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Synthesis and Antimicrobial Activity of Azetidin-2-one Containing Acetyl 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-hydroxyphenyl)-3-chloro-1-{4-[5-(Substituted phenyl)-1-acetyl-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-hydroxyphenyl)azetidin-2-one with Acetic acid. All these compounds were characterized by means of their IR, ^{1}H NMR, Spectroscopic data and microanalysis. All the compounds were tested for their antibacterial and antifungal activities by broth dilution method

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

Introduction:

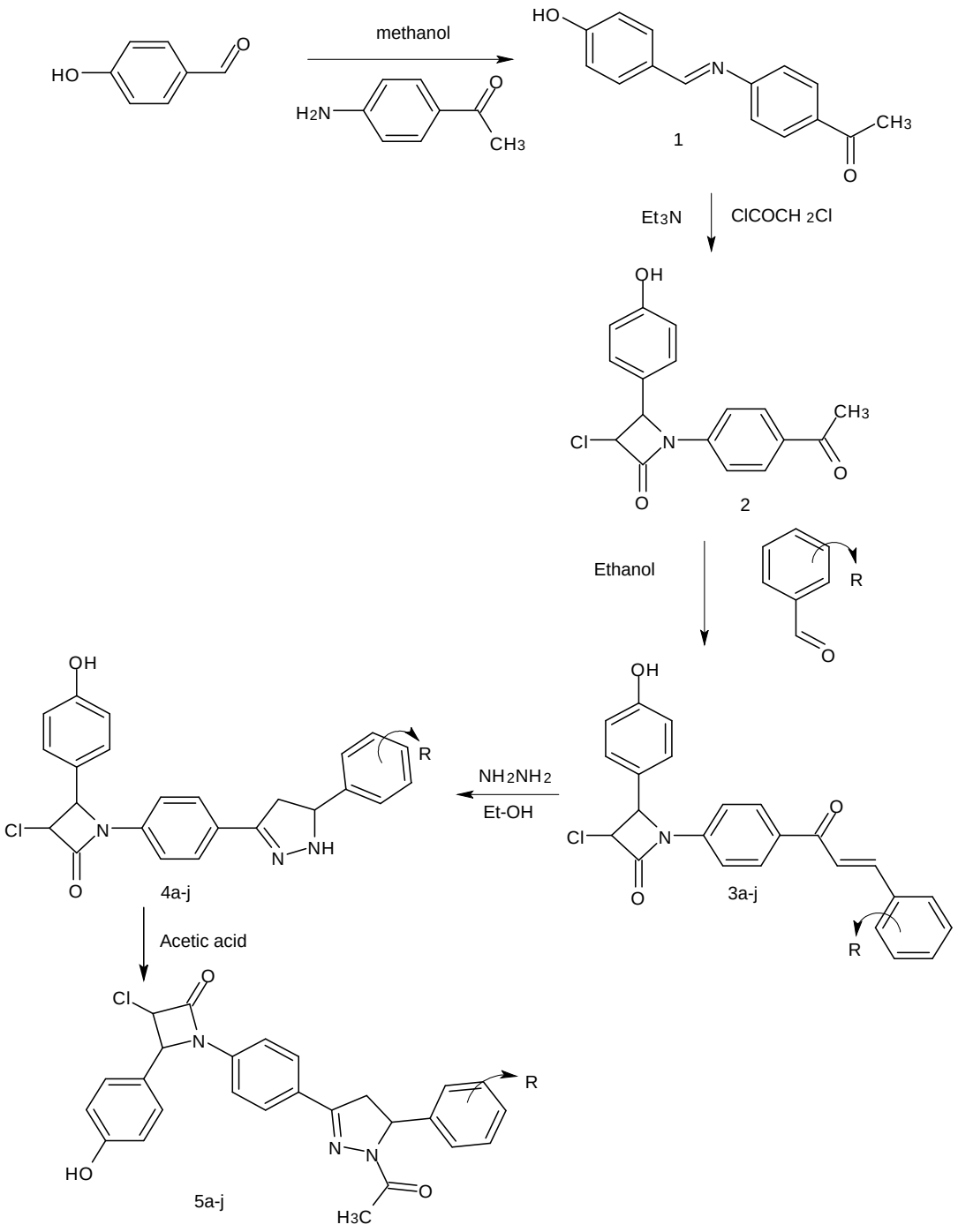
Pyrazolines have attracted attention of medicinal chemists for both with regard to heterocyclic chemistry and the pharmacological activities associated with them. Keeping in view the importance of these pharmaceutical activities, it was considered of interest to synthesize some new acetyl derivatives of pyrazoles.

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-hydroxyphenyl) azetidin-2-one with Acetic acid to form acetyl 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-hydroxyphenyl) methylene] amino} phenyl) ethanone (1)

A mixture of 4-hydroxy 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-hydroxy phenyl)methylene]amino}phenyl)ethanone respectively. The yield of the product was 75% and the product melts at 195^0C. Found: C(75.28%) H(5.45%) N(5.82%) , Calcd. for $C_{15}H_{13}NO_2$: C(75.30%) H(5.48%) N(5.85%). IR,cm^{-1}:3085 (-

OH), 3040 (=C-H), 2920(-C-H), 1676(>C=O), 1647(>C=N-), 1606 (>C=C<), 1363(-CH_3, bend), 1314(-C-N<), 1284 (-C-O-), 1240(-C-CO-C-).^{1}H-NMR (DMSO, δ, ppm): 2.5692 (3H, s, $COCH_3$), 6.5277-7.9774 (8H, m, Ar-H), 8.3820 (1H, s, -CH=N-), 9.6392 (1H, s, Ar-OH).

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

In a 100ml Round bottom flask 1-(4-{[(4-hydroxyphenyl) 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 119^0C. Found: C(64.64%) H(4.44%) N(4.42%), Calcd. for $C_{17}H_{14}ClNO_3$: C(64.67%) H(4.47%) N(4.44%). IR, cm^{-1}: 3300 (-OH), 3050(=C-H), 2950(-C-H), 1680(>C=O), 1600(>C=C<), 1375(-CH_3, bend), 1300(-C-N<), 1240(-C-CO-C-), 1220(-C-O), 560 (C-Cl). ^{1}H-NMR (DMSO, δ, ppm): 2.5392 (3H, s, $COCH_3$), 4.8954 (1H, d, >CH-Ar), 5.5151 (1H, d, >CH-Cl), 6.6720-8.0745 (8H, m, Ar-H), 9.7784 (1H, s, Ar-OH).

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

To the solution of 1-(4-acetylphenyl)-3-chloro-4-(4-hydroxyphenyl) 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(**3b**), cm^{-1}:3359(-OH), 3045(=C-H), 1728(>C=O), 1608(>C=C<), 1290(-C-N<), 1186 (-C-O-), 769(-C-Cl).^{1}H-NMR (**3c**-DMSO, δ, ppm): 3.8789 (6H, s, -OCH_3), 4.8613 (1H, d, >CH-Ar), 5.3413 (1H, d, >CH-Cl), 6.7340-7.8883 (11H, m, Ar-H), 7.9733 (2H, d, -CH=CH-), 9.8306 (1H, s, Ar-OH).

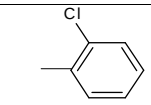
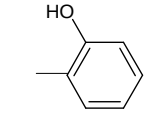
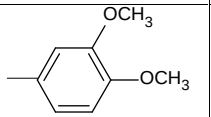
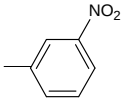
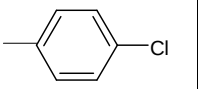
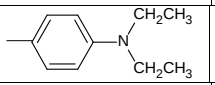
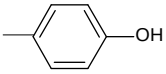
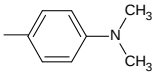
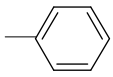
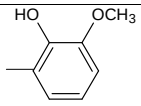
Preparation 3-chloro-1-{4-[5-(Substituted phenyl)-4,5-dihydro-pyrazol-3-yl]phenyl}-4-(4-hydroxyphenyl)azetidin-2-one.(4a-j)

A mixture of 3-chloro-1-{4-[3-(Substituted phenyl) prop-2-enoyl] phenyl}-4-(4-hydroxyphenyl) 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(**4f**), cm^{-1}: 3317 (-OH), 3080 (=C-H), 1718(>C=O), 1658(>C=N-), 1544 (>C=C<), 1460(-CH_3, bend), 1324(-C-N<),1284 (-N-N), 1234 (-C-O),641 (-C-Cl-), 3463 (>NH). ^{1}H-NMR (**4h**-DMSO, δ, ppm): 3.1699 (6H, s, $N(CH_3)_2$), 3.9462 (2H, d, CH_2- of Pyrazol), 4.3000 (1H, t, >CH-Ar of Pyrazol), 4.8268 (1H, d, >CH-Ar of Azetidine), 5.3981 (1H, d, >CH-Cl of Azetidine), 6.6114-7.9986 (13H, m, Ar-H, -NH-), 9.5428 (1H, s, Ar-OH).

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

A mixture of 3-chloro-1-{4-[5-(2-chlorophenyl)-4, 5-dihydro-pyrazol-3-yl] phenyl}-4-(4-hydroxy phenyl) azetidin-2-one (0.001M) and acetic acid (10ml) refluxed for 3 hours. The solution was then concentrated, on cooling, the resulting solid was filtered, washed with water and recrystallized from absolute ethanol. IR(**5h**), cm^{-1}: 3367 (-OH), 3048 (=C-H), 2945 (-C-H), 1735(>C=O), 1620(>C=N-), 1543 (>C=C<), 1460(-CH_2, bend), 1382(-CH_3-bend) 1284(-C-N<),1232 (-N-N), 1232 (-C-O), 844(-C-Cl-),. ^{1}H-NMR (**5f**-DMSO, δ, ppm): 1.2 (6H, s, -CH_3), 2.1 (3H, s, -$COCH_3$), 3.5 (4H, s, -CH_2), 3.7 (2H, d, CH_2- of Pyrazol), 4.3 (1H, t, >CH-Ar of Pyrazol), 4.8 (1H, d, >CH-Ar of Azetidine), 5.5 (1H, d, >CH-Cl of Azetidine), 6.6-8.0 (12H, m, Ar-H), 9.6 (1H, s, Ar-OH).

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

Compd	R	M.F.	Yield %	M.P. °C	Elemental Analysis		
					% C Found (Calcd)	% N Found (Calcd)	% H Found (Calcd)
5a		$C_{26}H_{21}Cl_2N_3O_3$	70	202	63.14 (63.17)	8.48 (8.50)	4.25 (4.28)
5b		$C_{26}H_{22}ClN_3O_4$	72	179	65.59 (65.62)	8.81 (8.83)	4.62 (4.66)
5c		$C_{28}H_{26}ClN_3O_5$	69	169	64.64 (64.68)	8.04 (8.08)	5.01 (5.04)
5d		$C_{26}H_{21}ClN_4O_5$	74	185	61.81 (61.85)	11.07 (11.10)	4.14 (4.19)
5e		$C_{26}H_{21}Cl_2N_3O_3$	67	192	63.12 (63.17)	8.47 (8.50)	4.24 (4.28)
5f		$C_{30}H_{31}ClN_4O_3$	70	114	67.81 (67.85)	10.52 (10.55)	5.84 (5.88)
5g		$C_{26}H_{22}ClN_3O_4$	76	168	65.59 (65.62)	8.81 (8.83)	4.62 (4.66)
5h		$C_{28}H_{27}ClN_4O_3$	68	101	66.84 (66.86)	11.11 (11.14)	5.37 (5.41)
5i		$C_{26}H_{22}ClN_3O_3$	77	185	67.86 (67.90)	9.11 (9.14)	4.78 (4.82)
5j		$C_{27}H_{24}ClN_3O_5$	75	238	64.07 (64.10)	8.28 (8.31)	4.75 (4.78)

Results and discussion

Antimicrobial activity

The MICs of synthesized compounds were carried out by broth micro dilution method as described by Ratan (2000). It is one of the non automated in vitro bacterial susceptibility tests. This classic method yields a quantitative result for the amount of antimicrobial agents that is needed to inhibit growth of specific microorganisms.

The invitro antimicrobial activity of test compounds were assessed against 24 hr cultures of several selected bacteria and fungi. The bacteria used were *E. coli, S.aureus, P. aeruginosa,* and *S. pyogenus*; the fungi used were *C. albicans, A. Niger, and A.clavatus.*

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.

Table: 2: Antimicrobial activity of 4-(4-hydroxyphenyl)-3-chloro-1-{4-[5-(Substituted phenyl)-1-acetyl-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	100	200	62.5	100	1000	1000	800
2	5b	-2-OH	175	200	175	250	800	700	700
3	5c	-3-OCH_3, -4-OCH_3	225	225	150	200	>1000	800	600
4	5d	-3-NO_2	175	225	200	150	700	600	1000
5	5e	-4-Cl	150	150	175	200	700	>1000	1000
6	5f	-4-$N(C_2H_5)_2$	125	200	100	100	250	1000	1000
7	5g	-4-OH	200	250	125	250	800	800	800
8	5h	-4-$N(CH_3)_2$	200	250	125	250	500	1000	1000
9	5i	-H	175	225	200	125	750	600	800
10	5j	-3-OCH_3, -4-OH	200	200	225	200	1000	800	**>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

Biological screening result of activities 4-(4-hydroxyphenyl)-3-chloro-1-{4-[5-(Substituted phenyl)-1-acetyl-4, 5-dihydro-pyrazol-3-yl] phenyl} azetidin-2-one based derivatives shows that compound (**5a**) have shown better activity against E. coli and S. pyogenus, while (**5f**) have shawn better activity against S. pyogenus, while rest of all compound possessed good activity against S.aureus in the range of 62.5-225 μg/ml. Compound (**5f** & **5h**) is found to be good antifungal activity against C. albicans, against standard drugs Greseofulvin. While rest of all derivatives are poor against A. Niger, and A.clavatus

Conclusion

The Main focus of this research work was to synthesize, characterize and evaluate antimicrobial activities of the newly synthesized acetyl 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-hydroxyphenyl)-3-chloro-1-{4-[5-(Substituted phenyl)-1-acetyl-4, 5-dihydro-pyrazol-3-yl] phenyl} azetidin-2-one MIC values revealed that amongst newly synthesized compound having chlorophenyl and Di-Ethyl amine and Di-Methyl amine type linkage has shown good activity against the bacterial strains..

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