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RESEARCH ARTICLE

Synthesis and Antimicrobial Activity of 2-Amino-4-(substitutedphenyl)-6-{4-[3-chloro-2-(4-chlorophenyl)-4-oxoazetidin -1-yl] phenyl}pyridine-3-carbonitrile

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Abstract: Pyridine the simplest and perhaps is the best-known heterocyclic compound. The credit for the discovery of pyridine goes to Anderson who first obtained it from bone oil. The simple pyridine compounds are prepared by the cyclization of aliphatic raw materials. A new series of 2-amino-4-(substitutedphenyl)-6-{4-[3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl] phenyl} pyridine-3-carbonitrile are synthesized by reacting 3-chloro-1-{4-[5-(substituted phenyl)-4,5-dihydro-pyrazol-3-yl]phenyl}-4-(4-chlorophenyl)azetidin-2-one with malanonitrile and ammonium acetate by using ethanol as a solvent**.** 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, Cyanopyridine, Azetidin-2-one, Antimicrobial activity

Introduction

Interest in the synthesis of pyridine containing compounds has increased in recent years because of their biological and pharmacological activities. Pyridine is the parent compound of the series of compounds that is important in pharmaceutical, agriculture and industrial chemistry. Among a wide range of pyridines 3-cyanopyridines acquired a special attention due to their wide range of therapeutic activities. Preparation of 3-cyanopyridines is available in the literature with different methods[1-5].

Some substituted pyridine-3-carbonitrile and their derivatives have been reported to possess some interesting biological activities such as Antifungal[6], Antiepileptic[7], Antibacterial[8], Anticonvulsant[9], Antitubercular[10], Analgesics[11], Insecticidal[12], Antipsoriasis[13] and Antihypertensive[14].

In the present study the reaction of 3-chloro-1-{4-[5-(substituted phenyl)-4, 5-dihydro-pyrazol-3-yl] phenyl}-4-(4-chlorophenyl) azetidin-2-one with malanonitrile and ammonium

acetate to form pyridine-3-carbonitrile (**4a-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 (Table 1). 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.

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

A mixture of 4-chloro benzaldehyde (0.01 M), 1-(4-aminophenyl) ethanone (0.01 M) and methanol (30 mL) 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 ^{0}C. 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-chloro phenyl)azetidin-2-one (**2**)*

In a 100 mL Round bottom flask 1-(4-{[(4-chloro phenyl) methylene] amino} phenyl) ethanone (0.01 M) in 70 mL benzene was taken. Chloro acetyl chloride (0.01 M) was added at room temperature with constant stirring and Triethylamine 1 mL was added and the reaction mixture was refluxed for 7 h. 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 ^{0}C. 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 (0.01 M) in absolute ethanol (50 mL), substituted Benzaldehyde (0.01 M) and 2% NaOH were added and refluxed for 10 h. 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 2-amino-4-(substitutedphenyl)-6-{4-[3-chloro-2-(4-Chlorophenyl)-4-oxoazetidin -1-yl]phenyl}pyridine-3-carbonitrile(**4a-j**)*

A mixture of 3-chloro-1-{4-[3-(substituted phenyl) prop-2-enoyl] phenyl}-4-(4-chlorophenyl) azetidin-2-one (0.01 M) in 30 mL alcohol then 0.011 mole of malanonitrile and 0.06 mole of ammonium acetate was added and refluxed for 7 h. Then the resulting product was cooled into crushed ice, filtered, washed by H_2O, dried and recrystallized by ethanol.

IR(**4f**), cm^{-1}: 3465 (>N-H), 3055 (=C-H), 2945 (-C-H), 1715(>C=O), 2245(C≅N-), 1615 (>C=N), 1555 (>C=C<), 1380(-CH_3),1285 (-C-N), 675 (-C-Cl).

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 *in vitro* antimicrobial activity of test compounds were assessed against 24 h 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 2-4.

Biological screening result of activities 2-amino-4-(substitutedphenyl)-6-{4-[3-chloro-2-(4-chlorophenyl)-4-oxoazetidin -1-yl] phenyl} pyridine-3-carbonitrile based derivatives shows that compound (**4g & 4j**) have shown better activity against *E. coli* and (**4d & 4g**) against *S. pyogenus*, while rest of all compound possessed good activity against *S.aureus* in the range of 100-500 µg/mL. Compound (**4b** & **4e**) 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.*

Table 1. Physical constant of 2-amino-4-(substitutedphenyl)-6-{4-[3-chloro-2-(4-chlorophenyl)-4-oxoazetidin -1-yl] phenyl} pyridine-3-carbonitrile

Compd	R	M.F.	Yield %	M.P. °C	Elemental Analysis		
					% C Found (Calcd)	% N Found (Calcd)	% H Found (Calcd)
4a	-2-Cl	$C_{27}H_{17}Cl_3N_4O$	58	220	62.35 (62.39)	10.73 (10.78)	3.26 (3.30)
4b	-2-OH	$C_{27}H_{18}Cl_2N_4O_2$	73	195	64.63 (64.68)	11.13 (11.17)	3.58 (3.62)
4c	-3,4-$(OCH_3)_2$	$C_{29}H_{22}Cl_2N_4O_3$	65	198	63.83 (63.86)	10.23 (10.27)	4.02 (4.07)
4d	-3-NO_2	$C_{27}H_{17}Cl_2N_5O_3$	62	178	61.11 (61.14)	13.16 (13.20)	3.19 (3.23)

Contd…

4e	-4-Cl	$C_{27}H_{17}Cl_3N_4O$	73	230	62.32 (62.39)	10.74 (10.78)	3.26 (3.30)
4f	-4-N(C_2H_5)$_2$	$C_{31}H_{27}Cl_2N_5O$	59	172	66.88 (66.91)	12.52 (12.58)	4.82 (4.89)
4g	-4-OH	$C_{27}H_{18}Cl_2N_4O_2$	65	191	64.64 (64.68)	11.14 (11.17)	3.58 (3.62)
4h	-4-N(CH_3)$_2$	$C_{29}H_{23}Cl_2N_5O$	63	210	65.88 (65.91)	13.21 (13.25)	4.32 (4.39)
4i	CHO	$C_{27}H_{18}Cl_2N_4O$	68	132	66.76 (66.81)	11.50 (11.54)	3.70 (3.74)
4j	-2-OH-3-OCH_3	$C_{28}H_{20}Cl_2N_4O_3$	67	200	63.22 (63.29)	10.49 (10.54)	3.72 (3.79)

Table 2. Antimicrobial activity of 2-amino-4-(substitutedphenyl)-6-{4-[3-chloro-2-(4-chlorophenyl)-4-oxoazetidin -1-yl] phenyl} pyridine-3-carbonitrile

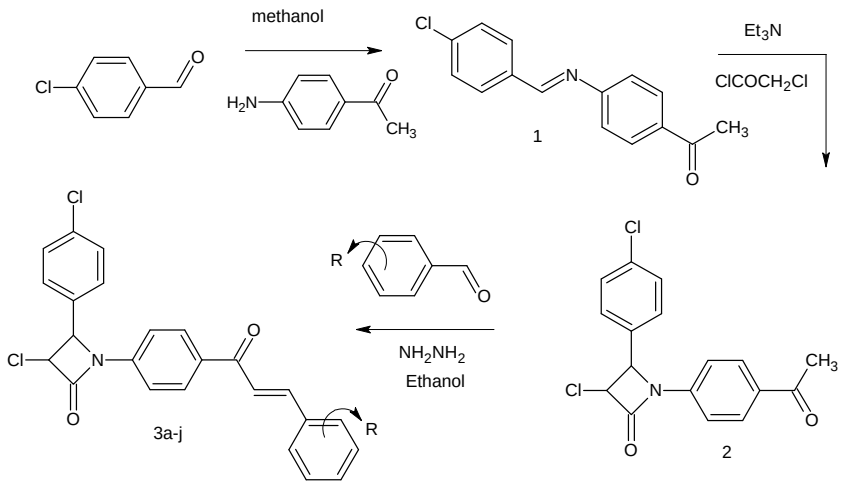
Comp	R	Antibacterial minimal concentration		Activity inhibition		Antifungal minimal concentration		Activity inhibition
		E.coli	*P.aeruginosa*	*S.aureus*	*S.pyogenus*	*C.albicans*	*A.niger*	*A.clavatus*
		MTCC 443	MTCC 1688	MTC C 96	MTCC 442	MTCC 227	MTC C 282	MTCC 1323
4a	-2-Cl	100	250	100	500	1000	500	>1000
4b	-2-OH	500	100	500	250	500	250	1000
4c	-3-OCH_3, -4-OCH_3	500	250	500	500	>1000	500	125
4d	-3-NO_2	500	250	125	100	250	1000	500
4e	-4-Cl	500	500	500	250	500	500	1000
4f	-4-N(C_2H_5)$_2$	100	200	500	125	>1000	500	>1000
4g	-4-OH	250	125	125	100	1000	500	>1000
4h	-4-N(CH_3)$_2$	125	500	125	250	1000	1000	500
4i	-H	100	250	100	125	1000	1000	>1000
4j	-3-OCH_3, -4-OH	250	100	500	125	1000	1000	500

Table 3. Antibacterial activities: Minimal inhibition concentration (The standard drugs)

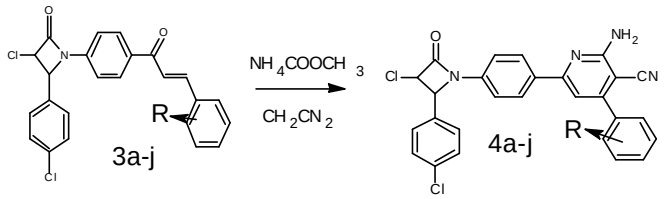
Drug	*E.coli*	*P.aeruginosa*	*S.aureus*	*S.pyogenus*
-	MTCC 443	MTCC 1688	MTCC 96	MTCC 442
(Microgramme/mL)				
Gentamycin	0.05	1	0.25	0.5
Ampicillin	100	--	250	100
Chloramphenicol	50	50	50	50
Ciprofloxacin	25	25	50	50
Norfloxacin	10	10	10	10

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



Scheme 1



Scheme 2

Conclusion

The main focus of this research work was to synthesize, characterize and evaluate antimicrobial activities of the newly synthesized pyridine-3-carbonitrile 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 2-amino-4-(substitutedphenyl)-6-{4-[3-chloro-2-(4-chlorophenyl)-4-oxoazetidin -1-yl] phenyl} pyridine-3-carbonitrile MIC values revealed that amongst newly synthesized compound having hydroxy and 3-methoxy, 2- hydroxy type linkage has shown good activity against the bacterial strains..

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