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Design, Synthesis and Biological Screening of Pyrazolo [3,4-b] Pyridines Containing Quinoline-4-One

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ABSTRACT
We would like to report herein synthesis of two new pyrazolo[3,4-b]pyridines. Furthermore the characterization of product is carried out by elemental analysis and spectral analysis. Products were evaluated for their in vitro biological assay for antimicrobial activity against various bacterial standard strains i.e. S. pyogenes MTCC-442, S. aureus MTCC-96, E. coli MTCC-443, and B. subtilis MTCC-441 and antifungal activity against Aspergillus niger MTCC-282 and Candida albicans MTCC-227 at different concentrations, results were compared with standard drugs.

Keywords : Heterocycles, Synthesis, Reflux, Spectroscopy, Antimicrobial Activity

I. INTRODUCTION

When pyrazolo and pyridine ring systems are fused together various condensed systems may arise from such fusion. The pyrazolopyridine comprise of five isomers.

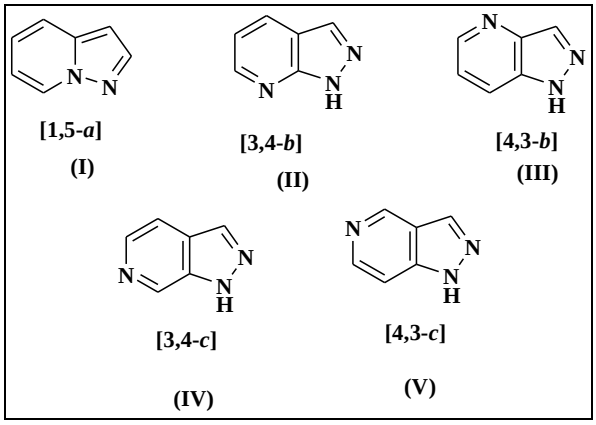

Figure 1

The pyrazolo[3,4-b]pyridine system has shown many interesting biological and pharmacological properties, such as antitubercular activity[1,2], activity against gram positive and negative bacteria[3]. Interest in the synthesis of condensed pyrazoles has recently revived because of the wide variety of their biological properties[4-6]. Although the pyrazolo[3,4-b]pyridine ring system has proved to be an interesting class in heterocyclic chemistry, it has received little attention in the literature. Some of its derivatives are important as anticancer agents with low toxicity[7,8], as antiinflammatories[9], as blood platelet aggregation inhibitors[9], as bone metabolism improvers[10] as adenosine antagonists[11,12] and as controlling herbicides[13]. They also show antifungal and antiparasitic activities[14,15]. From all the benefits mentioned above and in continuation of our interest directed towards the synthesis of new pyrazine heterocycles. We would like to report herein a synthesis of new pyrazolo[3,4-b]pyridine members along with studies of the effect of some of them as antifungal and as antibacterial agents.

Nitrogen-containing heterocycles are widely distributed in nature and essential for life, playing a vital role in the metabolism of all living cells. Among the many nitrogen containing heterocycles, the pyrazolo[3,4-b]pyrimidine nucleus many pharmacologically active compounds. This structure is an isostere of adenine, which is fundamental for every aspect of cell life as a constituent of DNA and RNA. Due to continuing interest in pyrazolo[3,4-b]pyrimidine derivatives as drugs with the potential to modulate purine activity or metabolism and purinergic receptor activation, researchers have dedicated much effort to investigating new approaches for the synthesis of these derivatives. On the basis of the above facts, we have synthesized two compounds bearing of pyrazolo[3,4-b]pyrimidine motif. The newly synthesized compounds were characterized by Mass, IR, ^{1}H NMR, ^{13}C NMR spectroscopy.

II. MATERIALS AND METHODS

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by UV and iodine. IR spectra were recorded Shimadzu FT-IR-8400 instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. ^{1}H NMR and ^{13}C NMR were determined in DMSO-d_6 solution on a Bruker Ac 400 MHz spectrometer.

A. General Synthesis

Diethyl 2-(((3-chloro-4-fluorophenyl)amino) methylene)malonate [Int 2]

A mixture of 3-chloro-4-fluoro aniline (0.01mol) and diethyl ethoxymethylenemalonate (0.01mol) in ethanol (20 ml) was heated under reflux condition for 5 hours. The reaction mass was cooled at 0 to 5 ^{o}C temperature. The reaction mixture was poured into water, filtered and washed with waterto gives diethyl 2-(((3-chloro-4-fluorophenyl)amino) methylene)malonate.

Ethyl-(7-chloro-6-fluoro-4-oxo-1,4-dihydroquinoline)-3-carboxylate [Int 3]

A mixture of diethyl 2-(((3-chloro-4-fluorophenyl)amino)methylene)malonate (Int 2) (0.01mol) and diphenyl ether (20 ml) was heated under stirring at 220 to 250 ^{o}C. The resulting mixture was then refluxed for 8 hours. The reaction mixture was allowed to cool at room temperature. Reaction mixture was poured into water, filtered and washed with water to yield ethyl-(7-chloro-6-fluoro-4-oxo-1,4-dihydroquinoline)-3-carboxylate.

Ethyl-(7-chloro-6-fluoro-1-(methyl or ethyl)-4-oxo-1,4-dihydroquinoline)-3-carboxylate [Int 4]

A mixture of ethyl(-7-chloro-6-fluoro-4-oxo-1,4-dihydroquinoline)-3-carboxylate (Int 3) (0.01 mol) and anhydrous potassium carbonate (0.02 mol) in dimethylsulphoxide (20 ml) was heated and stirred at 110 to 120 ^{o}C for 1 hour and then allowed to cool up to the temp 60 to 70 ^{o}C. To this reaction mixture, the solution of various alkyl halides (0.01mol) in dimethylsulphoxide (6 ml) was added drop wise and the temperature was maintained 95-100 ^{o}C for 8 hours. The resulting mixture was poured on to crushed ice, filtered, washed with water to afford ethyl-(7-chloro-6-fluoro-1-(methyl or ethyl)-4-oxo-1,4-dihydroquinoline)-3-carboxylate.

7-chloro-6-fluoro-1-(methyl or ethyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid [Int 5]

A mixture of ethyl-(7-chloro-6-fluoro-1-(methyl or ethyl)-4-oxo-1,4-dihydroquinoline)-3-carboxylate (Int 4) (0.01 mol) and anhydrous sodium hydroxide (2 N), in methanol (20 ml) was heated and stirred at 80 to 100 ^{o}C for 2 hours and then allowed to cool up to room temperature. Reaction mixture was neutralized by 1 N HCl, the solid product obtained is filtered to afford 7-chloro-6-fluoro-1-(methyl or ethyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.

6-fluoro-7-hydrazinyl-1-(methyl or ethyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid [Int 6]

A mixture of 7-chloro-6-fluoro-1-(methyl or ethyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(Int 5)** (0.01mol) and hydrazine hydrate 99% (0.1 mol) in ethanol (15 ml) was heated under reflux for 16 hours. The excess of the solvent was removed by distillation and reaction mass was poured in to ice-cold water. The precipitate was filtered out followed by washed with diethyl ether to yield 6-fluoro-7-hydrazinyl-1-(methyl or ethyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.

6-fluoro-1-(methyl or ethyl)-7-(3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)-4-oxo-1,4-dihydroquinoline -3-carboxylic acid [Int 7]

A mixture of 6-fluoro-7-hydrazinyl-1-(methyl or ethyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (Int 6)(0.01 mol) and 1,3-diketone (0.01 mol) in the presence of 2 drops of conc. HCl was heated under reflux for 25 min. The progress of reaction is monitored by TLC. After completion of reaction, The reaction mixture was poured into ice cold water, extracted using ethyl acetate followed by dried over sodium sulphate, and the solvent removed in vacuo to yield6-fluoro-1-(methyl or ethyl)-7-(3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.

7-(5-acetyl-3,6-dimethyl-4-substitutedphenyl-4,7-dihydro-1H-pyrazolo[3,4-b]pyridin-1-yl)-6-fluoro-1-(methyl or ethyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (7a &7b)

A mixture of 6-fluoro-1-(methyl or ethyl)-7-(3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)-4-oxo-1,4-

dihydroquinoline-3-carboxylic acid (Int 7) (0.01 mol), substituted aldehyde (0.01 mol) and ammonium acetate (0.02 mol) in ethanol (20 ml) was heated under reflux for 6-8 hours, progress of reaction is checked by TLC. After the completion, the reaction mixture was poured into ice cold water; the crude product was filtered, dried and purified using column chromatography.

B. Reaction Scheme

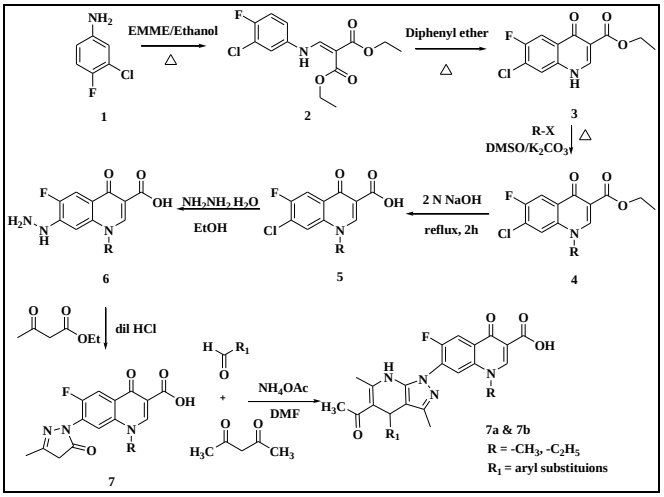

Figure 2

C. Physical Parameters Of Synthesized Compounds (Depicted Table 1.)

III. RESULTS AND DISCUSSION

A. Spectral Data

7-(5-acetyl-4-(4-chlorophenyl)-3,6-dimethyl-4H-pyrazolo[3,4-b]pyridin-1(7H)-yl)-6-fluoro-1,4-dihydro -1-methyl-4-oxoquinoline-3-carboxylic acid (7a)

Yield = 48 %, m.p. 225-227 ˚C; IR (KBr) cm^{-1}: 3298, 3234, 3026, 2849, 1710, 1610, 1600, 1583, 1521, 1309, 1471, 1143, 1193, 789, 704 ; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.68 (s, 3H), 1.93 (s, 3H), 3.05 (s, 3H), 3.26 (s, 3H), 5.86 (s, 1H), 7.26-7.28 (d, 2H. Ar-H), 7.36-7.38 (d, 2H, Ar-H), 7.86-7.89 (m, 2H), 8.24 (s, 1H, N-H), 11.43 (s, 1H, O–H); ^{13}C NMR ($CDCl_3$) δ:13.62, 16.30, 27.66, 31.10, 36.93, 106.58, 112.57, 113.93, 116.12, 117.02, 129.03, 130.20, 131.02, 132.59, 133.66, 138.29, 139.57, 142.09; 145.91, 147.76, 153.32, 153.40, 167.01, 176.56, 197.82, MS (m/z): 520, Anal. Calcd. For $C_{27}H_{22}ClFN_4O_4$: C, 62.25; H, 4.26; Cl, 6.81; F, 3.65; N, 10.75; O, 12.29 %; Found: C, 61.91; H, 4.05; Cl, 6.60; F, 3.55; N, 10.49; O, 12.07 %.

7-(5-acetyl-3,6-dimethyl-4-phenyl-4H-pyrazolo[3,4-b]pyridin-1(7H)-yl)-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (7b)

Yield = 61 %, m.p. >245 ˚C; IR (KBr) cm^{-1}: 3286, 3183, 2972, 1722, 1712, 1641, 1554, 1512, 1442, 1363, 1186, 1105, 1016, 812 ; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 0.83-0.89 (m, 6H), 1.70 (s, 3H), 2.09 (s, 3H), 4.09-4.15 (q, 2H), 5.02 (s, 1H), 6.92-7.02 (m, 3H. Ar-H), 7.08-7.10 (d, 2H, Ar-H), 7.18 (s,1H), 7.23-7.27 (t, 2H), 8.40 (s, 1H, N-H), 11.35 (s, 1H, O–H); ^{13}C NMR ($CDCl_3$) δ:12.41, 13.63, 16.30, 27.66, 31.15, 36.98, 106.59, 112.57, 112.26, 116.05, 117.02, 129.03, 130.20, 131.02, 132.59, 133.66, 138.44, 139.59, 142.10; 145.79, 147.52, 153.33, 153.43, 167.15, 176.57, 197.08, MS (m/z): 500, Anal. Calcd. For $C_{27}H_{22}ClFN_4O_4$: C, 67.19; H, 5.03; F, 3.80; N, 11.19; O, 12.79 %; Found: C, 66.93; H, 4.85; F, 3.75; N, 10.74; O, 12.61 %

B. Biological Evaluation

The synthesized compounds were tested for their antibacterial and antifungal activity (MIC) in vitro by broth dilution method[16-18] with two Gram-positive bacteria Staphylococcus aureus MTCC-96, Streptococcus pyogenes-MTCC-443, two Gram-negative bacteria Escherichia coli MTCC-442, Pseudomonas aeruginosa-MTCC-441 and three fungal strains Candida albicans MTCC-227, Aspergillus Niger MTCC-282, Aspergillus clavatus MTCC-1323 taking ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, nystatin, and greseofulvin as standard drugs. The standard strains were procured from the Microbial Type Culture Collection (MTCC) and Gene Bank, Institute of Microbial Technology, Chandigarh, India. The minimal inhibitory concentration (MIC) values for all the newly synthesized compounds, defined as the lowest concentration of the compound preventing the visible growth, were determined by using microdilution broth method according to NCCLS standards[16].

IV. CONCLUSIONS

In the present article, we report the synthesis, spectral studies, antibacterial and antifungal activities of a novel series of 1,2,4 triazole scaffold. The preliminary in vitro biological activities revealed that compounds **7a & 7b**

exhibited moderate to good antimicrobial activities. The results obtained from antimicrobial susceptibility testing are depicted in Table2.

Table 1. Physical parameters of synthesized compounds 7a & 7b

Code	R	R_1	M.F.	M.W.	M.P. ºC	Yield %	R_f
7a	$-CH_3$	$4\text{-}ClC_6H_4$	$C_{27}H_{22}ClFN_4O_4$	520	225-227	48	0.47
7b	$-C_2H_5$	C_6H_5	$C_{28}H_{25}FN_4O_4$	500	>245	61	0.56

Table 2. Antibacterial and antifungal activity of synthesized compounds 7a & 7b

Compounds	Minimum inhibition concentration (μg mL^{-1})						
	Gram-positive		Gram-negative		Fungal species		
	S.a.	*S.p.*	*E.c.*	*P.a.*	*C. a.*	*A. n.*	*A.c.*
P-7a	**62.5**	**100**	250	**100**	500	>1000	1000
P-7b	**62.5**	**100**	**100**	**125**	**100**	**100**	**100**
Ampicillin	250	100	100	100	-	-	-
Chloramphenicol	50	50	50	50	-	-	-
Ciprofloxacin	50	50	25	25	-	-	-
Norfloxacin	10	10	10	10	-	-	-
Nystatin	-	-	-	-	100	100	100
Griseofulvin	-	-	-	-	500	100	100

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