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Synthesis, cytotoxicity, antimicrobial and anti-biofilm activities of novel pyrazolo[3,4-*b*]pyridine and pyrimidine functionalized 1,2,3-triazole derivatives

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ABSTRACT

A series of novel pyrazolo[3,4-*b*]pyridine and pyrimidine functionalized 1,2,3-triazole derivatives **8a–g** and **9a–g** were prepared starting from 6-trifluoromethylpyridine-2(1*H*)one **2** via selective O-alkylation, followed by cyclisation using hydrazine hydrate to obtain 6-(trifluoromethyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine **4**. Compound **4** was diazotized followed by reaction with sodium azide, resulted in 3-azido-6-(trifluoromethyl)-1*H*-pyrazolo[3,4-*b*]pyridine **5**. Compound **5** was further cyclized with *N*-/*O*-propargylated pyrimidine derivatives under Sharpless conditions and obtained compounds **6** and **7**, respectively. Each set of compounds **6** and **7** were alkylated with different alkyl halides and obtained respective products **8** and **9**. All the products were screened for cytotoxicity against four human cancer cell lines such as A549-Lung (CCL-185), MCF7-Breast (HTB-22), DU145-Prostate (HTB-81) and HeLa-Cervical (CCL-2), compounds **9d**, **9e** and **9f** which showed promising activity have been identified. The products were also screened for antimicrobial, anti bio-film and MBC activities. Promising compounds in each case have been identified.

Cancer is a multifaceted disease that represents one of the leading causes of mortality in developed countries. Worldwide, one in eight deaths is due to cancer and it is the second most common cause of death in the US, exceeded only by heart disease.[1] Chemotherapy is the mainstay for cancer treatment, the use of available chemotherapeutics is often limited due to undesirable side effects. It is important to identify new agents and new targets for the treatment of cancer.

Nitrogen heterocycles have received a great deal of attention in the literature due to their role as active pharmacophores of historical significance. Among these heterocyclic systems, especially those containing pyridine rings are associated with diverse pharmacological properties such as anticancer,[2,3] antimicrobial,[4–6] anticonvulsant,[7] antiviral,[8] anti-HIV,[9] antifungal and antimycobacterial activities.[10] The pyrazolo[3,4-*b*]pyridine framework is a key structural fragment of many heterocyclic compounds showing a broad spectrum of biological activities.[11–13] In the last decade, some heterocycles of this class have been found to regulate the cardiovascular system and possess antiviral,[14,15] antileishmanial,[16] GSK-3 inhibitor (Fig. 1)[17] and antimicrobial properties.[18] Recently, it was found that the fluorine[19] or trifluoromethyl[20,21] group at a strategic position of an organic molecule dramatically alters the properties of molecule in terms of lipid solubility, oxidative thermal stability, permeability and oral bioavailability thereby showed an enhancement of transport mechanism.

During the last two decades, several pyrimidine derivatives have been developed as chemotherapeutic agents and found wide clinical applications.[22–24] Some of the promising pyrimidine derivatives such as Flufenerim,[25] Primsulfuron-methyl,[26] Diflumetorim[27] and Fluropyrimidol[28] were identified as insecticides, herbicides, fungicides, and plant growth regulators, respectively. The 5-fluorouracil[29] (Fig. 1) and their congeners belong to a family of the most potent anticancer and antiviral drugs.[30,31]

Based on the importance of the two scaffolds that is, the pyrazolo[3,4-*b*]pyridine and pyrimidine, it was proposed to conceive both the scaffolds in a single molecule to have promising activity. The two scaffolds were connected through the click reaction under Sharpless conditions[32,33] and obtained pyrazolo[3,4-*b*]pyridine and pyrimidine functionalized 1,2,3-triazoles. The products were evaluated for cytotoxic activity against four human cancer cell lines such as A549-Lung (CCL-185), MCF7-Breast (HTB-22), DU145-Prostate (HTB-81) and HeLa-Cervical (CCL-2). The products also screened for antimicrobial, anti biofilm and MBC activities.

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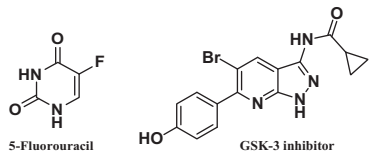


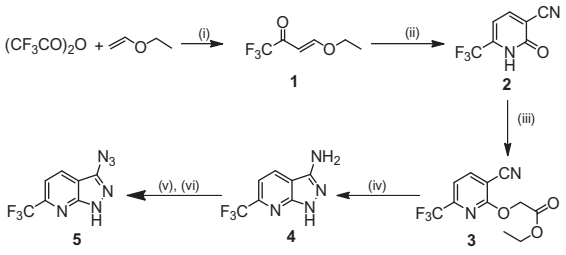
Figure 1. Bioactive compounds based on pyrimidine and pyridine derivatives.

The compounds which showed promising activity in each case have been identified.

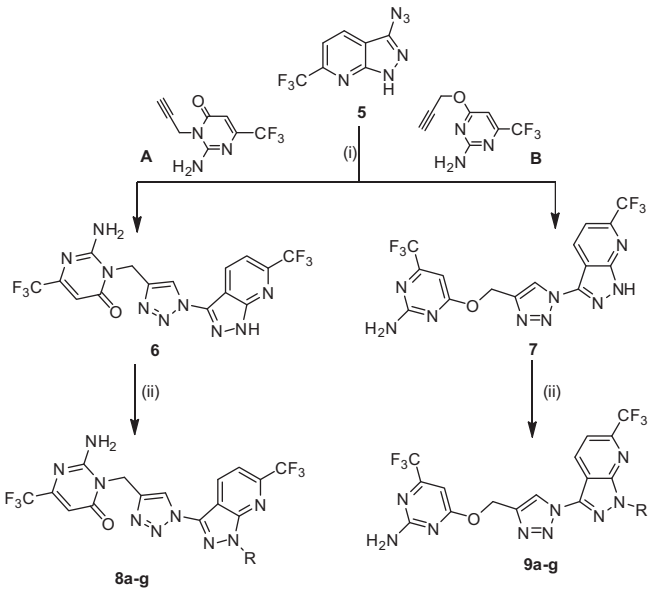
The 3-cyano-6-trifluoromethyl-2(1*H*) pyridone **2**[34] was prepared by acylation of vinyl ethyl ether in pyridine at room temperature, followed by reaction with cyanoacetamide in presence of sodium ethoxide in ethanol. Compound **2** was selectively *O*-alkylated[35] with α-bromoethylacetate in acetone reflux using potassium carbonate as a base, followed by reaction with hydrazine hydrate by an efficient method to obtain 6-(trifluoromethyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine **4** in high yield. Compound **4** was diazotized with sodium azide at room temperature as a result 3-azido-6-(trifluoromethyl)-1*H*-pyrazolo [3,4-*b*]pyridine **5** was formed. The synthetic sequence is depicted in Scheme 1.

The compounds 2-amino-3-prop-2-ynyl-6-trifluoromethyl-3*H*-pyrimidin-4-one (**A**) and 4-prop-2-ynyloxy-6-trifluoromethyl-pyrimidin-2-ylamine (**B**) reported[36] by us, was further reacted independently with compound **5** in THF using Cu(I) as catalyst under Sharpless conditions and obtained exclusively 1,4-disubstituted pyrazolo[3,4-*b*]pyridine and pyrimidine functionalized 1,2,3-triazole derivatives **6** and **7** respectively. Compounds **6**, **7** were further alkylated with different alkyl halides and obtained exclusively *N*-alkylated pyrazolo[3,4-*b*]pyridine and pyrimidine functionalized 1,2,3-triazole derivatives **8a–g** and **9a–g** respectively as outlined in Scheme 2. All the products formed have been tabulated in Table 1.

The cytotoxicity of all the synthesized compounds were screened against four human cancer cell lines namely, A549-Lung (CCL-185), MCF7-Breast (HTB-22), DU145-Prostate (HTB-81) and HeLa-Cervical (CCL-2) using MTT assay.[37] 5-Fluorouracil was used as a positive control. IC_{50} values of the test compounds for 24 h on each cell line was calculated and presented in Table 2. Most of the compounds were found to show significant cytotoxicity on all the cell lines. More specifically, compounds **6**, **8b**, **8d**, **8e**, **8f** and **9b** exhibited promising activity against all the four cell lines with IC_{50} value of ⩽21 μM, whereas compounds **8a**, **8c** and **9c** showed moderate activity against MCF7 and HeLa cell lines. Similarly, compound **7**, **9a**, **9d** and **9g** also showed specific activity against A549 and DU145 with IC_{50} for compounds **7** and **9a** in the range of 22.2–28.9 μM, while compound **9d** showed IC_{50} values of 4.1 and 4.7 μM, respectively. Among all the compounds, *O*-alkylated derivatives **9d**, **9e** and **9f** exhibited promising activity against A549 and DU145 cell lines with IC_{50} value of ⩽6.3 μM. The remaining compounds did not show cytotoxicity up to the concentration of 100 μg/mL.



Scheme 1. Synthesis of 3-azido-6-(trifluoromethyl)-1*H*-pyrazolo[3,4-*b*]pyridine. Reagents and conditions: (i) pyridine, DCM, rt, 4 h; (ii) cyanoacetamide, Na/EtOH, reflux, 6 h; (iii) ethyl 2-bromoacetate, K_2CO_3, acetone, reflux, 3 h; (iv) hydrazine hydrate, 100 °C, 4 h; (v) $NaNO_2$, concd HCl, H_2O; (vi) NaN_3, H_2O.



Scheme 2. Synthesis of pyrazolo[3,4-*b*]pyridine and pyrimidine functionalized 1,2,3-triazole derivatives **8a–g** and **9a–g**. Reagents and conditions: (i) CuI, THF, rt, 14 h; (ii) K_2CO_3, acetone, R-X, rt, 4 h.

Table 1
Physical properties of compounds **6**, **7**, **8a–g** and **9a–g**

Compds	R	Yield[a] (%)	Mp (°C)
6	H	71	221–223
8a	CH_3CH_2—	91	254–256
8b	$CH_3CH_2OC(O)CH_2$—	89	192–194
8c	CH_2=CH—CH_2—	93	240–242
8d	HC≡C—CH_2—	87	217–219
8e	$CH_3(CH_2)_6CH_2$—	94	140–142
8f	$CH_3(CH_2)_8CH_2$—	81	129–131
8g	$CF_3(CF_2)_7CH_2CH_2$—	79	111–113
7	H	73	164–166
9a	CH_3CH_2—	93	181–183
9b	$CH_3CH_2OC(O)CH_2$—	87	145–147
9c	CH_2=CH—CH_2—	91	136–138
9d	HC≡C—CH_2—	83	198–200
9e	$CH_3(CH_2)_6CH_2$—	93	102–104
9f	$CH_3(CH_2)_8CH_2$—	85	115–117
9g	$CF_3(CF_2)_7CH_2CH_2$—	89	161–163

[a] Isolated yields.

The structure–activity relationship studies revealed that the alkyl (decyl and octyl) chain on the nitrogen promotes cytotoxicity as observed in compounds **8e**, **8f**, **9e** and **9f** against A549 and DU145 cell lines. Whereas the perfluoroalkyl chain on nitrogen did not show any activity against MCF7, HeLa cell lines and decreased activity against A549 and DU145 cell lines compared to the hydrogen substituent on nitrogen in compounds **6** and **7**. There was an increase in activity, by increasing the n-alkyl chain length (ethyl–octyl–decyl) (**9b**, **9e** and **9f**). From the data, *O*-alkylated triazole derivatives were more potent than *N*-alkylated triazole derivatives on A549 and DU145 cancer cell lines. Alternatively, *N*-alkylated triazole derivatives were more active than *O*-alkylated triazole derivatives on MCF7 and HeLa cancer cell lines. Among all the tested compounds, the compounds **9d**, **9e** and **9f** were considered as promising candidates.

Compounds **6**, **8a–g**, **7** and **9a–g** were also tested for antimicrobial activity[38] against several bacterial organisms: *Micrococcus luteus* MTCC 2470, *Staphylococcus aureus* MTCC 96, *Staphylococcus*

Table 2
In vitro cytotoxicity of pyrazolo[3,4-*b*] pyridine and pyrimidine functionalized 1,2,3-triazole derivatives

Test compds	IC_{50} values (in μM)			
	A549	MCF7	DU145	HeLa
6	9.5 ± 0.19	14.4 ± 0.26	10.5 ± 0.22	16.1 ± 0.32
8a	—	95.2 ± 0.58	—	62.3 ± 0.46
8b	16.2 ± 0.22	12.2 ± 0.46	18.3 ± 0.16	11.4 ± 0.28
8c	—	20.2 ± 0.34	—	18.2 ± 0.31
8d	21.2 ± 0.32	12.4 ± 0.18	23.4 ± 0.32	10.2 ± 0.30
8e	10.1 ± 0.18	11.3 ± 0.22	12.2 ± 0.20	9.4 ± 0.26
8f	14.3 ± 0.21	15.7 ± 0.18	17.1 ± 0.35	13.4 ± 0.19
8g	68.2 ± 0.54	—	88.8 ± 0.52	—
7	22.8 ± 0.14	—	28.9 ± 0.48	—
9a	25.4 ± 0.22	—	27.7 ± 0.26	—
9b	16.3 ± 0.16	12.4 ± 0.22	18.2 ± 0.23	9.8 ± 0.26
9c	—	19.4 ± 0.24	—	18.4 ± 0.23
9d	4.1 ± 0.12	—	4.7±.18	—
9e	5.7 ± 0.22	24.7 ± 0.16	6.3 ± 0.21	22.7 ± 0.11
9f	4.2 ± 0.31	37.2 ± 0.31	5.8 ± 0.14	34.3 ± 0.32
9g	33.7 ± 0.25	—	37.7 ± 0.26	—
4	22.4 ± 0.22	58.4 ± 0.42	29.1 ± 0.33	60.2 ± 0.29
5-FU	1.3 ± 0.11	1.4 ± 0.09	1.5 ± 0.12	1.3 ± 0.14

IC_{50} = compound concentration required to inhibit tumor cell proliferation by 50% — activity >100 μM, A549-Lung cancer (ATCC No. CCL-185), MCF7-Breast cancer (ATCC No. HTB-22), DU145-Prostate cancer (ATCC No. HTB-81), HeLa-Cervical cancer (ATCC No. CCL-2).

aureus MLS-16 MTCC 2940, *Bacillus subtilis* MTCC 121, *Escherichia coli* MTCC 739, *Pseudomonas aeruginosa* MTCC 2453, *Klebsiella planticola* MTCC 530 and *Candida albicans* MTCC 3017 and the results are tabulated in Table 3. *O*-Alkylated triazole derivatives **7**, **9b** and **9f** showed promising activity (⩽15.6 μg/mL) against all the bacterial organisms except *Candida albicans* MTCC 3017. These conjugates were further studied for the bio-film inhibition and minimum bactericidal assays[39,40] and the results were tabulated in Tables 4 and 5. Compounds **7**, **9b** and **9f** exhibited good bio-film inhibitory activity against *Micrococcus luteus* MTCC 2470, *Staphylococcus aureus* MTCC 96, *Staphylococcus aureus* MLS-16 MTCC 2940, *Pseudomonas aeruginosa* MTCC 2453 and *Klebsiella planticola* MTCC 530 bacterial strains with IC_{50} values up to 7.8 ± 0.11 μg/mL (except **9b** which showed IC_{50} value of 15.6 ± 0.31 μg/mL). The compound **9f** was found to be more promising with IC_{50} value of 3.9 ± 0.14 μg/mL against *Staphylococcus aureus* MTCC 96, *Staphylococcus aureus* MLS-16 MTCC 2940, *Pseudomonas aeruginosa* MTCC 2453 and *Klebsiella planticola* MTCC 530) bacterial strains. Minimum bactericidal concentration (MBC) results showed that the compound **7** exhibited significant activity against *Escherichia coli* MTCC 739 (7.8 μg/mL), while compound **9f** showed promising activity (7.8 μg/mL) against *Staphylococcus aureus* MLS-16 MTCC 2940, and *Klebsiella planticola* (MTCC 530) organisms. Compound **9f** was found to be most potent among all the tested compounds.

A series of novel pyrazolo[3,4-*b*]pyridine and pyrimidine functionalized 1,2,3-triazole derivatives **6**, **7**, **8a–g** and **9a–g** were prepared and screened for their in vitro cytotoxicity against four human cancer cell lines as well as their antimicrobial, anti-bio-film and MBC activities. The compounds **9d**, **9e** and **9f** showed very good activity among all the compounds against A549 and DU145 cell lines. *O*-Alkylated triazole derivatives were more active than *N*-alkylated triazole derivatives on A549 and DU145 cancer cell lines, whereas *N*-alkylated triazole derivatives were more active than *O*-alkylated triazole derivatives on MCF7 and HeLa cancer cell lines. From the antimicrobial, anti bio-film and MBC data, the com-

Table 3
MIC values of the compounds **7** and **9a–g** against several standard strains

S. no.	Test compds	Minimum inhibitory concentration (μg/mL)							
		M.l[a]	*S.a*[b]	*S.a*[c]	*B.s*[d]	*B.s*[e]	*E.c*[f]	*P.a*[g]	*K.p*[h]
1	**7**	7.8	15.6	15.6	15.6	7.8	7.8	15.6	—[i]
2	**9a**	>250	>250	>250	>250	>250	>250	>250	—[i]
3	**9b**	>250	15.6	7.8	15.6	15.6	15.6	7.8	—[i]
4	**9c**	7.8	>250	>250	>250	>250	>250	>250	—[i]
5	**9d**	>250	>250	>250	>250	>250	>250	>250	—[i]
6	**9e**	>250	>250	>250	>250	>250	>250	>250	—[i]
7	**9f**	15.6	7.8	7.8	15.6	7.8	7.8	7.8	—[i]
8	**9g**	>250	>250	>250	>250	>250	>250	>250	—[i]
Ciprofloxacin		0.9	0.9	0.9	0.9	0.9	0.9	0.9	—[i]
Miconazole		—	—	—	—	—	—	—	7.8

[a] *Micrococcus luteus* MTCC 2470.
[b] *Staphylococcus aureus* MTCC 96.
[c] *Staphylococcus aureus* MLS-16 MTCC 2940.
[d] *Bacillus subtilis* MTCC 121.
[e] *Escherichia coli* MTCC 739.
[f] *Pseudomonas aeruginosa* MTCC 2453.
[g] *Klebsiella planticola* MTCC 530.
[h] *Candida albicans* MTCC 3017.
[i] No activity.

Table 4
Anti bio-film activity of compounds **7**, **9b** and **9f** against five different bacterial strains

S. no.	Test compds	IC_{50} values in (μg/mL)				
		Micrococcus luteus MTCC 2470	*Staphylococcus aureus* MTCC 96	*Staphylococcus aureus* MLS16 MTCC 2940	*Klebsiella planticola* MTCC 530	*Pseudomonas aeruginosa* MTCC 2453
1	**7**	7.8 ± 0.11	7.8 ± 0.22	7.8 ± 0.21	7.8 ± 0.34	3.9 ± 0.26
2	**9b**	7.8 ± 0.22	3.9 ± 0.19	7.8 ± 0.32	3.9 ± 0.15	15.6 ± 0.31
3	**9f**	3.9 ± 0.14	7.8 ± 0.36	3.9 ± 0.18	3.9 ± 0.24	3.9 ± 0.21
Erythromycin (standard)		0.24 ± 0.31	0.32 ± 0.14	0.32 ± 0.14	0.21 ± 0.12	0.26 ± 0.11

Table 5
MBC values of the compounds **7**, **9b** and **9f** against several bacterial strains

S. no.	Test compds	Minimum bactericidal concentration (μg/mL)						
		Micrococcus luteus MTCC 2470	*Staphylococcus aureus* MTCC 96	*Staphylococcus aureus* MLS-16 MTCC 2940	*Bacillus subtilis* MTCC 121	*Escherichia coli* MTCC 739	*Pseudomonas aeruginosa* MTCC 2453	*Klebsiella planticola* MTCC 530
1	**7**	15.6	31.2	31.2	31.2	7.8	15.6	31.2
2	**9b**	15.6	15.6	15.6	15.6	31.2	15.6	15.6
3	**9f**	15.6	15.6	7.8	31.2	15.6	15.6	7.8
Ciprofloxacin (standard)		0.58	0.58	1.17	1.17	0.58	0.58	1.17

pound **9f** was found to be the most potent amongst all the tested compounds.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.04.084.

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