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Der Pharma Chemica, 2010, 2(5):315-321
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X

Synthesis and Characterization of condensed pyrazole derivatives

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ABSTRACT

A series of quinolines bearing pyrazole nucleus have been prepared in one pot by condensing various quinolines and semicarbazide in presence of catalytic amount of PTSA. All the synthesised compound have been characterised by their percentage yield, melting points, elemental analysis, ^{1}H-NMR and ^{13}C-NMR and IR spectra. These compounds have been screened for their antimicrobial activities.

Keywords: quinolines, condensation, pyrazole, antibacterial, PTSA.

INTRODUCTION

Quinolines are the versatile nitrogen contain heterocyclic compound. The quinolines skeleton is a common structural motif in a broad range of biologically active compounds. Quinolines have been found to be associated diverse biological activities and numerous reports have appeared in the literature, which highlighted their chemistry and use.[1-4] The quinolines derivatives have remarkable pharmacological activity and widely used in field of anti-malarial drugs.

Pyrazoles have attracted a great deal of attention from synthetic community due to their diverse types of biological properties such as antibacterial[5], antiviral[6], anti-inflamatory[7], antidepresent[8], ant-tumor[9], anticancer[10], analgesic[11] and anti-herpetic properties[12].

Development and applications of microwave irradiations towards organic reactions has added a new dimension to solid phase synthesis[13]. Usually the application of microwave irradiation as a source of thermal energy in organic reactions[14,15] such as Aldol type condensation[16], Vilsmeier reaction[17], Knoevenagal condensation [18], preparation of aryl vinyl nitriles[19], rearrangements[20] is due to the reduction in reaction times, operational simplicity, cleaner reactions, easier work-up and better yields. In this context, the present work was mainly focused

on the construction of several newly fused quinoline system and to formulate some new synthetic route for the preparation of known quinoline derivatives based on microwave irradiation. It is well known that, the major synthetic routes leading to the formation of pyrano-, furo-, pyrido- pyrimido- and pyrazolo- quinoline alkaloids invariably involved some common intermediate. 2-Chloro-3-formyl quinolines (**1**) occupy a prominent position, as they are key intermediates for further [b]-annelation of a wide variety of rings and for various functional group interconversions.

In view of these observations and continuing our interest in the synthesis of heterocyclic compounds [21-27], it was envisaged in the present investigation to undertake the synthesis and evaluation of antimicrobial activity of pyrazoloquinolines with to find new and more potent antimicrobial agent.

MATERIALS AND METHODS

Melting points (mp) were determined using Boetieus micro heating table and are uncorrected. IR (KBr, cm^{-1}) spectra were obtained on Shimadzu–8201 spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker AMX-400 MHz spectrometer using TMS as an internal reference (Chemical shifts in δ, ppm). Elemental analyses were performed on Perkin Elmer CHN-analyzer. Mass spectra were recorded on Shimadzu GCMS-QP5050A (70 ev) mass spectrometer. For microwave irradiation a Kenstar (OM-20ESP, 2450 MHz) domestic microwave oven was used.

General procedure for the preparation of the 1-carboamidopyrazolo[3,4-*b*] quinolines (2a-g):

2-Chloro-3-formylquinolines (1 mmol), semicarbazide (1.25 mmol) and PTSA (120 mg) were taken in 100 ml beaker and mixed well. Then the reaction mixture was irradiated in microwave oven for the respective time (**Table 1**). After completion of the reaction, the mixture was poured into ice. The formed product was extracted with chloroform and purified using column chromatography.

1-carboamidopyrazolo[3,4-*b*]quinoline (3a): IR (KBr cm^{-1}): 2900-3450 (NH_2), 1699 (C=O), 1640 (>C=N), 1571 (>C=N); ^{1}H-NMR (DMSO-d_6): δ 8.07 (s, 1H, C_5-CH_3), 8.27 (s, 1H, C_3-H), 7.16-8.04 (m, 4H, Ar-H), 10.17 (s, 1H, NH_2); MS m/z: 212 ($M^{+\cdot}$); Anal. Calcd for $C_{11}H_8N_4O$: C, 62.25; H, 3.80; N, 26.41. Found: C, 62.28; H, 3.86; N, 26.39.

6-Methyl-1-carboamidopyrazolo[3,4-*b*]quinoline (3b): IR (KBr cm^{-1}): 2900-3400 (NH_2), 1685 (C=O), 1642 (>C=N), 1570 (>C=N); ^{1}H-NMR (DMSO-d_6): δ 2.58 (s, 3H, C_7-CH_3), 7.10-7.98 (m, 2H, Ar-H), 8.06 (s, 1H, C_6-H), 8.11 (s, 1H, C_5-H), 8.26 (s, 1H, C_3-H), 9.98 (s, 1H, NH_2); MS m/z: 226 ($M^{+\cdot}$); Anal Calcd for $C_{12}H_{10}N_4O$: C, 63.72; H, 4.46; N, 24.77. Found: C, 63.68; H, 4.42; N, 24.74.

8-Methyl-1-carboamidopyrazolo[3,4-*b*]quinoline (3c): IR (KBr cm^{-1}): 3000-3400 (NH_2), 1690 (C=O), 1640 (>C=N), 1565 (>C=N); ^{1}H-NMR (DMSO-d_6): δ 2.62 (s, 3H, C_9-CH_3), 7.22-7.83 (m, 3H, Ar-H), 8.10 (s, 1H, C_5-H), 8.21 (s, 1H, C_3-H), 9.92 (s, 1H, NH_2); MS m/z: 226 ($M^{+\cdot}$); Anal Calcd for $C_{12}H_{10}N_4O$: C, 63.72; H, 4.46; N, 24.77. Found: C, 63.70; H, 4.452; N, 24.71.

6-Methoxy-1-carboamidopyrazolo[3,4-*b*]quinoline (3d IR (KBr cm^{-1}): 2950-3400 (NH_2), 1701 (C=O), 1645 (>C=N), 1565 (>C=N); ^{1}H-NMR (DMSO-d_6): δ 3.90 (s, 3H, C_7-OCH_3), 7.22-7.91 (m, 2H, Ar-H), 8.00 (s, 1H, C_6-H), 8.15 (s, 1H, C_5-H), 8.28 (s, 1H, C_3-H), 10.05 (s, 1H, NH_2); MS m/z: 242 ($M^{+\cdot}$); Anal Calcd for $C_{12}H_{10}N_4O_2$: C, 59.50; H, 4.17; N, 23.14. Found: C, 58.90; H, 4.10; N, 23.11..

8-Methoxy-1-carboamidopyrazolo[3,4-*b*]quinoline (3e): IR (KBr cm^{-1}): 2955-3450 (NH_2), 1700 (C=O), 1640 (>C=N), 1570 (>C=N); ^{1}H-NMR (DMSO-d_6): δ 3.93 (s, 3H, C_7-CH_3), 7.28-8.00 (m, 3H, Ar-H), 8.16 (s, 1H, C_5-H), 8.30 (s, 1H, C_3-H), 10.15 (s, 1H, NH_2); MS m/z: 242 ($M^{+\cdot}$); Anal Calcd for $C_{12}H_{10}N_4O_2$: C, 59.50; H, 4.17; N, 23.14. Found: C, 59.30; H, 4.13; N, 23.10..

6-Bromo-1-carboamidopyrazolo[3,4-*b*]quinoline(3f): IR (KBr cm^{-1}): 2900-3440 (NH_2), 1700 (C=O), 1640 (>C=N), 1570 (>C=N); ^{1}H-NMR (DMSO-d_6): 7.22-7.98 (m, 4H, Ar-H), δ 8.10 (s, 1H, C_5-CH_3), 8.26 (s, 1H, C_3-H), 10.08 (s, 1H, NH_2); MS m/z: 290 ($M^{+\cdot}$); Anal. Calcd for $C_{11}H_7N_4OBrl$: C, 45.51; H, 2.43; N, 19.31. Found: C, 45.461; H, 2.40; N, 19.27. .

6-Chloro-1-carboamidopyrazolo[3,4-*b*]quinoline (3g): IR (KBr cm^{-1}): 2950-3400 (NH_2), 1690 (C=O), 1640 (>C=N), 1570 (>C=N); ^{1}H-NMR (DMSO-d_6): 7.18-7.92 (m, 4H, Ar-H), δ 8.08 (s, 1H, C_5-CH_3), 8.22 (s, 1H, C_3-H), 10.08 (s, 1H, NH_2); MS m/z: 246 ($M^{+\cdot}$); Anal. Calcd for $C_{11}H_7N_4OCl$: C, 53.66; H, 2.87; N, 22.74. Found: C, 53.61; H, 2.84; N, 22.68.

Evaluation of antimicrobial activity

All the synthesized compounds were screened for their antibacterial and antifungal activities. For preliminary screening, the antimicrobial tests were carried out by disc-diffusion method [28]. One hundred µl of suspension containing 10^8 CFU/ml of bacteria, 10^6 CFU/ml of fungi were spread on Mueller-Hinton agar medium (MHA) and Sabouraud's dextrose agar (SDA) medium respectively. The discs (6 mm in diameter), impregnated with 10 µl of the test compounds (500 µg/disc and 1000 µg/disc) at the concentration of 50 mg/ml and 100mg/ml were placed on the inoculated agar. Negative controls were prepared using the same solvent (DMSO) employed to dissolve the test compounds. Ofloxacin (5 µg/disc) and Clotrimazole (10 µg/disc) were used as positive reference standard to determine the sensitivity of each microbial species tested. The inoculated plates were incubated at 37˚ C for 24 hr and 27˚ C for 72 hr for bacteria and fungi strains respectively. Antimicrobial activity was evaluated by measuring the diameter of zone of inhibition against test organisms.

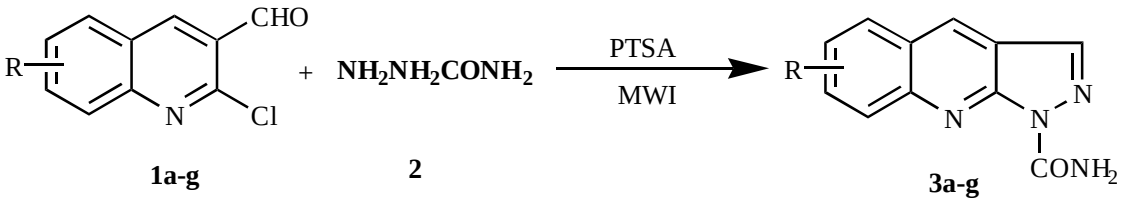
Minimum inhibitory concentration (MIC) of the compounds was also estimated by broth dilution assay [29] for the microorganisms, which were determined as sensitive to the compounds in disc-diffusion assay. Nutrient broth (NB) and Sabouraud's dextrose broth (SDB) were used to estimate the MIC values of the test compounds against bacteria and fungi respectively. A two fold serial dilution of test compounds were followed with 1ml of sterile broth in test tubes to provide various concentration ranges from 3.9-1000 µg/ml of the test compounds. Ten µl of the test organism was added to each tube and incubated at 37˚ C for 24 hr and 27˚ C for 72 hr for bacteria and fungi strains respectively. The highest dilution of the test compound completely inhibiting the test organism was considered as MIC value of the test compound respectively.

RESULTS AND DISCUSSION

The reaction sequence for the synthesis of title compounds are shown in **scheme 1.** The key intermediates 2-chloro-3-formylquinoline **1a-e** was prepared by Vilsmeier-Haack reaction on acetanilide. The quinolines intermediate **1a-e**, when irradiated with semicarbazide **2** in catalytic amount of PTSA under microwave reactor afforded pyrazoloquinoline **3a-e** in 85-92% yield (**Table 1**). All the synthesized compound were characterized by analytical, IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectral data. The synthesized compounds were screened for their antibacterial and antifungal activities and result of screening studies are tabulated in **Table 2** and **3**.

The formation of pyrazoloquinolines was confirmed by IR, ^{1}H & ^{13}C–NMR, Mass and elemental analysis. The IR Spectra of the compound **3a** exhibited peak at 2900-3450 cm^{-1}, 1699 due to group NH_2, C=O respectively and also registered the peak at 1640 , 1571 cm^{-1} due to >C=N groups. In its ^{1}H NMR Spectrum, two singlets observed at δ 8.27 and δ 8.07 for C_3- and C_5-protons and unresolved multiplet at δ 7.16-8.04 is accounted for all the aromatic protons. The spectrum also registered singlet at δ 10.17 for NH_2 protons. Mass spectrum showed the molecular ion peak at *m/z* 212 and elemental analysis Calcd. For $C_{11}H_8N_4O$; C 62.25, H 3.80, N 26.41: Found: C 62.28, H 3.86, N 26.39.

Scheme 1



1a-g **2** **3a-g**

R = H, CH_3, OCH_3,Br, Cl

Table 1. Microwave synthesis of pyrazolo[3,4-*b*]quinolines (3a-g) at 320W

Compound	Time (min)	Yield (%)	mp° C
3a	8	96	232
3b	8	84	236
3c	8	98	254
3d	12	96	240
3e	12	81	246
3f	14	85	272
3g	12	80	259

All the pyrazolo[3,4-*b*]quinolines derivatives synthesized in the present study were tested for antibacterial activity against five bacteria and five fungi stains.

Table 2: *In vitro* antimicrobial activity of pyrazolo[3,4-*b*]quinolines (3a-g) (µg/disc) by disc diffusion assay

Microorganisms	Diameter of zone of inhibition in mm																	
	3a µg/disc		**3b** µg/disc		**3c** µg/disc		**3d** µg/disc		**3e** µg/disc		**3f** µg/disc		**3g** µg/disc		**A** µg/disc	**B** µg/disc
	500	1000	500	1000	500	1000	500	1000	500	1000	500	1000	500	1000	5	10
Escherichia coli (NCIM 2065) [a]	10	11	8	12	9	13	9	14	10	13	8	12	10	14	23	-
Pseudomonas aeruginosa (NCIM 2200) [a]	11	13	7	11	7	9	7	9	7	10	7	10	9	11	22	-
Klebsiella aerogenes (NCIM 2239) [a]	10	13	-	-	-	-	-	-	-	8	-	-	-	8	21	-
Bacillus subtilis (NCIM 2063) [a]	16	18	8	13	-	-	-	11	-	10	-	7	8	10	24	-
Staphylococcus aureus (NCIM 2079) [a]	10	11	-	8	-	-	8	9	7	9	-	8	-	11	25	-
Aspergillus niger (NCIM 1196) [b]	8	9	-	-	9	12	-	-	-	-	-	-	-	-	-	16
Aspergillus flavus (NCIM 535) [b]	10	12	9	13	7	9	8	10	8	11	-	8	-	9	-	16
Rhodotorula rubra (NCIM 3174) [b]	11	13	9	12	9	13	-	-	-	11	-	10	-	7	-	17
Candida albicans (NCIM 3471) [b]	10	13	-	-	-	-	-	-	-	8	-	-	-	8	-	18
Lipomyces lopofera (NCIM 3252) [b]	16	18	9	12	-	-	-	-	-	-	-	-	10	13	-	18

[a] *bacteria* [b] *fungi* ***A*** = *Ofloxacin,* ***B*** = *Clotrimazole, - No inhibition*

Table 3: Minimum Inhibitory Concentration values of the pyrazoloquinolines 3a-g (μg/mL) against the microorganisms tested in broth dilution assay

Microorganisms	**3a**	**3b**	**3c**	**3d**	**3e**	**3f**	**3g**
Escherichia coli (NCIM 2065) [a]	3.9	15.6	7.8	7.8	7.8	15.6	3.9
Pseudomonas aeruginosa (NCIM 2200) [a]	15.6	125	62.5	125	15.6	62.5	7.8
Klebsiella aerogenes (NCIM 2239) [a]	31.2	-	-	-	125	-	125
Bacillus subtilis (NCIM 2063) [a]	31.2	125	-	125	62.5	-	31.2
Staphylococcus aureus (NCIM 2079) [a]	62.5	125	-	31.2	62.5	125	31.2
Aspergillus niger (NCIM 1196) [b]	62.5	-	125	-	-	-	-
Aspergillus flavus (NCIM 535) [b]	7.8	125	62.5	62.5	31.2	125	125
Rhodotorula rubra (NCIM 3174) [b]	15.6	31.2	31.2	-	15.6	62.5	125
Candida albicans (NCIM 3471) [b]	15.6	-	-	-	125	62.5	125
Lipomyces lopofera (NCIM 3252) [b]	7.8	15.6	-	-	-	-	31.2

[a] bacteria [b] fungi - Not tested

Based on the results (**Tables 2**), it is inferred that pyrazolo[3,4-*b*]quinolines **3a-g** have significant inhibition effect on the growth of bacteria like *Escherichia coli*, *Pseudomonas aeruginosa* and also the compounds **3a-g** exhibited good antifungal activity against *Aspergillus flavus* and *Rhodotorula rubra.* These compounds showed moderate activity against other bacteria and fungi (**Tables 2**).

Among them, compound **3a** registered good antibacterial activity against most of the bacteria and fungi. The compound **3e** & **3g** showed excellent *in vitro* activity against *Escherichia coli*, and displayed moderate inhibition against other bacteria such as *Pseudomonas aeruginosa*, *Klebsiella aerogenes*, *Bacillus subtilis*, and *Staphylococcus albus* (**Table 2**). The compounds **3b,c,d & f** have no activity against *Klebsiella aerogenes* and *Candida albicans* . The potency of **3c** and **3f** were found to be less against most of the bacteria and fungi.

The results of the MIC values of the compounds are listed in **Table 3**. The MIC values of the compounds range between 3.9 and 125 μg/mL in most of the cases. The compound 3a and 3g show the excellent activity against *Escherichia coli* i.e. MIC 3.9 μg/mL and displayed moderate activity against other bacteria. Excellent activity against all the bacteria and fungi range between 3.9-62.5 μg/mL.

CONCLUSION

In conclusion, the salient feature of our approach is coupling microwaves with solvent free technique keeping modernization and simplification of classical procedure, avoiding volatile and toxic organic solvents, corrosive, mineral acids which make it a clean, efficient and cheap technology to pyrazoloquinolines. These prepared quinolines have showed moderate to significant antibacterial and antifungal activities.

Acknowledgment
The author VN is grateful to Director of Collegiate Education, Govt. of Tamilnadu, India, for financial support. Authors thank NMR Research centre, Indian Institute of Science, Bangalore, INDIA for providing NMR spectral data.

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