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Original article

Synthesis and biological evaluation of novel 2-aralkyl-5-substituted-6-(4′-fluorophenyl)-imidazo[2,1-b][1,3,4]thiadiazole derivatives as potent anticancer agents

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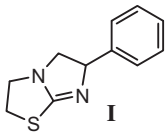
ABSTRACT

Levamisole, the imidazo[2,1-b]thiazole derivative has been reported as a potential antitumor agent. In the present study, we synthesized, characterized and evaluated biological activity of its novel analogues with substitution in the aralkyl group and on imidazothiadiazole molecules with same chemical backbone but different side chains namely 2-aralkyl-6-(4′-fluorophenyl)-imidazo[2,1-b][1,3,4]thiadiazoles (**SCR1**), 2-aralkyl-5-bromo-6-(4′-fluorophenyl)-imidazo[2,1-b][1,3,4]-thiadiazoles (**SCR2**), 2-aralkyl-5-formyl-6-(4′-fluorophenyl)-imidazo[2,1-b][1,3,4]-thiadiazoles (**SCR3**) and 2-aralkyl-5-thiocyanato-6-(4′-fluorophenyl)-imidazo[2,1-b][1,3,4]-thiadiazoles (**SCR4**) on leukemia cells. The cytotoxic studies showed that **3a**, **4a**, and **4c** exhibited strong cytotoxicity while others had moderate cytotoxicity. Among these we chose **4a** (IC_{50}, 8 μM) for understanding its mechanism of cytotoxicity. FACS analysis in conjunction with mitochondrial membrane potential and DNA fragmentation studies indicated that **4a** induced apoptosis without cell cycle arrest suggesting that it could be used as a potential chemotherapeutic agent.

1. Introduction

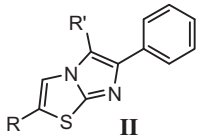
Development of anticancer drugs with fewer or no side effects is important for the treatment of cancer. The search for such potential anticancer drugs have led to the discovery of synthetic small molecules with anti-carcinogenic activity and limited harmful side effects particularly with respect to the immune system. Alternatively, stimulation of the body's immune system could provide a valuable support in cancer treatment, since it is capable of eradicating the neoplastic cells completely. Research in this area is expanding rapidly and some promising leads have emerged.

Levamisole (I) appears to be the most effective in patients with small tumor burdens and it acts by stimulating the responsiveness of lymphocytes to tumor antigens [1]. In addition, the imidazo[2,1-b]thiazole derivatives of Levamisole have been reported as potential antitumor agents (II) [2]. Later, antitumor activity of 5-formyl-6-arylimidazo-[2,1-b][1,3,4]thiadiazole sulfonamides (III) were also reported [3]. The promising results obtained in that study prompted us to prepare a new series of analogues including fluorine at position 4 of 6-phenyl in imidazo-[2,1-b]-1,3,4-thiadiazole (IV).



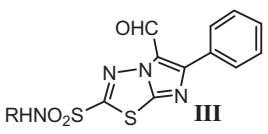
I

Levamisole



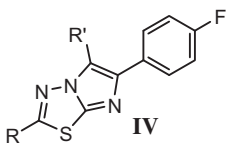
II

2,5-disubstituted-6-phenyl imidazo[2,1-b]thiazole



III

5-formyl-6-aryl-imidazo[2,1-b][1,3,4]thiadiazole sulfonamide



IV

6-(4'-fluorophenyl) imidazo[2,1-b][1,3,4]thiadiazoles (SCR1-SCR4)

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Previously, we have reported purification and synthesis of many natural and synthetic compounds, respectively. These were also studied for their biological activity and were shown to possess different levels of cytotoxicity on cancer cells [4–17]. In the present study, we synthesized, characterized and evaluated biological activity of new analogues of levamisole (I) with substitution in the aralkyl group and on fused imidazo[2,1-b][1,3,4]thiadiazole ring with same chemical backbone but different side chains namely 2-aralkyl-6-(4′-fluorophenyl)-imidazo[2,1-b][1,3,4]thiadiazoles (**SCR1**), 2-aralkyl-5-bromo-6-(4′-fluorophenyl)-imidazo[2,1-b][1,3,4]-thiadiazoles (**SCR2**), 2-aralkyl-5-formyl-6-(4′-fluorophenyl)-imidazo[2,1-b][1,3,4]-thiadiazoles (**SCR3**) and 2-aralkyl-5-thiocyanato-6-(4′-fluorophenyl)-imidazo[2,1-b][1,3,4]-thiadiazoles (**SCR4**). Based on our studies one of the molecules, **4a** was identified as the most promising lead compound.

2. Chemistry

We have synthesized series of 14 derivatives of imidazo[2,1-b][1,3,4]thiadiazoles containing aralkyl group at 2nd position by reacting 2-amino-5-aralkyl-1,3,4-thiadiazoles **X** with 4-fluoro phenacyl bromide as depicted in Scheme 1. Further compounds **SCR2**, **SCR3** and **SCR4** were obtained by bromination, formylation and thiocyanation respectively (Table 1). Structures of the synthesized compounds were established on the basis of IR, ^{1}H NMR and mass spectra analysis (Supplementary Figs. 1–3). All synthesized compounds showed absorption bands ranging from 3288 to 3025 cm^{-1} for C–H aromatic stretching, 2980 to 2840 cm^{-1} for C–H aliphatic stretching. Compounds **3a** and **3b** showed vibration bands at 1653–1676 cm^{-1} for C=O stretching. Compounds **4a**–**4c** showed vibration bands at 2163–2159 cm^{-1} for SCN in their respective IR spectra. Rf values of all the compounds were determined (Supplementary Table 1)

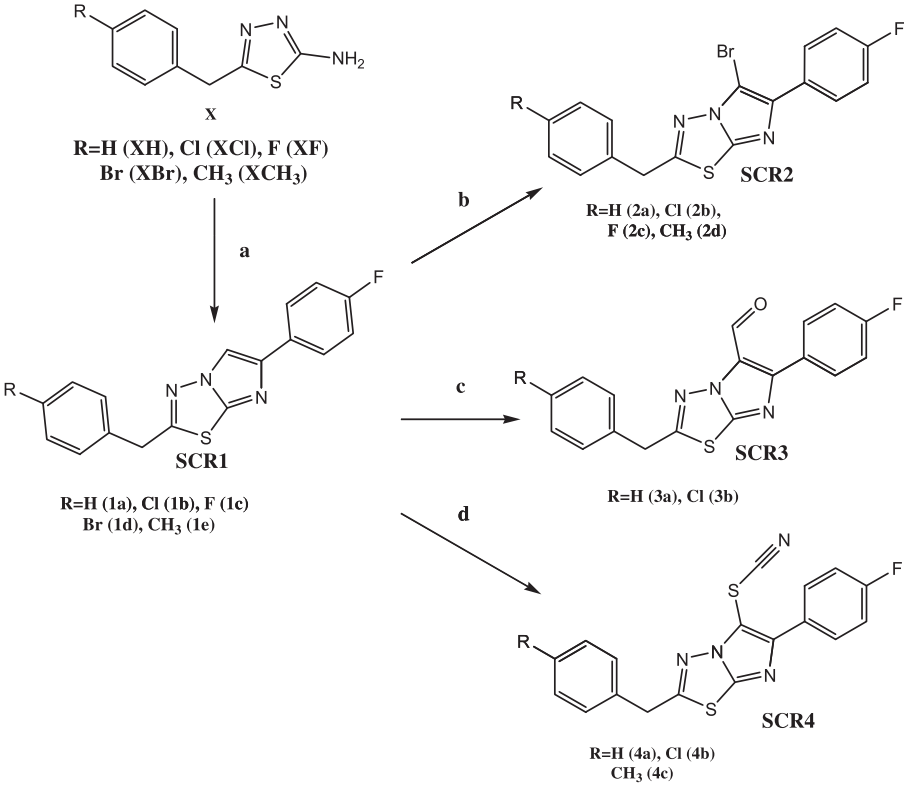
In ^{1}H NMR, the presence of singlet between δ 7.83 and 8.65 ppm for imidazole proton (C_5–H) confirmed the cyclization of **X** with 4-fluoro phenacyl bromide. All the electrophilic substitution reactions carried out on imidazo[2,1-b][1,3,4]thiadiazole derivatives (**SCR1**) and afforded the expected 5-substituted derivatives (**SCR2**, **SCR3**, and **SCR4**). All these 5-substituted derivatives showed the absence of C_5–H in their respective spectra, confirming the substitution at 5th position. Compounds **3a** and **3b** showed a singlet between δ 9.98 to 9.96 ppm for CHO proton respectively. All the compounds showed prominent signals for aromatic protons around δ 7.00–8.05 ppm. Methylene proton at C_2 appeared between δ 4.21–4.58 ppm for all synthesized derivatives. Compounds **1e**, **2d** and **4c** showed a singlet at δ 2.29 ppm for presence of methyl proton on phenyl ring. The structures of all the compounds were finally ascertained by mass spectra analysis (Table 1).

3. Pharmacology

The human T-cell leukemia cells, CEM were used for the study of preliminary anti-cancer screening of newly synthesized compounds. To assess the cytotoxicity, we used trypan blue dye exclusion and MTT assays. To test this, cells growing in log phase were treated with different concentrations of **SCR1** (**1a**–**1e**), **SCR2** (**2a**–**2d**), **SCR3** (**3a** and **3b**) and **SCR4** (**4a**–**4c**)). Further studies on lead cytotoxic compound, **4a**, in CEM cells were assessed by tritiated thymidine incorporation, cell cycle analysis, measurement of mitochondrial transmembrane potential ($\Delta\psi m$) and DNA fragmentation assays.

4. Results and discussion

In the present study we have investigated the cytotoxic effect of **1a**, **1b**,**1c**, **1d**, **1e** (**1a**–**1e**), **2a**, **2b**, **2c**, **2d** (**2a**–**2d**), **3a**, **3b** (**3a** and **3b**), **4a**, **4b**, **4c** (**4a**–**4c**) on T-cell leukemic cell line, CEM (Table 1). In order to estimate the effect of compounds, CEM cells were treated with increasing concentrations of compounds (10, 50, 100 and 250 μM)



Scheme 1. Reagents and conditions: (a) (i) F–C6H4–COCH2Br, EtOH, (ii) Na_2CO_3; (b) Br2, gl. acetic acid; (c) (i) POCl3, DMF, 80–90 °C, (ii) Na2CO3; (d) KSCN, gl. acetic acid, Br2 in gl. acetic acid, 0–5 °C.