Design, Synthesis and Antiproliferative Activity of Novel Heterobivalent Hybrids Based on Imidazo[2,1-*b*]1,3,4]Thiadiazole and Imidazo[2,1-*b*]1,3]Thiazole Scaffolds

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Abstract: Heterobivalent ligands constituted by two different pharmacophores that bind to different molecular targets or to two distinct sites on the same molecular target could be one of the methods used for the treatment of cancer. In view of the importance of imidazo[1,2-*b*][1,3]thiazole and imidazo[1,2-*b*][1,3,4]thiadiazole as privileged structures for the preparation of novel anticancer agents, we decided to explore the synthesis and biological evaluation of molecular conjugates comprising these fused bicyclic systems tethered at their C-6 position by a *meta*-(α-bromoacryloylamido)phenyl moiety. We found that most of the hybrid compounds displayed high antiproliferative activity toward a wide panel of cancer cell lines, with one-digit micromolar to submicromolar 50% inhibitory concentrations (IC_{50}). We have observed that selected compounds **7d**, **7e**, **7n** and **8c** induced apoptosis which was associated with the release of cytochrome *c* and cleavage of multiple caspases. Overexpression of the protective mitochondrial protein Bcl-2 did not confer protection to cell death induced by these compounds.

Keywords. Apoptosis, structure-activity relationship, imidazo[1,2-*b*][1,3,4]thiadiazole, *in vitro* antiproliferative activity, caspases.

1. Introduction

The discovery and optimization of hybrid structures, constituted by two different biologically active moieties incorporated in a single molecule, has attracted much attention in the last few years as one of the methods that is being useful to treat cancer [1-3]**.** The pharmacophores are generally selected on the basis of their biological target, so that the successive hybrid molecules may exhibit synergistic or additive pharmacological activities [4-9].

Molecules bearing an imidazo[2,1-*b*][1,3]thiazole and imidazo[2,1-*b*][1,3,4]thiadiazole scaffold have been recognized as antitumor agents with a broad spectrum of activity against many cancer cell lines (Chart 1) [10].

Noolvi et al. have reported the anticancer properties of a new series of 2,6-disubstituted imidazo[2,1-*b*][1,3,4]thiadiazoles, with 4-(2-(4-methoxyphenyl)imidazo[2,1-*b*][1,3,4]thiadiazol-6-yl)benzene-1,3-diol (**1**) as one of the most active compounds of the series [11]. This derivative exhibited significant *in vitro* anticancer activity effects on non small cell lung HOP-92, CNS SNB-75 and renal A-198 cancer cell lines, with IC_{50} values of 0.23, 0.33 and 0.36 μM, respectively. Recently, a new series of 2,6-disubstituted imidazo[2,1-*b*][1,3,4]thiadiazole derivatives were employed as templates for the design of activin receptor-like kinase 5 (ALK5) inhibitors [12]. Among them, compound **2** shows the highest ALK5 inhibition (IC_{50}=1.2 nM) and it was found to be selective toward the P38α kinase with significant inhibition (91%) at 10 μM.

Karki et al have reported a series of 6-(*p*-fluorophenyl)-2-benzylsubstituted imidazo[2,1-*b*][1,3,4]thiadiazole with different substituents (hydrogen, methyl, formyl, thiocyanate and bromine) at the C-5 position of imidazo[2,1-*b*][1,3,4]thiadiazole scaffold [13]. Among these, the C-5 formyl derivative **3a** showed maximum antiproliferative activity, with an IC_{50} value of 8 μM against the human leukemia cell line. The promising results obtained with compound **3a** prompted Kumar et al. to prepare second-generation derivatives, where the *p*-fluorophenyl moiety was replaced by other substituted phenyl rings or by a coumarin-3-yl group, maintaining the *p*-chlorobenzyl moiety at the C-2 position of the imidazo[2,1-*b*][1,3,4]thiadiazole system [14]. Among the tested compounds, derivative **4** emerged as the most potent against a panel of four different cancer cell lines, with an IC_{50} in the range of 0.75-0.90 μM. In a new series of 2,5,6-trisubstituted imidazo[2,1-*b*][1,3,4]thiadiazole analogues, the results confirmed that molecules with a benzyl group at the C-2 position exhibited an increase in activity by the introduction of a formyl moiety at the C-5 position [15].

3,6-Diaryl imidazo[1,2-*b*][1,3]thiazole analogues are reported as potential antitumor agents. Koppireddy et al. have reported a series of 3,6-diphenyl imidazo[1,2-*b*][1,3]thiazole

derivatives with general structure **5**, with the 3-(3-trifluoromethylphenyl)-6-phenyl imidazo[2,1-*b*][1,3]thiazole derivative **5a** as the most active compound of the series, with IC_{50} values ranging from 6.0 to 17.5 μM against a wide panel of human cancer cell lines [16].

The α-bromoacrylic acid is an alkylating moiety of low chemical reactivity and devoid of cytotoxic effects (IC_{50}>120 μM against murine leukemia L1210 cells) [17]. The α-bromoacryloyl moiety was present in a series of potent anticancer distamycin-like minor groove binders, including PNU-166196 (brostallicin, **6**), which was evaluated as first-line single agent chemotherapy in patients with advanced or metastatic soft tissue sarcoma [18, 19]. Nowadays the research is primarily focused on the usage of brostallicin in combination with other chemotherapeutic drugs to target triple-negative breast cancer [20]. Regarding the chemical reactivity of the α-bromoacrylic moiety, we speculated that an intracellular biological nucleophile could perform a first-step Michael attack on the double bond, possibly followed by a further reaction of the alpha halogen to the carbonyl, leading to a beta elimination or a second nucleophilic substitution [21].

The modification of 2,6-disubstituted imidazo[2,1-*b*][1,3,4]thiadiazole and 3,6-disubstituted imidazo[2,1-*b*][1,3]thiazole pharmacophores by the introduction of an additional α-bromoacryloyl moiety represents a strategy to obtain novel potential antiproliferative agents. The first series of compounds **7a-o** was characterized by the presence of an imidazo[2,1-*b*][1,3,4]thiadiazole nucleus substituted at its 2-position with a phenyl (**7a**), thien-2-yl (**7d**), benzyl (**7b**), phenylethyl (**7c**), phenyl and benzyl moieties with electron-releasing or electron-withdrawing groups (ERG and EWG, respectively). Another short set of compounds **8a-c**, the phenyl (**8a**), *p*-chlorophenyl (**8b**) and *p*-methoxyphenyl (**8c**) moieties were inserted at the 3-position of imidazo[2,1-*b*][1,3]thiazole template. All these new conjugates were characterized by the presence of a common *meta*-(α-bromoacryloylamido)phenyl moiety linked at the 6-position of imidazo[2,1-*b*][1,3,4]thiadiazole and imidazo[2,1-*b*][1,3]thiazole skeletons (Chart 2). The structure-activity relationship (SAR) was investigated by the insertion of different

substituents on the phenyl or benzene portion of the benzyl moiety at the C-2 and C-3 positions of the imidazo[2,1-*b*][1,3,4]thiadiazole and imidazo[2,1-*b*][1,3]thiazole systems, respectively. Besides hydrogen, the examined substituents included electron-donating alkyl and alkoxy or electron-withdrawing fluorine and chlorine groups.

2. Chemistry

Synthesis of derivatives **7a-o** and **8a-c** was carried out by the general methodology shown in Scheme 1. 2-Substituted-6-(*meta*-nitrophenyl) imidazo[2,1-*b*][1,3,4]thiadiazoles **11a-o** and 3-substituted-6-(*meta*-nitrophenyl) imidazo[2,1-*b*][1,3]thiazoles **12a-c** were obtained by the condensation reaction of 2-amino-5-substituted-1,3,4 thiadiazoles **9a-o** [22] and 2-amino-4-aryl-1,3 thiazoles **10a-c** [23], respectively, with *meta*-nitrophenacyl bromide in refluxing ethanol. The subsequent reduction of the nitro functionality of **11a-o** and **12a-c** using tin(II) chloride in refluxing ethanol yielded the related anilino derivatives **13a-o** and **14a-c**, respectively, which were converted to the hybrid compounds **7a-o** and **10a-c** by condensation with α-bromoacrylic acid using 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDCl) in dimethylformamide [24].

3. Biological results and discussion.

3.1. In vitro antiproliferative activities.

Table 1 summarizes the antiproliferative effects of the two novel series of 2-substituted-6-[*m*-(α-bromoacryloylamido)phenyl]imidazo[2,1-*b*][1,3,4]thiadiazoles **7a-o** and 3-aryl-6-[*m*-(α-bromoacryloylamido)phenyl]imidazo[2,1-*b*][1,3]thiazoles **8a-c** against the proliferation of murine leukemia (L1210), murine mammary carcinoma (FM3A), human T-lymphoblastoid (CEM) and human cervix carcinoma (HeLa) cells, using the alkylating agent melphalan as reference compound. All synthesized compounds possessed significant antiproliferative activity, comparable to or even higher than that of melphalan against L1210, FM3A and CEM cells, while only compounds **7d-e**, **7n-o** and **8c** proved clearly superior to melphalan against

HeLa cells. Among the hybrid compounds, four of them (**7d**, **7e**, **7n** and **8c**) exhibited potent antiproliferative activity with submicromolar IC_{50} values in the range of 0.17-0.67, 0.25-0.87, 0.042-0.61 and 0.20-0.86 μM, respectively, against all four cancer cell lines. The validity of the molecular hybridization approach was confirmed comparing the potency of conjugate derivatives **7a-o** and **8a-c** with those of the anilino precursors **13a-o** and **14a-c**, respectively. These latter compounds were 2- to 200-fold less active than the corresponding α-bromoacryloylamido analogues, demonstrating that the presence of the α-bromoacryloyl moiety significantly enhanced cell growth inhibitory activity (see Table 1s in Supplementary Data). The data shown in Table 1 indicate the importance of the substituents and their relative position on the phenyl ring at the C-2 or C-3 position of the imidazo[2,1-*b*][1,3,4]thiadiazole and imidazo[2,1-*b*][1,3]thiazole systems, respectively, for activity and selectivity against the different cancer cell lines.

The tumor cell growth inhibitory activities of benzyl and 2'-phenylethyl derivatives **7b** and **7c**, respectively, were very similar, but 2-5-fold reduced as compared to the phenyl analogue **7a**. Compound **7d** bearing the bioisosteric thien-2-yl moiety, with IC_{50} values ranging from 0.17 to 0.67 μM, was 2- to 4-fold more potent than its phenyl counterpart **7a**. Introduction of a weak EWG, a *para*-fluorine atom, to yield compound **7e**, further improved the cytostatic activity in comparison to that of the unsubstituted phenyl derivative **7a**. Cell growth inhibition activity decreased 3-6-fold by increasing the halogen size from fluorine to chlorine (**7f**). By comparing the effects of substituents with opposite electronic properties at the *para*-position of the phenyl ring, a marginal (~ 1.5-fold) increase in antiproliferative potency was observed replacing the electron-withdrawing chlorine atom with an electron-donating methyl group (compounds **7f** and **7h**, respectively).

The number and position of methoxy substituents on the phenyl ring (derivatives **7j**, **7l** and **7n**) had a major influence on the antiproliferative activity. A single methoxy substituent on the *para*-position of the phenyl ring (compound **7j**) led to a ≤ 2-fold reduction in

antiproliferative activity relative to **7a** on three of the four cancer cell lines, while **7a** and **7j** proved equipotent against FM3A cells. Moving the methoxy group from the *para*- to the *meta*-position, to furnish derivative **7l**, enhanced antiproliferative activity of 1.5-2-fold in three of the four cancer cell lines, with a minimal difference in potency between the two isomeric derivatives **7j** and **7l** against CEM cells. A 2-3-fold reduction in activity was observed replacing the *m*-methoxyphenyl with a *m*-methoxybenzyl moiety at the 2-position of the imidazo[2,1-*b*][1,3,4]thiadiazole system (compounds **7l** and **7m**, respectively).

Relative to the activity of derivative **7j**, the results suggest that the insertion of an additional methoxy group at the *meta*-position, to furnish the *m,p*-dimethoxy derivative **7n**, was well-tolerated and increased antiproliferative potency of 20-, 8-, 3- and 4-fold against L1210, FM3A, CEM and HeLa cells, respectively. Of all tested compounds, derivative **7n** possessed the highest potency with IC_{50} values ranging between 0.042 and 0.61 μM.

For compound **7j**, the introduction of a methylene spacer between the *para*-methoxyphenyl moiety and the C-2 position of the imidazo[2,1-*b*][1,3,4]thiadiazole skeleton, resulting in the derivative **7k**, did not alter the activity toward FM3A and HeLa cells, but showed a 1.5- and 2-fold reduction in activity against L1210 and CEM cells, respectively.

Replacement of the methoxy unit by the less electron-releasing methyl group (compound **7h**) maintained the antiproliferative activity, indicating that the methyl and methoxy moieties are bioequivalent at the *para*-position of the phenyl ring. The *para*-ethyl homologue **7i** was 1.5-3-fold less active than the methyl counterpart **7h** against the panel of cancer cell lines.

Replacing the *para*-methoxy (**7j**) with the ethoxy homologue (**7o**) resulted in a 2-fold increase in activity against HeLa cells, while the two compounds **7j** and **7o** were equipotent against L1210, FM3A and CEM cells.

For the *para*-chlorobenzyl derivative **7g**, the activity was 2-fold improved with respect to **7f** against FM3A, while an opposite effect was observed against L1210 cells. **7f** and its homologue **7g** were equipotent against CEM and HeLa cells.

In the case of the small series of 3-arylimidazo[2,1-*b*][1,3]thiazole derivatives **8a-c**, we found that there was no difference in activity between unsubstituted phenyl and its *para*-chloro analogue (derivatives **8a** and **8b**, respectively). Replacing the chlorine atom in **8b** with the strong electron-donating methoxy group (**8c**) led to a 2-9-fold increase in antiproliferative activity, with IC_{50} values ranging from 0.20 to 0.86 μM.

The exploration of the impact of the substitution at the *para*-position of the phenyl group at the 2- or 3-position of imidazo[2,1-*b*][1,3,4]thiadiazole or imidazo[2,1-*b*][1,3]thiazole, respectively, suggested that replacement of the lipophilic electron-withdrawing chlorine by a strong electron-releasing methoxy group (i.e. **7f** *vs.* **7j**, **7g** *vs.* **7k**, **8b** *vs.* **8c**) is beneficial for activity against the four cancer cell lines.

*3.2. Selected compounds **7d**, **7e**, **7n** and **8c** inhibit cell viability and induce caspase activation-mediated apoptosis in human leukemia cell cultures*

The four most active compounds **7d**, **7e**, **7n** and **8c** were highly cytotoxic against leukemia (HL-60 and U937), melanoma cells (SK-MEL-1) and even the human leukemia U937 cell line over-expressing Bcl-2 (U937/Bcl-2), with IC_{50} values between 0.2 and 1 μM (see Table 2s in Supplementary Data). Treatment with compounds **7d**, **7e**, **7n** and **8c** resulted in a concentration-dependent inhibition of cell viability, with no significant changes among the four cell lines (HL-60, U937, U937/Bcl-2 and SK-MEL-1).

It has been recognized that the antitumor efficacy of several chemotherapeutic agents is correlated with their ability to induce apoptosis [25]. Therefore, promoting apoptosis in cancer cells is a promising strategy that could lead to the discovery and development of new anticancer agents [26, 27]. In this context, in order to determine the possible mechanism of action mediating cell growth inhibition, we have examined the ability of conjugates **7d**, **7e**, **7n** and **8c** to induce apoptosis, using several leukemia cell lines as the experimental system. As shown in Fig. 1A, these molecules induced important morphological changes as well as an important reduction in the number of tumor cells [28]. Apoptosis is regulated by genetic

mechanisms and characterized by morphological and biochemical changes in the cell nucleus, including chromatin condensation and nuclear shrinking [29]. After 8 h of incubation with derivatives **7d**, **7e**, **7n** and **8c** at 3 μM-concentrations, characteristic apoptotic morphological changes were observed by fluorescent microscopy after DNA staining with Hoechst 33258, including cell shrinkage, an increase in condensed and fragmented chromatin and the formation of apoptotic bodies (Fig. 1B).

Evaluation of the number of hypodiploid cells by flow cytometry in HL-60 cells after 16 h of treatment with compounds **7d**, **7e**, **7n** and **8c** at a concentration of 1 μM, revealed that the percentage of apoptotic cells increased approximately 5.6-fold (14.5±4.3%), 4.6-fold (12.1±0.2), 6-fold (16±2.5%) and 4-fold (11.1±0.4%), respectively, compared to control (2.6±0.6%) (Fig. 2A and B) and this effect was dose-dependent. Although compounds **7d**, **7e**, **7n** and **8c** promote condensation and fragmentation of chromatin as well as an increase in the percentage of hypodiploid cells, there was no clear DNA laddering (Fig. 2C).

To determine whether apoptosis induced by compounds **7d**, **7e**, **7n** and **8c** in leukemia cells involves the release of cytochrome *c* from mitochondria to the cytosol, cells were treated with compounds **7d**, **7e**, **7n** and **8c** at 3 μM-concentrations and cytosolic preparations were analyzed by immunoblotting. As shown (Fig. 3A), a significant increase in the amount of cytochrome *c* in the cytosol was detected after treatment with these compounds, suggesting that the release of cytochrome *c* from mitochondria might be responsible for the induction of apoptosis by these molecules in the human leukemia cells [30]. The release of cytochrome *c* was also triggered in Bcl-2 over-expressing U937 cells (Fig. 3A).

From previous reports, it is well established that the main players for the initiation and execution of the apoptotic process are caspases, a family of intracellular cysteine proteases expressed as inactive zymogens in living cells and sequentially activated by specific proteolytic cleavage [31]. Initiator caspase-8 and -9 are usually the first to be stimulated in the apoptotic process, and then, they activate the executioner caspase-3 which is responsible for

specific cellular protein destruction during apoptosis [32]. Caspase-3 is one of the most important members of this family and is activated in the apoptotic cells both during extrinsic and intrinsic pathways [33]. The effect of derivatives **7d**, **7e**, **7n** and **8c** on proteolytic processing of caspases was also examined. To this end, HL-60 and U937 cells were treated with the compounds at 3 μM for 8 h and initiator (caspase-9 and -8) and the executioner (caspase-3) caspases were determined by immunoblotting using antibodies that bind both the proenzyme (caspase precursors) and the cleaved caspases. The results show that compounds **7d**, **7e**, **7n** and **8c** stimulate the cleavage of pro-caspase-9, -8 and -3 (Fig. 3B). Pro-caspase processing does not always correlate with activity, and so the enzymatic activity of caspase-3-like proteases (caspase-3/7) was also investigated in extracts of control or HL-60 cells treated with compounds **7d**, **7e**, **7n** and **8c** at two different concentrations (1 and 3 μM). Results indicated that caspase 3/7 activity increased 3-, 2.3-, 3.2- and 3.8-fold after treatment with 3 μM of compounds **7d**, **7e**, **7n** and **8c**, respectively (Fig. 3C). Taken together the results consistently suggest that these compounds are able to induce apoptosis by activation of the extrinsic and the intrinsic pathways leading to cell death.

4. Conclusions

The imidazo-based bicyclic systems, specifically imidazo[2,1-*b*][1,3]thiazole and the bioisosteric imidazo[2,1-*b*][1,3,4]thiadiazole were the privileged structures associated with a wide variety of compounds with potential antitumor activity. In a continuing study of hybrid compounds containing the α-bromoacryloyl unit as potential anticancer drugs, we synthesized two novel different series of molecules. They were characterized by the α-bromoacryloylamido moiety located at the *meta*-position on the phenyl linked at the C-6 position of 2-substituted imidazo[2,1-*b*][1,3,4]thiadiazole and 3-substituted imidazo[2,1-*b*][1,3]thiazole scaffolds, and correspond to derivatives **7a-o** and **8a-c**, respectively. Such hybrid derivatives demonstrated a significantly increased activity compared with the

corresponding aniline counterparts. In general, the antiproliferative activities of conjugates **7a-o** and **8a-c** were somewhat more pronounced against L1210 and CEM as compared with FM3A and HeLa tumor cells. The bioisosteric replacement of phenyl (**7a**) by a thien-2-yl (**7d**) greatly increased activity, with IC_{50} values of 0.17-0.67 μM *versus* 0.60-1.4 μM against the tumor cell lines. The antiproliferative activities of these hybrid molecules were influenced by the substituents on the phenyl ring linked at the bicyclic systems. Many of the conjugates prepared (**7d-e**, **7n** and **8f**) demonstrated pronounced submicromolar antiproliferative activity against a panel of eight cancer cell lines, being more active than the reference compound melphalan. The exploration of the impact of the substitution at the *para*-position of the phenyl group at the 2- or 3-position of imidazo[2,1-*b*][1,3,4]thiadiazole or imidazo[2,1-*b*][1,3]thiazole, respectively, suggested that replacement of the lipophilic electron-withdrawing chlorine by an electron releasing methoxy group (i.e. **7f** *vs.* **7j**, **7g** *vs.* **7k**, **8b** *vs.* **8c**) is beneficial for activity. The presence of a *para*-methoxy phenyl substituent at the C-3 position of 3-substituted imidazo[2,1-*b*][1,3]thiazole scaffold was superior to the phenyl and *para*-chlorophenyl substituents for the antiproliferative activity (compounds **8c**, **8a** and **8b**, respectively). Moreover, compounds **7d-e**, **7n** and **8c** were also evaluated for their effects on induction of apoptosis in HL-60 and U937 cells. These molecules caused an increase in the number of hypodiploid cells and cell death was found to be associated with the release of cytochrome *c* and activation of caspases.

5. Experimental protocols

5.1. Chemistry

5.1.1. Materials and Methods

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 200 and Varian 400 Mercury Plus spectrometer, respectively. Chemical shifts (δ) are given in ppm upfield from tetramethylsilane as internal standard, and the spectra were recorded in appropriate deuterated

solvents, as indicated. Positive-ion electrospray ionization (ESI) mass spectra were recorded on a double-focusing Finnigan MAT 95 instrument with BE geometry. Melting points (mp) were determined on a Buchi-Tottoli apparatus and are uncorrected. All products reported showed ^{1}H and ^{13}C NMR spectra in agreement with the assigned structures. The purity of tested compounds was determined by combustion elemental analyses conducted by the Microanalytical Laboratory of the Chemistry Department of the University of Ferrara with a Yanagimoto MT-5 CHN recorder elemental analyzer. All tested compounds yielded data consistent with a purity of at least 95% as compared with the theoretical values. All reactions were carried-out under an inert atmosphere of dry nitrogen, unless otherwise indicated. Standard syringe techniques were used for transferring dry solvents. Reaction courses and product mixtures were routinely monitored by TLC on silica gel (precoated F_{254} Merck plates), and compounds were visualized with aqueous $KMnO_4$. Flash chromatography was performed using 230-400 mesh silica gel and the indicated solvent system. Organic solutions were dried over anhydrous Na_2SO_4.

*5.1.2. General procedure A for the synthesis of compounds **9i** and **9m**.*

Phosphorus oxychloride (5 mL) was added to a stirring mixture of the appropriate benzoic acid (10 mmol) and thiosemicarbazide (10 mmol) and the mixture was heated at 80 °C for 2 hours. After cooling in a ice bath, water (10 mL) was added slowly and the reaction mixture was further refluxed for 4 h. The ice bath cooled solution was basified (pH=8) by dropwise addition of a 50% NaOH aqueous solution. The precipitate obtained was filtered, washed with cold water (10 mL) and recrystallized from ethanol.

*5.1.2.1. 5-(4-Ethylphenyl)-1,3,4-thiadiazol-2-amine (**9i**).* Following general procedure A, compound **9i** was obtained as white solid. Yield 78%, mp 188-190 °C. ^{1}H NMR (d_6-DMSO) δ:1.19 (t, J=7.8 Hz, 3H), 2.62 (q, J=7.8 Hz, 2H), 7.28 (d, J=8.0 Hz, 2H), 7.34 (s, 2H), 7.64 (d, J=8.0 Hz, 2H). MS (ESI): $[M+1]^{+}$=206.3.

*5.1.2.2. 5-(3-Methoxybenzyl)-1,3,4-thiadiazol-2-amine (**9m**).* Following general procedure A, compound **121** was obtained as a brown solid. Yield 83%, mp 179-181 °C. ^{1}H NMR (d_6-DMSO) δ:3.70 (s 3H), 4.08 (s, 2H), 6.78 (m, 2H), 6.81 (s, 1H), 6.99 (s, 2H), 7.22 (t, J=7.8 Hz, 1H). MS (ESI): $[M+1]^+$=222.3.

*5.1.3. General procedure B for the synthesis of compounds **11b-i**, **11k-o** and **12a-c**.*

A mixture of the appropriate 2-amino-5-substituted 1,3,4-thiadiazole **9b-o** or 4-arylthiazol-2-amine **10a-c** (2 mmol) and 2-bromo-1-(3-nitrophenyl)ethanone (488 mg, 2 mmol) in anhydrous EtOH (20 mL) was stirred at reflux for 18 h. The solution was cooled to room temperature, the solvent was removed *in vacuo*, and saturated aqueous $NaHCO_3$ solution was added to make the mixture basic (pH=8-9). The mixture was extracted with CH_2Cl_2 (3 x 15 mL), the combined organic phases were washed with brine (10 mL) and dried with anhydrous Na_2SO_4. After removal of the solvent, the residue was stirred with ethyl ether (10 mL) and filtered to afford the desired product.

*5.1.3.1. 2-Benzyl-6-(3-nitrophenyl)imidazo[2,1-b][1,3,4]thiadiazole (**11b**).* Following general procedure B, compound **11b** was isolated as a brown solid. Yield 54%, mp 155-157 °C. ^{1}H-NMR (200 MHz, d_6-DMSO) δ: 4.47 (s, 2H), 7.32 (m, 5H), 7.66 (t, J=8.0 Hz, 1H), 8.09 (dt, J= 8.0 and 1.8 Hz, 1H), 8.26 (d, J=8.0 Hz, 1H), 8.65 (t, J=1.8 Hz, 1H), 8.92 (s, 1H). MS (ESI): $[M+1]^+$=337.4.

*5.1.3.2. 6-(3-Nitrophenyl)-2-(2-phenylethyl)imidazo[2,1-b][1,3,4]thiadiazole (**11c**).* Following general procedure B, compound **11c** was obtained as a yellow solid. Yield 77%, mp 111-113 °C. ^{1}H-NMR (200 MHz, d_6-DMSO) δ: 3.08 (t, J=7.6 Hz, 2H), 3.38 (t, J=7.6 Hz, 2H), 7.12 (m, 1H), 7.26 (m, 4H), 7.68 (t, J=8.0 Hz, 1H), 8.08 (dt, J=8.0 and 2.0 Hz, 1H), 8.25 (d, J=8.0 Hz, 1H), 8.63 (t, J=2.0 Hz, 1H), 8.87 (s, 1H). MS (ESI): $[M+1]^+$=351.4.

*5.1.3.3. 6-(3-Nitrophenyl)-2-(2-thienyl)imidazo[2,1-b][1,3,4]thiadiazole (**11d**).* Following general procedure B, derivative **11d** was obtained as a yellow solid. Yield 68%, mp 243-245 °C. ^{1}H-NMR (200 MHz, d_6-DMSO) δ: 7.27 (dd, J=5.0 and 4.0 Hz, 1H), 7.69 (t, J=8.0 Hz, 1H), 7.89 (d, J=4.0 Hz, 1H), 7.92 (d, J=5.0 Hz, 1H), 8.11 (dt, J=8.0 and 2.4 Hz, 1H), 8.30 (dt, J=8.0 and 2.4 Hz, 1H), 8.69 (t, J=2.4 Hz, 1H), 8.98 (s, 1H). MS (ESI): $[M+1]^+$=329.4.

*5.1.3.4. 2-(4-Fluorophenyl)-6-(3-nitrophenyl)imidazo[2,1-b][1,3,4]thiadiazole (**11e**).* Following general procedure B, derivative **11e** was obtained as a yellow solid. Yield 76%, mp 227-229 °C. ^{1}H-NMR (200 MHz, d_6-DMSO) δ: 7.41 (t, J=8.8 Hz, 2H), 7.72 (t, J=8.2 Hz, 1H), 7.99 (dd, J=9.0 and 5.4 Hz, 2H), 8.11 (d, J=8.2 Hz, 1H), 8.30 (d, J=8.2 Hz, 1H), 8.68 (s, 1H), 9.02 (s, 1H). MS (ESI): $[M+1]^+$=341.4.

*5.1.3.5. 2-(4-Chlorophenyl)-6-(3-nitrophenyl)imidazo[2,1-b][1,3,4]thiadiazole (**11f**).* Following general procedure B, derivative **11f** was isolated as a white solid. Yield 67%, mp 256-258 °C. ^{1}H-NMR (200 MHz, d_6-DMSO) δ: 7.66 (d, J=8.8 Hz, 2H), 7.73 (t, J=8.0 Hz, 1H), 7.97 (d, J=8.8 Hz, 2H), 8.11 (dd, J=8.0 and 1.8 Hz, 1H), 8.31 (d, J=8.0 Hz, 1H), 8.70 (t, J=1.8 Hz, 1H), 9.04 (s, 1H). MS (ESI): $[M+1]^+$=357.8.

*5.1.3.6. 2-(4-Chlorobenzyl)-6-(3-nitrophenyl)imidazo[2,1-b][1,3,4]thiadiazole (**11g**).* Following general procedure B, derivative **11g** was isolated as a yellow solid. Yield 69%, mp 157-159 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 4.48 (s, 2H), 7.46 (s, 4H), 7.70 (t, J=8.0 Hz, 1H), 9.09 (dt, J=8.0 and 1.6 Hz, 1H), 8.27 (d, J=8.0 Hz, 1H), 8.66 (t, J=1.6 Hz, 1H), 8.92 (s, 1H). MS (ESI): $[M+1]^+$=371.9.

*5.1.3.7. 2-(4-Methylphenyl)-6-(3-nitrophenyl)imidazo[2,1-b][1,3,4]thiadiazole (**11h**).* Following general procedure B, compound **11h** was obtained as a yellow solid. Yield 82%, mp 214-216 °C. ^{1}H-NMR (200 MHz, d_6-DMSO) δ: 2.40 (s, 3H), 7.39 (d, J=8.0 Hz, 2H), 7.72

(t, J=7.8 Hz, 1H), 7.76 (d, J=8.0 Hz, 2H), 8.10 (dd, J=7.8 and 1.8 Hz, 1H), 8.29 (d, J=7.8 Hz, 1H), 8.67 (d, J=1.8 Hz, 1H), 8.99 (s, 1H). MS (ESI): $[M+1]^+$=337.4.

*5.1.3.8. 2-(4-Ethylphenyl)-6-(3-nitrophenyl)imidazo[2,1-b][1,3,4]thiadiazole (**11i**).* Following general procedure B, compound **11i** was isolated as a yellow solid. Yield 57%, mp 160-162 °C. ^{1}H-NMR (200 MHz, d_6-DMSO) δ: 1.22 (t, J=7.8 Hz, 3H), 2.68 (q, J=7.8 Hz, 2H), 7.42 (d, J=8.2 Hz, 2H), 7.72 (t, J=7.8 Hz, 1H), 7.85 (d, J=8.2 Hz, 2H), 8.09 (d, J=7.8 Hz, 1H), 8.30 (d, J=7.8 Hz, 1H), 8.68 (s, 1H), 8.99 (s, 1H). MS (ESI): $[M+1]^+$=351.4.

*5.1.3.9. 2-(4-Methoxybenzyl)-6-(3-nitrophenyl)imidazo[2,1-b][1,3,4]thiadiazole (**11k**).* Following general procedure B, compound **11k** was isolated as a green solid. Yield 85%, mp 125-127 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 3.75 (s, 3H), 4.38 (s, 2H), 6.92 (d, J=8.6 Hz, 2H), 7.32 (d, J=8.6 Hz, 2H), 7.70 (t, J=8.0 Hz, 1H), 8.09 (dt, J=8.0 and 2.0 Hz, 1H), 8.27 (d, J=8.0 Hz, 1H), 8.65 (t, J=2.0 Hz, 1H), 8.91 (s, 1H). MS (ESI): $[M+1]^+$=367.4.

*5.1.3.10. 2-(3-Methoxyphenyl)-6-(3-nitrophenyl)imidazo[2,1-b][1,3,4]thiadiazole (**11l**).* Following general procedure B, derivative **11l** was isolated as a yellow solid. Yield 57%, mp 197-199 °C. ^{1}H-NMR (200 MHz, d_6-DMSO) δ: 3.87 (s, 3H), 7.22 (m, 1H), 7.46 (s, 1H), 7.51 (d, J=5.2 Hz, 2H), 7.69 (t, J=8.0 Hz, 1H), 8.11 (dt, J=8.2 and 1.6 Hz, 1H), 8.31 (d, J=8.2 Hz, 1H), 8.69 (t, J=1.6 Hz, 1H), 9.02 (s, 1H). MS (ESI): $[M+1]^+$=353.4.

*5.1.3.11. 2-(3-Methoxybenzyl)-6-(3-nitrophenyl)imidazo[2,1-b][1,3,4]thiadiazole (**11m**).* Following general procedure B, compound **11m** was isolated as a brown solid. Yield 72%, mp 96-98 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 3.76 (s, 3H), 4.30 (s, 2H), 6.44 (m, 2H), 7.01 (m, 1H), 7.32 (t, J=7.6 Hz, 1H), 7.97 (t, J=8.0 Hz, 1H), 8.42 (dt, J=8.0 and 1.8 Hz, 1H), 8.61 (d, J=8.0 Hz, 1H), 8.73 (t, J=1.8 Hz, 1H), 8.89 (s, 1H). MS (ESI): $[M+1]^+$=367.4.

*5.1.3.12. 2-(3,4-Dimethoxyphenyl)-6-(3-nitrophenyl)imidazo[2,1-b][1,3,4]thiadiazole (**11n**).* Following general procedure B, derivative **11n** was obtained as a yellow solid. Yield 73%, mp

224-226 °C. ^{1}H-NMR (200 MHz, d_6-DMSO) δ: 3.86 (s, 3H), 3.88 (s, 3H), 7.12 (d, J=8.6 Hz, 1H), 7.47 (m, 2H), 7.71 (t, J=7.8 Hz, 1H), 8.10 (d, J=7.8 Hz, 1H), 8.29 (d, J=7.8 Hz, 1H), 8.67 (s, 1H), 8.94 (s, 1H). MS (ESI): $[M+1]^+$=383.4.

*5.1.3.13. 2-(4-Ethoxyphenyl)-6-(3-nitrophenyl)imidazo[2,1-b][1,3,4]thiadiazole (**11o**).* Following general procedure B, derivative **11o** was isolated as a yellow solid. Yield 73%, mp 201-203 °C. ^{1}H-NMR (200 MHz, d_6-DMSO) δ: 1.33 (t, J=7.0 Hz, 3H), 4.08 (q, J=7.0 Hz, 2H), 7.10 (d, J=8.8 Hz, 2H), 7.68 (t, J=8.2 Hz, 1H), 7.86 (d, J=8.8 Hz, 2H), 8.10 (d, J=8.2 Hz, 1H), 8.29 (d, J=8.2 Hz, 1H), 8.68 (s, 1H), 8.97 (s, 1H). MS (ESI): $[M+1]^+$=357.4.

*5.1.3.14. 6-(3-Nitrophenyl)-3-phenylimidazo[2,1-b][1,3]thiazole (**12a**).* Following general procedure B, compound **12a** was isolated as a yellow solid. Yield 38%, mp 182-184 °C. ^{1}H-NMR (200 MHz, d_6-DMSO) δ: 7.55 (m, 4H), 7.66 (t, J=8.0 Hz, 1H), 7.84 (dd, J=7.4 and 1.2 Hz, 2H), 8.09 (dd, J=8.0 and 1.2 Hz, 1H), 8.38 (d, J=8.0 Hz, 1H), 8.77 (t, J=1.2 Hz, 1H), 8.84 (s, 1H). MS (ESI): $[M+1]^+$=322.3.

*5.1.3.15. 3-(4-Chlorophenyl)-6-(3-nitrophenyl)imidazo[2,1-b][1,3]thiazole (**12b**).* Following general procedure B, **12b** was isolated as a yellow solid. Yield 72%, mp 202-204 °C. ^{1}H-NMR (200 MHz, d_6-DMSO) δ: 7.19 (s, 1H), 7.48 (d, J=8.6 Hz, 2H), 7.75 (t, J=7.6 Hz, 1H), 7.88 (d, J=8.6 Hz, 2H), 8.10 (d, J=7.6 Hz, 1H), 8.36 (d, J=8.0 Hz, 1H), 8.76 (d, 1H), 8.84 (s, 1H). MS (ESI): $[M+1]^+$=356.8.

*5.1.3.16. 3-(4-Methoxyphenyl)-6-(3-nitrophenyl)imidazo[2,1-b][1,3]thiazole (**12c**).* Following general procedure B, compound **12c** was obtained as an orange solid. Yield 97%, mp 100-102 °C. ^{1}H-NMR (200 MHz, d_6-DMSO) δ: 3.86 (s, 3H), 6.89 (d, J=8.6 Hz, 2H), 6.99 (s, 1H), 7.69 (t, J=8.0 Hz, 1H), 7.82 (d, J=8.6 Hz, 2H), 8.08 (d, J=8.0 Hz, 1H), 8.37 (d, J=8.0 Hz, 1H), 8.76 (s, 1H), 8.78 (s, 1H). MS (ESI): $[M+1]^+$=352.4.

*5.1.4. General procedure C for the synthesis of compounds **13b-i**, **13k-o** and **14a-c***. To a suspension of derivatives **11b-i**, **11k-o**or **12a-c** (1 mmol) in absolute ethanol (10 mL) was added $SnCl_2$ $2H_2O$ (1.13 g, 5 mmol), and the stirring mixture was refluxed for 1.5 h. After this time, the reaction was cooled to room temperature and treated with cold water (15 mL), and the suspension was carefully adjusted to pH 8 with $NaHCO_3$. The mixture was extracted with EtOAc (3 x 15 mL), the combined organic phase was washed with water (10 mL) and brine (10 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by crystallization with ethyl ether**.**

*5.1.4.1. 3-(2-Phenylimidazo[2,1-b][1,3,4]thiadiazol-6-yl)aniline (**13a**).* Following general procedure C, compound **13a** was obtained as a yellow solid. Yield 51%, mp 168-170 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 5.12 (bs, 2H), 6.48 (t, J=7.8 Hz, 1H), 7.02 (d, J=7.8 Hz, 1H), 7.12 (m, 4H), 7.26 (m, 2H), 8.23 (s, 1H), 8.51 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 110.21, 113.54, 118.32, 120.89, 126.54, 127.62 (2C), 128.55, 130.12 (2C), 132.12, 133.76, 134.56, 143.78, 144.77, 158.93. MS (ESI): $[M+1]^+$=293.4.

*5.1.4.2. 3-(2-Benzylimidazo[2,1-b][1,3,4]thiadiazol-6-yl)aniline (**13b**).* Following general procedure C, compound **13b** was isolated as a yellow solid. Yield 83%, mp 154-156 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 4.43 (s, 2H), 5.07 (bs, 2H), 6.46 (dt, J=8.0 and 1.6 Hz, 1H), 6.96 (m, 2H), 7.02 (t, J=1.6 Hz, 1H), 7.32 (m, 1H), 7.40 (m, 4H), 8.43 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 36.80, 109.60, 110.20, 112.56, 113.00, 126.73, 127.37, 128.53, 128.80, 128.93, 129.02, 134.26, 135.97, 144.34, 145.64, 148.71, 164.03. MS (ESI): $[M+1]^+$=307.3.

*5.1.4.3. 3-[2-(2-Phenylethyl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl]aniline (**13c**).* Following general procedure C, compound **13c** was obtained as a yellow solid. Yield 58%, mp 151-153 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 3.11 (t, J=7.6 Hz, 2H), 3.36 (t, J=7.6 Hz, 2H), 5.08 (bs, 2H), 6.46 (dt, J=8.0 and 2.0 Hz, 1H), 6.96 (m, 2H), 7.07 (t, J=2.0 Hz, 1H), 7.21 (m, 1H), 7.31 (m, 4H), 8.41 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 32.62, 33.61, 109.54, 110.13,

112.51, 112.96, 126.31, 128.21, 128.33 (2C), 128.39, 128.92 (2C), 134.28, 139.49, 145.65, 148.69, 163.79. MS (ESI): $[M+1]^+$=351.4.

*5.1.4.4. 3-[2-(2-Thienyl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl]aniline (**13d**).* Following general procedure C, derivative **13d** was obtained as a yellow solid. Yield 44%, mp 229-231 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 5.12 (bs, 2H), 6.48 (dt, J=7.6 and 2.0 Hz, 1H), 7.02 (m, 2H), 7.11 (t, J=2.0 Hz, 1H), 7.26 (dd, J=5.2 and 3.6 Hz, 1H), 7.85 (d, J=3.6 Hz, 1H), 7.92 (d, J=5.2 Hz, 1H), 8.52 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 110.02, 110.16, 112.58, 113.16, 128.61, 129.00, 130.35, 131.08, 131.55, 134.11, 143.48, 146.29, 148.78, 155.00. MS (ESI): $[M+1]^+$=299.4.

*5.1.4.5. 3-[2-(4-Fluorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl]aniline (**13e**).* Following general procedure C, derivative **13e** was obtained as a white solid. Yield 68%, mp 196-197 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 5.10 (s, 2H), 6.45 (dt, J=7.6 and 1.6 Hz, 1H), 7.01 (m, 2H), 7.11 (t, J=1.6 Hz, 1H), 7.42 (d, J=8.8 Hz, 2H), 7.99 (m 2H), 8.55 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 110.03, 110.23, 112.64, 113.18, 116.60, 116.82, 126.20, 129.00, 129.08, 129.17, 134.10, 143.97, 146.32, 148.78, 159.73, 165.18. MS (ESI): $[M+1]^+$=311.4.

*5.1.4.6. 3-[2-(4-Chlorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl]aniline (**13f**).* Following general procedure C, derivative **13f** was isolated as a yellow solid. Yield 69%, mp 234-236 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 5.10 (bs, 2H), 6.47 (dt, J=7.2 and 1.6 Hz, 1H), 7.01 (m, 2H), 7.12 (t, J=1.6 Hz, 1H), 7.65 (dd, J=6.8 and 2.0 Hz, 2H), 7.94 (dd, J=6.8 and 2.0 Hz, 2H), 8.56 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 110.59, 110.78, 113.19, 113.76, 128.82 (2C), 129.00, 129.54, 130.14 (2C), 134.59, 136.94, 144.52, 147.01, 149.33, 160.18. MS (ESI): $[M+1]^+$=327.8.

*5.1.4.7. 3-[2-(4-Chlorobenzyl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl]aniline (**13g**).* Following general procedure C, derivative **13g** was isolated as a white solid. Yield 64%, mp 175-177 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 4.45 (s, 2H), 5.08 (bs, 2H), 6.46 (dt, J=7.6 and 1.6 Hz,

1H), 6.96 (m, 2H), 7.08 (t, J=1.6 Hz, 1H), 7.44 (s, 4H), 8.44 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 35.96, 109.62, 110.17, 112.54, 112.99, 128.46, 128.70, 128.93, 130.43, 130.96 (2C), 132.07, 134.23, 134.98, 145.66, 148.71, 163.49. MS (ESI): $[M+1]^+$=341.9.

*5.1.4.8. 3-[2-(4-Methylphenyl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl]aniline (**13h**).* Following general procedure C, compound **13h** was obtained as a white solid. Yield 95%, mp 176-178 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 2.52 (s, 3H), 5.11 (bs, 2H), 6.49 (dt, J=7.2 and 2.0 Hz, 1H), 7.03 (m, 2H), 7.13 (t, J=2.0 Hz, 1H), 7.40 (d, J=8.0 Hz, 2H), 7.83 (d, J=8.0 Hz, 2H), 8.54 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 20.97, 109.95, 110.19, 112.61, 113.12, 126.44 (2C), 126.86, 128.98, 130.02 (2C), 134.14, 142.10, 143.76, 146.20, 148.75, 160.87. MS (ESI): $[M+1]^+$=307.4.

*5.1.4.9. 3-[2-(4-Ethylphenyl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl]aniline (**13i**).* Following general procedure C, compound **13i** was isolated as a yellow solid. Yield 76%, mp 158-160 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 1.22 (t, J=7.6 Hz, 3H), 2.69 (q, J=7.6 Hz, 2H), 5.11 (bs, 2H), 6.49 (dt, J=7.2 and 1.6 Hz, 1H), 7.02 (m, 2H), 7.13 (t, J=1.6 Hz, 1H), 7.43 (d, J=8.2 Hz, 2H), 7.85 (d, J=8.2 Hz, 2H), 8.54 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 15.12, 27.98, 109.95, 110.20, 112.61, 113.12, 126.57 (2C), 127.12, 128.88 (2C), 128.97, 134.14, 143.77, 146.22, 148.20, 148.75, 160.87. MS (ESI): $[M+1]^+$=321.4.

*5.1.4.10. 3-[2-(4-Methoxybenzyl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl]aniline (**13k**).* Following general procedure C, compound **13k** was isolated as a green solid. Yield 72%, mp 145-147 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 3.73 (s, 3H), 4.33 (s, 2H), 5.05 (bs, 2H), 6.43 (dt, J=8.0 and 1.8 Hz, 1H), 6.91 (m, 2H), 7.00 (dd, J=6.8 and 2.4 Hz, 2H), 7.06 (t, J=1.8 Hz, 1H), 7.29 (dd, J=6.8 and 2.4 Hz, 2H), 8.40 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 36.07, 55.07, 109.63, 110.27, 112.63, 113.06, 114.01, 114.26, 127.83, 129.02, 129.68, 130.29 (2C), 134.36, 145.65, 148.80, 158.60, 164.89. MS (ESI): $[M+1]^+$=321.4.

*5.1.4.11. 3-[2-(3-Methoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl]aniline (**13l**).* Following general procedure C, derivative **13l** was isolated as a yellow solid. Yield 71%, mp 195-197 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 3.87 (s, 3H), 5.12 (bs, 2H), 6.51 (dt, J=7.8 and 2.0 Hz, 1H), 7.03 (m, 2H), 7.13 (d, J=1.6 Hz, 1H), 7.20 (m, 1H), 7.45 (t, J=2.0 Hz, 1H), 7.52 (m, 2H), 8.57 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 55.41, 109.98, 110.22, 111.03, 112.64, 113.18, 117.91, 119.07, 128.99, 129.77, 130.78, 134.09, 143.91, 146.36, 148.77, 159.73, 160.60. MS (ESI): $[M+1]^+$=323.4.

*5.1.4.12. 3-[2-(3-Methoxybenzyl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl]aniline (**13m**).* Following general procedure C, compound **13m** was isolated as an orange solid. Yield 61%, mp 118-120 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 3.75 (s, 3H), 4.40 (s, 2H), 5.08 (bs, 2H), 6.46 (dt, J=8.0 and 1.6 Hz, 1H), 6.84 (m, 1H), 6.91 (dd, J=8.0 and 2.0 Hz, 1H), 6.97 (m, 3H), 7.02 (t, J=1.6 Hz, 1H), 7.30 (t, J=8.0 Hz, 1H), 8.44 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 36.79, 54.98, 109.59, 110.18, 112.53, 112.79, 112.99, 114.73, 121.14, 128.93, 129.62, 129.89, 134.25, 137.37, 145.61, 148.71, 159.44, 163.95. MS (ESI): $[M+1]^+$=337.4.

*5.1.4.13. 3-[2-(3,4-Dimethoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl]aniline (**13n**).* Following general procedure C, derivative **13n** was obtained as a yellow solid. Yield 67%, mp 162-164 °C. ^{1}H-NMR (200 MHz, d_6-DMSO) δ: 3.86 (s, 3H), 3.88 (s, 3H), 5.11 (bs, 2H), 6.48 (dt, J=8.6 and 2.0 Hz, 1H), 7.02 (m, 2H), 7.12 (d, J=8.4 Hz, 1H), 7.16 (s, 1H), 7.46 (t, J=2.0 Hz, 1H), 7.48 (dd, J=8.4 and 2.0 Hz, 1H), 8.51 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 55.62, 55.71, 108.65, 109.87, 110.19, 111.92, 112.60, 113.09, 120.34, 122.00, 128.97, 134.20, 143.74, 146.05, 148.77, 149.15, 151.84, 160.78. MS (ESI): $[M+1]^+$=353.4.

*5.1.4.14. 3-[2-(4-Ethoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl]aniline (**13o**).* Following general procedure C, derivative **13o** was isolated as a white solid. Yield 62%, mp 169-171 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 1.34 (t, J=7.2 Hz, 3H), 4.12 (q, J=7.2 Hz, 2H), 5.10 (bs, 2H), 6.50 (dt, J=7.2 and 2.0 Hz, 1H), 7.01 (m, 2H), 7.68 (t, J=8.2 Hz, 1H), 7.11 (dd, J=6.8 and

2.0 Hz, 2H), 7.86 (dd, J=6.8 and 2.0 Hz, 2H), 8.51 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 14.39, 48.62, 109.91, 110.17, 112.58, 113.07, 115.27 (2C), 121.81, 128.26 (2C), 128.97, 134.21, 143.68, 146.01, 148.74, 160.65, 161.26. MS (ESI): $[M+1]^+$=337.4.

*5.1.4.15. 3-(3-Phenylimidazo[2,1-b][1,3]thiazol-6-yl)aniline (**14a**).* Following general procedure C, compound **14a** was isolated as a yellow solid. Yield 56%, mp 180-182 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 5.05 (bs, 2H), 6.48 (dt, J=7.8 and 1.6 Hz, 1H), 7.02 (m, 2H), 7.17 (t, J=1.6 Hz, 1H), 7.46 (s, 1H), .7.57 (m, 3H), 7.82 (dd, J=8.4 and 1.6 Hz, 2H), 8.27 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 108.06, 109.14, 110.53, 112.86, 112.92, 126.61 (2C), 128.86, 129.22 (2C), 129.37, 131.71, 134.41, 134.62, 145.12, 147.38, 148.64. MS (ESI): $[M+1]^+$=292.4.

*5.1.4.16. 3-[3-(4-Chlorophenyl)imidazo[2,1-b][1,3]thiazol-6-yl]aniline (**14b**).* Following general procedure C, **14b** was isolated as a yellow solid. Yield 67%, mp 75-77 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 5.02 (bs, 2H), 6.44 (dt, J=7.2 and 1.6 Hz, 1H), 6.94 (m, 2H), 7.13 (t, J=1.6 Hz, 1H), 7.50 (s, 1H), 7.61 (dt, J=8.8 and 1.6 Hz, 2H), 7.83 (dt, J=8.8 and 1.6 Hz, 2H),), 8.26 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 108.23, 110.00, 110.62, 110.93, 112.93, 113.03, 128.10, 128.55 (2C), 128.95, 129.30 (2C), 130.65, 133.96, 134.46, 147.54, 148.74. MS (ESI): $[M+1]^+$=326.7.

*5.1.4.17. 3-[3-(4-Methoxyphenyl)imidazo[2,1-b][1,3]thiazol-6-yl]aniline (**14c**).* Following general procedure C, compound **14c** was obtained as a yellow solid. Yield 68%, mp 68-70 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 3.85 (s, 3H), 5.05 (bs, 2H), 6.46 (dt, J=7.6 and 1.6 Hz, 1H), 7.02 (m, 2H), 7.12 (s, 1H), 7.14 (dd, J=6.8 and 2.0 Hz, 2H), 7.31 (s, 1H), 7.76 (dd, J=6.8 and 2.0 Hz, 2H), 8.21 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 55.26, 107.40, 107.91, 110.50, 112.82, 114.57 (2C), 121.63, 125.12, 128.18 (2C), 128.85, 131.53, 133.12, 134.45, 147.31, 148.63, 159.92. MS (ESI): $[M+1]^+$=322.4.

*5.1.5. General procedure D for the synthesis of compounds **7a-o** and **8a-c***. To an ice-cooled solution of aniline derivative **13a-o** and **14a-c** (1.00 mmol) in dry DMF (5 mL) were added a mixture of EDCI (383 mg, 2.00 mmol) and α-bromoacrylic acid (2.00 mmol, 306 mg). The reaction mixture was stirred at room temperature for 18 h and then concentrated under reduced pressure. The residue was dissolved with a mixture of CH_2Cl_2 (15 mL) and water (5 mL), and the organic phase was washed with brine (5 mL), dried over Na_2SO_4 and evaporated to dryness *in vacuo.* The resulting crude residue was purified by column chromatography on silica gel.

*5.1.5.1. 2-Bromo-N-[3-(2-phenylimidazo[2,1-b][1,3,4]thiadiazol-6-yl)phenyl]acrylamide (**7a**).* Following general procedure D, the crude residue purified by flash chromatography, using ethyl acetate:petroleum ether 4:6 (v:v) for elution, furnished **7a** as a yellow solid. Yield 49%, mp 196-198 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 6.33 (d, J=3.2 Hz, 1H), 6.79 (d, J=3.2 Hz, 1H), 7.40 (t, J=7.8 Hz, 1H), 7.61 (d, J=7.8 Hz, 1H), 7.63 (m, 4H), 7.96 (m, 2H), 8.23 (s, 1H), 8.73 (s, 1H), 10.3 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 110.73, 115.54 116.93, 119.52, 120.73, 125.02, 125.86, 126.62 (2C), 128.94, 129.55 (2C), 131.99, 134.16, 138.61, 144.38, 145.17, 160.93, 161.29. MS (ESI): $[M+1]^+$=426.4 and 428.4. Anal. calcd for $C_{19}H_{13}BrN_4OS$ C, 53.66; H, 3.08; N, 13.17; found: C, 53.52; H, 2.93; N, 13.01.

*5.1.5.2. N-[3-(2-benzylimidazo[2,1-b][1,3,4]thiadiazol-6-yl)phenyl]-2-bromoacrylamide (**7b**).* Following general procedure D, the crude residue purified by flash chromatography, using ethyl acetate:petroleum ether 4:6 (v:v) for elution, furnished **7b** as a white solid. Yield 68%, mp 144-146 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 4.43 (s, 2H), 6.29 (d, J=3.2 Hz, 1H), 6.75 (d, J=3.2 Hz, 1H), 7.24 (t, J=7.8 Hz, 1H), 7.32 (m, 5H), 7.52 (m, 2H), 8.14 (s, 1H), 8.67 (s, 1H), 10.3 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 37.35, 110.85, 117.40, 119.89, 121.18, 125.56, 126.37, 127.96, 128.36, 129.36 (2C), 129.59 (2C), 134.82, 136.46, 139.10,

145.02, 154.73, 161.44, 165.12. MS (ESI): $[M+1]^+$=440.4 and 442.4. Anal. calcd for $C_{20}H_{15}BrN_4OS$ C, 54.68; H, 3.44; N, 12.75; found: C, 54.46; H, 3.31; N, 12.58.

*5.1.5.3. 2-Bromo-N-{3-[2-(2-phenylethyl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl]phenyl}acrylamide (**7c**).* Following general procedure D, the crude residue purified by flash chromatography, using ethyl acetate:petroleum ether 4:6 (v:v) for elution, furnished **7c** as a yellow solid. Yield 49%, mp 108-110 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 3.08 (t, J=7.6 Hz, 2H), 3.37 (t, J=7.6 Hz, 2H), 6.31 (d, J=3.2 Hz, 1H), 6.77 (d, J=3.2 Hz, 1H), 7.21 (m, 1H), 7.30 (m, 4H), 7.38 (t, J=7.8 Hz, 1H), 7.53 (dt, J=7.8 and 2.0 Hz, 1H), 7.59 (dt, J=7.8 and 2.0 Hz, 1H), 8.16 (t, J=2.0 Hz, 1H), 8.57 (s, 1H), 10.3 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 32.65, 33.60, 110.26, 116.83, 119.34, 120.61, 125.02, 125.83, 126.33, 128.35 (2C), 128.40 (2C), 128.87, 134.29, 138.55, 139.48, 144.52, 144.67, 160.90, 164.32. MS (ESI): $[M+1]^+$=454.3 and 456.4. Anal. calcd for $C_{21}H_{17}BrN_4OS$ C, 55.64; H, 3.78; N, 12.36; found: C, 55.48; H, 3.58; N, 12.21.

*5.1.5.4. 2-Bromo-N-{3-[2-(2-thienyl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl]phenyl}acrylamide (**7d**).* Following general procedure D, the crude residue purified by flash chromatography, using ethyl acetate:petroleum ether 1:1 (v:v) for elution, furnished **7d** as a white solid. Yield 44%, mp 166-168 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 6.32 (d, J=3.2 Hz, 1H), 6.78 (d, J=3.2 Hz, 1H), 7.28 (dd, J=5.2 and 4.0 Hz, 1H), 7.39 (t, J=7.8 Hz, 1H), 7.56 (d, J=7.8 Hz, 1H), 7.61 (d, J=7.8 Hz, 1H), 7.88 (d, J=4.0 Hz, 1H), 7.96 (d, J=5.2 Hz, 1H), 8.21 (s, 1H), 8.67 (s, 1H), 10.3 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 110.71, 116.87, 119.50, 120.67, 125.02, 125.85, 128.64, 128.95, 130.56, 131.26, 131.44, 134.14, 138.62, 143.97, 145.12, 155.44, 160.93. MS (ESI): $[M+1]^+$=432.3 and 434.3. Anal. calcd for $C_{17}H_{11}BrN_4OS_2$ C, 47.34; H, 2.57; N, 12.99; found: C, 47.18; H, 2.42; N, 12.78.

*5.1.5.5. 2-Bromo-N-{3-[2-(4-fluorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl]phenyl}acrylamide (**7e**).* Following general procedure D, the crude residue purified by flash

chromatography, using ethyl acetate:petroleum ether 4:6 (v:v) for elution, furnished **7e** as a white solid. Yield 48%, mp 198-200 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 6.31 (d, J=3.2 Hz, 1H), 6.76 (d, J=3.2 Hz, 1H), 7.36 (t, J=8.0 Hz, 1H), 7.42 (dt, J=8.8 and 2.0 Hz, 2H), 7.54 (dd, J=8.0 and 1.2 Hz, 1H), 7.61 (dd, J=8.0 and 1.2 Hz, 1H), 8.00 (dt, J=8.8 and 2.0 Hz, 2H), 8.21 (t, J=1.2 Hz, 1H), 8.70 (s, 1H), 10.3 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 110.27, 117.16, 117.38, 117.48, 120.07, 121.28, 125.57, 126.40, 129.48, 129.70 (2C), 129.80 (2C), 134.69, 139.16, 144.99, 145.70, 160.74, 161.48. MS (ESI): $[M+1]^+$=444.4 and 446.4. Anal. calcd for $C_{19}H_{12}BrFN_4OS$ C, 51.48; H, 2.73; N, 12.64; found: C, 51.36; H, 2.53; N, 12.48.

*5.1.5.6. 2-Bromo-N-{3-[2-(4-chlorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl]phenyl}acrylamide (**7f**).* Following general procedure D, the crude residue purified by flash chromatography, using ethyl acetate:petroleum ether 4:6 (v:v) for elution, furnished **7f** as a white solid. Yield 51%, mp 218-220 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 6.30 (d, J=3.2 Hz, 1H), 6.79 (d, J=3.2 Hz, 1H), 7.39 (t, J=8.0 Hz, 1H), 7.56 (d, J=7.6 Hz, 1H), 7.63 (d, J=7.6 Hz, 1H), 7.63 (dd, J=6.8 and 2.0 Hz, 2H), 7.98 (dd, J=6.8 and 2.0 Hz, 2H), 8.23 (s, 1H), 8.73 (s, 1H), 10.3 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 110.73, 116.94, 119.55, 120.74, 125.00, 125.85, 128.33 (2C), 128.93, 129.59 (2C), 131.03, 134.09, 136.51, 138.61, 144.43, 145.28, 160.09, 160.91. MS (ESI): $[M+1]^+$=460.7 and 462.7. Anal. calcd for $C_{19}H_{12}BrClN_4OS$ C, 49.64; H, 2.63; N, 12.19; found: C, 49.45; H, 2.52; N, 12.03.

*5.1.5.7. 2-Bromo-N-{3-[2-(4-chlorobenzyl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl]phenyl}acrylamide (**7g**).* Following general procedure D, the crude residue purified by flash chromatography, using ethyl acetate:petroleum ether 4:6 (v:v) for elution, furnished **7g** as a white solid. Yield 55%, mp 157-159 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 4.45 (s, 2H), 6.29 (d, J=3.2 Hz, 1H), 6.75 (d, J=3.2 Hz, 1H), 7.32 (t, J=8.0 Hz, 1H), 7.43 (s, 4H), 7.52 (dt, J=8.0 and 2.0 Hz, 1H), 7.58 (dt, J=8.0 and 2.0 Hz, 1H), 8.15 (t, J=2.0 Hz, 1H), 8.58 (s, 1H), 10.3 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 36.08, 110.41, 116.95, 119.44, 120.72, 125.10,

125.91, 128.82 (2C), 128.97, 131.07 (2C), 132.20, 134.36, 135.02, 138.65, 144.61, 144.88, 160.98, 164.12. MS (ESI): $[M+1]^+$=474.8 and 476.8. Anal. calcd for $C_{20}H_{14}BrClN_4OS$ C, 50.70; H, 2.98; N, 11.83; found: C, 50.49; H, 2.78; N, 11.65.

*5.1.5.8. 2-Bromo-N-{3-[2-(4-methylphenyl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl]phenyl}acrylamide (**7h**).* Following general procedure D, the crude residue purified by flash chromatography, using ethyl acetate:petroleum ether 4:6 (v:v) for elution, furnished **7h** as a white solid. Yield 54%, mp 192-194 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 2.40 (s, 3H), 6.32 (d, J=3.2 Hz, 1H), 6.79 (d, J=3.2 Hz, 1H), 7.37 (m, 3H), 7.56 (dd, J=7.8 and 2.0 Hz, 1H), 7.63 (d, J=7.8 and 2.0 Hz, 1H), 7.84 (d, J=6.8 and 2.0 Hz, 2H), 8.22 (s, 1H), 8.69 (s, 1H), 10.3 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 20.97, 110.65, 116.89, 119.46, 120.70, 125.01, 125.84, 126.51 , 126.78, 128.92, 129.04, 130.04 (2C), 134.18, 138.59, 142.23, 144.23, 145.03, 160.91, 161.34. MS (ESI): $[M+1]^+$=440.3 and 442.4. Anal. calcd for $C_{20}H_{15}BrN_4OS$ C, 54.68; H, 3.44; N, 12.75; found: C, 54.53; H, 3.28; N, 12.56.

*5.1.5.9. 2-Bromo-N-{3-[2-(4-ethylphenyl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl]phenyl}acrylamide (**7i**).* Following general procedure D, the crude residue purified by flash chromatography, using ethyl acetate:petroleum ether 6:4 (v:v) for elution, furnished **7i** as a white solid. Yield 54%, mp 168-170 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 1.34 (t, J=7.2 Hz, 3H), 2.82 (q, J=7.2 Hz, 2H), 6.39 (d, J=3.2 Hz, 1H), 6.86 (d, J=3.2 Hz, 1H), 7.44 (t, J=7.8 Hz, 1H), 7.51 (d, J=8.8 Hz, 2H), 7.63 (d, J=7.8 Hz, 1H), 7.69 (d, J=7.8 Hz, 1H), 7.94 (d, J=8.8 Hz, 2H), 8.29 (s, 1H), 8.77 (s, 1H), 10.4 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 15.23, 28.10, 110.76, 117.00, 119.57, 120.81, 125.11, 125.94, 126.74 (2C), 126.83, 127.13, 129.01 (2C), 134.29, 138.70, 144.36, 145.15, 148.42, 161.02, 161.44. MS (ESI): $[M+1]^+$=454.3 and 456.4. Anal. calcd for $C_{21}H_{17}BrN_4OS$ C, 55.64; H, 3.78; N, 12.36; found: C, 55.46; H, 3.56; N, 12.19.

*5.1.5.10. 2-Bromo-N-{3-[2-(4-methoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl]phenyl}acrylamide (**7j**).* Following general procedure D, the crude residue purified by flash chromatography, using ethyl acetate:petroleum ether 4:6 (v:v) for elution, furnished **7j** as a white solid. Yield 53%, mp 202-204 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 3.84 (s, 3H), 6.30 (d, J=3.2 Hz, 1H), 6.77 (d, J=3.2 Hz, 1H), 7.12 (d, J=8.8 Hz, 2H), 7.35 (t, J=8.0 Hz, 1H), 7.54 (d, J=7.8 Hz, 1H), 7.60 (d, J=7.8 Hz, 1H), 7.87 (d, J=8.8 Hz, 2H), 8.19 (s, 1H), 8.65 (s, 1H), 10.3 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 55.64, 110.73, 115.04 (2C), 116.99, 119.53, 120.79, 122.00, 125.15, 125.97, 128.47 (2C), 129.04, 134.37, 138.72, 144.30, 144.98, 161.05, 161.20, 162.18. MS (ESI): $[M+1]^+$=456.3, and 457.4. Anal. calcd for $C_{20}H_{15}BrN_4O_2S$ C, 52.76; H, 3.32; N, 12.30; found: C, 52.45; H, 3.19; N, 12.11.

*5.1.5.11. 2-Bromo-N-{3-[2-(4-methoxybenzyl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl]phenyl}acrylamide (**7k**).* Following general procedure D, the crude residue purified by flash chromatography, using ethyl acetate:petroleum ether 4:6 (v:v) for elution, furnished **7k** as a white solid. Yield 51%, mp 165-167 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 3.73 (s, 3H), 4.34 (s, 2H), 6.29 (d, J=3.2 Hz, 1H), 6.75 (d, J=3.2 Hz, 1H), 6.91 (d, J=8.8 Hz, 2H), 7.30 (d, J=8.8 Hz, 2H), 7.36 (t, J=8.0 Hz, 1H), 7.52 (d, J=7.8 Hz, 1H), 7.57 (d, J=7.8 Hz, 1H), 8.15 (s, 1H), 8.56 (s, 1H), 10.3 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 36.08, 55.07, 110.34, 114.27 (2C), 116.93, 119.40, 120.70, 125.11, 125.91, 127.76, 128.96, 130.32 (2C), 134.39, 138.64, 144.51, 144.90, 158.61, 160.97, 165.44. MS (ESI): $[M+1]^+$=470.9 and 472.0. Anal. calcd for $C_{21}H_{17}BrN_4O_2S$ C, 52.76; H, 3.32; N, 12.30; found: C, 52.58; H, 3.19; N, 12.16.

*5.1.5.12. 2-Bromo-N-{3-[2-(3-methoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl]phenyl}acrylamide (**7l**).* Following general procedure D, the crude residue purified by flash chromatography, using ethyl acetate:petroleum ether 4:6 (v:v) for elution, furnished **7l** as a white solid. Yield 62%, mp 153-155 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 3.86 (s, 3H), 6.31 (d, J=3.2 Hz, 1H), 6.77 (d, J=3.2 Hz, 1H), 7.22 (m, 1H), 7.38 (t, J=8.0 Hz, 1H), 7.45 (m, 1H),

7.50 (dd, J=4.0 and 2.4 Hz, 2H), 7.54 (dt, J=7.8 and 2:0 Hz, 1H), 7.61 (d, J=7.8 and 2.0 Hz, 1H), 8.20 (t, J=2.0 Hz, 1H), 8.70 (s, 1H), 10.3 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 55.42, 110.67, 111.10, 116.93, 118.00, 119.12, 119.52, 119.73, 120.74, 125.01, 125.85, 125.96, 128.93, 130.79, 134.13, 138.61, 144.38, 145.20, 159.73, 161.06. MS (ESI): $[M+1]^+$=456.2 and 458.3. Anal. calcd for $C_{20}H_{15}BrN_4O_2S$ C, 52.76; H, 3.32; N, 12.30; found: C, 52.51; H, 3.20; N, 12.15.

*5.1.5.13. 2-Bromo-N-{3-[2-(3-methoxybenzyl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl]phenyl}acrylamide (**7m**).* Following general procedure D, the crude residue purified by flash chromatography, using ethyl acetate:petroleum ether 4:6 (v:v) for elution, furnished **7m** as a white solid. Yield 42%, mp 68-70 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 3.75 (s, 3H), 4.41 (s, 2H), 6.31 (d, J=3.2 Hz, 1H), 6.77 (d, J=3.2 Hz, 1H), 6.88 (dt, J=8.0 and 1.6 Hz, 1H), 6.99 (m, 2H), 7.28 (t, J=7.6 Hz, 1H), 7.36 (t, J=8.0 Hz, 1H), 7.54 (d, J=8.0 Hz, 1H), 7.57 (d, J=8.0 Hz, 1H), 8.17 (s, 1H), 8.60 (s, 1H), 10.3 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 36.80, 54.98, 110.30, 112.82, 114.76, 116.85, 119.34, 120.62, 121.15, 125.02, 125.83, 128.88, 129.91, 134.26, 137.29, 138.56, 144.44, 146.40, 159.44, 160.88, 164.49. MS (ESI): $[M+1]^+$=470.8 and 472.0. Anal. calcd for $C_{21}H_{17}BrN_4O_2S$ C, 52.76; H, 3.32; N, 12.30; found: C, 52.54; H, 3.20; N, 12.18.

*5.1.5.14. 2-Bromo-N-{3-[2-(3,4-dimethoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl]phenyl}acrylamide (**7n**).* Following general procedure D, the crude residue purified by flash chromatography, using ethyl acetate:petroleum ether 6:4 (v:v) for elution, furnished **7n** as a white solid. Yield 51%, mp 78-80 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 3.86 (s, 3H), 3.88 (s, 3H), 6.32 (d, J=3.2 Hz, 1H), 6.79 (d, J=3.2 Hz, 1H), 7.15 (d, J=8.8 Hz, 1H), 7.39 (t, J=7.6 Hz, 1H), 7.47 (d, J=2.0 Hz, 1H), 7.50 (d, J=8.8 and 2.0 Hz, 1H), 7.57 (dd, J=7.8 and 2.0 Hz, 1H), 7.62 (dd, J=7.8 and 2.0 Hz, 1H), 8.21 (t, J=2.0 Hz, 1H), 8.66 (s, 1H), 10.3 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 55.72 (2C), 108.72, 110.56, 111.94, 116.89, 119.43, 120.44, 120.70, 121.89, 125.02, 125.85, 128.92, 134.23, 138.60, 144.22, 144.87, 149.16, 151.92, 160.93,

161.24. MS (ESI): $[M+1]^+$=486.3 and 488.3. Anal. calcd for $C_{21}H_{17}BrN_4O_3S$ C, 51.97; H, 3.53; N, 11.54; found: C, 51.78; H, 3.38; N, 11.32.

*5.1.5.15. 2-Bromo-N-{3-[2-(4-ethoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl]phenyl}acrylamide (**7o**).* Following general procedure D, the crude residue purified by flash chromatography, using ethyl acetate:petroleum ether 1:1 (v:v) for elution, furnished **7o** as a white solid. Yield 66%, mp 180-182 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 1.36 (t, J=7.2 Hz, 3H), 4.13 (q, J=7.2 Hz, 2H), 6.32 (d, J=3.2 Hz, 1H), 6.79 (d, J=3.2 Hz, 1H), 7.12 (d, J=8.8 Hz, 2H), 7.38 (t, J=8.0 Hz, 1H), 7.55 (d, J=8.0 Hz, 1H), 7.61 (d, J=8.0 Hz, 1H), 7.87 (d, J=8.8 Hz, 2H), 8.21 (s, 1H), 8.66 (s, 1H), 10.3 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 14.40, 54.82, 109.62, 110.61, 115.30 (2C), 116.89, 119.42, 120.68, 121.73, 125.03, 125.84, 128.35 (2C), 128.91, 134.26, 138.61, 144.85, 160.92, 161.12, 161.36. MS (ESI): $[M+1]^+$=470.4 and 472.4. Anal. calcd for $C_{21}H_{17}BrN_4O_2S$ C, 52.76; H, 3.32; N, 12.30; found: C, 52.52; H, 3.21; N, 12.09.

*5.1.5.16. 2-Bromo-N-[3-(3-phenylimidazo[2,1-b][1,3]thiazol-6-yl)phenyl]acrylamide (**8a**).* Following general procedure D, the crude residue purified by flash chromatography, using ethyl acetate:petroleum ether 4:6 (v:v) for elution, furnished **8a** as a white solid. Yield 53%, mp 124-126 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 6.32 (d, J=3.2 Hz, 1H), 6.79 (d, J=3.2 Hz, 1H), 7.37 (t, J=7.6 Hz, 1H), 7.51 (s, 1H), 7.57 (m, 4H), 7.68 (d, J=7.6 Hz, 1H), 7.84 (d, J=7.2 Hz, 2H), 8.20 (s, 1H), 8.44 (s, 1H), 10.3 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 108.89, 109.74, 117.20, 119.33, 120.97, 122.11, 125.02, 125.85, 126.70 (2C), 128.83, 129.05, 129.24 (2C), 131.81, 134.25, 138.48, 145.96, 147.33, 160.84. MS (ESI): $[M+1]^+$=425.4 and 427.4. Anal. calcd for $C_{20}H_{14}BrN_3OS$ C, 56.61; H, 3.33; N, 9.90; found: C, 56.38; H, 3.21; N, 9.77.

*5.1.5.17. 2-Bromo-N-{3-[3-(4-chlorophenyl)imidazo[2,1-b][1,3]thiazol-6-yl]phenyl}acrylamide (**8b**).* Following general procedure D, the crude residue purified by flash chromatography, using ethyl acetate:petroleum ether 1:1 (v:v) for elution, furnished **8b** as a

white solid. Yield 62%, mp 168-170 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 6.30 (d, J=3.2 Hz, 1H), 6.77 (d, J=3.2 Hz, 1H), 7.35 (t, J=7.6 Hz, 1H), 7.54 (s, 1H), 7.56 (d, J=7.6 Hz, 1H), 7.62 (d, J=8.0 Hz, 2H), 7.66 (m, 1H), 7.87 (d, J=8.0 Hz, 2H), 8.18 (t, J=1.6 Hz, 1H), 8.42 (s, 1H), 10.3 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 108.89, 110.35, 117.21, 119.30, 120.93, 125.04, 125.84, 127.93, 128.54 (2C), 128.80, 129.23 (2C), 130.60, 133.95, 134.42, 138.48, 146.27, 149.09, 160.84. MS (ESI): $[M+1]^+$=459.8 and 461.9. Anal. calcd for $C_{20}H_{13}BrClN_3OS$ C, 52.36; H, 2.86; N, 9.16; found: C, 52.19; H, 2.76; N, 9.01.

*5.1.5.18. 2-Bromo-N-{3-[3-(4-methoxyphenyl)imidazo[2,1-b][1,3]thiazol-6-yl]phenyl}acrylamide (**8c**).* Following general procedure D, the crude residue purified by flash chromatography, using ethyl acetate:petroleum ether 1:1 (v:v) for elution, furnished **8c** as a white solid. Yield 58%, mp 168-170 °C. ^{1}H-NMR (400 MHz, d_6-DMSO) δ: 3.86 (s, 3H), 6.32 (d, J=3.2 Hz, 1H), 6.78 (d, J=3.2 Hz, 1H), 7.13 (d, J=6.8 Hz, 2H), 7.35 (t, J=8.0 Hz, 1H), 7.38 (s, 1H), 7.56 (d, J=8.0 Hz, 1H), 7.69 (d, J=8.0 Hz, 1H), 7.77 (d, J=6.8 Hz, 2H), 8.19 (s, 1H), 8.37 (s, 1H), 10.3 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ: 55.29, 107.87, 108.67, 114.59 (2C), 117.15, 119.21, 120.91, 121.53, 125.04, 125.83, 128.26 (2C), 128.80, 131.59, 134.49, 138.47, 146.14, 149.06, 159.99, 160.84. MS (ESI): $[M+1]^+$=455.3 and 457.4. Anal. calcd for $C_{21}H_{16}BrN_3O_2S$ C, 55.51; H, 3.55; N, 9.25; found: C, 55.35; H, 3.38; N, 9.09.

5.2. Biological assays

5.2.1. Materials and Methods

Stock solutions of 100 mM of compounds were made in dimethyl sulfoxide (DMSO) and aliquots were frozen at -20 ºC. Poly(vinylidene difluoride) (PVDF) membranes were purchased from Millipore (Billerica, MA, USA). Acrylamide, bisacrylamide, ammonium persulfate and *N, N, N´, N´*-tetramethylethylenediamine were from Bio-Rad (Hercules, CA, USA). Antibodies for caspases-3, -8 and -9 were purchased from Assay Designs (Ann Arbor, MI, USA) and anti-cytochrome *c* was from–BD PharMingen (San Diego, CA, USA).

Secondary antibodies were from GE Healthcare (Little Chalfont, UK). All other chemicals were obtained from Sigma (Saint Louis, MO, USA).

5.2.2. Cell growth inhibitory activity.

Murine leukemia L1210, murine mammary carcinoma FM3A, human T-lymphocyte CEM and human cervix carcinoma (HeLa) cells were suspended at 300,000-500,000 cells mL^{-1} of culture medium, and 100 μL of the cell suspension was added to 100 μL of an appropriate dilution of the test compounds in wells of 96-well microtiter plates. After incubation at 37 °C for two (L1210, FM3A), three (CEM) or four (HeLa) days, the tumor cell number was determined using a Coulter counter. The IC_{50} was defined as the compound concentration required to inhibit cell proliferation by 50%. Data are expressed as the mean ± SE from the dose-response curves of at least two to three independent experiments.

5.2.3. Cell culture

The human leukemia HL-60, U937 and SK-MEL-1 cells were obtained from DSMZ (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) and grown in RPMI 1640 medium containing 10% (v/v) heat-inactivated fetal bovine serum, 100 units mL^{-1} penicillin and 100 μg mL^{-1} streptomycin at 37 ºC in a humidified atmosphere containing 5% CO_2. The U937 cell line overexpressing human Bcl-2 (kindly provided by Dr. Jacqueline Bréard) was cultured as described [34]. The cell numbers were counted by a hematocytometer, and the viability was always greater that 95% in all experiments as assayed by the trypan blue exclusion method. Further dilutions of stock solutions of compounds were made in culture media just before use. In all experiments, the final concentration of DMSO did not exceed 0.1% (v/v), a concentration which is non-toxic to the cells. The same concentration was present in the control groups.

*5.2.4. Cytotoxicity of compounds **7d-e**, **7n** and **8c** on human leukemia cells*

The cytotoxicity of compounds on human leukemia cells was analyzed by colorimetric 3-(4,5-dimethyl-2-thiazolyl-)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay as described [35]. Briefly, 10^4 exponentially growing cells were seeded in 96-well microculture plates in the presence of various compound concentrations. After the addition of MTT (0.5 mg mL^{-1}) the tumor cells were incubated at 37 ºC for 4 h. Sodium dodecyl sulfate (SDS) (10% w/v) in 0.05 M HCl was added to the wells and then incubated overnight at room temperature under dark conditions. The extension of reduction of MTT was quantified by absorbance measurement at 570 nm. Concentrations inducing a 50% inhibition of cell growth (IC_{50}) were determined graphically using the curve-fitting algorithm of the computer software Prism 4.0 (GraphPad). Values are means ± S.E. from three independent experiments, each performed in triplicate.

5.2.5. Evaluation of apoptosis

The rate of apoptotic cell death was analyzed by fluorescent microscopy and by flow-cytometric analysis of propidium iodide (PI)-stained nuclei as described below.

5.2.6. Fluorescent microscopy analysis

Cells were harvested and fixed in 3% paraformaldehyde and incubated at room temperature for 10 min. The fixative was removed and the cells were washed with phosphate-buffered saline (PBS), resuspended in 30-50 μL of PBS containing 20 μg mL^{-1} bis-benzimide trihydrochloride (Hoechst 33258) and incubated at room temperature for 15 min. Stained nuclei were visualized using a Zeiss fluorescent microscopy.

5.2.7. Quantification of apoptosis by flow cytometry

To study changes in the cell DNA content, histogram measurements of hypodiploid DNA formation was performed by flow cytometry using a BD FACSVerse™ cytometer (BD Biosciences, San Jose, CA, USA). Cells were collected and centrifuged at 500 x *g*, washed with PBS and resuspended in 50 μL of PBS. Following dropwise addition of 1 mL of ice-cold 75% ethanol, fixed cells were stored at -20 ºC for 1 h. Samples were then centrifuged at 500 x

g and washed with PBS before resuspension in 1 mL of PBS containing 50 μg mL^{-1} propidium iodide and 100 μg mL^{-1} RNase A and incubated for 1 h at 37 ºC in the dark. The percentage of cells with decreased DNA staining, composed of apoptotic cells resulting from either fragmentation or decreased chromatin, was determined for a minimum of 10,000 cells per experimental condition. Cell debris were excluded from analysis by selective gating based on anterior and right angle scattering.

5.2.8. Analysis of DNA fragmentation

A late biochemical hallmark of apoptosis is the fragmentation of the genomic DNA. It is an irreversible event and occurs before changes in plasma membrane permeability. DNA isolation and gel electrophoresis were performed as described previously [35]. Briefly, cells (10^5) were collected by centrifugation, washed with PBS and incubated in 30 μL of lysis buffer [50 mM Tris-HCl (pH 8.0), 10 mM EDTA, 0.5% sodium dodecyl sulfate], containing 1 μg μL^{-1} RNase A at 37 ºC for 1 h. Then, 3 μL of proteinase K (10 μg μL^{-1}) was added and the mixture was incubated at 50 ºC for an additional 2 h. DNA was extracted with 100 μL of phenol-chloroform-isoamyl alcohol (24:24:1) and mixed with 5 μL of loading solution [10 mM EDTA, 1% (w/v) low melting-point agarose, 0.25% bromophenol blue and 40% sucrose, pH 8.0]. Samples were separated by electrophoresis in 2% agarose gels in TAE buffer [40 mM Tris-acetate (pH 8.0), 1 mM EDTA], visualized by ultraviolet illumination after ethidium bromide (0.5 μg mL^{-1}) staining and the images were captured by a digital camera (Digi Doc system, Bio-Rad).

5.2.9. Western blot analysis

Cells (1 x 10^6 mL^{-1}) were treated in the absence or presence of selected compounds and immunoblot analysis of caspases and cytochrome *c* was performed as previously described [34]. Briefly, after compound treatment, cells were washed twice with PBS and then resuspended in ice-cold buffer [20 mM HEPES (pH 7.5), 1.5 mM $MgCl_2$, 10 mM KCl, 1 mM

EDTA, 1 mM EGTA, 1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride and 5 μg mL^{-1} leupeptin, aprotinin, and pepstatin A] containing 250 mM sucrose. After 15 min incubation on ice, cells were lysed by pushing them several times through a 22-gauge needle and the lysate was spun down at 1,000 x *g* for 5 min at 4 ºC. The supernatant fraction was centrifuged at 105,000 x *g* for 45 min at 4 ºC and the resulting supernatant was used as the soluble cytosolic fraction. Protein concentration was measured by the Bradford method and samples containing equal amounts of proteins were boiled in sodium dodecyl sulfate sample buffer for 5 min before loading on a sodium dodecyl sulfate-polyacrylamide gel (12.5% for caspases and 15% for cytochrome *c*). Proteins were electrotransferred to poly(vinylidenedifluoride) (PVDF) membranes, blocked with 5% fat-free dry milk in Tris-buffered saline [50 mM Tris-HCl (pH 7.4), 150 mM NaCl] with 0.1% Tween 20 and then incubated with specific primary antibodies overnight at 4 ºC. After washing and incubation with an appropriate horseradish peroxidase-conjugated secondary antibody, the antigen-antibody complexes were visualized by enhanced chemiluminescence (Millipore) using the manufacturer´s protocol.

5.2.10. Assay of caspase activity

Caspase-3 like protease activity was evaluated by measuring proteolytic cleavage of the chromogenic substrate DEVD-*p*NA as previously described [34].

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Supplementary data. Antiproliferative activity for compounds **13a-o** and **14a-c** (Table 1s) and for selected hybrid compounds **7d-e**, **7n** and **8c** on human leukemia and melanoma cells (Table 2s). Supplementary data associated with this article can be found in the online version**.**

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Table 1. In vitro inhibitory effects of compounds **7a-o** and **8a-c** on the proliferation of murine leukemia (L1210), murine mammary carcinoma (FM3A), human T-lymphocyte (CEM) and human cervix carcinoma (HeLa) cells

Compound	IC_{50} (μM)[a]			
	L1210	FM3A	CEM	HeLa
7a	0.60±0.48	0.90±0.06	0.96±0.19	1.4±1.1
7b	3.0±1.5	3.0±0.8	4.2±0.6	4.5±1.2
7c	2.2±1.7	2.6±0.3	4.2±0.5	3.3±1.3
7d	0.17±0.01	0.37±0.17	0.41±0.24	0.67±0.24
7e	0.25±0.06	0.61±0.02	0.83±0.08	0.87±0.39
7f	1.4±1.1	2.0±1.0	3.2±0.3	3.0±1.8
7g	2.3±2.2	1.1±0.0	3.3±1.6	2.7±2.0
7h	0.92±0.48	1.2±0.3	1.7±1.2	2.0±1.5
7i	2.2±1.3	3.3±0.0	3.4±0.2	3.2±0.9
7j	0.94±0.58	0.89±0.28	1.2±0.6	2.3±1.5
7k	1.4±1.2	0.85±0.26	2.3±1.9	2.3±2.1
7l	0.58±0.41	0.50±0.18	1.2±0.4	1.1±0.7
7m	2.0±1.3	1.1±0.3	3.1±0.6	2.5±2.4
7n	0.042±0.00	0.11±0.02	0.38±0.36	0.61±0.39
7o	0.79±0.13	1.1±0.1	1.4±0.1	1.1±0.2
8a	1.3±0.9	1.3±0.8	2.8±1.7	3.4±1.0
8b	1.9±1.7	1.1±0.6	2.2±1.2	3.0±0.2
8c	0.20±0.03	0.62±0.10	0.50±0.22	0.86±0.17
melphalan	8.6 ± 0.3	3.6 ± 0.3	3.5 ± 0.1	1.9 ± 0.1

[a]IC_{50}= compound concentration required to inhibit tumor cell proliferation by 50%. Data are expressed as the mean ± SE from the dose-response curves of at least two to three independent experiments.

Figure Legends

Figure 1. Morphological changes and apoptosis induction by compounds **7d**, **7e**, **7n** and **8c** on human leukemia cells. (A) Upper panel: HL-60, U937 and U937/Bcl-2 cells were incubated with medium alone (control), or the indicated compounds. Images of cells in culture were obtained using an inverted phase contrast microscope. (B) Photomicrographs of representative fields of U937 and U937/Bcl-2 cells stained with Hoechst 33258 to evaluate nuclear chromatin condensation (i.e. apoptosis) after treatment with the indicated compounds (3 μM) for 8 h. Etoposide and staurosporine were used as positive controls.

Figure 2. (A) HL-60 cells were treated with compounds **7d**, **7e**, **7n** and **8c** for 24 h and subjected to flow cytometry. (B) HL-60 cells were incubated with the indicated concentrations of compounds **7d**, **7e**, **7n** and **8c** and apoptotic cells were quantitated as percentage of cells in the sub-G_1 region by flow cytometry. Values represent means ± SE of three independent experiments each performed in triplicate. (C) HL-60 and U937 cells were incubated with the indicated concentrations of compounds **7d**, **7e**, **7n** and **8c** for 24 h and genomic DNA was extracted, separated on an agarose gel and visualized under UV light by ethidium bromide staining. Etoposide was used as a positive control.

Figure 3. Involvement of caspases in the induction of apoptosis in human leukemia cells. (A) Cells were incubated in the presence of 3 μM of compounds **7d**, **7e**, **7n** and **8c** for 8 h and cytosolic extracts were assayed by immunoblotting. β-Actin was used as a loading control. (B) Cells were incubated as above and cell lysates were assayed by immunoblotting for the cleavage of procaspases-9, -8 and -3. (C) Caspase activation and response to compounds **7d**, **7e**, **7n** and **8c**. Cells were incubated with the indicated concentrations of compounds for 8 h and cell lysates were assayed for caspase-3/7 activity using the DEVD-*p*NA colorimetric substrate. Results are expressed as fold-increase in caspase activity compared with control.

Values represent means±S.E. This histogram is representative of two independent experiments each performed in triplicate.

Chart 1. Structure of imidazo[1,2-*b*][1,3,4]thiadiazole derivatives **1-4**, 3,6-diaryl imidazo[1,2-*b*][1,3]thiazole **5** and brostallicin (**6**).

Chart 2. Structure of α-bromoacryloyl hybrid derivatives **7a-o** and **8a-c**.

Scheme 1. **Reagents**: **a**: *m*-nitrophenacyl bromide, EtOH, reflux, 18 h; **b**: $SnCl_2$ H_2O, EtOH, reflux; **c**: α-bromoacrylic acid, EDCI, HOBt, DMF, rt, 18 h.

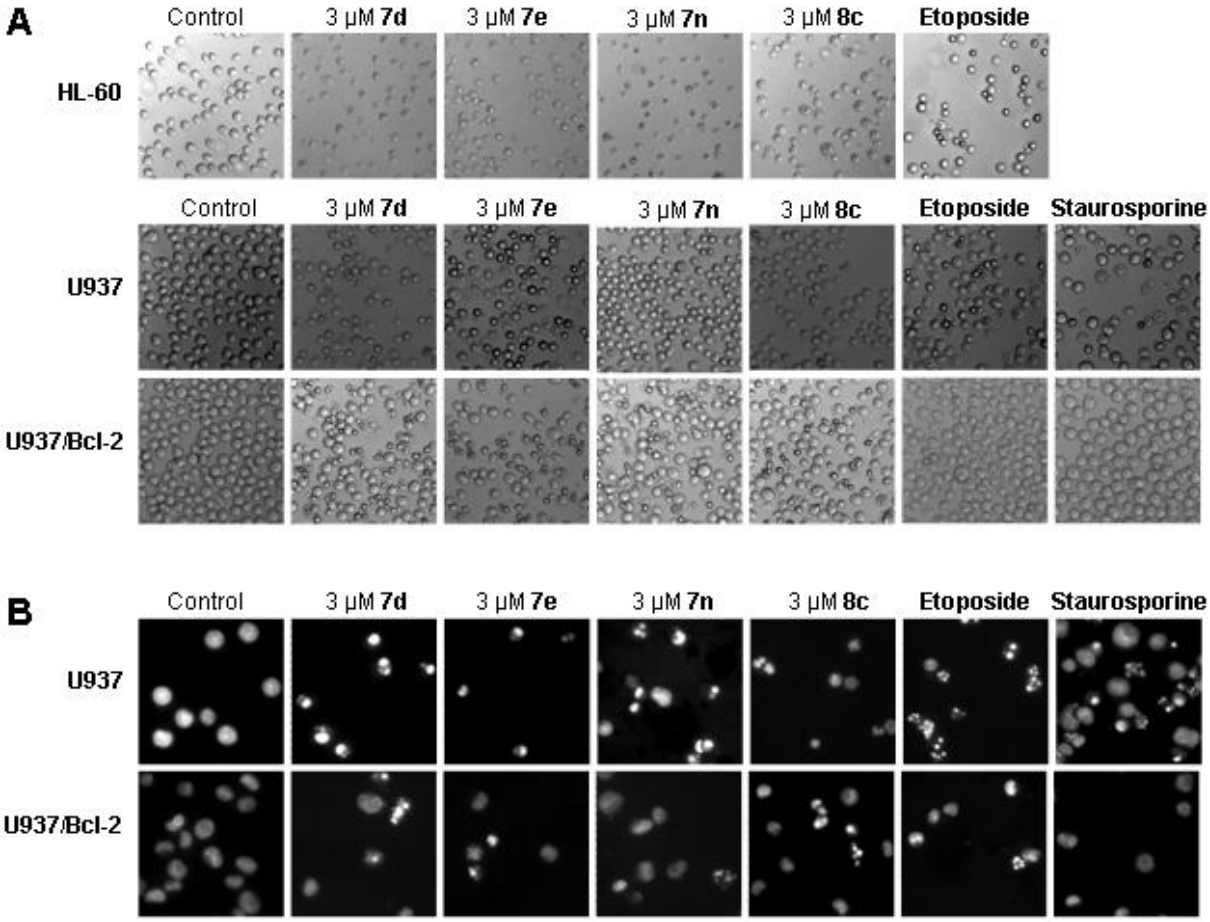

A
Control
3 μM 7d
3 μM 7e
3 μM 7n
3 μM 8c
Etoposide
HL-60
Control
3 μM 7d
3 μM 7e
3 μM 7n
3 μM 8c
Etoposide
Staurosporine
U937
U937/Bcl-2
B
Control
3 μM 7d
3 μM 7e
3 μM 7n
3 μM 8c
Etoposide
Staurosporine
U937
U937/Bcl-2

Figure 1

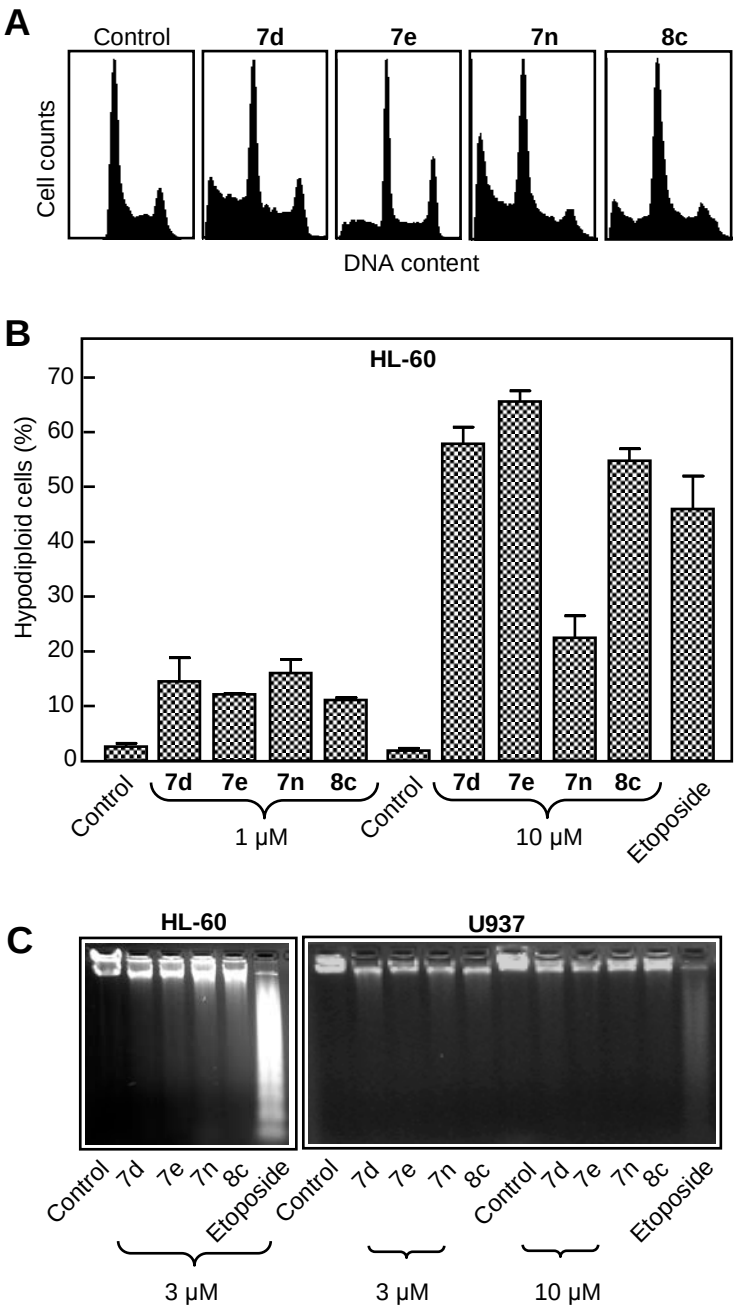

A
Control
7d
7e
7n
8c
Cell counts
DNA content
B
HL-60
Hypodiploid cells (%)
0
10
20
30
40
50
60
70
Control
7d
7e
7n
8c
1 μM
Control
7d
7e
7n
8c
10 μM
Etoposide
C
HL-60
U937
Control
7d
7e
7n
8c
Etoposide
Control
7d
7e
7n
8c
Control
7d
7e
7n
8c
Etoposide
3 μM
3 μM
10 μM

Figure 2

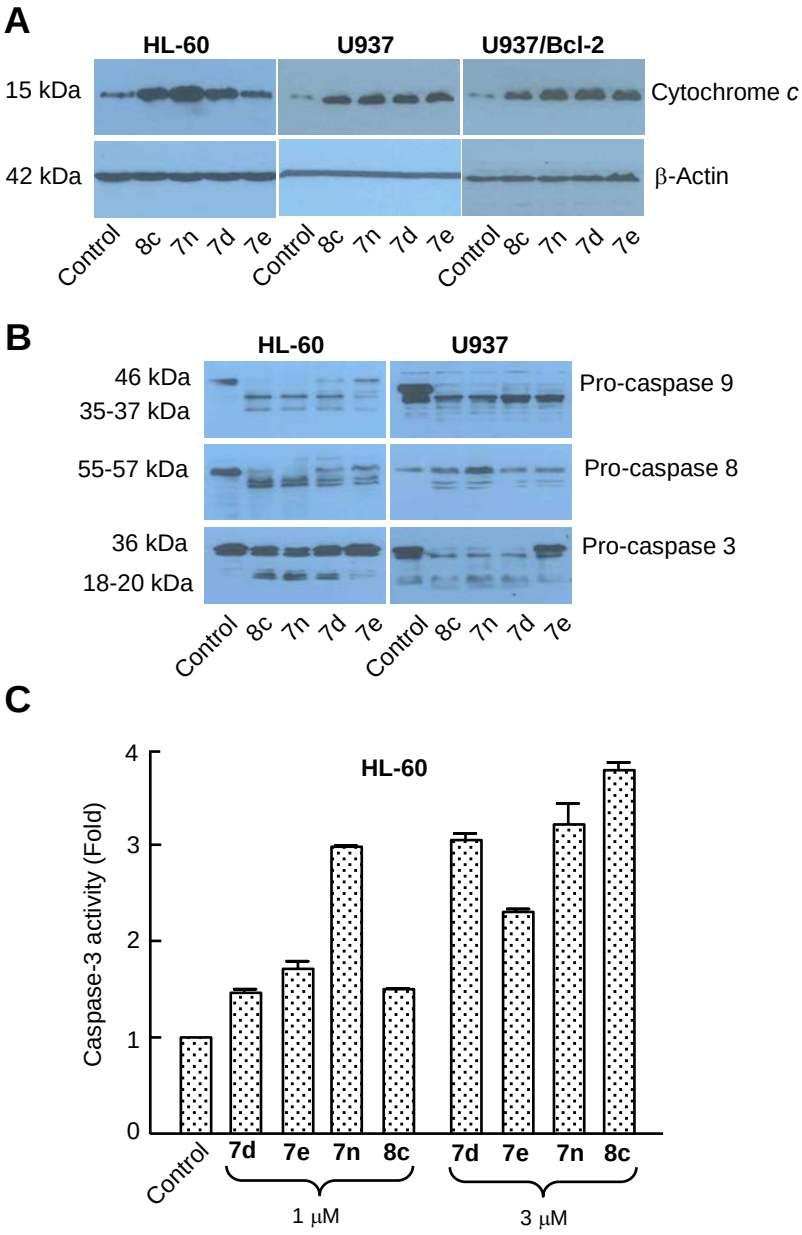
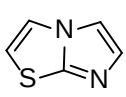
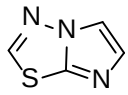

A
HL-60
U937
U937/Bcl-2
15 kDa
Cytochrome c
42 kDa
β-Actin
Control
8c
7n
7d
7e
B
HL-60
U937
46 kDa
35-37 kDa
Pro-caspase 9
55-57 kDa
Pro-caspase 8
36 kDa
18-20 kDa
Pro-caspase 3
C
HL-60
Caspase-3 activity (Fold)
0
1
2
3
4
1 μM
3 μM

Figure 3

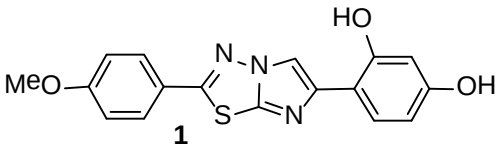
Chart 1.



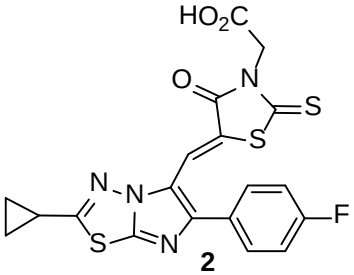
Imidazo[2,1-b][1,3]thiazole



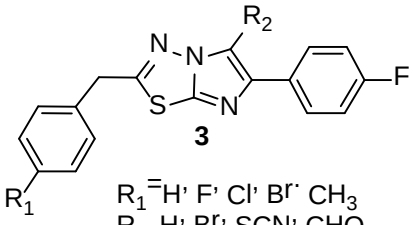
Imidazo[2,1-b][1,3,4]thiadiazole



1

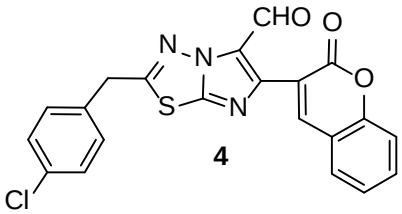


2



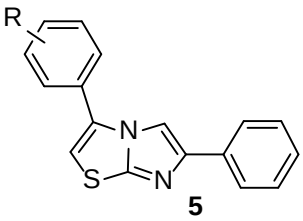
3

R_1 = H, F, Cl, Br, CH_3
R_2, H, Br, SCN, CHO
3a, R_1 = H, R_2 = CHO



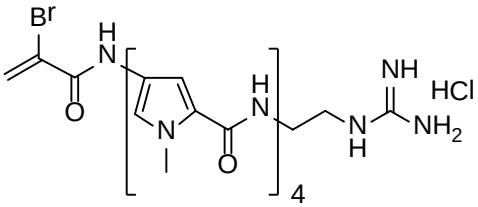
4

Cl



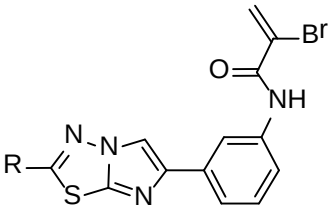
5

R = halogen, alkyl, alkoxy, CF_3
5a, R = *m*-CF_3



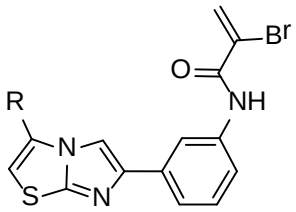
PNU-166196 or Brostallicin (**6**)

Chart 2



7a-o

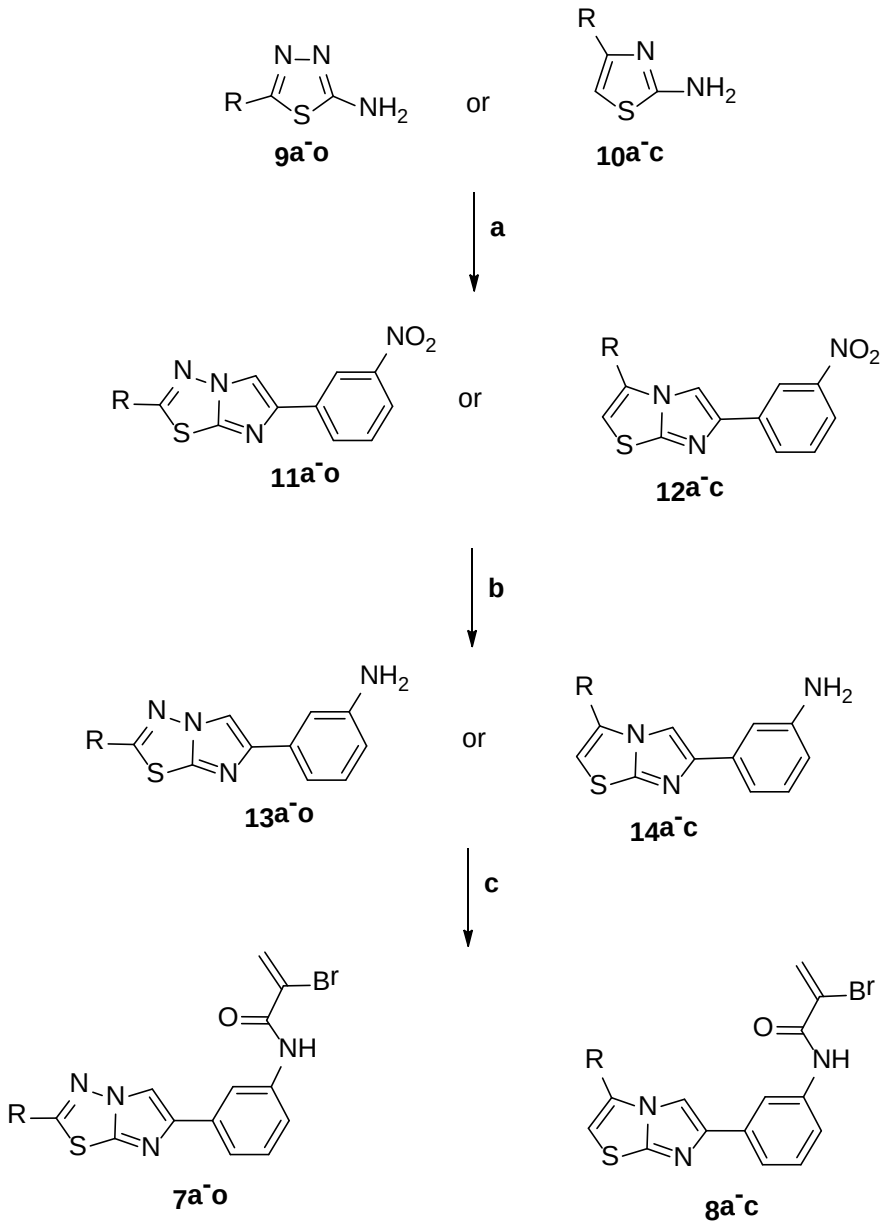
7a, R = C_6H_5
7b, R = $C_6H_5CH_2$
7c, R = $C_6H_5(CH_2)_2$
7d, R = Thien-1-yl
7e, R = *p*-F-C_6H_4
7f, R = *p*-Cl-C_6H_4
7g, R = *p*-Cl-$C_6H_4CH_2$
7h, R = *p*-CH_3-C_6H_4
7i, R = *p*-C_2H_5-C_6H_4
7j, R = *p*-OCH_3-C_6H_4
7k, R = *p*-OCH_3-$C_6H_4CH_2$
7l, R = *m*-OCH_3-C_6H_4
7m, R = *m*-OCH_3-$C_6H_4CH_2$
7n, R = *m*,*p*-$(OCH_3)_2$-C_6H_3
7o, R = *p*-OC_2H_5-C_6H_4



8a-c

8a, R = C_6H_5
8b, R = *p*-Cl-C_6H_4
8c, R = *p*-OCH_3-C_6H_4

Scheme 1



9a-o **10a-c**

11a-o **12a-c**

13a-o **14a-c**

7a-o **8a-c**

7a, **9a**, **11a**, **13a**, R = C_6H_5
7b, **9b**, **11b**, **13b**, R = $C_6H_5CH_2$
7c, **9c**, **11c**, **13c**, R = $C_6H_5(CH_2)_2$
7d, **9d**, **11d**, **13d**, R = Thien-1-yl
7e, **9e**, **11e**, **13e**, R = *p*-F-C_6H_4
7f, **9f**, **11f**, **13f**, R = *p*-Cl-C_6H_4
7g, **9g**, **11g**, **13g**, R = *p*-Cl-$C_6H_4CH_2$
7h, **9h**, **11h**, **13h**, R = *p*-CH_3-C_6H_4
7i, **9i**, **11i**, **13i**, R = *p*-C_2H_5-C_6H_4
7j, **9j**, **11j**, **13j**, R = *p*-OCH_3-C_6H_4
7k, **9k**, **11k**, **13k**, R = *p*-OCH_3-$C_6H_4CH_2$
7l, **9l**, **11l**, **13l**, R = *m*-OCH_3-C_6H_4
7m, **9m**, **11m**, **13m**, R = *m*-OCH_3-$C_6H_4CH_2$
7n, **9n**, **11n**, **13n**, R = *m*,*p*-$(OCH_3)_2$-C_6H_3
7o, **9o**, **11o**, **13o**, R = *p*-OC_2H_5-C_6H_4

8a, **10a**, **12a**, **14a**, R = C_6H_5
8b, **10b**, **12b**, **14b**, R = *p*-Cl-C_6H_4
8c, **10c**, **12c**, **14c**, R = *p*-OCH_3-C_6H_4