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Research paper

Synthesis of novel trifluoromethyl substituted furo[2,3-*b*]pyridine and pyrido[3′,2′:4,5]furo[3,2-*d*]pyrimidine derivatives as potential anticancer agents

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ABSTRACT

A series of novel trifluoromethyl substituted furo[2,3-*b*]pyridine and pyrido[3′,2′:4,5]furo[3,2-*d*] pyrimidine derivatives **3a-b**, **6a-k**, **9**, **10a-b, 11a-c** and **12a-c** were prepared from 2-carbethoxy-3-amino-6-trifluoromethyl furo[2,3-*b*]pyridine **1** under different set of conditions. Compounds functionalized with oxadiazole **11a-c** were also prepared from 2-carbohydrazide-3-amino-6-trifluoromethyl furo[2,3-*b*] pyridine **4**. All the final products were screened for anticancer activity against four human cancer cell lines such as Neuro-2a, Hela, A549 and COLO 205 as well as normal human lung cell line, IMR-90. All the compounds showed promising anticancer activity against all the tested cell lines at <25 μM concentration except **5b, 6d, 6e** and **6k**. The selectivity index (SI) values have also been calculated for all the tested compounds in comparison to the normal cell line. Compounds **6g, 10a, 10b**, and **11a** were considered as potential leads which showed cytotoxicity with IC_{50} values of 10, 10.7, 11.0 and 10.5 μM, respectively. Compounds **7** and **12a** were considered as highly potent exhibiting promising cytotoxicity with IC_{50} value of 5.8 and 3.6 μM, respectively.

1. Introduction

Cancer is the second most leading disease all over the world. Among all the cancer types, breast cancer is most common in women. Early detection and the introduction of new therapies resulted decline in mortality [1–4]. Most of the synthetic anticancer drugs made from molecules which occupy a central position in medicinal chemistry [5–7] and are integral part of the chemical and life sciences across the globe. Heterocyclic ring systems have emerged as powerful scaffolds for many biological evaluations [8] and play an important role in the design and discovery of new physiological/pharmacologically active molecules [9]. Specifically, oxygen heterocycles exhibit diverse biological and pharmacological activities due to the similarities with many natural and synthetic molecules [10].

Recently, the furo[2,3-*b*]pyridine/pyrido[3′,2′:4,5]furo[3,2-*d*] pyrimidine with different substitutions have gained renewed interest as templates for drug discovery. Furo[2,3-*b*]pyridine ring system, because of a formal iso-electronic relationship with purine has numerous pharmacological and agrochemical applications viz. antimalarial [11], antifolate [12–16], antiviral [17] as well as potential radiation protection agents [18]. Some furo[2,3-*b*]pyridine derivatives considered to be potent VEGFR2 (vascular endothelial growth factor receptor 2) and EGFR (epidermal growth factor receptor) inhibitors [19]. Pyrido[3′,2′:4,5]furo[3,2-*d*]pyrimidine derivatives also identified as potent PI3K p110a inhibitors [20–23]. Based on the importance of fused heterocyclic compounds, we have focused our attention on the synthesis of tricyclic and bicyclic ring systems and their evaluation to find novel potent anticancer agents.

Comparative molecular field analysis (CoMFA) [24] has emerged as a very important method in ligand based drug design strategies is a

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combination of reasonable molecular descriptors, statistical analysis and graphical representation of results. Molecular structures are described with molecular interaction energies as steric and electrostatic fields surrounding the molecules, the statistics is computed by PLS (partial least squares) regression analysis and the output is displayed as contours superimposed on the molecules. The CoMFA methodology assumes that a suitable sampling of steric and electrostatic fields around a set of aligned molecules provides all the information necessary for understanding their biological properties. CoMFA is usually employed to increase the binding affinity [25]. When used in a comparative investigation on the same series of compounds acting on multiple targets, such methodology is valuable in identifying the structural basis of the observed quantitative differences in the pharmacotoxicological properties. Considering these facts, we developed the 3D QSAR (Quantitative Structure Activity Relationship) CoMFA models of the Neuro-k2a cell line inhibitors in the anticipation of getting a model that would account for the quantitative differences in biological activity seen in this series and to capitalize upon the insights to design ligands with pronounced inhibitory potency and selectivity. Considering the importance of the furo[2,3-*b*]pyridine/pyrido[3′,2′:4,5]furo[3,2-*d*]pyrimidine derivatives from a medicinal chemistry perspective, the present study was undertaken and in addition the CoMFA study was carried out on the furo[2,3-*b*]pyridine derivatives.

2. Results and discussion

2.1. Chemistry

Ethyl 3-amino-6-(trifluoromethyl)furo[2,3-*b*]pyridine-2-carboxylate **1** on reaction with *n*-hexyl amine in a sealed tube resulted in 3-amino-*N*-hexyl-6-(trifluoromethyl)furo[2,3-*b*]pyridine-2-carboxamide **2**. Further reaction with triethyl orthoformate or triethyl orthoacetate in presence of catalytic amount of glacial acetic acid produced pyrido[3′,2′:4,5]furo[3,2-*d*]pyrimidine derivatives **3**. Similarly, compound **1** was treated with hydrazine hydrate in ethanol under reflux conditions and obtained 3-amino-6-(trifluoromethyl) furo[2,3-*b*]pyridine-2-carbohydrazide **4**. Compound **4** was further reacted with aromatic aldehydes in ethanol using triethylamine (TEA) as base at reflux temperature selectively formed benzylidene furo[2,3-*b*]pyridine carbohydrazide derivatives **5**. Compound **5** was cyclized with triethyl orthoformate/triethyl orthoacetate at 120–135 ^{O}C resulted in substituted pyrido[3′,2′:4,5]furo[3,2-*d*]pyrimidine derivatives **6**. Alternatively, the compound **1** was also reacted with triethyl orthoformate in the presence of ammonium acetate to obtain pyrido[3′,2′:4,5]furo[3,2-*d*]pyrimidine derivative **7** and on *N*-alkylation with bromoethyl acetate in dry acetone selectively resulted in compound **8**. Compound **8** on reaction with hydrazine hydrate in ethanol at reflux temperature formed pyrido[3′,2′:4,5]furo[3,2-*d*] pyrimidine acetohydrazide **9**. The 3-amino-6-(trifluoromethyl)furo [2,3-*b*]pyridine-2-carbohydrazide **4** reacted with different reagents such as triethyl orthoformate/triethyl orthoacetate or acid chlorides resulted respective derivatives **10, 11** and **12**. Synthetic pathways for all these derivatives are represented in Schemes 1 and 2. The physical properties of the products are tabulated in Table 1.

2.2. Pharmacology

2.2.1. Anticancer activity

Compounds **2**, **3a-b**, **5a-h**, **6a-k**, **7**, **8**, **9**, **10a-b**, **11a-c**, and **12a-c** were screened against a panel of four human cancer cell lines including Neuro-2a (brain cancer), HeLa (cervical cancer), A549 (lung cancer) and Colo 205 (colon cancer) using 5-fluorouracil as standard. The data is tabulated in Table 2. All the compounds showed promising anticancer activity against all the cell lines at <25 μM concentration except **5b**, **6d**, **6e** and **6k**. Compounds **7** and **12a** were considered as promising leads which exhibited promising cytotoxicity with IC_{50} values 5.8 and 3.6 μM respectively. While, the compounds **6g, 10a, 10b**, and **11a** were considered as potential anticancer leads with IC_{50} values 10, 10.7, 11.0 and 10.5 μM respectively. From the structure verses activity relationship study, the cytotoxicity of compound **7** is attributed to the presence of pyrimidinone ring over furo[2,3-*b*]pyridine ring system, while the substitution on N^3 with nitrogen in compounds **10a** and **10b** marginally reduced the cytotoxicity. Alternatively, di-amide functionalized furo[2,3-*b*] pyridine derivatives such as **12a**, **12c** and **12b** showed cytotoxicity in a decreasing manner, which clearly suggests that with an increase in the nature of electron withdrawing groups on amide carbonyl of furo[2,3-*b*]pyridine, increased the cytotoxicity. One of the amides functionalized with oxadiazole **11a** resulted in decrease of cytotoxicity. Based on the data of **5a-h** series, the presence of electron donating substituents on phenyl ring (**5a**, **5c** and **5e**) showed no activity; and remaining derivatives with electron withdrawing substituents showed good activity. As in case of **6a-k** series, irrespective of the nature of substituents on phenyl ring showed reduced activity as compared with the derivatives having no substituents (**6a**, **6g**) on phenyl ring of pyrido[3′,2′:4,5]furo[3,2-*d*] pyrimidines. Among all the compounds screened, compound **12a** was considered as highly potent lead candidate. While, all the tested compounds were considered non-cytotoxic since the IC_{50} values were >80 μM against the normal cell line. Further, the selectivity index (SI) values were also determined and the values to this regard are represented in Table 2.

3. Computational studies

The 3D-QSAR CoMFA studies were carried out using furo[2,3-*b*] pyridine and pyrido[3′,2′:4,5] furo[3,2-*d*] pyrimidine derivatives which are reported as Neuro-2a cell line inhibitors. In the study, apart from the molecules which do not have bioactivity in exact numerical form for the inhibition for both enzymes were removed from the analysis. This helped us to make a comparative investigation about the structural requirements for interaction with the respective Neuro-2a cell line. A total of 30 molecules were taken up for the current study which showed cytotoxicity. The total compounds were partitioned into a training set of 23 and a test set of 7 compounds as 3:1 ratio (test set is 1 percent and training set is 3 percent) were selected randomly. Despite the ambiguity of ligand-receptor interactions in general, a statistically robust models were obtained from the CoMFA study. Experimental and predicted activities are given in Table 3.

The CoMFA PLS analysis is summarized in Table 4. The cross-validated correlation coefficient is used as a measure of goodness of prediction whereas the non cross-validated conventional correlation coefficient indicates goodness of fit of a QSAR model. The F value indicates the degree of statistical confidence. A cross-validated correlation coefficient of 0.512 was obtained using 5 as optimum number of components for the present model. The r^2cv obtained indicated a good internal predictive ability of the models. The models developed also exhibited a very good non-cross validated correlation co-efficient of 0.970. The test set compounds are generally used to evaluate the external predictive capabilities of QSAR models. For this purpose, a randomly selected 7 compounds from the series were set-aside during model development. The bootstrapping analysis was done for 100 runs. The r^2bs value obtained 0.965 of bootstrapping by 100 runs which further supports the statistical validity of the developed models and absence of chance correlation. The contributions of steric to electrostatic fields were found to be 66.7% for steric and 33.3% for electrostatic. The plots of actual versus predicted pIC_{50} values are shown in Fig. 1.