Bioorganic & Medicinal Chemistry Letters xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and biological evaluation of novel alkyl amide functionalized trifluoromethyl substituted pyrazolo[3,4-*b*]pyridine derivatives as potential anticancer agents

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ARTICLE INFO

Article history:
Received 25 June 2013
Revised 19 August 2013
Accepted 23 August 2013
Available online xxxx

Keywords:
Pyrazolo[3,4-*b*]pyridine
Alkylation
Primary aliphatic amines
Cyclic secondary amines
L-Amino acids
Anticancer activity

ABSTRACT

A series of novel alkyl amide functionalized trifluoromethyl substituted pyrazolo[3,4-*b*]pyridine derivatives **5**, **6** and **7** were prepared starting from 6-phenyl-4-(trifluoromethyl)-1H-pyrazolo[3,4-*b*]pyridin-3-amine **3** via selective N-alkylation, followed by reaction with different primary aliphatic amines, cyclic secondary amines or L-amino acids under different set of conditions. All the synthesized compounds **5**, **6** and **7** were screened for anticancer activity against four cancer cell lines such as A549—Lung cancer (CCL-185), MCF7—Breast cancer (HTB-22), DU145—Prostate cancer (HTB-81) and HeLa—Cervical cancer (CCL-2). The compounds **5i** and **6e** are found to have promising bioactivity at micro molar concentration.

The pyrazolo[3,4-*b*]pyridine ring system[1] present in a number of pharmaceutically important compounds and some of them as anticancer agents,[2,3] glycogen synthase kinase-3 (GSK-3) inhibitors,[4–6] A_1 adenosine receptor antagonist,[7] phosphodi esterase 4 (PDE4) inhibitors,[8] anti-pyretic and ACTH (adreno corticotropic hormone) releasing factor antagonist activity. CRF (Corticotrophin-releasing factor) antagonists are believed to be effective in the treatment of a wide variety of stress-related illness, such as depression, Alzheimer's disease, gastrointestinal diseases, anorexia nervosa, haemorrhaged stress and alcohol withdrawal symptoms, drug addiction and infertility.[9] Amide derivatives were associated with broad spectrum of biological activities including antituberculosis,[10] anticonvulsant,[11] analgesic, anti-inflammatory,[12] insecticidal,[13] antifungal,[14] and antitumor[15] properties.

Recently, it was found that the presence of fluorine[16] or trifluoromethyl[17,18] group in heterocycles can modulate the physical, chemical and biological properties. It is also well documented that the influence of the trifluoromethyl substituent in a molecule on physiological activity is mainly due to the increased lipophilicity, greater cell permeability and resistance to enzyme degradation.[19] Based on the importance and further to our ongoing research on the synthesis of trifluoromethyl substituted hetero ring fused pyridines as potential molecules,[20–22] we developed a synthetic strategy towards the synthesis of a number of novel N-alkyl amide functionalized trifluoromethyl substituted pyrazolo[3,4-*b*]pyridine derivatives and screened for anticancer activity against four cancer cell lines such as A549, MCF7, DU145 and HeLa. The promising compounds **5i** and **6e** which showed high activity at micro molar concentrations comparable to the standard have been identified and reported here for the first time.

The 2(1*H*) pyridone **1** was reacted with 2-chloroacetamide in acetone using K_2CO_3 as base and formed exclusively 2-*O*-acetamido-3-cyano-4-trifluoromethyl-6-phenyl pyridine **2** which was reacted with excess hydrazine monohydrate at 100 °C to afford 6-phenyl-4-(trifluoromethyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine **3**.[23] Compound **3** was reacted with bromo ethyl acetate in acetone using potassium carbonate as a base and obtained exclusively *N*-alkyl ester pyrazolo[3,4-*b*]pyridin-3-amine **4**. It was identified based on IR spectrum in which characteristic absorption bands at 3326, 3454 cm^{-1} for NH_2 group and ^1H NMR spectrum in which NH_2 appeared as broad signal at δ 4.36 ppm. Compound **4** was further reacted with different primary aliphatic amines, cyclic secondary amines or *L*-amino acids under various set of conditions and obtained *N*-alkyl acetamide pyrazolo[3,4-*b*]pyridine **5**, secondary amine ethanone tagged pyrazolo[3,4-*b*]pyridine **6** and α-amino acid functionalised pyrazolo[3,4-*b*]pyridine **7** derivatives respectively. However, reaction of compound **4** with aromatic amines under different set of conditions such as (i) DMF, K_2CO_3, 110–120 °C, 12–14 h. (ii) Ethanol, Zn Dust, sealed tube, 120 °C, 10–12 h. (iii)

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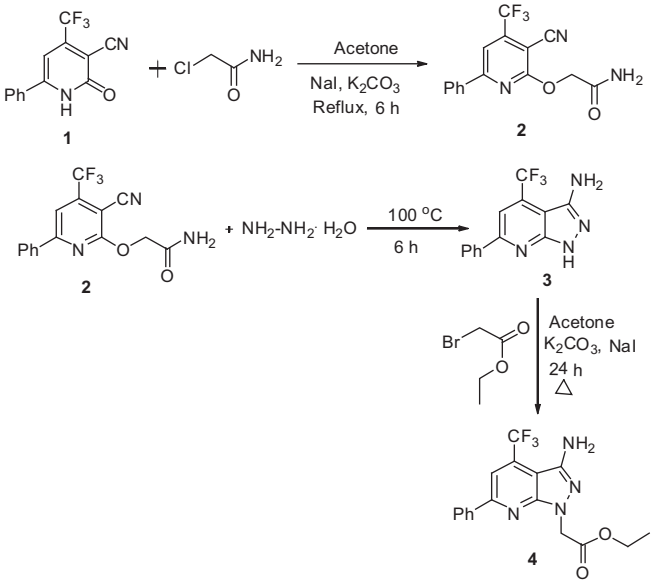
http://dx.doi.org/10.1016/j.bmcl.2013.08.089



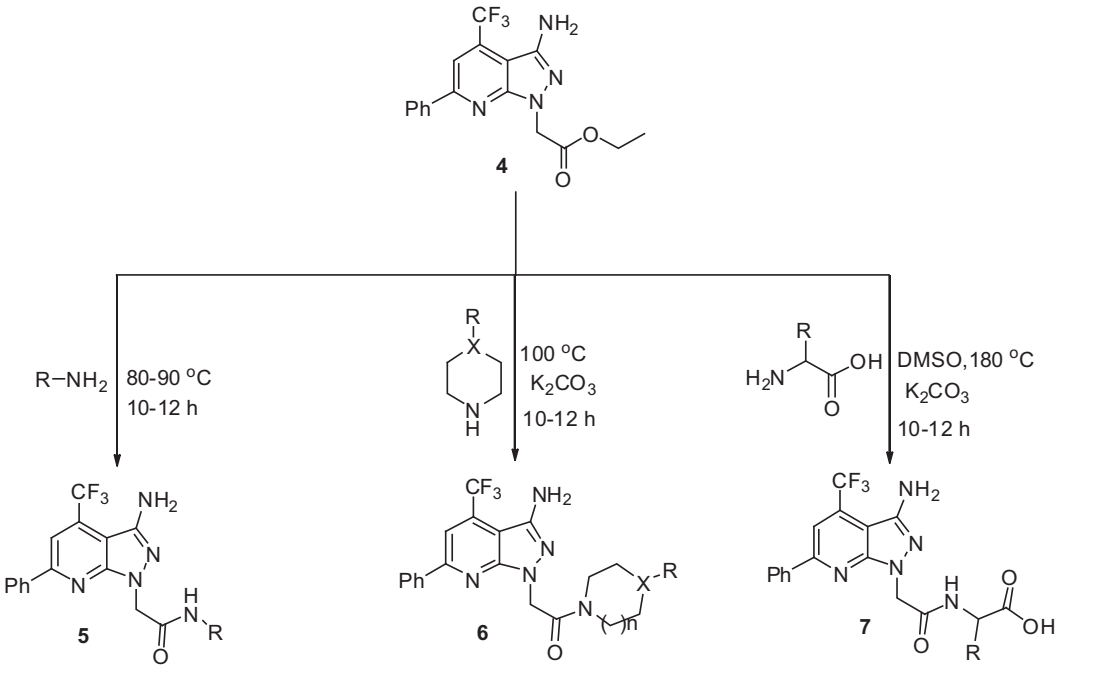
Scheme 1. Synthesis of N-alkyl ester pyrazolo[3,4-*b*]pyridine.

DMSO, K_2CO_3, 150–160 °C, 10–12 h could not give the product and the starting material was recovered. It is attributed to the less basicity of aromatic amines compared to aliphatic amines. The synthetic sequence is drawn in Schemes 1 and 2 and products are tabulated in Table 1.

Table 1
Preparation of pyrazolo[3,4-*b*]pyridine derivatives **4, 5a–j, 6a–f** and **7a–c**

Compound no.	*R*	m.p. (°C)	Yield (%)
4	–	138–140	90
5a	CH_3	237–239	92
5b	CH_3 CH_2	232–234	95
5c	CH_3 CH_2 CH_2	204–206	93
5d	NH_2	276–278	89
5e	CH_2 CH_2 NH_2	196–198	78
5f	CH_2 CH_2 OH	220–222	76
5g	Cyclopropyl	234–236	87
5h	Furfuryl	248–250	85
5i	Cyclo hexyl	268–270	91
5j	Benzyl	238–240	88
6a	-	158–160	83
6b	CH_3	161–163	79
6c	HO CH_2 CH_2	178–180	71
6d	Ph	191–193	73
6e	H	152–154	85
6f	H	196–198	87
5a–j	7a–c	188–90	75
7b	CH_3	200–202	72
7c	CH_2 Ph	190–192	70

Compounds **5a–j**, **6a–f** and **7a–c** were screened for *in vitro* against four cancer cell lines such as A549 (Lung cancer, CCL-185), MCF7 (Breast cancer, HTB-22), DU145 (Prostate cancer, HTB-81) and HeLa (Cervical cancer, CCL-2) using MTT assay.[24] IC_{50} values of the test compounds for 24 h on each cell line was calculated and presented in Table 2. All the compounds except **5g** showed activity against four cancer cell lines at micro molar



5a) R = -CH_3, **5b**) R = -CH_2-CH_3,

5c) R = CH_3-CH_2-CH_2-, **5d**) R = -NH_2,

5e) R = -CH_2-CH_2-NH_2, **5f**) R = -CH_2-CH_2-OH,

5g) R = Cyclo propyl, **5h**) R = Furfuryl,

5i) R = Cyclo hexyl, **5j**) R = Benzyl

6a) X = O, n = 1

6b) R = CH_3, X = N, n = 1

6c) R = -CH_2-CH_2-OH, X = N, n = 1

6d) R = Ph-, X = N, n = 1

6e) R=H, X = CH, n = 1

6f) R=H, X = CH, n = 0

7a) R = H, **7b**) R = CH_3,

7c) R = CH_2-Ph

Scheme 2. Synthesis of alkyl amide functionalized trifluoromethyl substituted pyrazolo[3,4-*b*]pyridine derivatives.

Table 2
In vitro cytotoxicity of alkyl amide functionalized pyrazolo[3,4-*b*]pyridine derivatives against A549, MCF7, DU145 and HeLa cancer cell lines

Compound no.	IC_{50} values in (μM)			
	A549	MCF7	DU145	HeLa
5a	15.3	11.8	14.4	12.2
5b	18.8	21.3	17.9	20.2
5c	16.5	14.9	15.5	15.9
5d	53.6	35.4	52.4	48.4
5e	50.2	42.9	38.9	44.5
5f	127.5	78.2	96.3	69.5
5g	—	—	—	—
5h	7.7	5.0	5.6	7.1
5i	2.3	5.2	3.1	3.9
5j	17.3	16.3	15.6	17.1
6a	5.4	5.9	5.1	6.6
6b	18.4	16.6	18.1	17.7
6c	14.9	15.6	3.2	2.9
6d	20.7	19.7	18.9	19.8
6e	1.2	4.1	3.2	2.9
6f	18.7	15.9	12.2	16.1
7a	19.9	21.2	18.8	19.1
7b	25.6	19.9	22.4	20.1
7c	30.1	42.1	32.3	31.3
5-Fluorouracil (Standard control)	1.1	1.2	1.3	1.1

IC_{50} = compound concentration required to inhibit tumor cell proliferation by 50%.

concentration. Among all the compounds **5h**, **5i**, **6a** and **6e** showed promising activity, while the compounds **5a**, **5b**, **5c**, **6b**, **6c**, **6d** and **6f** showed moderate activity. The order of toxicity over A549 and HeLa cell lines is **6e** > **5i** > **6a** > **5h**. Compound **6e** was considered as the more potent. The structure–activity relationship studies revealed that the increase in chain length on the aliphatic primary amine had no additional advantage in promoting cytotoxicity, while cyclohexyl on primary amine or cyclic secondary amine promoted the cytotoxicity as experienced in compound **5i** and **6e** against all the four cancer cell lines. Thus, the structure of promising compounds **5i** and **6e** will be further optimized in order to find the lead molecule.

In conclusion, we have synthesized a series of novel alkyl amide functionalized pyrazolo[3,4-*b*]pyridine derivatives **5**, **6** and **7** by selective N-alkylation of compound **4** followed by reaction with different primary aliphatic amines or cyclic secondary amines or *L*-amino acids. All the products were screened for anticancer activity and promising compounds **5i** and **6e** have been identified.

Acknowledgements

Authors are thankful to the Director, IICT for her constant encouragement and the authors C. Kurumurthy, P. Sambasiva Rao, B. Veera swamy, G. Santhosh kumar, G. Jitender Dev and Y. Poornachander Rao are thankful to Council of Scientific and Industrial Research (CSIR), India for providing financial assistance in the form of Senior Research Fellowship and contingency grant.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.08.089.

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