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Original article

2,6-Disubstituted imidazo[2,1-*b*][1,3,4]thiadiazoles: Search for anticancer agents

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ABSTRACT

In this study, some novel 2,6-disubstituted imidazo[2,1-*b*][1,3,4]thiadiazoles **4** (**a**–**i**), **7** (**a**–**p**) and **11** (**a**–**i**) were synthesized from 5-substituted-1,3,4-thiadiazol-2-amine. The newly synthesized compounds **4a**, **4b**, **4c**, **4e**, **4g**, **7j**, **7l**, **11b** and **11c** were evaluated in the National Cancer Institute for single dose in vitro primary cytotoxicity assay. Among the tested nine compounds, compound **4b** (107166/760239) and **4c** (107168/760240) were passed the criteria for activity in this assay and scheduled automatically for evaluation against the full panel of 60 human tumor cell lines at a minimum of five concentrations at 10-fold dilutions. 3-(2-(4-methoxyphenyl)imidazo[2,1-*b*][1,3,4]thiadiazol-6-yl)aniline (**4c**) exhibited significant in vitro anticancer activity against Non Small Cell Lung Cancer HOP-92 cell line (GI_{50}: 0.114 μM) and Renal Cancer CAKI-1 cell line (GI_{50}: 0.743 μM).

1. Introduction

Cancer is undoubtedly a serious and potentially life-threatening illness. Cancer chemotherapy has entered a new era of molecularly targeted therapeutics, which is highly selective and not associated with the serious toxicities of conventional cytotoxic drugs [1]. A wide range of heterocyclic ring systems has been studied for the development of novel chemical entities as a lead molecule in the drug discovery paradigm. The anti-tumor potential of the 2-amino-1,3,4-thiadiazole skeleton was recognized in the early 1950s [2] and subsequently its fusion with the imidazo[2,1-*b*] ring system has resulted in compounds with potential anticancer [3], antitubercular [4], antibacterial [5], antifungal [6], anticonvulsant, analgesic [7], and antisecretory [8] activities. Consequently, a large number of imidazo[2,1-*b*][1,3,4]thiadiazole derivatives have been reported to possess antitumor property [9–12].

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*]thiadiazole 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] (Fig. 1).

In view of the above facts and in continuation of our search for novel anticancer agents [13–17], we have further investigated the potential of imidazo[2,1-*b*]thiadiazole as a supporting moiety for a number of selected pharmacophoric functions. Since this heterocycle constitutes the main part of levamisole, a well known immunomodulator [18], the possibility of reducing the harmful effects of the cytotoxic agents on the immune system could be seriously taken into account. In fact, the aim of this research was to discover a lead compound with a 2-fold action: first it could show antitumor activity and later, through one of its metabolites, it could restore the depressed immune system. With this in mind, in the present study a new series of 2,6-disubstituted imidazo[2,1-*b*][1,3,4]thiadiazoles have been synthesized and screened in vitro at NCI (National Cancer Institute)–USA for their anticancer properties (Fig. 1).

2. Chemistry

The synthetic route of the compound **4** (**a**–**i**), **7** (**a**–**p**) and **11** (**a**–**i**) is outlined in Schemes1–3 respectively. The 2,6-disubstituted imidazo[2,1-*b*][1,3,4]-thiadiazoles **4** (**a**–**i**), **7** (**a**–**p**) and **11** (**a**–**i**) were prepared by refluxing 5-substituted-1,3,4-thiadiazol-2-amine (**2**), (**6**) and (**9**) with substituted α-bromoketones in dry

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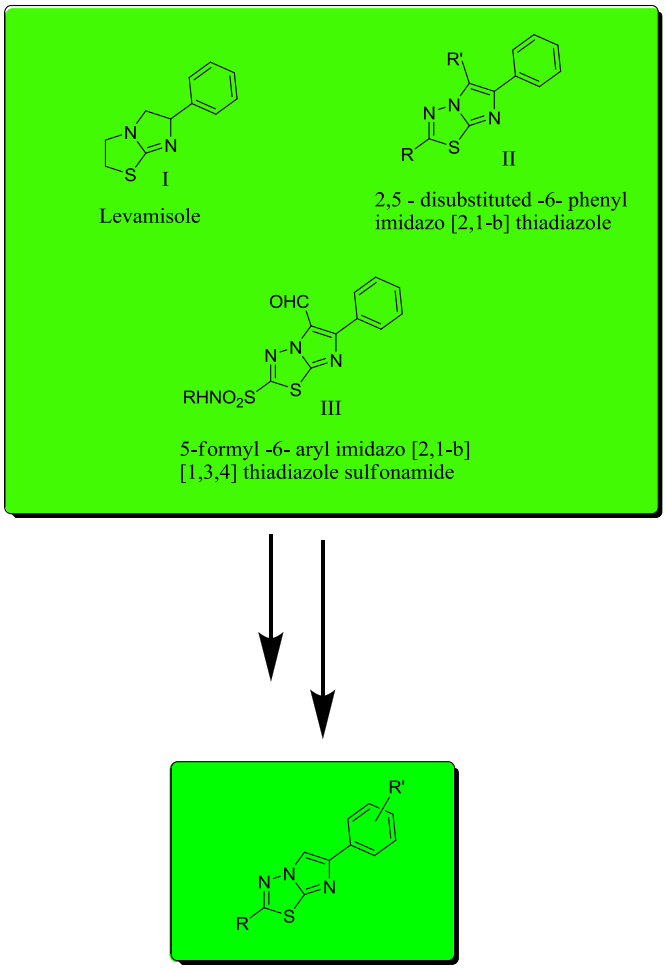


Fig. 1. Reported and proposed antitumor 2,6-disubstituted imidazo[2,1-*b*][1,3,4] thiadiazoles.

ethanol. The ring nitrogen of the imino form of thiadiazole is involved preferably on the nucleophilic displacement of bromine of the α-bromoketones forming an intermediate. It undergoes further cyclodehydration on heating with a suitable medium like ethanol, DMF to afford imidazothiadiazoles in good yields. The cyclodehydration involves intramolecular nucleophilic addition of the 2-amino group to carbonyl functions of the intermediate followed by the elimination of water as shown in Scheme 4. The physical data of all the synthesized compounds is shown in Table 1.

The structures of synthesized compounds were established on the basis of their spectral data. The formation of 2-aminothiadiazoles **2**, **6** and **9** was supported by the presence of *ν* N–H band in the IR spectra ~3200 and absence of carbonyl stretching band of the carboxylic acid function. The formation of imidazothiadiazoles **4** (**a**–**i**), **7** (**a**–**p**) and **11** (**a**–**i**) was indicated by the absence of *ν* N–H band ~3200 in the IR spectra and appearance of imidazo proton (C–H) around *δ* ~8.00 in ^{1}H NMR spectra. The ^{13}C NMR and Mass spectra of these compounds further confirmed the assigned structures.

3. Pharmacology

3.1. Anticancer activity (NCI 60 DTP human tumor cell line screening)

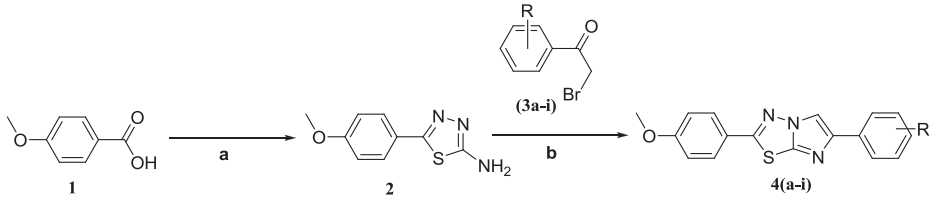
The screening of the compounds operated with the in vitro Cell Line Screening Project (IVCLSP) which is a dedicated service, providing direct support to the DTP anticancer drug discovery program. It is a two stage process, beginning with the evaluation of all compounds against the 60 cell lines at a single dose of 10^{-5} M. The output from the single dose screen is reported as a mean graph and is available for analysis by the compare program. Compounds which exhibit significant growth inhibition are evaluated against the 60 cell panel at five concentration level.

The tumor growth inhibition properties of the selected nine compounds **4a**, **4b**, **4c**, **4e**, **4g**, **7j**, **7l**, **11b** and **11c** with the NCI NSC-codes 107164/760238, 107166/760239, 107168/760240, 107180/760241, 108144/760535, 105053/758274, 105054/758274, 106805/759798 and 10806/759799 were screened on human tumor cell lines at 10^{-5} M concentration at the NCI, NIH, Bethesda, Maryland, USA, under the drug discovery program of the NCI. Among the tested compounds, compound **4b** (107166/760239) and **4c** (107168/760240) were further screened for 5-log dose molar range as they have shown prominent cell growth inhibition at 10^{-5} M concentration against variety of cell lines.

3.2. Methodology of the in vitro cancer screen

The human cancer cell lines of the cancer screening panel are grown in RPMI 1640 medium containing 5% fetal bovine serum at 2 mM L-glutamine. For a typical screening experiment, cells are inoculated into 96 well microtiter plates in 100 μl at plate densities ranging from 5000 to 40,000 cells/well depending upon the doubling time of individual cell lines. After cell inoculation, the microtiter plates are incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs.

After 24 h, two plates of each cell lines are fixed *in situ* with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs are solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate is thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 μg/ml gentamicin. Additional four,



Reagents: **a)** Thiosemicarbazide, $POCl_3$; **b)** EtOH, DMF

R= **a)** H; **b)** 2,4-di-OH; **c)** 3-NH_2; **d)** 4-Br; **e)** 4-OCH_3; **f)** 2,4-di-Cl; **g)** 4-NH_2; **h)** 2-OH; **i)** 4-Cl

Scheme 1. Synthesis of target compound **4**(**a**–**i**).