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Microwave Assisted Synthesis, Characterization, DFT Studies and Antimicrobial Activities of Novel Pyrazole Derivatives

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ABSTRACT

A simple and highly efficient procedure has been described for the synthesis of 3-bromo-1-phenyl-pyrazole derivatives. The cyclization of pyrazole ring is carried under microwave irradiation using chloramine-T as catalyst. The products were obtained in very good yields and characterized by elemental, U.V. Vis, I.R., NMR and mass spectra. HOMO and LUMO studies were carried by the density functional theory (DFT). Calculations have been performed at the B3LYP/ 6-31^{+}G(d,p) level of theory. The antimicrobial and antifungal activities of all compounds have been screened against Bacillus subtillis, Escherichia coli, Aspergillus niger and Candida albicans.

***Key words**: pyrozoles, microwave synthesis, antimicrobial activity, HOMO &LUMO studies.*

1. INTRODUCTION

Abundant studies have been reported on the synthesis of a variety of pyrazole derivatives covering a wide range of bioactivities [1]. As a result, a large number of pyrazole derivatives have been developed for clinical application. The early success of antipyrine in the treatment of headache, neuralgia, myalgia and chronic rheumatism has focused the light on pyrazoles as potential classical antipyretic, analgesic and anti-inflammatory agents[2-4]. Celecoxib, has been introduced clinically and proved to act as a potent and gastrointestinal-safe anti-inflammatory drug, through the inhibition of cyclooxygenase- 2 enzyme (COX-2)[5]. Among other biological properties reported to be associated with some pyrazole derivatives are their antipyrestic, antitubercular[6], anticonvulsant[7] activities. Over the past 2 decades, much interest has been focussed on the chemotherapeutic activity of pyrazole derivatives as antimicrobial [8], antibacterial [9], antiviral[10] and potential anticancer agents[11-13].

Green chemistry was developed to meet the increasing demand for environmentally benign chemical processes. Microwave irradiation technique have an importance in the search for green synthesis because of their use as an efficient alternative heating source for organic reactions[14]. The main advantage of microwave assisted synthesis is the shorter reaction time, simple experimental procedure, very high yields and clean reaction, eco-friendly, requires no special apparatus, non-hazardous, operationally simple and convenient. The present study describes the microwave assistant synthesis of pyrazole derivatives containing electron donating and withdrawing substituents on phenyl ring, HOMO and LUMO and their antimicrobial studies were discussed.

2. EXPERIMENTAL

2.1. Material and Instrument:

All chemicals and reagents were obtained analytical grade and used without further purification. Melting points were determined by open capillary method using sisco apparatus. The IR spectra (in KBr pellets) were recorded on a Perkin-Elmer FTIR spectrophotometer. Microwave reactions were carried out in a CATA-2R microwave reactor by irradiation (200 W) at 70ºC, intermittently at 30 sec. ^{1}H NMR spectra were recorded in DMSO-d_6 on a Bruker 400 MHz, NMR spectrometer using TMS as an internal standard. The mass spectra were recorded on a MASPEC/FAB mass spectrometer operating at 70 eV. Elemental analysis carried with flash EA 1112 Series CHNS instrument. The reactions were monitored by thin layer chromatography (TLC) on Merck pre-coated silica GF254 plates. The purity of the compounds was checked by thin layer chromatography (TLC) on silica gel plates using a mixture of n-Hexane and ethyl acetate. HOMO and LUMO studies were carried

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2.2. Synthesis

2.2.1. Preparation of methyl 2-(4-chlorophenyl)-5-oxopyrazolidine-3-carboxylate (3)

A mixture of dimethyl maleate (0.01), (4-chlorophenyl) hydrazine (a) (0.01 mole), chloramine-T and DMF (5 ml) was exposed to microwaves at 200 W (85ºC) intermittently at 30 sec intervals for 5.5 min. On completion of reaction (monitored by TLC), the reaction-mixture was cooled and poured into crushed ice. The compound is extracted with ethyl acetate (thrice) washed with water and brine solution and dried over Na_2SO_4. Solvent is evaporated to get crude product. The product is purified by column using ethylacetate: n-hexane (20%) Yield: 78%, M.P.:- 156ºC, I.R. (KBr, cm^{-1}):- 3155(N-H), 3012(Ar-H), 1705(C=O),1655 cm^{-1}, ^{1}H-NMR($CDCl_3$)δ:- 2.76 (S, 2H), 3.14(S, 3H), 3.62(S, 1H) 6.98(d, 2H) 7.24(d 2H). ES-MS: m/z (m+1): 254.72.

2.2.2. Preparation of methyl 3-bromo-1-(4-chlorophenyl)-1H-pyrazole-5-carboxylate (5):

The methyl-2-(4-chlorophenyl)-5-oxopyrazolidine-3-carboxylate (***3***) (0.001 moles) compound is dissolved in 100ml of acetonitrile and the reaction mixture is cooled to 10ºC. To this $POBr_3$ (0.001 moles) was added slowly to the reaction mixture. The temperature is raised to 70ºC and maintained for 6 hrs. Completion of reaction is monitored by TLC (Ethylacetate: n-Hexane). The solvent is removed completely by rotavapour at low temperatures. The crude product was extracted by ethyl acetate and washed with water. The solution is dried over Na_2SO_4 and concentrated the solution to get the product. The crude product (***4***) is dissolved in 100 ml of acetonitrile, to this $Na_2S_2O_8$ (0.001 moles) followed by H_2SO_4 was added. The reaction temperature is raised to 80ºC and maintained for 4 hrs. The reaction is cooled and pH was adjusted to 8 using $NaHCO_3$. The reaction mass was extracted with 100 ml of ethyl acetate by three times. The organic layer is washed with water and brine solution and dried over Na_2SO_4. The solution is concentrated and crude product (***5***) is purified by column. Yield: 78%, M.P.:-188ºC, I.R. (KBr, cm^{-1}):- 3145(N-H), 3012(Ar-H), cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ:- 3.75(S, 3H), 6.92(S, 1H), 7.24(d, 2H), 7.39(d, 2H). ES-MS: m/z (m+1): 313.11.

2.2.3. Preparation of 3-bromo-1-(4-chlorophenyl)-1H-pyrazole-5-carboxylic acid(6)

The purified compound ***5*** is taken into 100 ml RBF and dissolved in THF : H_2O (2:1). The reaction is cooled to -5 to 0ºC. Add LiOH : H_2O is added under cooled temperature and RM is stirred for overnight at RT. The reaction is made acidic using concentrated HCl solution to get white solid compound (***6***).

6a. I.R. (KBr, cm^{-1}):- 3423 (broad, -COOH), 3147, 3094 cm^{-1} Ar-H, 1722 cm^{-1} >C=O, 1603, 1559, 1512, 1459. ^{1}H-NMR ($CDCl_3$):- 7.1 (S, 1H) 7.33 - 7.48 (m, 4H).

6c. I.R. (KBr, cm^{-1}):- 3405 (broad, -COOH), 3177, 3082 cm^{-1} Ar-H, 1732 cm^{-1} >C=O, 1613, 1562, 1522, 1450. ^{1}H-NMR ($CDCl_3$) :- 6.9 (S, 1H)7.26 - 7.52 (m, 3H).

6f. I.R. (KBr, cm^{-1}):- 3454 (broad, -COOH), 3185, 3095 cm^{-1} Ar-H, 1720 cm^{-1} >C=O, 1625,1570, 1533, 1459. ^{1}H-NMR ($CDCl_3$):- 6.7 (S, 1H); 7.45 - 7.81 (m, 3H).

6h. I.R. (KBr, cm^{-1}):- 3400 (broad, -COOH), 3256, 3044 cm^{-1} Ar-H, 2225 (>CN), 1678 cm^{-1} >C=O, 1598, 1535, 1482, 1456. ^{1}H-NMR ($CDCl_3$):- 6.8 (S, 1H); 7.33 -7.50 (m, 3H).

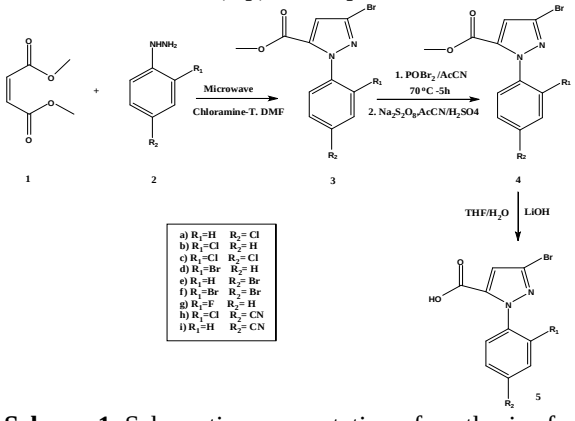
3. RESULTS AND DISCUSSION

3.1. Physical and spectral analysis

The reaction sequence employed for the synthesis of pyrazole derivatives is shown in **scheme-1.** Cyclization of pyrazole ring is carried in solvent free condition with dimethyl maleate and (4-chlorophenyl) hydrazine in presences of chloroamine-T and catalytic amount of DMF by microwave irradiation. Further the keto group in ***3*** is replaced by Br using $POBr_3$, under reflux in acetonitrile solvent, this is confirmed by IR spectra, by absence of 1730 cm^{-1} band. The crude product is dehydrogenated in presence of $Na_2S_2O_8$ under acidic medium by refluxing in acetonitrile to give ***4***. H^1-NMR and IR confirms the structure. The purified compound ***4*** is added with LiOH : H_2O under cooled temperature in THF, then RM is stirred for overnight at RT to give white solid compound ***6(a-i),*** which is characterized by elemental, H^1-NMR, I.R and mass spectra. The physical and analytical data of compounds ***6(a-i)*** are given in Table 1.

3.2. DFT Studies

Optimized minimized energy of all derivatives (5a-i) were carried initially, and HOMO and LUMO studies were carried by the density functional theory (DFT), calculations have been performed at the B3LYP/ 6-31$^+$G(d,p) level optimized



Scheme 1. Schematic representation of synthesis of pyrazole derivatives.

Table 1. Physical and analytical data of 3-bromo-1-phenyl-pyrazole -5-carboxylic acid derivatives ***6(a-i).***

Compound	M.P	Yield	Mol. Formulae	Elemental Analysis Observed (calculated)		
				C	H	N
6a	112-114ºC	72	$C_{10}H_6BrClN_2O_2$	39.79(39.83)	1.90(2.01)	9.23(9.29)
6b	113-115ºC	74	$C_{10}H_6BrClN_2O_2$	39.81(39.83)	1.97(2.01)	9.22(9.29)
6c	116-118ºC	68	$C_{10}H_5BrCl_2N_2O_2$	35.66(35.75)	1.38(1.50)	8.23(8.34)
6d	109-110ºC	65	$C_{10}H_6Br_2N_2O_2$	34.56(34.72)	1.69(1.75)	8.05(8.10)
6e	107-108ºC	66	$C_{10}H_6Br_2N_2O_2$	34.66(34.72)	1.72(1.75)	7.98(8.10)
6f	98-102ºC	55	$C_{10}H_5Br_3N_2O_2$	28.13(28.27)	1.12(1.19)	6.45(6.59)
6g	111-113ºC	72	$C_{10}H_6BrFN_2O_2$	42.08(42.13)	2.08(2.12)	9.76(9.83)
6h	132-134ºC	58	$C_{11}H_5BrClN_3O_2$	40.35(40.46)	1.48(1.54)	12.68(12.87)
6i	130-131ºC	51	$C_{11}H_6BrN_3O_2$	45.16(45.23)	1.98(2.07)	14.25(14.39)

Table 2. Calculated HOMO (eV), LUMO (eV), Δ*E*gap (LUMO-HOMO) (eV), Dipole moments (Debye) and total energies (u.a.)

Compound	Optimized energy	Energy (eV)		ΔE_{gap}	Dipole (Debye)	Log P
		HOMO	LUMO			
6a	0.06870	-0.36668	-0.02645	0.34023	1.5025	2.08
6b	0.06748	-0.36725	-0.02632	0.34093	1.9298	2.48
6c	0.06173	-0.37084	-0.02922	0.34162	2.0017	3.48
6d	0.08831	-0.36606	-0.02306	0.34300	0.7111	2.84
6e	0.08596	-0.36628	-0.02685	0.33943	1.8842	2.98
6f	0.09824	-0.36978	-0.03102	0.38760	1.9631	4.02
6g	0.00776	-0.36631	-0.02351	0.34280	0.9335	3.08
6h	0.13487	-0.3800	-0.03887	0.34113	3.4554	3.68
6i	0.14061	-0.37904	-0.03224	0.3468	3.8746	3.45

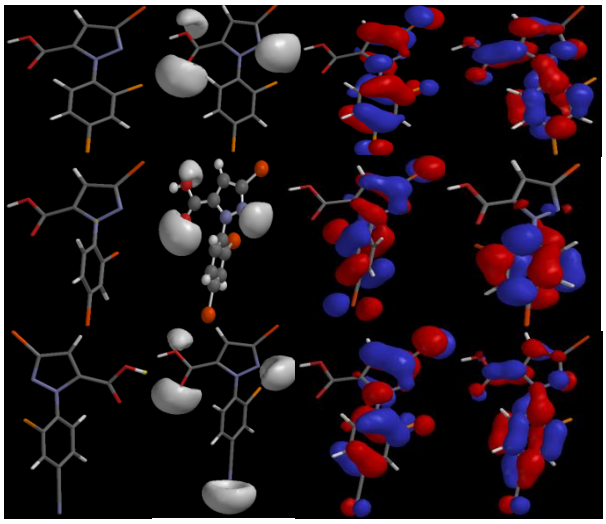


Figure 2. Optimized energies, electronic potential, HOMO and LUMO structure of 6c, 6f and 6h

Table 3. Antibacterial activity of the compounds 5a-i.

Compound No.	*Bacillus subtillis*		*Escherichia coli*	
	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml
6a	14	17	12	13
6b	16	20	12	14
6c	13	16	12	15
6d	15	18	13	17
6e	12	14	12	14
6f	19	22	18	19
6g	19	21	14	17
6h	18	21	16	20
6i	17	20	17	19
Ampicillin	24	28	28	34

Table 4. Antifungal activity of the compounds 5a-i.

Compound No.	*Aspergillus niger*		*Candida albicans*	
	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml
6a	12	16	12	14
6b	12	18	10	14
6c	13	15	12	14
6d	12	17	13	16
6e	10	14	10	13
6f	18	21	15	19
6g	16	17	14	17
6h	17	20	16	18
6i	17	20	15	19
Sertaconazole	25	30	24	30

minimized energy structure showed that, as expected, both benzene ring and pyrazole rings are perpendicular to each other to minimize the stearic interactions (Figure 3). Subsequently, a single-point energy *ab initio*

calculation was performed at the 6-31^{+}G* level in order to derive electronic properties, such as highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) energy values, molecular dipole moment (µ), ΔE_{gap} (difference between *E*HOMO and *E*LUMO) and total energies were calculated and obtained results are presented in table 2.

It is known that the value of E_{HOMO} is often associated with the electron donating ability of inhibitor molecule, higher values of E_{HOMO} is an indication of the greater ease of donating electrons to the unoccupied d orbital of the receptor. The value of E_{LUMO} is related to the ability of the molecule to accept electrons, lower values of E_{LUMO} shows the receptor would accept electrons. Consequently, the value of *Egap* provides a measure for the stability of the formed complex on the metal surface. The lower value of ΔE has, the higher stability is for the formed complex. The value of ΔE for dichloro (5c) and dibromo(5f) substitutents are 0.341 and 0.387 eV respectively; the values of dipolar moment are 2.0µ and 1.9 µ; the values of total energies are -697 and -982(kilojoules). The values of Gap energy and total energy are proportional to the biological activity.

3.3. Antimicrobial Activity

The newly synthesized compounds were screened for their antibacterial activities against *Bacillus subtillis* (ATCC-11774) and *Escherichia coli* (ATCC-25922) bacterial strains by disc diffusion method [15,16]. *Ampicillin* was used as standard drug. The results of antibacterial studies are given in Table 3. The antifungal activities of the compounds were evaluated by disc diffusion method against *Aspergillus niger* (NCIM no. 617) and *Candida albicans* (NCIM no. 300). Sertaconazole was used as standard drug. The results of antifungal studies are given in Table 3.

4. CONCLUSION

In conclusion, a new class of pyrazole derivatives were synthesized and evaluated as antimicrobial agents. The newly synthesized hetreocyclics exhibited mordarate antibacterial activity aganist *S. aureus*, and *B. subtilis*, and significant antifungal activity against *C. albicans* and *A. niger*. Quantum-chemical and physicochemical calculations indicate that antibacterial activity correlates well with calculated log P and HOMO-LUMO energy difference of molecules. The promising antibacterial activity of compounds 6c, 6f and 6h,the results of computational studies would be helpful in synthesis of a large library of 3-*bromo-1-(phenyl)-1H-pyrazole-5-carboxylic acid* analogues for extensive antimicrobial studies, which would be used to develop a more appropriate drug candidate. It can be concluded that these classes of compounds certainly holds great promise towards good active leads in medicinal chemistry.

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