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Synthesis and antiproliferative activity of imidazo[2,1-*b*][1,3,4]thiadiazole derivatives

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ABSTRACT

A series of 2,5,6-substituted imidazo[2,1-*b*][1,3,4]thiadiazole derivatives have been prepared and were tested for antiproliferative activity on cancer cells at the National Cancer Institute. Results showed that molecules with a benzyl group at position 2, exhibited an increase in activity for the introduction of a formyl group at the 5 position. The compound 2-benzyl-5-formyl-6-(4-bromophenyl)imidazo [2,1-*b*][1,3,4]thiadiazole **22** has been chosen for understanding the mechanism of action by various molecular and cellular biology studies. Results obtained from cell cycle evaluation analysis, analysis of mitochondrial membrane potential and Annexin V-FITC by flow cytometric analysis, ROS production and expression of apoptotic and DNA-repair proteins suggested that compound **22** induced cytotoxicity by activating extrinsic pathway of apoptosis, however, without affecting cell cycle progression.

Cancer is a disease of uncontrolled cell growth and is one of the prime diseases to be cured at the present generation.[1] Among different cancers, leukemia, and lymphoma account for 8% of all cancer.

Development of novel small molecule inhibitors against different cancers, particularly leukemia and lymphoma is an area of active investigation.[2] Kinases, a target involved also in chronic myelogenous leukemia, have been widely studied, leading to the development of several inhibitors.[3,4] Besides, novel small molecule inhibitors against DNA repair is also investigated by us and others. Recently, we have shown that small molecule inhibitor, SCR7, can interfere with the binding of Ligase IV to the broken DNA and prevent nonhomologous DNA end joining.[5]

Although there are many drugs currently under trial against cancer and particularly haematological malignancies, only few molecules have emerged as promising drugs. To overcome this, it is important to develop potent anticancer drugs with novel chemical backbones. Gadad et al. in 1999 reported on the antitumor effects of imidazo[2,1-*b*][1,3,4]thiadiazoles[6] and in 2003 Terzioglu et al. reported some hydrazone derivatives of 2,6-dimethyl imidazo [2,1-*b*][1,3,4]thiadiazole-5-carbohydrazide as anticancer agents against ovarian cancer cell line OVCAR.[7] Moreover, a large number of imidazothiadiazole derivatives have been reported to possess diverse pharmacological properties such as antitubercular,[8] antibacterial,[9] antifungal,[10] anticonvulsant, analgesic,[11] antisecretory,[12] anti-inflammatory,[13] cardiotonic,[14] diuretic[15] and herbicidal[16] activities.

Considering the importance of this scaffold, we focused our research on the synthesis of imidazo[2,1-*b*][1,3,4]thiadiazole derivatives endowed with anticancer activity.[17] Among them, compound **4a** (Chart 1) exhibited maximum cytotoxicity by inducing extrinsic pathway of apoptosis without affecting the cell cycle.

On the basis of these results, using **4a** as lead compound, we synthesized a series of analogs in order to investigate their antiproliferative activity. Most of the planned compounds bear the same substituent as compound **4a** at the 2 position (benzyl group), and at the 5 position (formyl group), whereas other derivatives bear different groups such as cyclohexylethyl or ethyl at the 2 position, Br and SCN at the 5 position. Furthermore, the substituent at the *para* position of the 6-phenyl ring was changed. In order to study the SAR, the antiproliferative activity of previously reported compounds was also investigated.

We have synthesized a series of derivatives of imidazo[2,1-*b*] [1,3,4]thiadiazole bearing the above mentioned substituents at 2,

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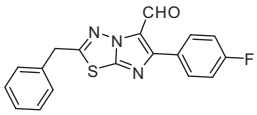


Chart 1. Cytotoxic agent described as **4a** in Ref. [17].

5 and 6 positions. The 2-aralkyl/alkyl/-6-aryl-imidazo[2,1-*b*][1,3,4]thiadiazole derivatives **5**–**12**, reported in Scheme 1, were prepared by reaction of 2-amino-5-substituted-1,3,4-thiadiazoles **1**–**3**, with the appropriate phenacyl bromide (**4**). The compounds **5**–**9** were previously reported in the literature[18,19] and compound **12** reported in literature[20] without spectral data. For the compound **11**, although commercial, we set up a procedure for its synthesis.

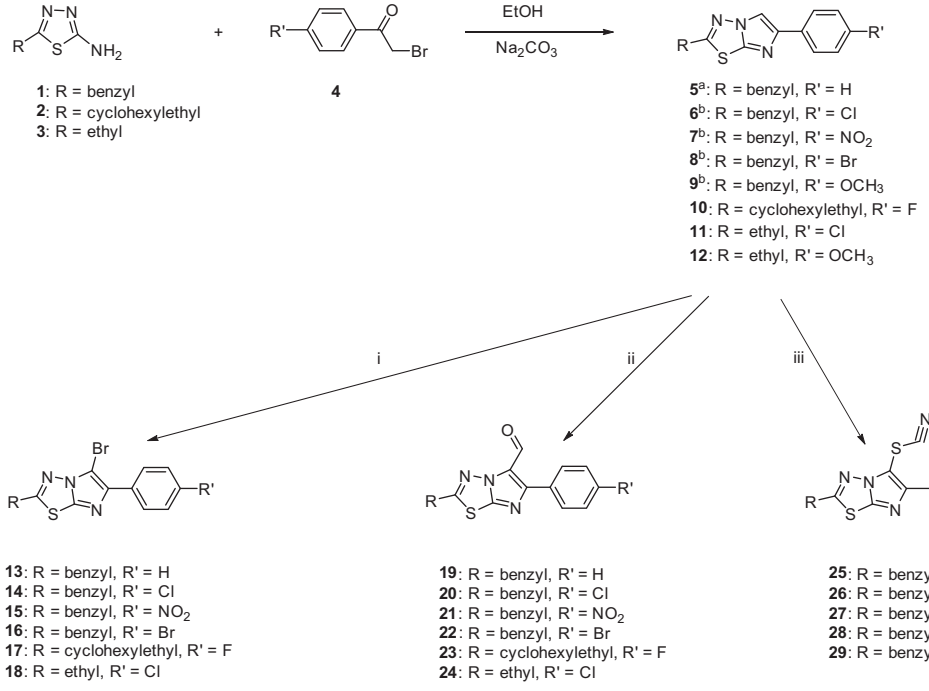
The starting 2-amino-5-substituted-1,3,4-thiadiazoles were prepared according to the literature,[21–23] whereas the various phenacyl bromides are commercially available or prepared by bromination of the corresponding ketones in glacial acetic acid. The 2-aralkyl/alkyl/-6-aryl-imidazo[2,1-*b*][1,3,4]thiadiazole derivatives (**5**–**8**, **10** and **11**) were subjected to electrophilic substitution at position 5 with bromine in the presence of sodium acetate in acetic acid to obtain the 5-bromo derivatives **13**–**18** in good yield. The 2-benzyl-6-phenylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (**19**) was reported in a patent[24] without spectral values. For the aldehydes **20**, **21**, **23** and **24** although commercial, we synthesized by means of the Vilsmeier reaction. Introduction of thiocyanate functional group at the 5 position was carried out by reaction with potassium thiocyanate in glacial acetic acid by drop wise addition of bromine in glacial acetic acid to get **25**–**29** in good yield.

Structures of the synthesized compounds were established on the basis of IR, ^{1}H NMR and mass spectra and CHN data. All synthesized compounds showed absorption bands ranging from 3172 to 3019 cm^{-1} for C—H aromatic stretching, 2999–2825 cm^{-1} for C—H aliphatic stretching. Compounds **19**–**24** showed peaks at 1683–1671 cm^{-1} for C=O stretching. Compounds **25**–**29** showed vibration bands at 2164–2159 cm^{-1} for SCN in their respective IR spectra.

In ^{1}H NMR, the presence of singlet between δ 8.64 and 7.88 ppm for imidazole proton (C_5—H) confirmed the cyclization of 2-amino-5-substituted-1,3,4-thiadiazole **1**–**3** with respective phenacyl bromide. All 5-substituted derivatives showed the absence of C_5—H in their respective spectra confirmed the substitution at 5th position. Compounds **19**–**24** showed a singlet between δ 10.20 and 10.03 ppm for CHO proton. All the compounds showed prominent signals for aromatic protons around δ 8.64–6.94 ppm. Bridge headed methylene proton at C_2 appeared between δ 4.48 and 4.21 ppm for **13**–**16**, **19**–**22**, **25**–**29** derivatives. For the compounds **10**, **17**, and **23** alkyl protons appeared between δ 1.80 and 0.93 ppm. The compounds **12** and **27** showed OCH_3 proton between δ 4.33 and 3.87 ppm. Compounds **11**, **12**, **18** and **24** showed ethyl protons between δ 1.49 and 1.31 ppm for CH_3 as triplet and δ 3.21–3.05 ppm for CH_2 as quartet, respectively.

As a preliminary test, the compounds were tested at a single high concentration (10^{-5} M) in the full NCI 60 cell panel (NCI 60 Cell One-Dose Screen). This panel is organized into subpanels representing leukemia, melanoma and cancers of lung, colon, kidney, ovary, breast, prostate and central nervous system. Only compounds with pre-determined threshold inhibition criteria in a minimum number of cell lines progress to the full 5-concentration assay. These criteria were selected to efficiently capture compounds with anti-proliferative activity based on careful analysis of historical Developmental Therapeutics Program (DTP) screening data. The one-dose data is a mean graph of the percent growth of treated cells (unpublished results).

Compounds **6**, **15**, **21**, **22**, **23**, **25** and **27** were active at a high concentration (10^{-5} M) and therefore entered the 5-concentration test. They were dissolved in DMSO and evaluated using five concentrations at 10-fold dilutions, the highest being 10^{-4} M. Table 1 shows the results obtained, expressed as micromolar concentration at three assay endpoints: the 50% growth inhibitory power (GI_{50}), the cytostatic effect (TGI = Total Growth Inhibition) and



Scheme 1. Synthesis of 2,5,6-substituted imidazo[2,1-*b*][1,3,4]thiadiazole derivatives. [a]Ref. 18; [b]Ref. 19. Reagents and conditions: (i) Br_2, CH_3COOH. (ii) DMF, $POCl_3$, 80–90 °C—Na_2CO_3. (iii) KSCN, Br_2, CH_3COOH.