Research Article

Synthesis, Characterization and Biological Activity of Azetidin-2-one based Benzoyl Pyrazoline Derivatives

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ABSTRACT
Pyrazolines are well-known and important nitrogen containing 5-membered heterocyclic compounds and various methods have been worked out for their synthesis. A new series of 4-(4-Chlorophenyl)-3-chloro-1-{4-[5-(Substituted phenyl)-1-benzoyl-4,5-dihydro-pyrazol-3-yl]phenyl}azetidin-2-one are synthesized by reacting 3-chloro-1-{4-[5-(Substituted phenyl)-4,5-dihydro-pyrazol-3-yl]phenyl}-4-(4-Chlorophenyl)azetidin-2-one (0.001M) with Benzoyl Chloride in presence of Pyridine. All these compounds were characterized by means of their IR, ^{1}H NMR, and Spectroscopic data and were tested for their antibacterial and antifungal activities by broth dilution method.

Keywords: Chalcones, Benzoyl-Pyrazolines, azetidin-2-one, Antimicrobial activity.

INTRODUCTION

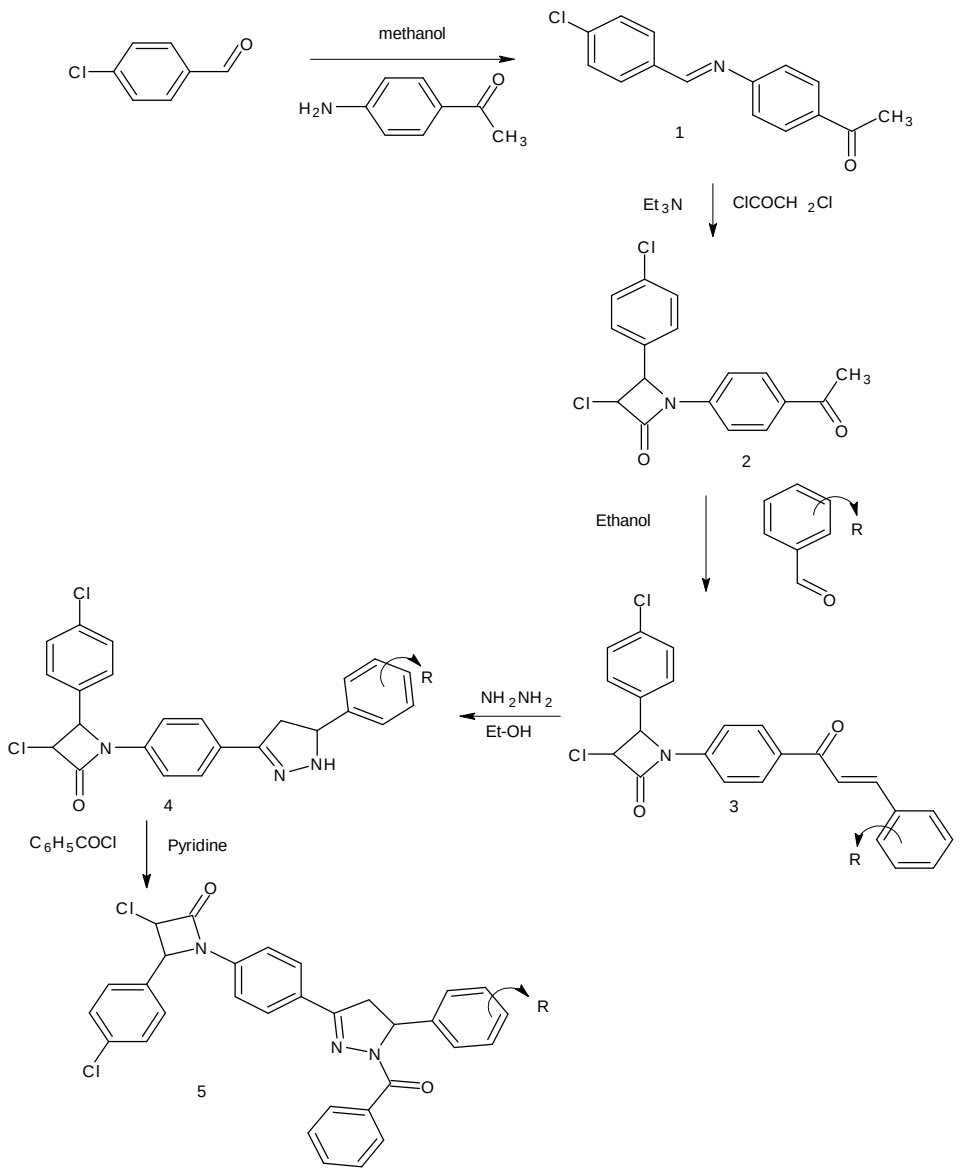
As evident from the literature, in recent years a significant portion of research work in heterocyclic chemistry has been devoted to Pyrazolines containing different aryl groups as substituents.

Some substituted Pyrazolines and their derivatives have been reported to possess some interesting biological activities such as anti-inflammatory[1], insecticidal[2], anti-tubercular[3], antitumor[4], tranquilizing[5], immunosupressive[6], diuretic[7], anticonvulsant[8], antifungal[9], antidepressant activities[10], antibacterial activitie[11], molluscidal[12]. In the present study we report the reaction of 3-chloro-1-{4-[5-(Substituted phenyl)-4, 5-dihydro-pyrazol-3-yl] phenyl}-4-(4-Chlorophenyl) azetidin-2-one (0.001M) with Benzoyl Chloride in presence of Pyridine to form Benzoyl Pyrazoline (5a-j). The structures of the various synthesized compounds were assigned on the basis of IR, ^{1}H-NMR spectral data and elemental analysis. These compounds were also screened for their antimicrobial activity.

Experimental

The IR spectra were recorded on IR affinity-1, DRS-8000A, Shimadzu, Ptc. Ltd., Japan spectrophotometer. The ^{1}H-NMR was recorded in DMSO on Bruker Advance II 400 MHz spectrometer using TMS as an internal standard. Melting points were determined in open capillary tubes and are uncorrected. The purity of the compounds was checked by TLC-using Silica gel-G (Merck). Column chromatography was performed on silica gel. All the compounds were tested for their antibacterial and antifungal activities by broth dilution method.

Reaction Scheme



Preparation of 1-(4-{[(4-Chlorophenyl)methylene]amino}phenyl) ethanone (1)

A mixture of 4-Chloro Benzaldehyde (0.01M), 1-(4-aminophenyl) ethanone (0.01M) and methanol (30ml) was heated for about 5 min. in a beaker (250 ml) to get a clear solution. The solution was kept overnight at room temperature to get the respective crude solid which was recrystallized from ethanol to obtain the pure crystals of 1-(4-{[(4-chloro phenyl)methylene]amino}phenyl)ethanone respectively. The yield of the product was 75% and the product melts at 120^0C. Found: C(69.88%) H(4.65%) N(5.41%) , Calcd. for $C_{15}H_{12}ClNO$: C(69.91%) H(4.69%) N(5.43%). IR, cm^{-1}: 3084(=C-H), 2922(-C-H), 1678(>C=O), 1628(>C=N-), 1595 (>C=C<), 1408(-CH_3, bend), 1301(-C-N<), 1240(-C-CO-C-), 738(-C-Cl). ^{1}H-NMR (DMSO, δ, ppm): 2.5785 (3H, s, $COCH_3$), 6.5144-7.7992 (8H, m, Ar-H), 8.803 (1H, s, -CH=N-).

Preparation of 1-(4-acetylphenyl)-3-chloro-4-(4-Chlorophenyl) azetidin-2-one (2)

In a 100ml Round bottom flask 1-(4-{[(4-Chloro phenyl) methylene] amino} phenyl) ethanone (0.01M) in 70ml benzene was taken. Chloro acetyl chloride (0.01M) was added at room temperature with constant stirring and triethylamine 1ml was added and the reaction mixture was refluxed for 7 hours. After the

completion of reaction, solvent was removed by vacuum distillation. The solid was filtered, dried and recrystallized from toluene. The yield of the product was 60% and the product melts at 108^0C. Found: C(61.07%) H(3.88%) N(4.17%), Calcd. for $C_{17}H_{13}Cl_2NO_2$: C(61.10%) H(3.92%) N(4.19%). IR, cm^{-1}: 3041(=C-H), 2921(-C-H), 1712(>C=O), 1548(>C=C<), 1365(-CH_3, bend), 1292(-C-N<), 1197(-C-CO-C-),642(-C-Cl). ^{1}H-NMR (DMSO, δ, ppm): 2.5550 (3H, s, $COCH_3$), 4.8102 (1H, d, >CH-Ar), 5.4594 (1H, d, >CH-Cl), 7.3170-8.0618 (8H, m, Ar-H).

Preparation of 3-chloro-1-{4-[3-(Substituted phenyl)prop-2-enoyl] phenyl}-4-(4-Chlorophenyl) azetidin-2-one (3a-j)

To the solution of 1-(4-acetylphenyl)-3-chloro-4-(4-Chloro phenyl) azetidin-2-one (0.01M) in absolute ethanol (50 ml), substituted Benzaldehyde (0.01M) and 2% NaOH were added and refluxed for 10 hours. After refluxing the reaction mixture was concentrated, cooled, filtered and neutralized with dil. HCl. The solid residue thus obtained was crystallized by absolute ethanol. IR(3d), cm^{-1}: 3043(=C-H), 1722(>C=O), 1624(>C=C<), 1451(-N=O), 1286(-C-N<), 1232 (-C-O-), 684(-C-Cl). ^{1}H-NMR (3g-DMSO, δ, ppm): 4.8757 (1H, d, >CH-Ar), 5.4224 (1H, d, >CH-Cl), 6.3621-8.5674 (12H, m, Ar-H), 7.9978 (2H, d, -CH=CH-), 9.9660 (1H, s, Ar-OH).

Preparation 3-chloro-1-{4-[5-(Substituted phenyl)-4,5-dihydro-pyrazol-3-yl]phenyl}-4-(4-Chlorophenyl)azetidin-2-one.(4a-j)

A mixture of 3-chloro-1-{4-[3-(Substituted phenyl) prop-2-enoyl] phenyl}-4-(4-Chlorophenyl) azetidin-2-one (0.01M) and 99% hydrazine hydrate (0.015M) in ethanol (50ml) refluxed gently for 3 hours. Then the mixture was concentrated and allowed to cool. The resulting solid was filtered, washed with ethanol and recrystallized from ethanol to give a pale brown solid. IR(**4e**), cm^{-1}: 3041 (=C-H), 2931 (-C-H), 1728(>C=O), 1643(>C=N-), 1552 (>C=C<), 1452 (-CH_2, bend), 1313(-C-N<),1290 (-N-N), 663 (-C-Cl-),. ^{1}H-NMR (**4b**-DMSO, δ, ppm): 3.61 (2H, d, CH_2- of Pyrazol), 4.33 (1H, t, >CH-Ar of Pyrazol), 4.80 (1H, d, >CH-Ar of Azetidine), 5.32 (1H, d, >CH-Cl of Azetidine), 6.56-7.92 (13H, m, Ar-H), 9.65 (1H, s, Ar-OH).

Preparation of 4-(4-Chlorophenyl)-3-chloro-1-{4-[5-(Substituted phenyl)-1-benzoyl-4, 5-dihydro-pyrazol-3-yl] phenyl} azetidin-2-one (5a-j)

A mixture of 3-chloro-1-{4-[5-(Substituted phenyl)-4,5-dihydro-pyrazol-3-yl]phenyl}-4-(4-Chlorophenyl)azetidin-2-one (0.001M) and Benzoyl chloride (0.0011M) dissolved in dry pyridine (25ml) and stirred at room temperature for 1 hours, after which the reaction mixture treated with cold dilute HCl (2N). The resulting solid was filtered and washed successively with water, cold NaOH (2%) and water, and recrystallized from glacial acetic acid. IR(**5d**), cm^{-1}: 3062(=C-H),1720(>C=O),1668(>C=N-),1539 (>C=C<),1454 (-CH_2, bend),1303(-C-N-),1286(-N-N),1445 (-N=O),686(-C-Cl-),^{1}H-NMR (**5c**-DMSO, δ, ppm): 3.7 (6H, s, OCH_3), 4.1 (2H, d, CH_2- of Pyrazol), 4.3 (1H, t, >CH-Ar of Pyrazol), 4.7 (1H, d, >CH-Ar of Azetidine), 5.3 (1H, d, >CH-Cl of Azetidine), 7.0-8.2 (16H, m, Ar-H).

RESULTS AND DISCUSSION

Antimicrobial activity

The MICs of synthesized compounds were carried out by broth micro dilution method[13] against four different strains, viz. Gram positive bacteria and Gram negative bacteria and compared with standard drug. Antifungal activity against *C. albicans, A. Niger, and A.clavatus* organisms was determined by same method and compared with standard drug.

The antimicrobial activity was performed by broth dilution method in DMSO. Gentamycin, Ampicilin, Chloramphenicol, Ciprofloxacin, Norfloxacin, Nystatin and Greseofulvin were used as standard for the evaluation of antibacterial and antifungal activities respectively. The activity was reported by Minimal Inhibition Concentration. The results are summarized in Table-2

Biological screening result of activities 4-(4-Chlorophenyl)-3-chloro-1-{4-[5-(Substituted phenyl)-1-benzoyl-4, 5-dihydro-pyrazol-3-yl] phenyl} azetidin-2-one based derivatives shows that compounds **5d, 5f,5g,** showed good to very good activity against S.aureus; whereas compounds **5f** showed good activity (100μg/ml) against S.pyogenes compared with standard drugs. In Gram negative bacterial strains: the result shows that compound **5g & 5h** showed good activity (100-125μg/ml) against E.coli. All others compound show moderately active or less-active against all bacterial strains. From the screening results of Antifungal activity compound **5d & 5g** shows very good activity against C.albicans.

Table 1: Physical constant of 4-(4-Chlorophenyl)-3-chloro-1-{4-[5-(Substituted phenyl)-1-benzoyl-4, 5-dihydro-pyrazol-3-yl] phenyl} azetidin-2-one

Comp	R	M.F.	Yield %	M.P. °C	Elemental Analysis		
					% C Found (Calcd)	% N Found (Calcd)	% H Found (Calcd)
5a	-2-Cl	$C_{31}H_{22}Cl_3N_3O_2$	68	104	64.72 (64.77)	7.28 (7.31)	3.85 (3.86)
5b	-2-OH	$C_{31}H_{23}Cl_2N_3O_3$	70	118	68.87 (66.91)	7.51 (7.55)	4.12 (4.17)
5c	-3,4-$(OCH_3)_2$	$C_{33}H_{27}Cl_2N_3O_4$	67	122	65.96 (66.00)	6.98 (7.00)	4.50 (4.53)
5d	-3-NO_2	$C_{31}H_{22}Cl_2N_4O_4$	72	140	63.57 (63.60)	9.51 (9.57)	3.74 (3.79)
5e	-4-Cl	$C_{31}H_{22}Cl_3N_3O_2$	65	115	64.72 (64.77)	7.28 (7.31)	3.84 (3.86)
5f	-4-$N(C_2H_5)_2$	$C_{35}H_{32}Cl_2N_4O_2$	68	110	68.71 (68.74)	9.12 (9.16)	5.25 (5.27)
5g	-4-OH	$C_{31}H_{23}Cl_2N_3O_3$	74	148	66.88 (66.91)	7.51 (7.55)	4.11 (4.17)
5h	-4-$N(CH_3)_2$	$C_{33}H_{28}Cl_2N_4O_2$	66	185	67.90 (67.93)	9.57 (9.60)	4.81 (4.84)
5i	CHO	$C_{31}H_{23}Cl_2N_3O_2$	75	178	68.85 (68.89)	7.76 (7.78)	4.25 (4.29)
5j	-2-OH-3-OCH_3	$C_{32}H_{25}Cl_2N_3O_4$	72	109	65.52 (65.54)	7.12 (7.16)	4.27 (4.30)

Table 2: Antimicrobial activity of 4-(4-Chlorophenyl)-3-chloro-1-{4-[5-(Substituted phenyl)-1-benzoyl-4, 5-dihydro-pyrazol-3-yl] phenyl} azetidin-2-one

SR. NO.	COMP. NO.	R	ANTIBACTERIAL ACTIVITY MINIMAL INHIBITION CONCENTRATION				ANTIFUNGAL ACTIVITY MINIMAL INHIBITION CONCENTRATION		
			E.COLI	P.AERUGINOSA	S.AUREUS	S.PYOGENUS	C.ALBICANS	A.NIGER	A.CLAVATUS
			MTCC 443	MTCC 1688	MTCC 96	MTCC 442	MTCC 227	MTCC 282	MTCC 1323
1	5a	-2-Cl	500	500	125	200	1000	>1000	>1000
2	5b	-2-OH	200	200	225	200	1000	800	>1000
3	5c	-3-OCH_3, -4-OCH_3	250	250	200	250	>1000	>1000	>1000
4	5d	-3-NO_2	200	200	250	250	500	500	500
5	5e	-4-Cl	200	200	125	200	1000	1000	1000
6	5f	-4-$N(C_2H_5)_2$	200	200	250	100	800	1000	>1000
7	5g	-4-OH	100	250	250	200	500	500	1000
8	5h	-4-$N(CH_3)_2$	125	200	100	250	1000	1000	1000
9	5i	-H	150	150	175	200	1000	>1000	1000
10	5j	-3-OCH_3, -4-OH	200	250	200	200	1000	1000	1000

Table 3: Antibacterial Activity: Minimal Inhibition Concentration (The Standard Drugs)

DRUG	E.COLI	P.AERUGINOSA	S.AUREUS	S.PYOGENUS
-	MTCC 443	MTCC 1688	MTCC 96	MTCC 442
(MICROGRAMME/ML)				
GENTAMYCIN	0.05	1	0.25	0.5
AMPICILLIN	100	--	250	100
CHLORAMPHENICOL	50	50	50	50
CIPROFLOXACIN	25	25	50	50
NORFLOXACIN	10	10	10	10

Table 4: Antifungal Activity: Minimal Inhibition Concentration (The Standard Drugs)

DRUG	C.ALBICANS	A.NIGER	A.CLAVATUS
-	MTCC 227	MTCC 282	MTCC 1323
(MICROGRAMME/ML)			
NYSTATIN	100	100	100
GRESEOFULVIN	500	100	100

CONCLUSION

The Main focus of this research work was to synthesize, characterize and evaluate antimicrobial activities of the newly synthesized benzoyl Pyrazoline derivatives, structures of synthesized compounds were confirmed and characterized with the help of analytical data's such as IR and ^{1}H-NMR. In summary, we have described the synthesis and antimicrobial activity of some new 4-(4-Chlorophenyl)-3-chloro-1-{4-[5-(Substituted phenyl)-1-benzoyl-4, 5-dihydro-pyrazol-3-yl] phenyl} azetidin-2-one MIC values revealed that amongst newly synthesized compound having Hydroxy type linkage has shown good activity against the bacterial strains.

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