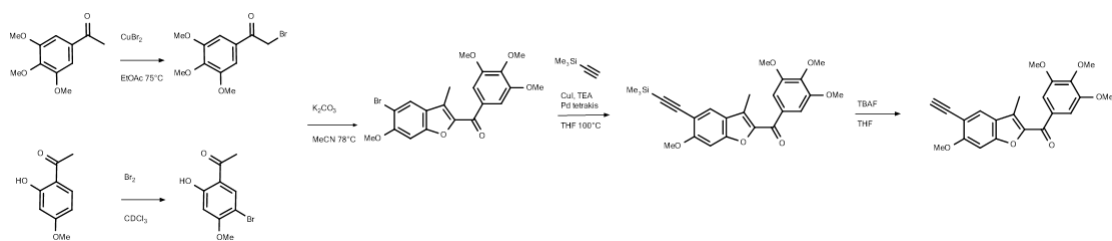


Figure 2.75: route for CP033

DCM, filtered and concentrated. Crude was purified by flash chromatography (silica 25g + 25g, Cy/EtOAc from 10:0 to 6:4) affording a mixture containing the target compound, [6-methoxy-3-methyl-5-(2-trimethylsilylethynyl)-1-benzofuran-2-yl]-(3,4,5-trimethoxyphenyl)methanone (N0758-53-1: 118 mg, 0.261 mmol, 56.74% yield) as yellowish foam.

LCMS acidic r.t. 1.52 min, MS (ESI)  $m/z = 453.27$  [M+H]<sup>+</sup>.

$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  ppm 0.25 - 0.26 (9 H, m) 2.52 - 2.54 (3 H, m) 3.79 - 3.81 (3H, m) 3.86 - 3.89 (6 H, m) 3.92 (3 H, s) 7.33 (2 H, s) 7.45 (1 H, s) 7.94 (1 H, s).

Tetrabutylammonium fluoride (0.22 mL, 0.220 mmol) was added to a stirred solution of [6-methoxy-3-methyl-5-(2-trimethylsilylethynyl)-1-benzofuran-2-yl]-(3,4,5-trimethoxyphenyl)methanone (118.0 mg, 0.220 mmol) in THF (2.278 mL). The reaction mixture was stirred at RT for 30 min, then brine and EtOAc were added: phases were separated, the organic one dried over sodium sulfate, filtered and concentrated. Crude was purified by flash chromatography (silica 10g + 10g, Cy/EtOAc from 10:0 to 6:4) affording (5-ethynyl-6-methoxy-3-methyl-benzofuran-2-yl)-(3,4,5-trimethoxyphenyl)methanone (N0758-54-1: 42.6 mg, 0.112 mmol, 51.75% yield) as yellowish solid.

LCMS acidic r.t. 1.26 min, MS (ESI)  $m/z = 381.26$  [M+H]<sup>+</sup>

$^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  ppm 2.51 - 2.54 (3 H, m) 3.79 (3 H, s) 3.84 - 3.87 (6 H, m) 3.92 (3 H, s) 4.26 (1 H, s) 7.29 - 7.33 (2 H, m) 7.43 - 7.47 (1 H, m) 7.93 - 7.97 (1 H, m).

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# CHAPTER 3

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## BIOLOGICAL RESULTS AND DISCUSSION

The first assay performed by biologists for each class reported below was the *in vitro* antiproliferative activities against six different cancer cell lines: on HeLa, MDA-MB-231, HL-60 and SEM cell lines CA-4 had nanomolar activity, while HT-29 and MCF-7 cells were more resistant to CA-4 inhibitory activity [40].

For the selected highly active compounds of appropriate classes, further tests were performed to first confirm the mechanism of action (through the *inhibition of tubulin polymerization*), then assessing how cell death is induced and, finally, evaluating the *in vivo* potency.

### 3.1 (3,4,5-trimethoxyphenyl)-1H-pyrazoles

Two different regioisomeric series of 3,4-diarylsubstituted 1H-pyrazole derivatives with general structures **3** and **4** (fig 3.1) were synthesised as previously described. In these two series of analogues, obtained by interchanging the substitution pattern of rings A and B, was fixed one of the aryl groups as the 3',4',5'-trimethoxyphenyl motif, identical with the A-ring of CA-4, and the modifications were focused on variation of the substituents at the *para*- position on the second phenyl ring, corresponding to the B-ring of CA-4, with electron-withdrawing (OCF<sub>3</sub>, CF<sub>3</sub>) or electron-releasing (Me, Et, n-Pr, MeO, EtO, MeS, EtS) groups (EWG and ERG, respectively). Since the methyl, methoxy and ethoxy groups proved to be favorable for bioactivity, an additional substituent (F, Cl, Me or MeO) was introduced at the *meta*-position of the *para*-methyl/methoxy/ethoxyphenyl ring.

In order to understand whether the substituents at the 3- and 4-positions on the 1H-pyrazole nucleus can be interchanged without affecting biological activity, starting from the 4'-tolyl (**3b**), 4'-methoxyphenyl (**3c**) and 4'-ethoxyphenyl (**3p**) derivatives, switching the aryl and 3',4',5'-trimethoxyphenyl moieties, the corresponding regioisomeric derivatives **4a**, **4b** and **4c**, respectively, were synthesized.

In fig 3.2 is reported the table of *in vitro* inhibitory effects of compounds **2c** (where R is naphthyl group), **3a-r**, **4a-c** and **CA-4**. As shown, the antiproliferative activities of most of the tested compounds were less pronounced against MCF-7 cells as compared with the other cell lines. Nevertheless, compounds **3a**, **3i**, **3p**, **3r**, **4b** and, especially, **4c** had excellent activity against MCF-7 cells (the IC<sub>50</sub> values ranged from 0.2 to 17 nM). Excluding compounds **3e**, **3h**, **3n** and **3o**, the tested compounds showed excellent antiproliferative activity against CA-4 resistant HT-29 cells, with IC<sub>50</sub> values ranging from double-digit to subnanomolar concentrations.

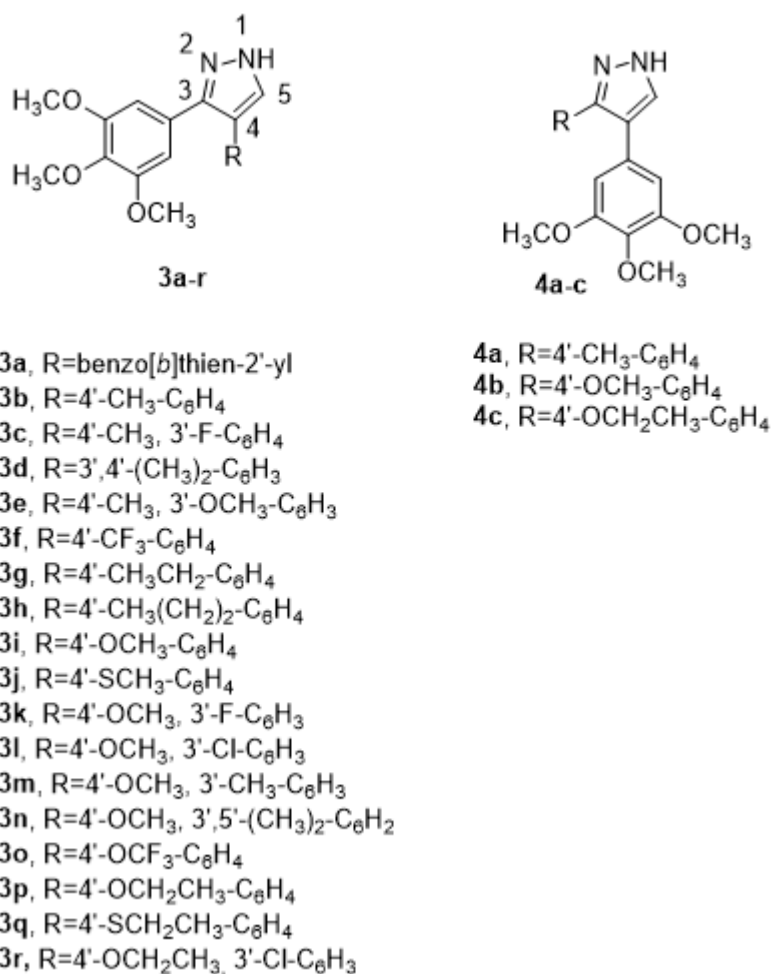


Figure 3.1: Structures of tested compounds

Two of the synthesized compounds, **3p**, bearing a 4'-ethoxyphenyl at the C-4 position of the 1H-pyrazole ring, and its isomeric analogue **4c**, exhibited the best antiproliferative activity among the tested derivatives, with IC<sub>50</sub> values of 0.05-4.5 and 0.06-0.7 nM, respectively, in the six cell lines, as compared with a range of 1-3100 and 3.3-28.4 nM obtained with the reference compounds CA-4 (**1a**) and **2c**. Compound **4c** was the only one more active than CA-4 against all cell lines, while the isomeric derivative **3p** was less active than CA-4 against HL-60 cells only.

In addition to highly active compounds **3p** and **4c**, derivatives **3a** and **4b** were more active than CA-4 in five of the six cell lines, again with the exception of the HL-60 cells.

To note was that the position of the 3',4',5'-trimethoxyphenyl moiety at either the 3- or 4-position of the 1H-pyrazole ring had little effect on antiproliferative activity: the potency of 3-(3',4',5'-trimethoxyphenyl)-1H-pyrazole derivatives **3b**, **3i** and **3p** was comparable and not dramatically reduced as compared with their regioisomers **4a**, **4b** and **4c**, respectively.

Replacement of the 2'-naphthyl by the bioisosteric 2'-benzo[*b*]thienyl ring (compounds **2c** and **3a**, respectively) resulted in a 4- to 15-fold increase in antiproliferative activity against five of the six cell lines. Except in HL-60 cells, **3a** had greater antiproliferative activity than CA-4, indicating that 2'-benzo[*b*]thienyl moiety, like the 2-naphthyl ring, was a good surrogate for the CA-4 B-ring.

Replacement of methyl with the more electron-withdrawing trifluoromethyl moiety (compounds **3b** and **3f**, respectively) resulted in a 2.4-72-fold reduction in antiproliferative activity, which was least pronounced (2-fold) with the HT-29 cells.

Encouraged by the activity obtained with compound **3b**, compounds **3c-e** were synthesized to determine whether various electron-withdrawing or electron-releasing substituents on the

### 3. BIOLOGICAL RESULTS AND DISCUSSION

Compound	IC <sub>50</sub> (nM)					
	HeLa	HT-29	MCF-7	MDA-MB-231	HL-60	SEM
<b>3a</b>	1.7±0.3	7.4±1.9	1.4±0.4	2.5±0.5	2.3±0.3	0.5±0.05
<b>3b</b>	11.1±1.7	28.2±9.1	127.0±34.2	10.6±1.6	11.9±1.1	2.6±0.2
<b>3c</b>	26.2±3.7	67.4±14.1	1695±450	481±26.3	31.0±4.5	23.5±2.0
<b>3d</b>	35.9±4.5	68.4±9.5	769±181	2606±124	51.3±6.2	28.5±3.8
<b>3e</b>	286.7±53.2	316.1±42.5	>10000	5283±365	160.2±22.3	305.3±24.8
<b>3f</b>	227.4±45.1	67.4±14.1	4375±672	523±45.6	179.0±18.4	187.2±26.1
<b>3g</b>	33.5±4.0	41.3±7.1	>10,000	3271±326	23.3±2.5	18.7±1.5
<b>3h</b>	361.3±35.1	540.9±55.2	2790±535	1553±101	553±49.1	406.1±56.2
<b>3i</b>	6.0±1.2	10.5±3.3	16.6±4.5	8.6±2.1	4.4±0.7	1.0±0.1
<b>3j</b>	28.7±3.7	51.3±8.7	>10,000	3650±254	40.9±7.6	30.1±3.2
<b>3k</b>	7.3±1.1	15.8±2.6	805.3±230	327±45	5.1±0.4	2.7±0.25
<b>3l</b>	1.4±0.2	0.2±0.05	1389±385	21.5±1.6	17.0±1.9	2.4±0.2
<b>3m</b>	32.9±4.0	37.3±5.2	>10000	2680±125	17.7±1.8	17.2±1.4
<b>3n</b>	>10,000	>10,000	>10,000	>10,000	>10,000	3340±275
<b>3o</b>	3376±647	2416±382	>10000	5740±435	1748±270	2470±181
<b>3p</b>	0.05±0.0	0.3±0.1	1.5±0.6	4.5±0.9	3.2±0.4	0.2±0.05
<b>3q</b>	12.5±1.5	5.4±1.3	492±110	31.4±5.6	29.7±3.8	13.9±1.8
<b>3r</b>	1.6±0.25	4.2±0.2	4.5±0.08	6.9±1.1	1.5±0.2	1.4±0.2
<b>4a</b>	25.2±3.8	44.4±6.3	700±165	262±15.8	31.4±4.2	14.7±1.2
<b>4b</b>	1.9±0.3	0.8±0.2	2.6±0.8	0.9±0.2	2.1±0.25	0.4±0.05
<b>4c</b>	0.07±0.0	0.06±0.0	0.21±0.07	0.7±0.1	0.25±0.03	0.06±0.0
<b>2c</b>	6.6±1.4	8.1±2.3	21.4±6.5	28.4±1.2	9.4±0.9	3.3±0.7
<b>CA-4 (1a)</b>	4.0±1.1	3100±100	370±100	4.5±0.2	1.0±0.2	5.1±0.1

<sup>a</sup>IC<sub>50</sub>= compound concentration required to inhibit tumor cell proliferation by 50%. Values are the mean ± SE from the dose-response curves of at least three independent experiments carried out in triplicate.

Figure 3.2: In vitro inhibitory effects of compounds **2c**, **3a-r**, **4a-c** and **CA-4**

phenyl ring would further enhance activity (without exception **3c-e** were less active than **3b** in all cell lines).

In an effort to further understand the steric effect of the alkyl substituent at the *para*-position, the ethyl and *n*-propyl derivatives (**3g** and **3h**, respectively) were prepared, but biological data showed that lengthening the 4'-alkyl chain was not tolerated and caused a significant reduction in antiproliferative activity in all cell lines.

Replacement of the methyl group both of **3b** and its regioisomeric derivative **4a** with a more electron-releasing methoxy moiety (to furnish **3i** and **4b**, respectively) resulted in enhanced antiproliferative activity for **3i**, which was much more pronounced (13-291-fold) for **4b** against all six cell lines relative to **3b** and **4a**, respectively.

A substantial reduction of activity was also observed when the methoxy group of **3i** was replaced with a weak electron-releasing thiomethyl group (**3j**), or with the strong electron-withdrawing and bulkier trifluoromethoxy moiety (**3o**). Relative to the activity of **3i**, the insertion of an additional electron-withdrawing (F or Cl) or electron-releasing methyl group at the 3'-position of the 4'-methoxyphenyl ring had varying effects on antiproliferative activity. The introduction of a methyl group at the *meta*-position of **3i** (**3m**) resulted in potency reduction.

The *para*-ethoxyphenyl derivative **3p** was 2- to 120-fold more potent than its methoxy counterpart **3i** in five of the six cancer cell lines. For the two isomeric derivatives **4b** and **4c**, the replacement of methoxy with ethoxy (**4c**) improved antiproliferative activity 7-27-fold relative

### 3. BIOLOGICAL RESULTS AND DISCUSSION

to 4b against five of the six cell lines, while the replacement of ethoxy with thioethyl (**3q**) caused a decrease in activity in all cell lines. The 4'-ethoxy group was favorable for potency, but the introduction of an additional *meta*-EWG chlorine group in compound **3p** (**3r**) produced a 1.5-32-fold reduction in antiproliferative activity against five of the six cell lines. Compound **3r**, as with the corresponding 3-Cl, 4-OMe congener **3l**, was more active than CA-4 in four of the six cell lines, the exceptions being HL-60 and MDA-MB-231 cells.

#### 3.1.1 Other *in vitro* and *in vivo* results

To predict a good therapeutic index is important to demonstrate that a compound is safe for not target cells: with this purpose, the cytotoxicity of the most active compounds (**3p** and **4c**) was evaluated *in vitro* against peripheral blood lymphocytes (both in quiescent lymphocytes and in an active phase of proliferation induced by phytohematoagglutinin -a mitogenic stimulus-) and in normal human astrocytes. **3p** and **4c**, both, were safe on these cell lines. Details of this study and other biological experiments carried on by collaborators are reported in detail on the published manuscript "Design, synthesis and biological evaluation of novel vicinal diaryl-substituted 1H-Pyrazole analogues of combretastatin A-4 as highly potent tubulin polymerization inhibitors" [40].

To note, **3p** and **4c** are not substrate of P-gp, drug efflux pumps that are responsible for drug resistance in tumoral cells: this result is a key step in the development of new chemotherapeutic agent.

Compound	Tubulin assembly <sup>a</sup>	Colchicine binding <sup>b</sup>	
	IC <sub>50</sub> ±S.D (µM)	% ±S.D	
		5 µM drug	0.5 µM drug
<b>3a</b>	0.30±0.01	93±2	69±2
<b>3b</b>	0.37±0.04	88±0.8	n.d
<b>3c</b>	0.48±0.06	91±2	49±2
<b>3d</b>	0.61±0.05	85±5	n.d.
<b>3g</b>	0.52±0.03	85±1	n.d.
<b>3i</b>	0.37±0.06	86±0.6	n.d.
<b>3j</b>	0.57±0.02	85±4	n.d.
<b>3k</b>	0.42±0.05	93±0.8	55±2
<b>3l</b>	0.52±0.09	85±3	n.d.
<b>3m</b>	0.44±0.09	81±0.1	n.d.
<b>3p</b>	0.36±0.01	95±0.9	69±5
<b>3q</b>	0.48±0.02	85±1	n.d.
<b>3r</b>	0.39±0.05	90±0.3	62±2
<b>4a</b>	0.57±0.01	86±5	n.d.
<b>4b</b>	0.34±0.05	93±0.8	79±2
<b>4c</b>	0.35±0.01	96±0.7	90±1
<b>2c</b>	0.41±0.04	91±4	68±0.2
CA-4 ( <b>1a</b> )	0.54±0.06	97±0.8	82±2

<sup>a</sup> Inhibition of tubulin polymerisation. Tubulin was at 10 µM.

<sup>b</sup> Inhibition of [<sup>3</sup>H] colchicine binding. Tubulin and colchicine were at 0.5 and 5 µM concentrations, respectively.

n.d.=not determined

Figure 3.3: Inhibition of tubulin polymerization and colchicine binding by potent compounds

In fig 3.2 are reported data concerning the cytotoxicity of our compounds: to demonstrate that the induction of cell death occur through their interaction with tubulin, for compounds

with good IC<sub>50</sub> were evaluated their inhibition of tubulin polymerization and their binding to colchicine. Results are reported in fig 3.3: compound **3a** was found to be the most active and it was almost twice as potent as CA-4, with several compounds (**3b**, **3i**, **3p**, **3r**, **4b** and **4c**) which were 1.5-fold more potent than CA-4, while all remaining compounds showed activity comparable to that of CA-4.

Comparing inhibition of tubulin polymerization with the growth inhibitory effects, there was a correlation for the majority of the active compounds: while **3b** was generally less potent than **3a** as an antiproliferative agent, the two compounds were similar as inhibitors of tubulin assembly. In the colchicine binding studies, all compounds when tested at the higher concentration (5 μM) had quantitatively similar effects and showed potency comparable to that of CA-4, which in these experiments inhibited colchicine binding by 97%. For the most active compounds (90% or higher inhibition when present in the reaction mixture at the same concentration as colchicine), even when tested at a ten-fold reduced concentration (0.5 μM), compounds **4b** and **4c** were as potent as CA-4: the potent inhibition observed with these compounds indicates that they bind to tubulin at a site overlapping the colchicine site (data confirmed also by molecular modeling studies).

Further experiments were carried on only with **3p** and **4c**, the two most active compounds of this series:

- Their effects on cell cycle progression was examined by flow cytometry: both induced a G2/M arrest (with **4c** more potent of **3p**), along with alteration of cell cycle checkpoint proteins (like a substantial increase in the expression of p21, a reduced expression of cdc25c and a drop in the phosphorylation of cdc2).
- Their mechanism of cell death involves apoptosis induction, that follows the mitochondrial pathway (so ROS production was induced by the treatment). A reduction in the expression of the anti-apoptotic proteins Mcl-1 and Xiap was also observed.
- An induced reduction in the migration of MDA-MB-231 cells (that are highly metastatic) was observed after the treatment with the two compounds.

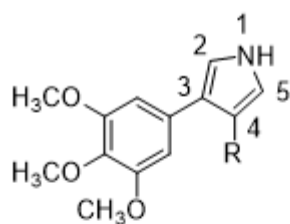
From the previous experiments, **4c** gave better results: for this reason in was chosen to be administered *in vivo*. The antitumor effect was evaluated at 5 and 15 mg/kg in an allograft tumor model developed in mice: the compound is able to significantly reduce tumor burden in a dose-dependent manner, even at the lower dose tested. Even at the higher dose (15 mg/kg), **4c** did not show any sign of toxicity and did not cause a decrease in animal body weight.

### 3.2 4-(3,4,5-trimethoxyphenyl)-1H-pyrroles

This serie, containing eleven vicinal diaryl pyrroles (fig 3.4) as *cis*-restricted analogues of combretastatin A-4 (CA-4), was evaluated for its *in vitro* antiproliferative activity against six different cancer cell lines and, for selected highly active compounds, inhibitory effects on tubulin polymerization, cell cycle effects and *in vivo* potency.

The 3',4',5'-trimethoxyphenyl moiety remained as ring A of CA-4, and a structure-activity relationship (SAR) was performed by preparing derivatives with general structure **11**, characterized by the presence of a common 3',4',5'-trimethoxyphenyl ring at the 3-position of the pyrrole ring: were synthesized and tested naphth-2-yl (**11a**), its biososteric 2'-benzo[b]thienyl (**11b**), and electron-releasing and electron withdrawing substitutions (**11c-o**) on the phenyl at the 4-position of the pyrrole ring. The effect of a *meta*-electron withdrawing group (chlorine or fluorine atom) was also examined in combination with the *para*-methoxy/ethoxy group of compounds **8** and **11i**, respectively.

In fig 3.5 are reported data concerning the antiproliferative activities against six different human cancer cell lines (CCRF-CEM, SEM, HL-60, A549, Hela and HT-29). CA-4 had single-digit nanomolar activity (IC<sub>50</sub>:1-5 nM) against four of the six lines, while A549 and HT-29 cells were more resistant to CA-4, with IC<sub>50</sub> values of 180 and 3100 nM, respectively.



11 a-o

- 11a**, R=naphth-2-yl  
**11b**, R=benzo[b]thien-2'-yl  
**11c**, R=4'-CH<sub>3</sub>-C<sub>6</sub>H<sub>4</sub>  
**11d**, R=4'-CF<sub>3</sub>-C<sub>6</sub>H<sub>4</sub>  
**11e**, R=4'-C<sub>2</sub>H<sub>5</sub>-C<sub>6</sub>H<sub>4</sub>  
**11f**, R=4'-OCF<sub>3</sub>-C<sub>6</sub>H<sub>4</sub>  
**11g**, R=4'-OCH<sub>3</sub>, 3'-F-C<sub>6</sub>H<sub>4</sub>  
**11h**, R=4'-OCH<sub>3</sub>, 3'-Cl-C<sub>6</sub>H<sub>4</sub>  
**11i**, R=4'-OCH<sub>2</sub>CH<sub>3</sub>-C<sub>6</sub>H<sub>4</sub>  
**11j**, R=4'-OCH<sub>2</sub>CH<sub>3</sub>, 3'-F-C<sub>6</sub>H<sub>3</sub>  
**11k**, R=4'-OCH<sub>2</sub>CH<sub>3</sub>, 3'-Cl-C<sub>6</sub>H<sub>3</sub>  
**11l**, R=4'-SCH<sub>2</sub>CH<sub>3</sub>-C<sub>6</sub>H<sub>4</sub>  
**11m**, R=4'-OCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>-C<sub>6</sub>H<sub>4</sub>  
**11n**, R=4'-OCH<sub>2</sub>OCH<sub>3</sub>-C<sub>6</sub>H<sub>4</sub>

Figure 3.4: Structures of tested compounds

Seven of the compound 11 derivatives (**11a-c**, **11g** and, especially, **11i-k**), had IC<sub>50</sub> values smaller than 100 nM against three-five of the six cell lines, as compared with four cell lines with the reference compounds CA-4 (**1a**).

Most of the 3-(3',4',5'-trimethoxyphenyl)-4-substituted-1H-pyrrole analogues **11a-n** had submicromolar activity against the A549 cells, with derivatives **11c** and 11i-k being the most potent, with IC<sub>50</sub> values from 28 to 98 nM. These compounds were thus more active than CA-4 in A549 cells.

The replacement of the 2'-naphthyl by the bioisosteric 2'-benzo[b]thienyl ring in the compound 11 series (compounds **11a** and **11b**, respectively) had contrasting effects on the 6 cell lines.

The *para*-tolyl derivative **11c** had IC<sub>50</sub> values ranging from 17 to 54 nM against four of the six cell lines, but A549 and HT-29 cells were somewhat less sensitive, with IC<sub>50</sub> values of 0.098 and 0.13 μM, respectively. Replacement of the *para*-methyl of 11c with the more electron-withdrawing trifluoromethyl moiety (compound **11d**) resulted in a 3-28-fold reduction in antiproliferative activity, most pronounced with the CCRF-CEM cells. Lengthening the alkyl chain from methyl (**11c**) to ethyl (**11e**) at the 4'-position of the phenyl ring resulted in a loss of activity in all cell lines.

Replacement of the methyl group of **11c** with a more electron-releasing methoxy moiety was beneficial for activity against HeLa and HT-29 cells, and the insertion of an additional electron-withdrawing (F or Cl) at the *m*-position of the *p*-methoxyphenyl ring had varying effects on antiproliferative activity. Introduction of a fluorine atom furnished the *p*-OMe, *m*-F derivative **11g**, while the addition of a *meta*-chlorine atom (**11h**) produced a 2-10-fold reduction in activity in five cell lines (most pronounced in HeLa and HT29 cells).

The *para*-ethoxyphenyl derivative **11i** was about 2-12-fold more potent than its methoxy counterpart: this means that that the *p*-ethoxyphenyl ring is a good surrogate for the B-ring of CA-4. The replacement of the ethoxy group with a weak electron releasing thioethyl moiety,

### 3. BIOLOGICAL RESULTS AND DISCUSSION

Compound	IC <sub>50</sub> (μM)					
	CCRF-CEM	HL-60	SEM	HeLa	A549	HT-29
<b>11a</b>	0.0879±0.031	0.054±0.009	0.032±0.002	0.137±0.019	0.228±0.036	0.255±0.036
<b>11b</b>	0.155±0.004	0.0353±0.004	0.0373±0.002	0.043±0.007	0.228±0.041	0.383±0.045
<b>11c</b>	0.047±0.001	0.0317±0.003	0.0172±0.003	0.054±0.012	0.098±0.008	0.13±0.026
<b>11d</b>	1.34±0.14	0.933±0.039	0.343±0.017	0.445±0.054	0.408±0.026	0.865±0.095
<b>11e</b>	0.226±0.031	0.164±0.015	0.048±0.002	0.297±0.046	0.257±0.036	0.289±0.069
<b>11f</b>	6.26±0.43	5.95±0.33	4.793±0.139	5.23±0.95	4.231±0.789	5.46±0.87
<b>11g</b>	0.053±0.014	0.0336±0.004	0.0053±0.005	0.033±0.009	0.203±0.36	0.048±0.008
<b>11h</b>	0.28±0.021	0.177±0.012	0.0693±0.017	0.297±0.061	0.243±0.031	0.279±0.012
<b>11i</b>	0.0166±0.002	0.149±0.022	0.0028±0.004	0.037±0.008	0.081±0.012	0.042±0.009
<b>11j</b>	0.0075±0.001	0.168±0.017	0.0017±0.005	0.026±0.005	0.028±0.009	0.028±0.006
<b>11k</b>	0.153±0.032	0.144±0.021	0.039±0.003	0.037±0.007	0.079±0.008	0.064±0.005
<b>11l</b>	3.07±0.29	2.66±0.11	0.48±0.026	0.386±0.051	0.336±0.061	0.630±0.087
<b>11m</b>	4.24±0.43	3.62±0.52	0.913±0.086	0.37±0.042	0.683±0.055	0.703±0.092
<b>11n</b>	>10	>10	>10	>10	8.0±1.1	>10
<b>CA-4 (1a)</b>	0.002±0.001	0.001±0.0002	0.005±0.0001	0.004±0.001	0.180±0.050	3.1±0.1

<sup>†</sup>IC<sub>50</sub>= compound concentration required to inhibit tumor cell proliferation by 50%. Values are the mean ± SE from the dose-response curves of at least three independent experiments carried out in triplicate.

Figure 3.5: In vitro inhibitory effects of compounds **11a-n** and **CA-4**

resulting in compound **11l**, produced a strong drop in potency against all cell lines. The reduction of potency was even more pronounced when the 4'-ethoxy group of **11i** was replaced with the n-propoxy homologue **11m** and its methoxymethoxy bioisosteric analogue **11n**.

While the 4'-ethoxy group was favorable for potency, the introduction of an additional electron-withdrawing *meta*-fluorine group in compound **11i** (resulting in compound **11j**) produced a 1.5-3-fold increase in antiproliferative activity against five of the six cell lines. In contrast, the compound with an alternative EWG, a *m*-Cl group (**11k**), had less antiproliferative activity in three cell lines as compared with both **11i** and **11j**. However **11k** and **11i** were equipotent against HL-60, HeLa and A549 cells, and **11k** was slightly more active than **11j** in HL60 and slightly less active in HeLa and A549 cells. In this series, the methoxy and ethoxy groups at the *para*-position of the phenyl ring (compounds **8** and **11i**, respectively) generally enhanced biological activity, and the introduction of an additional electron-withdrawing fluorine atom at the *meta*-position of the *para*-methoxy/ethoxy phenyl ring (compounds **11g** and **11j**) usually increased antiproliferative activity.

#### 3.2.1 Other *in vitro* and *in vivo* results

As above, the most active compound (**11j**) was evaluated *in vitro* against peripheral blood lymphocytes (both in quiescent lymphocytes and in an active phase of proliferation induced by phytohematoagglutinin -a mitogenic stimulus-): with IC<sub>50</sub> greater than 10 μM, the compound was safe on these cell lines. Details of this study and other biological experiments carried on by collaborators are reported in detail on the published manuscript "A facile synthesis of diaryl pyrroles led to the discovery of potent colchicine site antimetabolic agents" [39].

In fig 3.5 are reported data concerning the cytotoxicity of our compounds: to demonstrate that the induction of cell death occur through their interaction with tubulin, for compounds with good IC<sub>50</sub> were evaluated their inhibition of tubulin polymerization and their binding to colchicine. Results are reported in fig 3.6: compounds **11i-k** strongly inhibited tubulin



### 3. BIOLOGICAL RESULTS AND DISCUSSION

Compound	Tubulin assembly <sup>a</sup>	Colchicine binding <sup>b</sup>	
	IC <sub>50</sub> ±S.D (μM)	% ±S.D	
		5 μM drug	0.5 μM drug
<b>11c</b>	1.2±0.07	83±1	n.d.
<b>11i</b>	0.82±0.1	92±1	80±2
<b>11j</b>	0.66±0.004	93±4	83±0.9
<b>11k</b>	0.71±0.07	90±2	68±2
CA-4 ( <b>1a</b> )	1.2±0.04	98±1	89±2

<sup>a</sup>Inhibition of tubulin polymerization. Tubulin was at 10 μM.  
<sup>b</sup>Inhibition of [<sup>3</sup>H]colchicine binding. Tubulin and colchicine were at 0.5 and 5 μM concentrations, respectively.  
n.d.=not determined

Figure 3.6: Inhibition of tubulin polymerization and colchicine binding by potent compounds

assembly, with compounds **11j** and **11k** found to be the most active, and they were almost twice as potent as CA-4, while derivative **11i** was 1.5-fold more active than CA-4. In the colchicine binding studies, compounds **11c** and **11i-k**, when tested at 5 μM, had quantitatively similar effects, varying within a narrow range (83-93% inhibition), and derivatives **11i-k** showed potency comparable to that CA-4, which in these experiments inhibited colchicine binding by 98%. For the most active compounds **11i-k** (90% or greater inhibition when present in the reaction mixture at the same concentration as colchicine), even when tested at a ten-fold reduced concentration (0.5 μM), compounds **11i** and **11j** were almost as potent as CA-4, which in these latter experiments inhibited colchicine binding by 89%, while derivative **11k** was less potent (68% inhibition).

Two compounds (**11i** and **11j**) had activities superior to that of CA-4 as inhibitors of tubulin assembly and, less frequently observed, potency similar to that of CA-4 as inhibitors of colchicine even though **11i** and **11j** were less active as antiproliferative agents than CA-4 in three cell lines (CCRF-CEM, HL-60 and HeLa cells).

Further experiments were carried on only with **11j**, the most active compound of this series:

- As other tubulin inhibitors, it caused mitotic arrest in cells. In particular, it induced cell cycle arrest in the G2/M phase in HeLa cells, in a concentration-dependent manner, along with alteration of cell cycle checkpoint proteins (like a substantial increase in the expression of B1, a reduced expression of cdc25c and a drop in the phosphorylation of cdc2): the cdc2/cyclin B1 complexes cannot be activated, blocking cells from exiting mitosis and leading to apoptotic cell death.
- Its mechanism of cell death involves apoptosis induction in HeLa cells, that follows the mitochondrial pathway. A reduction in the expression of the anti-apoptotic proteins Bcl-2, Mcl-1 and Xiap was also observed.
- Its antitumor effect *in vivo* was evaluated in an allograft tumor model developed in mice at 5 and 15 mg/kg: the compound is able to significantly reduce tumor volume in a dose-dependent manner, even at the lower dose tested. At the higher dose (15 mg/kg), it did not show any sign of toxicity and did not cause a decrease in animal body weight, suggesting low toxicity for this compound.

### 3.3 (3,4,5-trimethoxyphenyl)pyrrol-1-yl esters

On the basis of the previous series, a small series of derivatives of the two most potent compounds was designed and synthesized: the aim was to get a dual inhibitor that was able to

act as tubulin inhibitor (and thus to induce cell death) and also as HDAC inhibitor.

In this series, the free nitrogen of pyrazol was alkylated with short chains ending with an ethyl ester, with the purpose of understand if the ability of these compounds to induce cell death was maintained. If the hypothesis was validated, the ester function would be substituted by the hydroxamic acidic one (that is more challenging to synthesise). Unfortunately, the derivatives were not active against against different tumoral cell lines, and thus the series was abandoned.

#### 3.4 1H-1,2,4-triazole-3,5-diamines

This series, containing seventeen compounds (synthesized as intermediate that had to be acylated from other members of the research group) as cis-restricted analogues of combretastatin A-4 (CA-4), was evaluated for its in vitro antiproliferative activity against six different cancer cell lines.

The series was thought as small modification (the insertion of a benzylic methylene) of the previous active series reported in published manuscripts "Synthesis, antimitotic and antivasular activity of 1-(3,4,5-trimethoxybenzoyl)-3-arylamino-5-amino-1,2,4-triazoles" [41] and "3-Aryl/Heteroaryl-5-amino-1-(3,4,5-trimethoxybenzoyl)-1,2,4-triazoles as antimicrotubule agents. Design, synthesis, antiproliferative activity and inhibition of tubulin polymerization" [38].

Unfortunately, data were not good as the ones of the two parental series: because of the insertion of a benzylic methylene, compounds probably assumed an incorrect three-dimensional conformation that puts ring A and ring B in a way different from the cis-orientation present and fundamental for the action of CA-4. The series was abandoned and the following one was thought as a new rigidification of this one: in that way, rings A and B should have the correct orientation.

#### 3.5 7-N-(3,4,5-trimethoxyphenyl)-[1,2,4]triazolo [1,5-a]pyrimidine- 2,7-diamines

From the previous series were developed different triazolopyrimidine congeners that, as explained in our manuscript that will be soon published, may interact with tubulin/microtubules in different manners and with different activities depending on the particular substitution pattern at their 2-, 5-, 6- and 7-positions, thereby producing different effects on microtubules structure and function.

In fig 3.7 is reported the general structure of some of the compounds involved in this study.

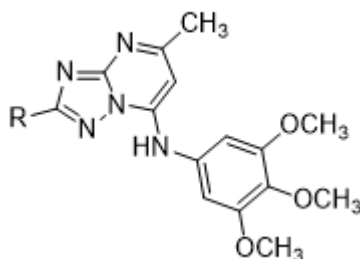


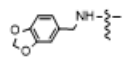


Figure 3.7: Chemical structures based on the [1,2,4]triazolo[1,5-a]pyrimidine scaffold

In this series, characterized by a common 7-(3',4',5'-trimethoxyanilino)-[1,2,4]triazolo [1,5-a]pyrimidine scaffold, the structure-activity relationship (SAR) was explored by examining various substitutions with electron-withdrawing (F, Cl, CN and CF<sub>3</sub>) or

### 3. BIOLOGICAL RESULTS AND DISCUSSION

electron-releasing (Me and MeO) groups (ERG's and EWG's, respectively) on the phenyl ring of aryl, aniline, benzylamino, pyridylmethylamino, 2-phenethylamino and 3-phenylpropylamino moieties at the 2-position of triazolopyrimidine scaffold. In fig 3.8 are reported the in vitro inhibitory effects of compounds **7a-ad** and CA-4 (**1a**); I've synthesized only 5 of these compounds: **7j**, **7k**, **7l**, **7m** and **7o**. Overall, our compounds are less potent than CA-4.

Compound	R	IC <sub>50</sub> (nM) <sup>a</sup>				
		A549	MDA-MB-231	HeLa	HT-29	Jurkat
<b>7a</b>	4'-F-C <sub>6</sub> H <sub>4</sub>	2707±249	2251±195	3760±187	>10,000	3575±4474
<b>7b</b>	4'-Cl-C <sub>6</sub> H <sub>4</sub>	>10,000	>10,000	5790±423	>10000	>10,000
<b>7c</b>	4'-CH <sub>3</sub> -C <sub>6</sub> H <sub>4</sub>	>10,000	2604±267	2079±189	>10,000	4231±1152
<b>7d</b>	C <sub>6</sub> H <sub>5</sub> NH	2300±120	1434±145	516±63	818±74	587±12
<b>7e</b>	4'-F-C <sub>6</sub> H <sub>4</sub> NH	1020±79	715±67	387±24	688±59	825±1
<b>7f</b>	4'-Cl-C <sub>6</sub> H <sub>4</sub> NH	289±32	471±45	92±11	325±39	>10,000
<b>7g</b>	4'-CH <sub>3</sub> -C <sub>6</sub> H <sub>4</sub> NH	1550±186	1513±137	501±41	944±111	846±25
<b>7h</b>	4'-OCH <sub>3</sub> -C <sub>6</sub> H <sub>4</sub> NH	3760±141	1168±135	520±26	917±87	1008±1
<b>7i</b>	C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> NH	3746±259	2713±310	3201±238	3535±138	4401±379
<b>7j</b> ★		>10000	59±13	3007±301	2534±248	9637±798
<b>7k</b> ★		5278±651	556±67	2978±321	3570±479	6850±629
<b>7l</b> ★	4'-F-C <sub>6</sub> H <sub>4</sub> CH <sub>2</sub> NH	5894±481	61±9	1863±127	1139±89	4681±479
<b>7m</b> ★	3'-F-C <sub>6</sub> H <sub>4</sub> CH <sub>2</sub> NH	2185±311	77±9	929±21	1526±156	5547±478
<b>7n</b>	4'-Cl-C <sub>6</sub> H <sub>4</sub> CH <sub>2</sub> NH	106±219	190±21	31±7	54±8	323±19
<b>7o</b> ★	3'-Cl-C <sub>6</sub> H <sub>4</sub> CH <sub>2</sub> NH	933±111	60±7	742±83	792±91	4722±361
<b>7p</b>	4'-CH <sub>3</sub> -C <sub>6</sub> H <sub>4</sub> CH <sub>2</sub> NH	422±69	301±45	60±12	110±21	427±1
<b>7q</b>	3'-CH <sub>3</sub> -C <sub>6</sub> H <sub>4</sub> CH <sub>2</sub> NH	3883±285	1681±178	1613±127	3462±368	7020±619
<b>7r</b>	2'-CH <sub>3</sub> -C <sub>6</sub> H <sub>4</sub> CH <sub>2</sub> NH	1925	2996	>10000	>10000	4437±469
<b>7s</b>	4'-OCH <sub>3</sub> -C <sub>6</sub> H <sub>4</sub> CH <sub>2</sub> NH	807±78	1268±117	217±34	489±56	900±86
<b>7t</b>	3'-OCH <sub>3</sub> -C <sub>6</sub> H <sub>4</sub> CH <sub>2</sub> NH	1654±137	1782±148	882±89	1039±79	1646±115
<b>7u</b>	2'-OCH <sub>3</sub> -C <sub>6</sub> H <sub>4</sub> CH <sub>2</sub> NH	>10000	4399	>10000	>10000	>10000
<b>7v</b>	3',4'-(OCH <sub>3</sub> ) <sub>2</sub> -C <sub>6</sub> H <sub>3</sub> CH <sub>2</sub> NH	1005±92	1217±152	557±67	>10000	1218±89
<b>7w</b>	4'-CF <sub>3</sub> -C <sub>6</sub> H <sub>4</sub> CH <sub>2</sub> NH	1278±135	556±34	516±21	589±256	961±62
<b>7x</b>	4'-CN-C <sub>6</sub> H <sub>4</sub> CH <sub>2</sub> NH	>10000	1551	9013	>10000	>10000
<b>7y</b>		656±31	303±19	114±15	168±19	778±86
<b>7z</b>	C <sub>6</sub> H <sub>5</sub> (CH <sub>2</sub> ) <sub>2</sub> NH	2008±161	941±89	859±99	1891±162	1986±238
<b>7aa</b>	4'-F-C <sub>6</sub> H <sub>4</sub> (CH <sub>2</sub> ) <sub>2</sub> NH	>10000	>10000	2497±267	2831±218	9615±891
<b>7ab</b>	4'-Cl-C <sub>6</sub> H <sub>4</sub> (CH <sub>2</sub> ) <sub>2</sub> NH	>10000	>10000	5807	>10000	5943±499
<b>7ac</b>	4'-OCH <sub>3</sub> -C <sub>6</sub> H <sub>4</sub> (CH <sub>2</sub> ) <sub>2</sub> NH	8881±876	1650±186	5047±629	6880±489	9592±1175
<b>7ad</b>	C <sub>6</sub> H <sub>5</sub> (CH <sub>2</sub> ) <sub>3</sub> NH	190±21	91±8	67±7	142±11	2724±129
<b>CA-4</b>	-	180±50	4.5±0.2	4±1	3100±29	1.0±0.2

<sup>a</sup>IC<sub>50</sub>= compound concentration required to inhibit tumor cell proliferation by 50%. Values are the mean ± SE from the dose-response curves of at least three independent experiments carried out in triplicate.

Figure 3.8: In vitro inhibitory effects of compounds **7a-ad** and CA-4 (**1a**)

Derivatives **7j**, **7l-m** and **7o** were characterized by a potent antiproliferative activity at double-digit nanomolar levels on MDA-MB-231 cell line, with IC<sub>50</sub> values of 59, 61, 77 and 60 nM, respectively, resulting less active (IC<sub>50</sub> higher than 100 nM) against the other cell lines.

Bioisosteric replacement of phenyl with 4'-pyridinyl in the benzylamino derivative **7i**, to furnish derivative **7j**, was detrimental for the activity against A549 and Jurkat cells. While **7i** and **7j** were equipotent against HeLa and HT-29, a 46-fold increase of activity was observed for **7j** relative to **7i** against MDA-MB-231 cancer cells, with a IC<sub>50</sub> value of 59 nM. On this

### 3. BIOLOGICAL RESULTS AND DISCUSSION

latter cancer cell lines, the isomeric 3'-pyridinyl compound **7k** was 10-fold less potent than the 4'-pyridinyl counterpart **7j**, while the two compounds were equipotent against the other four cancer cells.

For the p-fluoroaniline derivative **7e**, the corresponding p-fluorobenzylamino homologue **7l** resulted 2-6-fold less active on four of the five cancer cells, while in MDA-MB-231 cells, **7l** was 12-fold more potent than **7e**, with  $IC_{50}$  of 61 nM. Starting from **7l**, moving the fluorine atom from the *para*- to the *meta*-position (compound **7m**) maintained the activity against MDA-MB-231 cells, with a two-fold increased activity against A549 and HeLa cells, while **7l** and **7m** were equipotent against HT-29 and Jurkat cells.

The chlorine atom in meta position (compound **7o**) caused a 9-24-fold reduction in activity against four of the five cancer cell lines, with a 3-fold increase in potency against MDA-MB-231 ( $IC_{50}$ : 60 nM). On this latter cancer cell line, the cell growth inhibitory activity of m-chlorobenzylamino derivative **7o** was comparable to those observed for the two isomeric *para*- and *meta*-fluorobenzylamino derivatives **7l** and **7m**, respectively.

The SAR described above is relative to compounds I've synthesized; anyway, the most potent (but not enough to proceed with other tests) compounds of this series were **7n**, **7p**, **7y** and **7ad**.

### 3.6 Hydroxamic acids

The first compound of this series is the one reported below. Since the synthetic route was very challenging and laboured, only this compounds was tested as progenitor of this series.

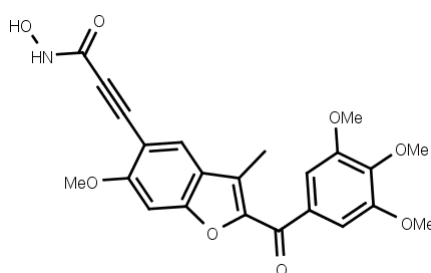


Figure 3.9: CP016

Data concerning in vitro inhibitory effects of compound CP016 were very good, and for this reason it was chosen to synthesize this series, removing one or more methoxy group from the trimethoxyphenyl ring and/or from the core ring. Also data concerning tubulin inhibition was very good:  $0.53 \pm 0.06M$  for CP016 and  $0.75 \pm 0.06M$  for CA - 4.

Compd	$IC_{50}$ (nM)							SEM
	A549	MDA-MB-231	HeLa	HT-29	HL-60	Jurkat	RS4;11	
NO-423-74	46.5	0.9	0.3	5.6	5.7	9.3	4.4	3.7

\*After 72 h of incubation

Figure 3.10: In vitro inhibitory effects of compound **CP016**

The antiproliferative activity was then evaluated also for the other eight derivatives, but data were not very good, included the one concerning **CP016**. For this reason, tests had to be repeated, but data are not still available.

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# CHAPTER 4

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## CONCLUSION

This PhD project is part of an older and bigger project whose purpose is to design and synthesise compounds selectively active against tumoral cell lines, so the aim is to get new antitumoral drugs.

Amongst diverse cancer therapeutic targets, drugs targeting microtubules represent one of the most effective classes of cancer chemotherapeutic compounds available to date, since they act as disruptors of mitotic spindle assembly (for this reason they are called anti-mitotic agents). As results, the tumoral cell is not able to complete the mitotic process and its fate is death.

They disrupt microtubule dynamics in distinct ways and they are classified into two main groups: microtubule destabilizing agents (MDAs) and microtubule stabilizing agents (MSAs). At relatively high concentrations, MTAs either inhibit microtubule polymerization, destabilizing microtubules and decreasing microtubule polymer mass, or promote microtubule polymerization, stabilizing microtubules and increasing the polymer mass.

Combretastatin A-4 (CA-4) is a potent and natural compounds that, *in vitro*, binds tubulin at the colchicine binding site, that is one of the most important pockets that have been focused on to design tubulin-binding agents. Although CA-4 is a promising clinical candidate, with very low side effects associated, it bears some very important issues: it has a very low solubility, a short biological half-life and undergoes to cis-trans isomerization in heat, light and protic media, forming the totally inactive trans form from the active cis form. To improve the solubility of CA-4, its phosphate salt (CA-4P) has been synthesized and this compound is still in clinical development for the treatment of ovarian and other cancer.

Anyway, a very huge amount of compounds that come from CA-4 rigidification have been synthesised and tested from different research groups, and some of this kind of small molecules have been designed and synthesised in this project.

- The first two regioisomeric series of 3,4-diaryl pyrazole derivatives were characterized by the presence of a common 3',4',5'-trimethoxyphenyl ring at either the C-3 or C-4 position of the 1H-pyrazole ring. The rationale in this design was that the 3,4-diarylsubstituted 1H-pyrazole ring could serve as a suitable mimic to retain the bioactive configuration afforded by the cis-double bond present in CA-4. For both these series of compounds, the 3',4',5'-trimethoxyphenyl and 1H-pyrazole rings mimic the ring A and cis-double bond of CA-4, respectively, while a 2'-benzo[b]thienyl or phenyl ring substituted with electron-releasing or electron-withdrawing groups was utilized as a B-ring surrogate to mimic the 3'-hydroxy-4'-methoxyphenyl group in CA-4. The flexible synthetic method developed permitted the generation of a large family of 4-substituted-3-(3',4',5'-trimethoxyphenyl)-1H-pyrazole derivatives starting from a common 1-tosyl-3-(3',4',5'-trimethoxyphenyl)-4-bromo-1H-pyrazole intermediate. In the second series of compounds, starting from derivatives **3b**, **3i** and **3p**, the two

## 4. CONCLUSION

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aromatic rings at the 3- and 4-positions of the 1H-pyrazole ring were switched and the corresponding isomers **4a**, **4b** and **4c**, respectively were synthesized.

The substitution pattern on the phenyl either at the 3- or 4-position of the 1H-pyrazole ring plays an important role for antitubulin and antiproliferative activities. The activity of most of the synthesized compounds was not affected by the relative resistance of HT-29 and MCF-7 cells to CA-4. Generally, either two (**3c-e**, **3k-m** and **3q**) or three substituents (**3n**) on the phenyl group led to a reduction in antiproliferative activity, suggesting that steric factors account for the loss of activity observed with these compounds. The results demonstrated that the 4'-ethoxy substituent on the second phenyl ring either at the 3- or the 4-position of the 1H-pyrazole nucleus (compounds **3p** and **4c**) could replace the B-ring of CA-4. Derivatives **3p** and **4c** had the best antiproliferative activities against the cell lines that were examined and, overall, were more active than CA-4, with the exception of derivative **3p**, which was 3-fold less active than CA-4 but only against HL-60 cells. In particular, the 3-(4'-ethoxyphenyl)-4-(3',4',5'-trimethoxyphenyl)-1H-derivative **4c** exhibited IC<sub>50</sub> values ranging from 0.05 to 0.7 nM against the cell lines examined, as compared with the range of 1-3100 nM obtained with CA-4. Compound **4c** was one the most potent inhibitors of tubulin polymerization and an exceptionally potent inhibitor of colchicine binding (IC<sub>50</sub>=0.35 μM for assembly, 96 and 90% inhibition of the binding of [3H]colchicine at the concentrations of 5 and 0.5 μM, respectively).

These studies identified tubulin as the molecular target of these compounds, since those with the greatest inhibitory effects on cell growth strongly inhibited tubulin assembly and the binding of colchicine to tubulin. In the series of tested compounds (**3a-d**, **3g**, **3i-m**, **3p-r** and **4a-c**), inhibition of [3H]colchicine binding correlated more closely with inhibition of tubulin assembly than with antiproliferative activity. Comparing pairs of regioisomeric derivatives (**3b** vs. **4a**, **3i** vs. **4b** and **3p** vs. **4c**), it is evident that the presence of the 3',4',5'-trimethoxyphenyl moiety at the 3- or 4-position of the pyrazole ring seemed not critical for antiproliferative as well as for tubulin polymerization inhibitory activity, with the two isomeric compounds **3p** and **4c** exhibiting potent tubulin polymerization inhibitory activity as well as antiproliferative activity superior to that of CA-4.

- In the series of 3-(3',4',5'-trimethoxyphenyl)-4-aryl-1H-pyrrole analogues **11a-n**, SAR analysis indicated that compounds **11g** (3'-fluoro-4'-methoxyphenyl), **11i** (4'-ethoxyphenyl), **11j** (3'-fluoro-4'-ethoxyphenyl) and **11k** (3'-chloro-4'-ethoxyphenyl) exhibited maximal antiproliferative activity, which correlated with inhibition of tubulin polymerization inhibition and of colchicine binding to tubulin. By comparing compounds with small (C1 or C2) alkyl or alkoxy groups, the CF<sub>3</sub> and OCF<sub>3</sub> substitution on the phenyl B ring reduced antiproliferative activity against all cancer cell lines. Substituents at the *para*-position of the phenyl B ring showed antiproliferative activity in the order of OEt>Me>OMe>C<sub>2</sub>H<sub>5</sub>>CF<sub>3</sub>>SCH<sub>2</sub>CH<sub>3</sub>>n-OC<sub>3</sub>H<sub>7</sub>>OCF<sub>3</sub>.

Comparison of the halogenated compounds (**11g** vs. **11h** and **11j** vs. **11k**), obtained by the introduction of electron-withdrawing substituents such as F or Cl at the *meta*-position of *para*-methoxy and ethoxyphenyl derivatives **8** and **11i**, the data indicated that the order of influence of halogen atoms on antiproliferative activity was F>Cl, so that antiproliferative activity decreased with increasing size of the halide substituent. Inhibitor potency on tubulin polymerization of selected 3,4-pyrrole analogues were generally from 1.5- to 2-fold more active than CA-4 (see compounds **11i-k**), although these derivatives exhibited antiproliferative activity generally lower to that of CA-4 on the CCRF-CEM, HL-60, SEM and HeLa cell lines. Compounds **11j** and **11k** were the only compounds that showed activity as inhibitors of colchicine binding comparable to that CA-4.

In this series of compounds, inhibition of tubulin assembly correlated more closely with inhibition of [3H]colchicine binding to tubulin than with antiproliferative activity.

## 4. CONCLUSION

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From a pharmacological point of view, compound 11j was able to block the cell cycle in metaphase and to induce apoptosis at low concentrations (25 nM), following the mitochondrial pathway. More importantly, the compound exerted good activity in vivo, being able to significantly reduce tumor growth in a syngeneic murine tumor model with low toxicity.

- Both the series of (3,4,5-trimethoxyphenyl)pyrrol-1-yl esters and 1H-1,2,4-triazole-3,5-diamines don't hold the ability to induce cell death in an appreciable way and for this reason they must be abandoned.
- In the series of 7-(3',4',5'-trimethoxyanilino)-[1,2,4]triazolo [1,5-a]pyrimidine, the synthesised compounds previously described are in general less active than the reference CA-4, although some are potent enough on MDA-MB-231 cell line. These compounds are only few among the 52 synthesised in the group. The manuscript is not still published, but it is possible to state that, in this series, the investigation demonstrated the possibility to replace the 3',4',5'-trimethoxyanilino moiety with 4'-fluoroaniline, 3'-chloro,4'-fluoroanilino, 4'-chloroanilino and 4'-bromoanilino.
- The first compound of the series of hydroxamic acids, N-hydroxy-3-[6-methoxy-3-methyl-2-(3,4,5-trimethoxybenzoyl)-1-benzofuran-5-yl]prop-2-ynamide, was tested on 8 different cell lines and results are very promising: with nanomolar IC<sub>50</sub> (between 0.3 nM for HeLa cells and 46.5 nM for A549 cells) it is very potent, but we have to wait for biological results prior to define their SAR.

Overall, in this project were design, synthesized and tested different classes of compounds as derivative of CA-4. Most of them were found to be active selectively against tumor cell lines, so these compounds act as antitumoral agents.

Other modifications can be evaluated, especially in the design of multi-targeting compounds and, for this reason, an huge number of small molecules targeting tubulin (and, thus, the mitotic spindle formation) can be design and synthesise.

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