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Discovery of novel pyrazoles as potent antimicrobial and antimalarial agents

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ABSTRACT

Some 1-aryl-3-alkyl-4-substituted aryl (or heteroaryl)-5 amino pyrazole derivatives have been synthesized by the reaction of substituted aryl or hetero aryl acetonitrile with alkyl acetate(ethyl acetate or ethyl trifluoroacetate) followed by reaction with phosphorous oxychloroide and further by cyclisation with substituted aryl hydrazine in presence of triethyl amine in ethanol . All the synthesized compounds were characterized by elemental analysis, ^1H NMR and LCMS. These were screened for in-vitro antimicrobial activity against two gram positive (Streptococcus pyogenes and Staphylococcus aureus) and two gram negative bacteria (Pseudomonas aeruginosa and Escherichia coli) along with antifungal and antimalarial activity.

Keywords: Pyrazole, Phenyl hydrazine, Antimicrobial and antimalarial activity.

INTRODUCTION

Pyrazole and its derivatives, occupy an important position in medicinal chemistry with a wide range of bioactivities. They possess antiobesity [1], receptor antagonists [2], HIV reverse transcriptase inhibitors [3], and anti-hyperglycemic activities [4]. They are also used as anti-inflammatory [5,6], antipyretic [7], antiarrhythmic [8], antitumor [9,10], monoamine oxidase inhibiting [11] and antibacterial agents [12]. Considering the important biological properties of pyrazole compounds, numerous methods toward pyrazoles syntheses have been developed over the past decades [13-17]. The 5-aminopyrazole system represents an important heterocyclic template that has attracted considerable interest because of its long history of application in the pharmaceutical and agrochemical industries [18- 21]. These compounds have been extensively investigated over the past one hundred years and their chemistry has been reviewed in two books published in 1964 [22] and in 1967 [23].

Looking at the importance of these heterocyclic nuclei, it is thought of interest to devote some attention for the synthesis of new substituted 5- amino pyrazole derivatives and to evaluate these derivatives for antimicrobial and antimalarial activity.

MATERIALS AND METHODS

3.1 General Procedures:

Reagent grade chemicals were used without further purification. All the melting points were taken in open capillaries and are uncorrected. The purity and mass of the synthesized compounds were checked by LCMS . ^1H NMR spectral was recorded in CDCl3 /DMSO with tetramethylsilane (TMS) as the internal standard at 400 MHz on a Bruker DRTX-400 spectrophotometer. The chemical shifts are reported as parts per million (ppm). Elemental analysis was performed using a (EURO EA 3000 instrument). Acme silica gel-G and Merck silica gel (100 to 200, 60 to 120 meshes) were used for analytical TLC and Column chromatography respectively.

3.2 Chemistry:
We have prepared the novel pyrazoles in three steps, using substituted aryl or heteroaryl acetonitrile and substituted aryl hydrazine as the starting materials. Substituted aryl or heteroaryl acetonitriles were treated with ethyl acetate or ethyl trifluoroacetate to obtain 3-oxo-2-aryl(or heteroaryl) butanenitrile or 4,4,4-trifluoro-3-oxo-2-aryl(or heteroaryl) butanenitrile which on reaction with phosphorous oxychloride and further by cyclisation with substituted aryl hydrazine in presence of triethyl amine in ethanol results the desired 5-amino pyrazoles. The clear procedure for the preparation of desired pyrazoles was given below.

4.Preparation of new substituted pyrazoles
4.1 General procedure for the synthesis of 3-oxo-2-aryl(or heteroaryl) butanenitrile or 4,4,4-trifluoro-3-oxo-2-aryl(or heteroaryl) butanenitrile
A solution of substituted aryl (or heteroaryl) acetonitrile (0.01 mole) in DMF 5ml was added to a solution of NaH (0.012 mole) in 10 ml DMF at 0 ^{o}C . Then to it was added a solution of ethyl acetate or ethyl trifluoro acetate (0.011 mole) dropwise at 0 ^{o}C. Then it was warmed to room temperature and stirred for 2hrs at room temperature. Then the reaction was quenched with 1N HCl (20 ml) at 0 ^{o}C and diluted with water 50 ml. The compound was extracted with ethyl acetate and washed with water. The organic layer was dried over Na_2SO_4 and evaporated which results to give dark brown viscous oil which was used as such for next step without any purification.

Spectral data of intermediate:
3-oxo-2-(thiophen-3-yl)butanenitrile :
1H-NMR (400MHz, $CDCl_3$): 7.43-7.41 (m, 2H, Het-H), 7.08-7.01 (m,1H, Het-H), 4.75 (s, 1H, -CHCN), 2.30 (s, 3H, -$COCH_3$), MS: 166 (M+). Anal. Calcd for C_8H_7NOS: C- 58.16%, H- 4.27%, N-8.48%, O-9.68%, S-19.41, Found : C- 58.14%, H- 4.25%, N-8.44%, O-9.67%, S-19.40.

4.2 General procedure for the synthesis of 3-alkyl -1-(substituted aryl)-4-Substituted aryl(or heteroaryl)-1H-pyrazole-5-amine
The solution of 3-oxo-2-aryl(or heteroaryl) butanenitrile or 4,4,4-trifluoro-3-oxo-2-aryl(or heteroaryl) butanenitrile (0.01 mole) in phosphorous oxychloride (5 mL) was refluxed for 2hrs. The reaction mixture was evaporated and dissolved in ethanol . To it was added triethyl amine (0.02 mole) followed by solution of substituted aryl hydrazine (0.015 mole) in ethanol at 0 ^{o}C dropwise. Then it was refluxed at 80 ^{o}C for 2hrs. The solvent of reaction mixture was evaporated , diluted with ethyl acetate and washed with water. The organic layer was dried over Na_2SO_4 and evaporated. The crude compound was purified by using column chromatography with 100-200 silica gel to give compound 2(a-h) Scheme-1.

Spectral data of Pyrazoles :
1-(2,4-dichlorophenyl)-4-(3-fluorophenyl)-3-methyl-1H-pyrazol-5-amine (2a) :
1H-NMR (400MHz, $CDCl_3$): 7.84 (d, J = 2.4 Hz, 1H, Ar-H), 7.56 (dd, J_1 = 2.2 Hz, J2 = 8.6 Hz, 1H, Ar-H), 7.50 (d, J = 8.4 Hz,1H, Ar-H), 7.43-7.38 (m,1H, Ar-H), 7.20 (d, J = 7.6 Hz, 1H, Ar-H), 7.17-7.14 (m,1H, Ar-H), 7.02-6.98 (m,1H, Ar-H), 5.33 (s,2H, NH_2), 2.16(s,3H, CH_3), LCMS: 336.4 (M+). Purity: 96.7 %,Anal. Calcd for $C_{16}H_{12}Cl_2FN_3$: C- 57.16%, H- 3.60%, Cl- 21.09% , F-5.65%, N-12.50%,Found :C- 57.10%, H- 3.56%, Cl- 21.10% , F-5.60%, N-12.46%.

3-methyl-4-(thiophen-3-yl)-1-(4-(trifluoromethyl)phenyl)-1H-pyrazol-5-amine (2b) :
1H-NMR (400MHz, $CDCl_3$): 7.89-7.82 (m,4H, Ar-H), 7.64-7.62 (m,1H, Het-H), 7.40 (bs,1H, Het-H), 7.27-7.25 (m,1H, Het-H), 5.34 (s,2H, NH_2), 2.20(s,3H, CH_3), LCMS: 324.2 (M+). Purity: 98.4 % ,Anal. Calcd for $C_{15}H_{12}F_3N_3S$: C- 55.72%, H- 3.74%, F-17.63%, N-13.00%, S-9.92%, Found :C- 55.68%, H- 3.70%, F-17.60%, N-12.97%, S-9.89%.

4-(3-chlorophenyl)-1-(2,4-dichlorophenyl)-3-methyl-1H-pyrazol-5-amine (2c) :
1H-NMR (400MHz, $CDCl_3$): 7.84 (d, J = 2.4 Hz, 1H, Ar-H), 7.56 (dd, J_1 = 2.2 Hz, J2 = 8.6 Hz, 1H, Ar-H), 7.51 (d, J = 8.4 Hz,1H,Ar-H), 7.42-7.38 (m,2H, Ar-H), 7.32 (d, J = 7.6 Hz, 1H, Ar-H), 7.23 (d,J = 8.0Hz,1H, Ar-H), 5.35 (s,2H,NH_2), 2.15(s,3H,CH_3), LCMS: 352.5 (M+). Purity: 97.9%,Anal. Calcd for $C_{16}H_{12}Cl_2FN_3$: C-54.49%, H-3.43%, Cl-30.16% , N-11.92%, Found :C- 54.46%, H- 3.41%, Cl- 30.13% , N-11.90%.

1-(2,4-dichlorophenyl)-4-(2,4-difluorophenyl)-3-methyl-1H-pyrazol-5-amine (2d) :
1H-NMR (400MHz, $CDCl_3$): 7.83 (d, J = 2.8 Hz, 1H, Ar-H), 7.56-7.50 (m,2H, Ar-H), 7.42-7.35 (m,1H, Ar-H), 7.27 (dt, J1 = 2.4 Hz, J2 = 10.2 Hz,1H, Ar-H), 7.11 (dt, J1 = 2.4, J2 = 8.4Hz,1H, Ar-H), 5.16 (s,2H,NH_2), 2.00(s,3H,CH_3), LCMS:354.5 (M+). Purity: 97.2%, Anal. Calcd for $C_{16}H_{11}Cl_2F_2N_3$: C-54.26%, H-3.13%, Cl-20.02% , F-10.73, N-11.86%, Found C- 54.22%, H- 3.10%, Cl- 19.98% , F- 10.71%, N-11.82%.

1-(4-methoxyphenyl)-3-methyl-4-(thiophen-3-yl)-1H-pyrazol-5-amine (2e) :
1H-NMR (400MHz, $CDCl_3$): 7.60 (bs,1H, Het-H), 7.44 (d, J = 8.4Hz,2H, Ar-H), 7.35-7.34(m,1H, Het-H), 7.27-7.26 (m,1H, Het-H), 7.04 (d,J = 8.4 Hz,2H, Ar-H), 4.96 (s,2H, NH_2),3.80 (s,3H, Ar-OCH_3) 2.18(s,3H, CH_3),LCMS :286.2 (M+). Purity: 99.69 % , Anal. Calcd for $C_{15}H_{15}N_3OS$: C- 63.13%, H- 5.30%, N-14.73%, O-5.61%, S-11.24%, Found : C- 63.10%, H- 5.32%, N-14.71%, O-5.57%, S-11.21%.

1-(4-methoxyphenyl)-4-(thiophen-3-yl)-3-(trifluoromethyl)-1H-pyrazol-5-amine (2f) :
1H-NMR (400MHz, $CDCl_3$): 7.64-7.62 (m,1H, Het-H), 7.49 (d, J = 8.8Hz,2H, Ar-H), 7.47-7.46(m,1H, Het-H), 7.16-7.14 (m,1H, Het-H), 7.09 (d,J = 8.8 Hz,2H, Ar-H), 5.36 (s,2H, NH_2),3.82 (s,3H, Ar-OCH_3).LCMS :340.18 (M+). Purity: 99.79 % , Anal. Calcd for $C_{15}H_{12}F_3N_3OS$: C- 53.09%, H- 3.56%, F-16.80%, N-12.38%, O-4.71%, S-9.45%, Found : C-53.06%, H- 3.53%, F-16.77%, N-12.39%, O-4.67%, S-9.42%.

4-(thiophen-3-yl)-3-(trifluoromethyl)-1-(4-(trifluoromethyl)phenyl)-1H-pyrazol-5-amine (2g) :
1H-NMR (400MHz, $CDCl_3$): 7.93 (d, J = 8.8Hz,2H, Ar-H), 7.89 (d,J = 8.8Hz,2H, Ar-H),7.67-7.65 (m,1H, Het-H),7.50-7.49 (m,1H, Het-H), 7.15 (d, J = 4.8Hz,1H, Het-H), 5.72 (s,2H, NH_2). LCMS :378.10 (M+). Purity: 93.62 % , Anal. Calcd for $C_{15}H_9F_6N_3S$: C- 47.75%, H- 2.40%, F-30.21%, N-11.14%, S-8.50%, Found : C- 47.72%, H- 2.36%, F-30.17%, N-11.10%, S-8.52%.

1-(4-fluorophenyl)-4-(thiophen-3-yl)-3-(trifluoromethyl)-1H-pyrazol-5-amine (2h) :
1H-NMR (400MHz, $CDCl_3$): 7.66-7.62 (m,3H, Ar-H), 7.48-7.47 (m,1H, Het-H),7.41-7.37 (m,2H, Ar-H), 7.15 (d, J = 4.8Hz,1H, Het-H), 5.51 (s,2H, NH_2). LCMS :328.19 (M+). Purity: 95.76 % , Anal. Calcd for $C_{14}H_9F_4N_3S$: C- 51.37%, H- 2.77%, F-23.22%, N-12.84%, S-9.80%, Found : C- 51.33%, H- 2.74%, F-23.17%, N-12.85%, S-9.76%.

5.Antimicrobial activity
All the synthesized compounds were tested against two gram positive bacteria (Staphylococcus aureus ,Streptococcus Pyogenes) and two gram negative bacteria (Escherichia coli ,Pseudomonas aeruginosa) using micro broth dilution method [24-27] for the determination of minimal inhibition concentration . For the antifungal activity the common standard strains that were used, are C.Albicans, A,Niger and A.Clavatus. Muller Hinton broth (Microcare laboratory & Tuberculosis Research Centre, Surat-3, India) was used as nutrient medium to grow and dilute the drug suspension for the test bacteria. Inoculum Size for Test Strain was adjust to 10^8 Cfu [Colony Forming Unit] per milliliter by comparing the turbidity. DMSO was used as diluents / vehicle to get desired concentration of drugs to test upon Standard bacterial strains. Serial dilution were prepared in primary and secondary screening. In primary screening 1000 micro/ml, 500 micro/ml, and 250 micro/ml concentrations of the synthesized compounds were taken. The active synthesized compounds found in this primary screening were further tested in a second set of dilution against all microorganisms. The highest dilution showing at least 99 % inhibition zone is taken as MIC. The test mixture should contain 10^8 organism/ml. Standard drugs Ampicillin and Chloramphenicol were used as antibacterial for comparison. Standard drugs Nystatin and Greseofulvin were used as antifungal for comparison.

6. Antimalarial activity
The in vitro antimalarial assay was carried out in 96 well microtitre plates according to the microassay protocol reference. The cultures of P. falciparum strain were maintained in medium RPMI 1640 supplemented with 25 mM HEPES, 1% D-glucose, 0.23% sodium bicarbonate and 10% heat inactivated human serum. The asynchronous parasites of P. falciparum were synchronized after 5% D-sorbitol treatment to obtain only the ring stage parasitized cells. For carrying out the assay, an initial ring stage parasitaemia of 0.8 to 1.5% at 3% haematocrit in a total volume of 200 µ l of medium RPMI-1640 was determined by Jaswant Singh Bhattacharya (JSB) staining to assess the percent parasitaemia (rings) and uniformally maintained with 50% RBCs (O+). A stock solution of 5mg/ml of each of the test samples was prepared in DMSO and subsequent dilutions were prepared with culture medium. The diluted samples in 20 µ l volume were added to the test wells so as to obtain final concentrations (at five fold dilutions) ranging between 0.4 µ g/ml to 100 µ g/ml in duplicate well containing parasitized cell preparation. The culture plates were incubated at 37oC in a candle jar. After 36 to 40 h incubation, thin blood smears from each well were prepared and stained with JSB stain. The slides were microscopically observed to record maturation of ring stage parasites into trophozoites and schizonts in presence of different concentrations of the test agents. The test concentration which inhibited the complete maturation into schizonts was recorded as the minimum inhibitory concentrations (MIC). Quinine was taken as the reference drug.

RESULTS AND DISCUSSION

7.Chemistry

Substituted aryl or hetero aryl acetonitrile on reaction with ethyl acetate (or ethyl trifluoro acetate) gives 3-oxo-2-aryl(heteroaryl) butanenitrile or 4,4,4-trifluoro-3-oxo-2-aryl(heteroaryl) butanenitrile. The obtained compound (1) on reaction with phosphorous oxychloride and further by cyclisation with substituted aryl hydrazine gives the desired substituted 5- amino pyrazoles. The list of synthesized compound are represented by Table-1.

Scheme:

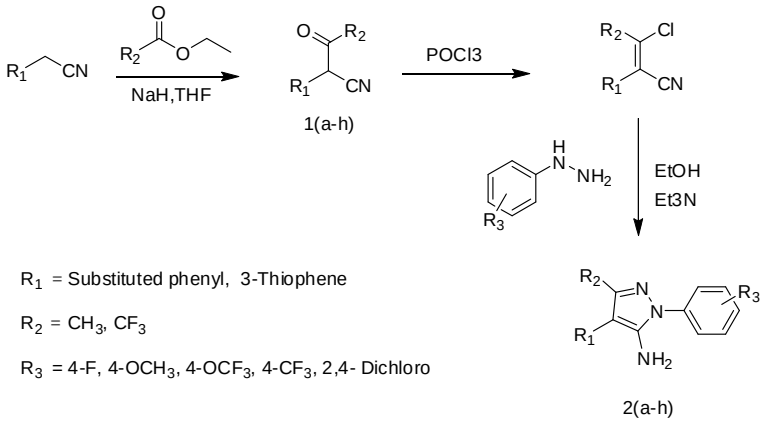

Table-1 List of Synthesized compound

Compound	R1	R2	R3	M.P (°C)	Yield (%)
2a	3F- Phenyl	CH3	2,4-Dichloro	140-142	39.6
2b	3-Thiophene	CH3	4-CF3	125-126	31.2
2c	3Cl-Phenyl	CH3	2,4-Dichloro	167-168	45.4
2d	2,4 –Difluoro phenyl	CH3	2,4-Dichloro	189-191	43.6
2e	3-Thiophene	CH3	4-OCH3	131-132	29.1
2f	3-Thiophene	CF3	4-OCH3	145-146	25.5
2g	3-Thiophene	CF3	4-CF3	117-118	28.7
2h	3-Thiophene	CF3	4-Fluoro	125-126	36.3

Antibacterial activity:

The antibacterial activity of all the synthesized compounds were tested in-vitro against pathogenic E. coli, P.aeruginosa, S.aureus and S.pyogenus and the results were compared with standard drugs (Ampicillin and Chloramphenicol). In case of S.aureus compounds 2b,2c,2e and 2f exhibit good activity while 2a, 2d, 2g and 2h show moderate activity. In case of S.pyogenus compounds 2a exhibit higher activity while 2b, 2c, 2d, 2e, 2f,2g and 2h shows moderate activity. In case of E. coli Compound 2b shows higher activity and 2a shows moderate activity while rest of the compounds possess less activity. In case of P.aeruginosa compounds 2b shows good activity than the rest of the compounds. The results are given in Table-2.

Table-2: Antibacterial activity of pyrazoles

Compound	*S.AUREUS*	*S.PYOGENUS*	*E.COLI*	*P.AERUGINOSA*
2a	200	62.5	100	125
2b	100	100	62.5	100
2c	100	125	250	500
2d	200	500	250	500
2e	100	500	200	500
2f	100	250	200	125
2g	125	125	250	250
2h	125	500	125	500
Ampicillin	250	100	100	100
Chloramphenicol	50	50	50	50

Antifungal activity:

The antifungal activity of all the synthesized compounds were tested in-vitro against fungi C.Albicans, A,Niger and A.Clavatus and the results were compared with standard drugs (Nystatin and Greseofulvin). In case of C.Albicans compounds 2c and 2d exhibit good activity while 2a, 2b, 2e, 2f 2g and 2h show moderate activity. In case of A,Niger and A.Clavatus all the compounds possess less activity . The results are given in Table-3.

Antimalarial activity:
For antimalarial activity, Compounds 2a and 2b exhibit good activity closer to reference compound Quinine while rest of the compounds possess less activity. The results are given in Table-4.

Table-3 Antifungal activity of pyrazoles

Compound	*C.Albicans*	*A.Niger*	*A.Clavatus*
2a	>1000	1000	>1000
2b	1000	>1000	>1000
2c	250	500	500
2d	250	500	500
2e	500	500	500
2f	500	1000	500
2g	1000	500	250
2h	500	250	500
Nystatin	100	100	100
Greseofulvin	500	100	100

Table-4 Antimalarial activity

Compound	Mean IC50 (microgame/ml)
2a	0.70
2b	0.66
2c	1.20
2d	0.90
2e	0.76
2f	0.86
2g	1.40
2h	0.85
Quinine	0.268

CONCLUSION

All the newly synthesized compounds were screened for antibacterial, antifungal and antimalarial activity. The data in the Table 2 indicate that among the synthesized compound 2a and 2b possesses good antibacterial activity. However, the activities of the tested compounds are much less than those of standard agents used. These compounds also show potent antimalarial activity. From the results of various biological activities it is clear that these compounds would be of better use in drug development to combat bacterial infections and as antimalarial agents in the future.

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