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Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL5-(2-CHLOROPHENYL)-2-(METHYLTHIO)-6-NITRO-7-(2-OXO-1, 2-DIHYDROQUINOLIN-3-YL)-4, 7-DIHYDROPYRAZOLO [1,5-A]PYRIMIDINE-3-CARBONITRILE DERIVATIVES

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ABSTRACT

A novel heterocyclic library was synthesized, characterized and tested for biological evaluation against bacteria and fungus. This novel series of pyrazole pyrimidine derivatives of 5-(2-chlorophenyl)-2-(methylthio)-6-nitro-7-(2-oxo-1, 2-dihydroquinolin-3-yl)-4,7-dihydropyrazolo [1,5-a]pyrimidine-3-carbonitrile in the presence of methanoland hydrochloric acid in good yield. The title compounds have been synthesized with several structural variations. The synthesized compounds were screened for antimicrobial activity against standard drugs. The structure of synthesized compounds were characterized by their spectral (IR, ^{1}H NMR and Mass) data. The purity of the synthesized compounds was confirmed by TLC.

Keywords: Pyrimidine, Pyrazole, Methanol, Antimicrobial activity.

1. INTRODUCTION

Biaryls and heterobiaryls have attracted significant attention from the scientific community because of their relevance in medicinal chemistry. Heterobiaryls frequently can be observed in numerous bioactive small molecules, and in particular, heterobiaryls fused with various heterocycles, such as pyrazole, pyridine, and pyrimidine, have been used as key pharmacophores [1]. Pyrazolo [1,5-*a*] pyrimidines have attracted considerable interest because of their biological activity. For instance, this heterocyclic system is found as purine analogues and has useful properties as antimetabolites in purine biochemical reactions [2]. Several compounds of this class display interesting antitrypanosomal [3] and anti-schistosomal activities [4]. They are used as HMG-CoA reductaseinhibitors [5], COX-2 selective inhibitors [6], 30, 50-cyclic-AMP phosphodiesteraseinhibitors [7], CRF_1 antagonists [8a-d, selective peripheral benzodiazepine receptor ligands [9a-c], potassium channel [10] and histamine-3 receptor ligands [11]and antianxiety agents [12].

In the present work, we report the synthesis of this novel series of pyrazole pyrimidine derivatives of 5-(2-chlorophenyl)-2-(methylthio)-6-nitro-7-(2-oxo-1,2-dihydroquinolin-3-yl)-4,7-dihydropyrazolo[1,5-a]pyrimidine-3-carbonitrile and their antimicrobial activity against fungi, gram positive and gram negative bacteria. The main significance of the work is to provide more potent stable molecule for biological response as most of pyrazole pyrimidine derivatives has significant biological activity. As we mentioned above, the significance and biological profile of this class of molecule so our continue efforts towards the synthesis of potential heterocyclic molecules.

2. EXPERIMENTAL

2.1. Material

Anhydrous solvents and all other reagents were purchased from, Spectrochem, Sigma-Aldrich, Loba-chemie and Merck. Involving air or moisture-sensitive compounds were performed under a nitrogen atmosphere using oven-dried glassware and syringes to transfer solutions. Thin-layer chromatography (TLC) was conducted by using aluminum plates 20x20 cm coated by silica gel 60 F254 purchased from Merck. Melting points were determined by melting point apparatus (uncorrected) using an open capillary method. Solvents evaporated by the help of a BUCHI rotary evaporator. IR spectra were recorded on FTIR-8400 spectrometer using DRS prob. which expressed in ν (cm^{-1}). Shimadzu GCMS-QP-2010 model was used to achieve Mass spectra of the products. Nuclear magnetic resonance spectra ^{1}HNMR spectra were determined in $CDCl_3$/DMSO-d_6

(in 3/1 ratio) or DMSO-d_6 and were recorded on a Bruker AVANCE II 400 MHz. Chemical shifts (δ scale) were reported in ppm (parts per million) downfield from tetramethylsilane (TMS) used as an internal standard. Splitting patterns are designated as followings: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet; brs, broad singlet; dd, double doublet. Shimadzu GCMS-QP-2010 model was used to achieve Mass spectra of the products.

2.2. Synthesis of various substituted 2-chloro-quinoline-3-carbaldehyde (INT-01)

Substituted Aniline (1) (1 mol) was taken in round bottom flask (RBF) in cooling conditions at 0°C-5°C and dropwise addition of acetic anhydride (1.5 mol) was done into RBF with continuous stirring. After addition, sulfuric acid (H_2SO_4) was added in catalytic amount to produce acidic medium. Reaction progress was reported by TLC. After the completion of the reaction, the reaction mass was poured in ice-cooled water and produced solid (4) was filtered, washed with cold water and dried (white solid product yield: 80.02%). In next step, Phosphorus oxychloride ($POCl_3$) (9 mol) was taken in RBF at 0°C-5°C, DMF (3 mol) was added drop wise into $POCl_3$ very slowly by maintaining the temperature with continuous stirring. Yellow colored salt was observed after completion of addition and at that stage calculated amount of N-phenylacetamide (3) (1mol) was added into it and reaction mass was refluxed in oil bath for 16-24 hours. By observing the completion of reaction on TLC, reaction mass was poured into crushed ice to get solid precipitate (Yellow solid product; yield 83.76%).

2.3. Synthesis of 1-(2-chlorophenyl)-2-nitroethanol (Int-2)

A mixture of 2-chlorobenzaldehyde (0.1mol), nitromethane (0.1mol) and sodium acetate (0.2mol) was stirred at RT for 24 h. The reaction was monitored by TLC. After completion of reaction, the solvent was removed under reduced pressure. The residue was poured in water and extracted with ethylacetate. The organic layer was dried and evaporated to afford Int-a in form of viscous oil. This oil was forwarded to next step without further purification.

2.4. Synthesis of 1-(2-chlorophenyl)-2-nitroethanone (Int-3)

To the suspension of $K_2Cr_2O_7$ (24.8 mmol) in 15ml water, Int-2 was added drop wise at 0°C. This mixture was allowed to stir for 30 min and then solution of sulphuric acid (10 ml con H_2SO_4 and 6 ml water) was drop wise added at same temperature. Here the exothermicity was controlled by keeping addition rate very slow. After completion of addition, reaction mixture was stirred for 15 min at the same temp. Colour of the reaction mixture turned dark green and it was poured over crushed ice. Separated solid was immediately filtered before temperature rose and was dissolved in saturated $NaHCO_3$ solution. Filtration was again carried out to separate non-dissolved matter. Filtrate was acidified with con HCl. Precipitated solid was filtered and washed with distilled water. Crystallization was carried out from methanol to afford pure Int-3.

2.5. Synthesis of 2-(bis(methylthio) methylene) malononitrile (Int-5)

A 100mL conical flask equipped with magnetic stirrer and septum was charged with a solution of malononitril (**4**), (10 mmol) in DMF (10 mL). Dry K_2CO_3 (10 mmol) was added and the mixture was stirred at RT for 2 h. CS_2 (30 mmol) was added and the mixture was stirred for an additional 2 h at room temperature. Then, methyl iodide (20 mmol) was added at 0°C-5°C and the mixture was stirred for 4 h at room temperature. The progress of the reaction was monitored by thin-layer chromatography. After completion of the reaction, it was poured into 50 ml cold water. The precipitated crude product was purified by filtration followed by crystallization from EtOH.

2.6. Synthesis of 5-amino-3-(methylthio)-1*H* pyrazole-4-carbonitril (Int-6)

To the solution of 2-(bis(methylthio)methylene) malononitrile (**Int-05**) (0.1mol) in isopropyl alcohol (100mL), hydrazine hydrate (0.1mol) was added. The reaction mixture was stirr to 0°C for 2 h. After completion of the reaction, it was poured into 50mL cold water. The precipitated crude product was purified by filtration followed by crystallization from EtOH.

2.7. General synthesis of pyrazolopyrimidine (MS-20 to MS-29)

In 50ml RBF Int-3 (2.5mmol), Int-6 (2.5mmol) and substituted aldehyde were suspended in 20 ml methanol under stirring on magnetic stirrer. Few (2-3) drops of acid was added into the reaction mixture and reaction mixture was refluxed for 10 to 12 h. The reaction was monitored by TLC. After the completion of the reaction, water was decanted and the solid residue was triturated with methanol to afford pure compound.

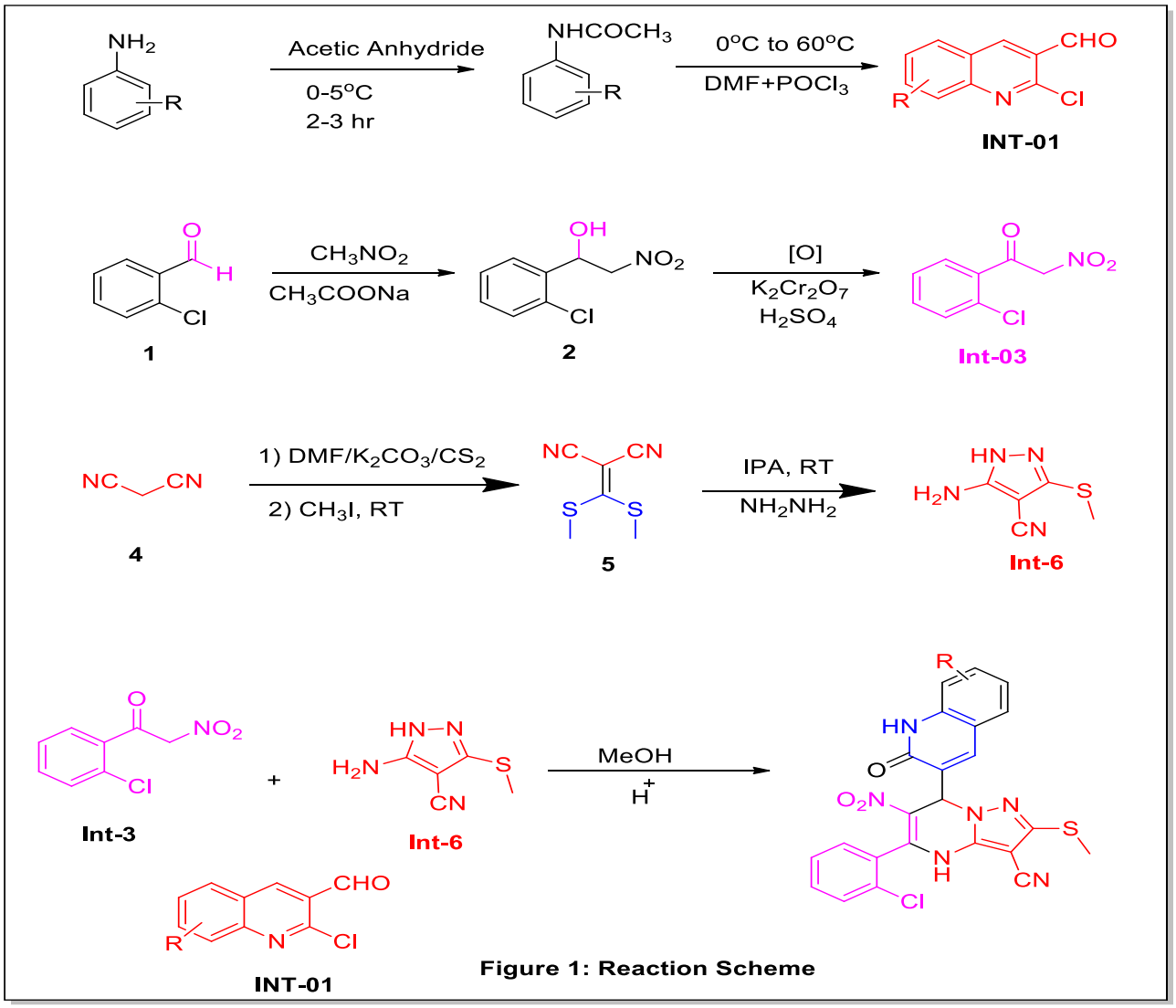

Figure 1: Reaction Scheme

2.8. Antibacterial evaluation

In our current study, antibacterial and antifungal activity was tested by standard agar cup method. All the synthesized compound were tested for their *in vitro* antimicrobial activity against Gram+*ve (Bacillus megaterium, Micrococcus spp.*), Gram -*ve (E.coli, S. typi) and fungal spps. (Ganoderma spp., A. niger, A. flavus and Penicillium spp.*), taking streptomycin, ciprofloxacin, and nystatin as standard drugs. Suspension of 24 to 48 hrs. grown fresh bacterial and fungal culture was prepared in N-broth and potato dextrose broth respectively. All the bacterial and fungal suspension were equally spreaded on to the sterile Muller Hinton and PDA plates respectively with the help of sterile swabs. Wells were made in the plates (1 cm) with the help of sterile cork borer. The standard antibiotics were dissolved in sterile distilled water to make the final concentration of 200µg/ml. The synthesized compounds to be tested were dissolved in DMSO up to the final concentration of 1 mg/ml and 0.1 ml of it was loaded in the well. The plate was incubated at 4°C for 20 minutes for proper diffusion of a compound in agar and then the plates were incubated in the upward position for 24 hrs at 37°C for bacterial culture and 48 hrs. at 25°C for fungal cultures. The control activity against DMSO was also performed. After incubation zone of inhibition was observed and measured.

3. RESULTS AND DISCUSSION

The structures of all the newly synthesized derivatives were confirmed by chromatographic and spectroscopic (IR, 1H-NMR, and mass) methods. We have prepared a series of novelpyrazole pyrimidine derivatives of 5-(2-chlorophenyl)-2-(methylthio)-6-nitro-7-(2-oxo-1,2-dihydroquinolin-3-yl)-4,7-dihydropyrazolo[1,5-a]pyrimidine-3-carbonitrile.

3.1. Spectral data of synthesized compounds

3.1.1. Spectral data of 5-(2-chlorophenyl)-2-(methylthio)-6-nitro-7-(2-oxo-1,2-dihydro-quinolin-3-yl)-4,7-dihydropyrazolo[1,5-a] pyrimidine-3-carbonitrile (MS-20)

Yellow Solid, Rf value 0.42 (Ethyl acetate 8: Hexane 2), IR (KBr pallet) in cm^{-1} :3108, 3040, 2990, 2890, 2813, 2214, 1738, 1608, 1569, 1486, 1327, 1287, 1144, 1056, 954, 819, 749, 646 cm^{-1}, ^1H NMR (DMSO) in δ ppm: 2.31 (3H, Triplet), 6.59 to 7.61 (9H, Complex), 8.86 (1H, Singlet broad), 10.89 (1H, Singlet broad), 7.74 (1H, Singlet), Mass (m/z): 490 (M^+), Ana. Calculated for Molecular formula $C_{23}H_{15}ClN_6O_3S$ is C; 56.27%, H; 3.08%, N; 17.12% Found C; 56.20%, H; 3.02%, N; 17.05%

3.1.2. Spectral data of 5-(2-chlorophenyl)-7-(6-methyl-2-oxo-1,2-dihydroquinolin-3-yl)-2-(methylthio)-6-nitro-4,7-dihydropyrazolo [1,5-a] pyrimidine-3-carbonitrile (MS-21)

White Solid, Rf value 0.41 (Ethyl acetate 8: Hexane 2), IR (KBr pallet) in cm^{-1}: 3155.51, 2983.51, 2899.03, 2745.57, 2244.43, 1686.73, 1599.54, 1408.65, 1272. 39, 1199.93, 1034.17, 936.55, 825.81, 750.67, 697.23, ^1H NMR (DMSO) in δ ppm: 2.30 to 2.41 (6H, Singlet), 7.17 to 7.69 (8H, Complex), 8.95 (1H, Singlet), 11.07 (1H, Singlet), 6.58 to 6.59 (1H, Singlet), Mass (m/z): 504 (M^+), Ana. Calculated for Molecular formula $C_{24}H_{17}ClN_6O_3S$ is C; 57.09%, H; 3.39%, N; 16.64% Found C; 57.01%, H; 3.32%, N; 16.63%

The synthesized products MS-20 to MS-29 (table 1) were tested for their antimicrobial activity by Cup-plate method against Gram positive bacteria *B. cocous* and *B. subtillis* and Gram negative bacteria *Proteus vulgaris, Escherichia coli* and antifungal activity against *Aspergillus niger*. The antimicrobial activity was compared with standard drugs viz. Amoxycillin, Benzoylpenicillin, Ciprofloxacin, Erythromycin, and antifungal activity was compared with Greseofulvin. Synthesized compounds (MS-20 to MS-29) showed (table 2) adequate to good and remarkable activities with compared to standard known drugs at same concentration.

The result includes that MS-21, MS-26 and MS-28 exhibited potent antibacterial activity against *B. megaterium, S. typi, Micrococcus* spp. and *E. coli*. Hence further investigation can be done, MIC can be identified and such compounds can further be tested and can be used as a potent drug in coming time.

Table 1: Physical constant of synthesized library

Code	Molecular formula	Substitution	Molecular Weight	M.P. °C	Percentage of Yield
MS-20	$C_{23}H_{15}ClN_6O_3S$	-H	490	154-156	81
MS-21	$C_{24}H_{17}ClN_6O_3S$	6-Me	504	170-172	79
MS-22	$C_{23}H_{14}Cl_2N_6O_3S$	6-Cl	524	152-154	74
MS-23	$C_{24}H_{17}ClN_6O_4S$	6-OMe	520	160-162	85
MS-24	$C_{23}H_{14}BrClN_6O_3S$	6-Br	569	178-180	82
MS-25	$C_{25}H_{19}ClN_6O_3S$	6,8- Di Me	518	180-182	75
MS-26	$C_{23}H_{14}ClN_7O_5S$	6-NO_2	535	156-158	78
MS-27	$C_{23}H_{14}Cl_2N_6O_3S$	8-Cl	524	144-146	80
MS-28	$C_{24}H_{17}ClN_6O_3S$	8-Me	504	184-186	82
MS-29	$C_{24}H_{17}ClN_6O_4S$	8-OMe	520	182-184	86

Table 2: Antibacterial and antifungal activity of synthesized compounds MS-20 to MS-29

Code	Antibacterial activity				Antifungal activity			
	Antibacterial activity (zone in cm), concentration: 1 mg/ml.				Antifungal activity (zone in cm), concentration: 1mg/ml			
	Gram + ve bacteria		Gram-ve bacteria					
	B. Megaterium	*Micrococcus spp.*	*S. typhi.*	*E. coli.*	*Penicillium spp.*	*Ganoderma spp.*	*A. niger*	*A. flavus*
MS-20	0.2	0.1	1.4	0.2	1.0	1.1	0.4	0.2
MS-21	**1.9**	**1.6**	**1.2**	**3.1**	0.2	1.2	0.6	0.2
MS-22	2.0	2.0	1.0	-	-	1.1	1.9	2.9
MS-23	1.9	1.6	1.2	0.1	0.2	1.2	0.6	0.2
MS-24	1.2	0.7	0.2	1.5	1.7	1.0	0.3	0.9
MS-25	-	-	1.4	1.2	0.1	1.8	0.8	0.9
MS-26	**2.1**	**2.1**	**1.4**	**1.0**	2.0	1.2	2.8	2.4

MS-27	1.1	2.0	1.5	0.1	1.5	1.2	-	1.8
MS-28	**2.4**	**2.1**	**1.9**	**1.2**	0.8	1.8	1.3	0.1
MS-29	-	0.1	1.4	1.0	2.0	3.0	1.7	1.6
Streptomycin (200µg/ml)	3.0	2	2	3.2	-	-	-	-
Ciprofloxacin (200µg/ml)	3.8	4	4	3	-	-	-	-
Nystatin (200µg/ml)	-	-	-	-	3.2	4	3.5	3.8

4. CONCLUSION

In summary, our protocol is a practical approach which uses reliable or easily performed reaction condition. The reaction was carried out clean and the products were obtained in excellent yields without any further formation of any side products or purification needed. Total ten compounds were synthesized and structure all the compounds were confirmed on the basis of the spectroscopic technique. The present work is important for the synthesis of a wide variety of novel entities.

5. ACKNOWLEDGEMENTS

The authors are heartily thankful to Department of Chemistry, Saurashtra University (DST-FIST Funded & UGC-SAP Sponsored) & NFDD (National Facility for Drug Discovery) for providing laboratory for synthetic research work and analytical data support, evaluation of antimicrobial activity.

Conflict of interest

None declared

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