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Research Article

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Synthesis of *N*-alkylated pyrazolopyridines and study of their antimicrobial activities

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ABSTRACT

N-alkylated Pyrazolopyridines was achieved by the reaction of pyrazolo-pyridines with different anilides in basic media and studied for their biological activity.

Keywords: *N*-alkylated Pyrazolopyridines, Anilides, antibacterial activity and antifungal activity, MIC

INTRODUCTION

The therapeutic importance of pyrazolopyridine is well documented. Fused heterocyclic containing pyrazolopyridine systems have been described and associated with several biological and medicinal activities including antibacterial [5], anxiolytic [6], antiviral [7-8], antileishmanial [9], anti-inflammatory [10] and adenosine A1-receptor antagonist [11-12]. Although the importance of ring systems in the drug discovery process is known, the current bioactive molecules described in literature include a limited functional group.

To address this point and our ongoing interest towards the synthesis of such nitrogen containing heterocycles [13], we intended to develop the convenient synthetic approaches for the synthesis of some new N-alkylated pyrazolo-pyridines that might be of pharmacological importance.

EXPERIMENTAL SECTION

Melting points were determined on a Gallenkamp melting point apparatus. The ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on a Varian NMR Mercury 300 spectrometer. Chemical shifts were reported in ppm relative to tetramethylsilane (TMS), and multiplicities are given as s (singlet), brs (broad singlet), d (doublet), t (triplet), q (quartet), or m (multiplet). Infrared spectra were recorded as KBr pellets on a Shimadzu FTIR-408 spectrophotometer. Mass spectra were recorded on a Shimadzu LC-MS: EI QP 2010A mass spectrometer with an ionization potential of 70eV. Elemental analyses (C, H and N) were performed on Thermo Finnigan Eager 300 EA 1112 series analyzer. Reactions were monitored by thin layer chromatography (TLC), carried out on 0.2 mm silica gel 60 F_{254} (Merck) plates using UV light (254 and 366 nm) for detection

1) Synthesis of 3-(4-chlorophenyl)-4-methyl-1-phenyl-1H-pyrazolo[3,4-b]pyridin-6(7H)-one ***2****.*
A mixture of 3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-5-amine **1** (0.001 mol)**,** substituted ethyl aceto acetate (0.001 mol) in acetic acid (20 mL) was refluxed for 28 hours. After completion of reaction (TLC check hexane: ethyl acetate 6:4) reaction mass was cooled to room temperature. The obtained solid was suction filtered, washed with water and dried to afford **1**.

Colourless solid: mp: 184-186^{o}C, IR (KBr) 3321 (-NH), 1682 (C=O), 1600 (C=C) cm^{-1}: ^{1}HNMR(DMSO d6) δ ppm: 1.98(s, 3H, Ar-CH_3), 6.92(s, 1H, Ar-H), 7.40-7.83 (m, 10H, Ar-H), 10.04 (s,1H, -NH): MS (*m*/*z*) : 335 (M^+), 337 (M+2): *Anal*. calcd for $C_{19}H_{14}ClN_3O$: C, 67.96; H, 4.20; N, 12.51. Found: C, 67.65; H, 4.14; N, 12.76.

General procedure for the synthesis of pyrazolo-pyridine-N-acetamide:-
A mixture of 3-(4-chlorophenyl)-4-methyl-1-phenyl-1H-pyrazolo[3,4-b]pyridin-6(7H)-one **2** (0.001 mol)**,** substituted anilides **3a-h** (0.001 mol) and K_2CO_3(0.001 mol) in DMF (15 mL) was stirred at room temperature for 15-24 hours. After completion of reaction (TLC check hexane: ethyl acetate 6:4) reaction mass was charged in ice cold water (20 mL) stirred for further 30 min. The obtained solid was suction filtered, washed with water and dried to afford **4a-h**.

2) *Synthesis of 2-(3-(4-chlorophenyl)-4-methyl-6-oxo-1-phenyl-1H-pyrazolo[3,4-b]pyridin-7(6H)-yl)-N-phenyl acetamide **4a**.*
Colourless solid: mp: 203-205^{o}C, IR (KBr) 3323 (-NH), 1680 (C=O), 1610 (C=C) cm^{-1}: ^{1}HNMR(DMSO d6) δ ppm: 1.82(s, 3H, Ar-CH_3), 4.23(s, 2H, CH_2), 7.10(s, 1H, Ar-H), 7.48-7.80 (m, 14H, Ar-H), 10.06 (s,1H, -NH): ^{13}C NMR ($CDCl_3$): δ ppm: 24.4, 53.4, 110.3, 120.5 (2Cs), 122.2 (2Cs), 124.4, 126.3, 126.7, 128.3 (2Cs), 129.7 (2Cs), 129.9 (2Cs), 131.1, 132.5, 133.7, 134.2, 134.5, 138.3, 139.2, 139.5, 148.4, 149.7, 165.7: MS (*m*/*z*) : 468 (M^+), 470 (M+2): *Anal*. calcd for $C_{27}H_{21}ClN_4O_2$: C, 69.15; H, 4.51; N, 11.95. Found: C, 69.23; H, 4.34; N, 12.16.

3) *Synthesis of 2-(3-(4-chlorophenyl)-4-methyl-6-oxo-1-phenyl-1H-pyrazolo[3,4-b]pyridin-7(6H)-yl)-N-p-tolyl acetamide **4b**.*
Colourless solid: mp: 207-209^{o}C, IR (KBr) 3322 (-NH), 1681 (C=O), 1500 (C=C) cm^{-1}: ^{1}HNMR(DMSO d6) δ ppm: 1.81(s, 3H, Ar-CH_3), 2.01(s, 3H, Ar-CH_3), 4.25(s, 2H, CH_2), 7.11(s, 1H, Ar-H), 7.38-7.65 (m, 13H, Ar-H), 10.07 (s,1H, -NH): MS (*m*/*z*) : 482 (M^+), 484 (M+2): *Anal*. calcd for $C_{28}H_{23}ClN_4O_2$: C, 69.63; H, 4.80; N, 11.60. Found: C, 69.43; H, 4.54; N, 11.86.

4) *Synthesis of 2-(3-(4-chlorophenyl)-4-methyl-6-oxo-1-phenyl-1H-pyrazolo[3,4-b]pyridin-7(6H)-yl)-N-(4-methoxy phenyl)acetamide **4c**.*
Colourless solid: mp:209-211^{o}C, IR (KBr) 3329 (-NH), 1682 (C=O), 1610 (C=C) cm^{-1}: ^{1}HNMR(DMSO d6) δ ppm: 1.80 (s, 3H, Ar-CH_3), 3.81 (s, 3H, OCH_3), 4.26(s, 2H, CH_2), 7.10 (s, 1H, Ar-H), 7.33-7.58 (m, 13H, Ar-H), 10.09 (s,1H, -NH): MS (*m*/*z*) : 498 (M^+), 500 (M+2): *Anal*. calcd for $C_{28}H_{23}ClN_4O_3$: C, 67.40; H, 4.65; N, 11.23. Found: C, 67.63; H, 4.44; N, 10.98.

5) *Synthesis of 2-(3-(4-chlorophenyl)-4-methyl-6-oxo-1-phenyl-1H-pyrazolo[3,4-b]pyridin-7(6H)-yl)-N-(4-chloro phenyl)acetamide **4d**.*
Colourless solid: mp:208-210^{o}C, IR (KBr) 3321 (-NH), 1676 (C=O), 1600 (C=C) cm^{-1}: ^{1}HNMR(DMSO d6) δ ppm: 1.82 (s, 3H, Ar-CH_3), 4.21(s, 2H, CH_2), 7.13 (s, 1H, Ar-H), 7.31-7.60 (m, 13H, Ar-H), 10.10 (s,1H, -NH): MS (*m*/*z*) : 502 (M^+), 504 (M+2): *Anal*. calcd for $C_{27}H_{20}ClN_4O_2$: C, 64.42; H, 4.00; N, 11.13. Found: C, 64.63; H, 3.84; N, 10.89.

6) *Synthesis of N-(4-chlorophenyl)-2-(3-(4-bromophenyl)-4-methyl-6-oxo-1-phenyl-1H-pyrazolo[3,4-b]pyridin-7(6H)-yl)acetamide **4e**.*
Off white solid: mp:213-215^{o}C, IR (KBr) 3331 (-NH), 1682 (C=O), 1610 (C=C) cm^{-1}: ^{1}HNMR(DMSO d6) δ ppm: 1.83 (s, 3H, Ar-CH_3), 4.27(s, 2H, CH_2), 7.15 (s, 1H, Ar-H), 7.36-7.66 (m, 13H, Ar-H), 10.02 (s,1H, -NH): MS (*m*/*z*) : 546 (M^+), 548 (M+2): *Anal*. calcd for $C_{27}H_{20}BrClN_4O_2$: C, 59.20; H, 3.68; N, 10.23. Found: C, 58.98; H, 3.84; N, 10.49.

7) *Synthesis of 2-(3-(4-chlorophenyl)-4-methyl-6-oxo-1-phenyl-1H-pyrazolo[3,4-b]pyridin-7(6H)-yl)-N-(4-fluoro phenyl)acetamide **4f**.*
Colourless solid: mp:214-216^{o}C, IR (KBr) 3325 (-NH), 1684 (C=O), 1500 (C=C) cm^{-1}: ^{1}HNMR(DMSO d6) δ ppm: 1.79 (s, 3H, Ar-CH_3), 4.23(s, 2H, CH_2), 7.19 (s, 1H, Ar-H), 7.31-7.73 (m, 13H, Ar-H), 10.08 (s,1H, -NH): MS (*m*/*z*) : 486 (M^+), 588 (M+2): *Anal*. calcd for $C_{27}H_{20}FClN_4O_2$: C, 66.60; H, 4.14; N, 11.51. Found: C, 66.66; H, 3.91; N, 11.73.

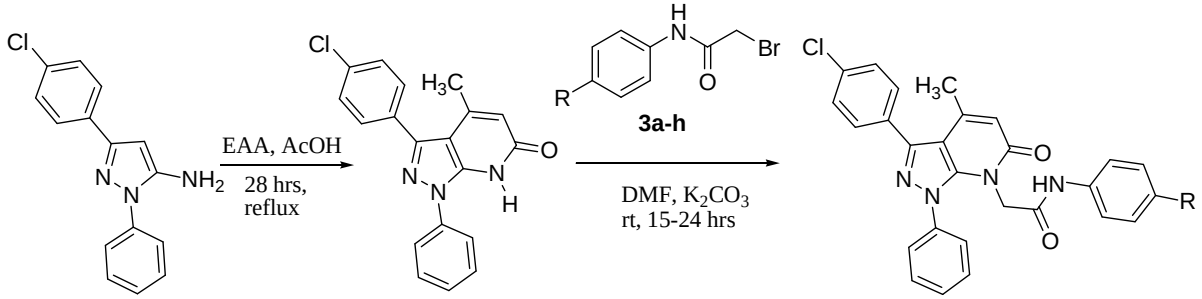
8) *Synthesis of 2-(3-(4-chlorophenyl)-4-methyl-6-oxo-1-phenyl-1H-pyrazolo[3,4-b]pyridin-7(6H)-yl)-N-(4-(trifluoro methyl)phenyl)acetamide **4g**.*
Colourless solid: mp:221-223^{o}C, IR (KBr) 3321 (-NH), 1682 (C=O), 1600 (C=C) cm^{-1}: ^{1}HNMR(DMSO d6) δ ppm: 1.83(s, 3H, Ar-CH_3), 4.23(s, 2H, CH_2), 7.13 (s, 1H, Ar-H), 7.27-7.83 (m, 13H, Ar-H), 10.07 (s,1H, -NH): MS (*m*/*z*) : 536 (M^+), 538 (M+2): *Anal*. calcd for $C_{28}H_{20}ClF_3N_4O_2$: C, 62.63; H, 3.75; N, 10.43. Found: C, 62.46; H, 3.91; N, 10.63.

9) *Synthesis of 2-(3-(4-chlorophenyl)-4-methyl-6-oxo-1-phenyl-1H-pyrazolo[3,4-b]pyridin-7(6H)-yl)-N-(4-nitro phenyl)acetamide **4h**.*
Yellow solid: mp:231-233^{o}C, IR (KBr) 3333 (-NH), 1668 (C=O), 1600 (C=C) cm^{-1}: ^{1}HNMR(DMSO d6) δ ppm: 1.88(s, 3H, Ar-CH_3), 4.27(s, 2H, CH_2), 7.18 (s, 1H, Ar-H), 7.19-7.88 (m, 13H, Ar-H), 10.13 (s,1H, -NH): MS (*m*/*z*) : 513 (M^+), 515 (M+2): *Anal*. calcd for $C_{27}H_{20}ClN_5O_4$: C, 63.10; H, 3.92; Cl, 6.90; N, 13.63. Found: C, 62.96; H, 4.22; N, 13.43.

RESULTS AND DISCUSSION

The reaction sequence employed for synthesis of key scaffolds, 3-(4-chlorophenyl)-4-methyl-1-phenyl-1H-pyrazolo[3,4-b]pyridin-6(7H)-one **2** from 3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-5-amine **1** is given in scheme 1**.**

Thus, the reaction of compound 1 with ethylacetoacetate in acetic acid at reflux temperature for 28 hours yielded the compound **2** in good yield. N-alkylated pyrazolo-pyridines were achieved by the reaction of compound **2** with substituted anilides **3a-h** in dimethyl formamide (15 mL) and K_2CO_3 as a base stirred at room temperature for 15-24 hours yielded compounds **4a-h** in good yields. The structural assignment of compounds **4a-h** is based upon spectroscopic and analytical data. For instance, the ^{1}H NMR of compound **4a** showed broad singlet at δ 1.82, 7.10 and 10.06 corresponds to –CH_3, -C=CH (pyridine) and -NH proton respectively. While the IR spectrum showed strong absorption bands at 3323, and 1680 cm^{-1} for -NH and –C=O respectively. The mass spectrum of **4a** revealed a molecular ion peak [M^+] at *m*/*z* = 468 (M^+), 470 (M+2).



4a)R=H, 4b)R=CH_3, 4c)R=Cl, 4d)R=F,
4e)R=CF_3, 4f)R=Br, 4g)R=OCH_3,
4h)R=NO_2

Scheme-1

It was noted that, during the synthesis of compound **4a-h** if the substituent present at para position of (benzene ring) anilides show the effect on reaction time and yield. Thus, the substituent at present para position is the electron donating then the reaction rate is high and also yield is high. Similarly if the substituent at present para position is the electron withdrawing then the reaction rate is poorer and also yields.

Table-1: Optimization of time and yield of synthesized compounds 4a-h

Comp. No	Time (h)	Yield (%)
4a	20	86
4b	18	88
4c	21	84
4d	22	83
4e	23	82
4f	21	84
4g	15	90
4h	24	80

Biological assays

The antimicrobial activity of the synthesized compounds was evaluated by the agar cup plate method. The antibacterial and antifungal assays were performed in Muller-Hinton broth and Czapek Dox broth respectively. Evaluation was performed using the bacteria reseeded in broth for 24 h at 37 ^{o}C, and the fungi were reseeded in broth for 48 h at 25 ^{o}C. The antibacterial activity of tested samples was studied against one Gram positive bacteria *Bacillus subtilis* NCIM 2250, one Gram negative bacteria *Escherichia Coli* ATCC 25922 while *Candida albicans* MTCC 277, *Candida tropicalis* MTCC 184, *Aspergillus niger* MCIM 545 and *Aspergillus clavatus* MTCC 1323

were used as standard fungal strain. The compounds were diluted in DMF with required concentration for bioassay. DMF was also loaded as control. Streptomycin and griseofluvin was used as standard to evaluate the potency of the tested compounds under same conditions. The zone of inhibition was determined from the diameter of the zone of inhibition using calliper. Each inhibition zone was measured three times to get average value. The minimum inhibitory concentration (MIC) values were determined on MH agar plates by pouring the molted agar in Petri dishes according to National Committee for Clinical Laboratory Standards (NCCLS, M7-A5 January 2000), containing the following concentrations (mg/mL): 0 (control), 5, 10, 15, 20, 30, 40. The MIC was defined as the lowest concentration tested samples showing no visible bacterial growth after 24 h incubation period at 37ºC.

Antimicrobial activity

In vitro antibacterial and antifungal activity of all newly synthesized compounds was screened by considering zone of inhibition of growth. The synthesized compounds (**4a-h)** were screened with their different concentrations along with standard antibiotics such as Streptomycin (5 µg/mL) and Griseofluvin (5 µg/mL) (Table-2). The results showed that compounds (**4a, 4b, 4c, 4f, 4g and 4h**) had very low or no significant antimicrobial activity while compounds (**4d** and **4e**) showed excellent antibacterial and antifungal activity with MIC value in between (10 and 15 µg/mL).

From the data it is clear that antimicrobial activity of the compounds (**4d-4e**) influences by changing the substituent's on the aromatic ring. Compound 4d having fluorine substituent while, 4d having trifluoro. Hence, F and CF_3 substituent's was showed consistently excellent antimicrobial activity against antibacterial and antifungal strains.

Table-2: Antibacterial and antifungal activity of compounds (4a-h)

Entry	*Bacillus subtilis* NCIM 2250	*Escherichia Coli* ATCC 25922	*Candida albicans* MTCC 277	*Candida tropicalis* MTCC 184	*Aspergillus niger* MCIM 545	*Aspergillus clavatus* MTCC 1323
	ZI[a] (MIC)[b]	ZI (MIC)	ZI (MIC)	ZI (MIC)	ZI (MIC)	ZI (MIC)
4a	17.1(25)	16.4(20)	-	15.4(20)	-	14.4(20)
4b	16.1(20)	13.2(25)	18.2(25)	-	-	-
4c	14.1 (20)	15.1(20)	16.4(20)	16.8(20)	14.4(20)	12.4(20)
4d	**14.6(15)**	**12.2(10)**	**14.5(15)**	**13.7(20)**	**15.2(15)**	**13.1(15)**
4e	**14.1 (10)**	**17.1(10)**	**16.8(10)**	**13.4(15)**	**17.1(10)**	**13.1(10)**
4f	15.2(20)	14.8(20)	12.4(20**)**	16.2(20)	16.9(25)	15.3(20)
4g	17.2(20)	16.6(25)	18.7(20)	15.2(20)	12.4(20)	16.4(20**)**
4h	16.1(20)	16.1(20)	16.1(20)	16.1(20)	16.1(20)	16.1(20)
Strept.	16.2(05)	16.4(05)	n.t.[c]	n.t.	n.t.	n.t.
Gris.	n.t.	n.t.	16.8(05)	17.3(05)	16.9(05)	17.6(05)

Bold values indicates better results; [a]Zone of inhibition in mm.; [b]Minimum inhibitory concentration in µg/ml.
[c]n.t. not tested

CONCLUSION

We synthesized new N-alkylated Pyrazolo-Pyridines by the reaction of pyrazolo-pyridines with different anilides in basic media. The result from biological activity study proved that **4d** and **4e** showed good antibacterial as well as antifungal activity.

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