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Synthesis, Characterization and Antimicrobial Activity of New *N*-Substituted-3-Chloro-2-Azetidinones.

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Various substituted 4(m-hydroxy-p-methoxy phenyl)-1[(6'-fluoro-7'-substituted (1,3)-benzothiazol-2'-yl) amido-2-phenyl] 3-chloro azetidin–2–one containing different functional groups have been synthesized by treating fluorochloroaniline with KSCN in presence of bromine in glacial acetic acid and ammonia to get 2- amino–6-fluoro-7-chloro (1,3)- benzothiozole, which was treated with anthranillic acid in presence of dry pyridine to get 2-(o-amino phenyl amido) 6–fluoro-7-chloro (1,3) benzothiazole. To the above, refluxed with vanillin and alcohol in presence of Conc.HCl to get 2-(3-hydroxy-4-methoxy benzylidene amino phenyl amido) 6-fluoro-7-chloro- (1, 3) benzothiazole or Schiff's base. A Solution of Schiff's base in 1, 4-dioxane was added to well-stirred mixture of Chloroacetyl Chloride and Triethylamine to get Azetidinone. To the above product different aromatic aniline, PABA, piperzino, diphenylamine, N- methyl piperzino, o-toludine in presence of DMF were treated to get newly targeted compound through replacing at 7th position chlorine.

The lead compounds were characterized by melting point, TLC, calculated elemental analysis, UV, IR and ^{1}HNMR spectral studies. The compounds were tested for antimicrobial studies and showed significant activity at low and high concentration compared to standard; still further studies are requested.

KEYWORDS: Anti-microbial activity, Azetidinone, Benzothiazole, Fluorine.

INTRODUCTION

2-Azetidinones, commonly known as β-lactams, are well-known heterocyclic compounds among the organic and medicinal chemists.[1] the activity of the famous antibiotics such as penicillins, cephalosporins and carbapenems are attributed to the presence of 2-azetidinone ring in them. Recently, some other types of biological activity besides the antibacterial activity have been reported in compounds containing 2-azetidinone ring.[2, 3] such biological activities include antimicrobial,[4] anti-tuburcular,[5] carbonic anhydrase inhibitors,[6] local anaesthatics,[7] anti-inflammatory,[8] anthelmintic,[9] anticonvulsant,[10] hypoglycemic agents activity.[11] The β-lactams also serve as synthons for many biologically important classes of organic compounds.[12] Due to this, the investigation of chemistry and biology of these compounds continue to appeal the synthetic and medicinal organic chemists.[1-4]

It is well known that the introduction of fluorine atom into an organic molecule causes dramatic changes in its biological profile, mainly due to high electro negativity of fluorine, the strong carbon-fluorine bond and increased solubility in lipids.[13] Therefore it was thought worthwhile to synthesize better kinds of drugs by incorporating azetidinone in benzothiazole moiety.

In search for new biodynamic potent molecule, it was thought worthwhile to incorporate some additional heterocyclic moieties in the β-lactam nucleus and study their biological and pharmacological activity.[14] The review of literature reveal prompted us to synthesize substituted fluorobenzothiazole, azetidinone targeted

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Table No. 1 Antibacterial activity

Sl. No	Name of the compounds	Mean zone of inhibition (in mm*)							
		S. aureus		*E. coli*		*B. Subtilis*		*P. aeruginosa*	
		50µg	100µg	50µg	100µg	50µg	100µg	50µg	100µg
01	Procaine penicillin	19	22	-	-	-	-	-	-
02	Streptomycin	-	-	19	24	-	-	-	-
O3	Cefazolin Sod	-	-	-	-	20	25	-	-
04	Sporafloxin	-	-	-	-	-	-	20	27
05	A_1	13(0.68)	17(0.77)	14(0.74)	16(0.66)	11(0.55)	13(0.52)	13(0.65)	18(0.66)
06	A_2	13(0.68)	17(0.77)	13(0.68)	15(0.71)	11(0.55)	14(0.56)	15(0.75)	19(0.70)
07	A_3	12(0.63)	17(0.77)	15(0.79)	18(0.75)	11(0.55)	16(0.64)	13(0.65)	15(0.55)
08	A_4	14(0.74)	18(0.82)	14(0.74)	19(0.80)	12(0.60)	14(0.56)	14(0.70)	19(0.70)
09	A_5	15(0.79)	18(0.82)	13(0.68)	18(0.75)	11(0.55)	14(0.56)	18(0.90)	21(0.77)
10	A_6	13(0.68)	18(0.82)	14(0.74)	16(0.66)	14(0.70)	17(0.68)	15(0.75)	20(0.74)
11	A_7	16(0.84)	19(0.86)	13(0.68)	16(0.66)	11(0.55)	14(0.56)	10(0.50)	13(0.48)
12	A_8	13(0.68)	18(0.82)	13(0.68)	17(0.71)	13(0.65)	15(0.60)	13(0.65)	17(0.63)
13	A_9	12(0.63)	16(0.84)	13(0.68)	16(0.66)	12(0.60)	15(0.60)	13(0.65)	17(0.63)
14	A_{10}	12(0.63)	16(0.84)	14(0.74)	18(0.75)	12(0.60)	16(0.64)	13(0.65)	17(0.63)
15	A_{11}	10(0.52)	12(0.55)	13(0.68)	16(0.66)	17(0.85)	24(0.96)	11(0.55)	13(0.48)
16	A_{12}	11(0.58)	15(0.68)	12(0.63)	15(0.71)	18(0.90)	22(0.88)	13(0.65)	17(0.63)

Activity Index = Test Compound / Standard compound

Table No. 3 ANALYTICAL DATA

Sl. No	Compound Code	M.P/ B.P°C	% Yield	MOL. FORM	M.Wt.	Calculated %		
						C	H	N
1	A_1	190	78%	$C_{30}H_{21}O_6SN_5FCl$	634	56.83	3.34	11.05
2	A_2	178	82%	$C_{30}H_{21}O_6SN_5FCl$	634	56.83	3.34	11.05
3	A_3	183	75%	$C_{30}H_{21}O_6SN_5FCl$	634	56.83	3.34	11.05
4	A_4	164	72%	$C_{30}H_{21}O_4SN_4FCl_2$	623	57.79	3.39	8.99
5	A_5	132	74%	$C_{30}H_{21}O_4SN_4FCl_2$	623	57.79	3.39	8.99
6	A_6	126	73%	$C_{30}H_{21}O_4SN_4FCl_2$	623	57.79	3.39	8.99
7	A_7	112	76%	$C_{30}H_{22}O_4SN_4FCl$	589	61.17	3.76	9.51
8	A_8	124	65%	$C_{31}H_{24}O_5SN_4FCl$	619	60.14	3.91	9.05
9	A_9	118	69%	$C_{31}H_{24}O_5SN_4FCl$	619	60.14	3.91	9.05
10	A_{10}	158	83%	$C_{31}H_{24}O_5SN_4FCl$	619	60.14	3.91	9.05
11	A_{11}	260	77%	$C_{31}H_{22}O_6SN_4FCl$	633	58.82	3.50	8.85
12	A_{12}	308	85%	$C_{28}H_{25}O_4SN_5FCl$	582	57.78	4.33	12.03

Table No. 2 Antifungal activity

Sl. No	Name of the compounds	Mean zone of inhibition (in mm*)			
		C. albicans		*A. flavus*	
		50µg	100µg	50µg	100µg
01	Griseofulvin	18	23	19	24
02	A_1	15(0.83)	20(0.87)	15(0.79)	19(0.79)
03	A_2	14(0.77)	19(0.83)	14(0.74)	17(0.71)
04	A_3	14(0.77)	19(0.83)	13(0.68)	14(0.58)
05	A_4	15(0.83)	20(0.87)	16(0.84)	19(0.79)
06	A_5	13(0.72)	17(0.74)	15(0.79)	19(0.79)
07	A_6	12(0.67)	16(0.70)	15(0.79)	19(0.79)
08	A_7	11(0.61)	15(0.65)	160.84)	20(0.83)
09	A_8	13(0.72)	19(0.83)	14(0.74)	19(0.79)
10	A_9	16(0.89)	20(0.87)	13(0.68)	16(0.66)
11	A_{10}	14(0.77)	19(0.83)	12(0.63)	16(0.66)
12	A_{11}	11(0.61)	14(0.61)	11(0.58)	15(0.63)
13	A_{12}	11(0.61)	17(0.74)	14(0.74)	15(0.63)

Activity Index = Test Compound / Standard compound

Table No. 4 Characteristics IR absorption bands:

Compound	Ar-NH (in cm^{-1})	C=0 Stretching (in cm^{-1})	C=N Stretching (in cm^{-1})	C=C Stretching (in cm^{-1})	NO_2 (in cm^{-1})	C-F (in cm^{-1})	C – S Stretching (in cm^{-1})	Sec.Ar. Amine (in cm^{-1})	C – Cl Stretching (in cm^{-1})	C-O-C Stretching (in cm^{-1})	Ar-OH Stretching (in cm^{-1})
A_1	3350	1750	1550	1710	1450	1130	720	1300	840	1250	1390
A_2	3370	1710	1525	1680	1450	1160	720	1340	840	1250	1390
A_3	3370	1700	1540	1660	1420	1160	725	1310	850	1255	1380
A_4	3380	1730	1540	1680	-	1155	720	1300	850	1250	1380
A_5	3400	1765	1540	1690	-	1170	725	1310	820	1250	1380
A_6	3290	1720	1530	1680	-	1160	725	1300	840	1250	1380
A_7	3390	1755	1510	1690	-	1150	720	1255	840	1220	1380
A_8	3350	1720	1540	1685	-	1165	725	1310	830	1250	1390
A_9	3310	1730	1550	1650	-	1130	725	1310	840	1245	1380
A_{10}	3400	1750	1560	1660	-	1170	730	1300	850	1230	1385
A_{11}	3320	1700	1530	1640	-	1165	730	1310	840	1270	1380

compounds and those will be screened for antimicrobial activity.

MATERIALS AND METHODS

Chemicals and Reagents

4-fluoro-3-chloro aniline, Potassium thiocyanate, Glacial acetic acid, Bromine, Anthranillic acid, Pyridine, Vanillin, Ethanol, Conc. Hydrochloric acid, Chloroacetyl chloride, Triethylamine, N,N' -dimethyl formamide (DMF), various substituted aniline, morpholine, piperazine and diphenylamine.

Experimental Section

Step I: 4-fluoro-3-chloro aniline was treated with potassium thiocyanate (KSCN) in presence of glacial acetic acid and bromine to get 2-amino-6-fluoro-7-chloro-benzothiazole.

Step II: 2-amino-6-fluoro-7-chloro-benzothiazole treated with Anthranillic acid in presence of Pyridine to get 2 (o-amino phenyl amido) 6–fluoro -7-chloro (1, 3) benzothiazole.

Step III: 2 (o-amino phenyl amido) 6–fluoro -7-chloro (1,3) benzothiazole reflexed with vanillin and alcohol in presence of Conc.HCl to get 2 (3-hydroxy-4-methoxy benzylidene amino phenyl amido) 6-fluoro-7-chloro-(1,3) benzothiazole or Schiff's base.

Step IV: A Solution of Schiff's base (0.01 mol) in 1,4-dioxane (50ml) was added to well-stirred mixture of Chloroacetyl Chloride (0.95 ml, 0.012 mol) and Triethylamine (1.08 ml, 0.02 mol) at 0^{o} C. The reaction mixture was then stirred for 18 - 20 hrs and kept aside for 3 days at room temperature. The product was recrystallised from N,N' Dimethyl formamide (DMF).

Step V: Azetidine were treated with double the quantities of various substituted aniline, piperazine, diphenyl amine, refluxed for 2 hours in presence of N,N' -dimethyl formamide (DMF). The mixture was cooled and poured in to crushed ice. The solid separated was filtered off, dried and crystallized from alcohol and benzene.

General Procedures

Melting points were determined in open capillaries and are uncorrected. IR spectra (KBr pellet technique) were recorded using a Perkin-Elmer 237 spectrophotometer. ^{1}H NMR spectra were recorded on Bruker AM 400 instrument (at 400 MHz) using tetramethylsilane (TMS) as an internal standard and DMSO-*d6* as a solvent. Chemical shifts are given in parts per million (ppm). Splitting patterns are designated as follows: s- singlet, d- doublet, t- triplet, q- quartet and m-multiplet. Mass spectra (MS) were recorded on Schimadzu GC-MS operating at 70eV. All the synthesized compounds were purified by recrystallization The reactions were followed up and the purity of compounds was monitored on pre-coated TLC plates and visualizing the spots in ultraviolet light.

In vitro antimicrobial study

Synthesised compounds were screened for antibacterial and antifungal activities at two different conc (50μg/ml, 100μg/ml) against *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus flavus* by cup plate method (diffusion technique) using Procaine penicillin, Streptomycin, Cefazolin Sodium, Sporafloxin and Griseofulvin respectively as standards.[16] The results are the mean value of zone of inhibition measured in millimeter of two sets. The results are tabulated.

Table-4. NMR Spectral Data

Sl no	Compound Code	Hydrogen	δ(ppm)	Multiplity	Solvent
1	A_3	-Ar-H- -NH- β lactum 2H – Proton	7.0 – 7.8 5.4 6.6	Multiplet Singlet Doublet	$CDCl_3$
2	A_6	-Ar-H- - NH - β lactum 2H – Proton	7.0 – 7.8 5.3 6.6	Multiplet Singlet Doublet	$CDCl_3$

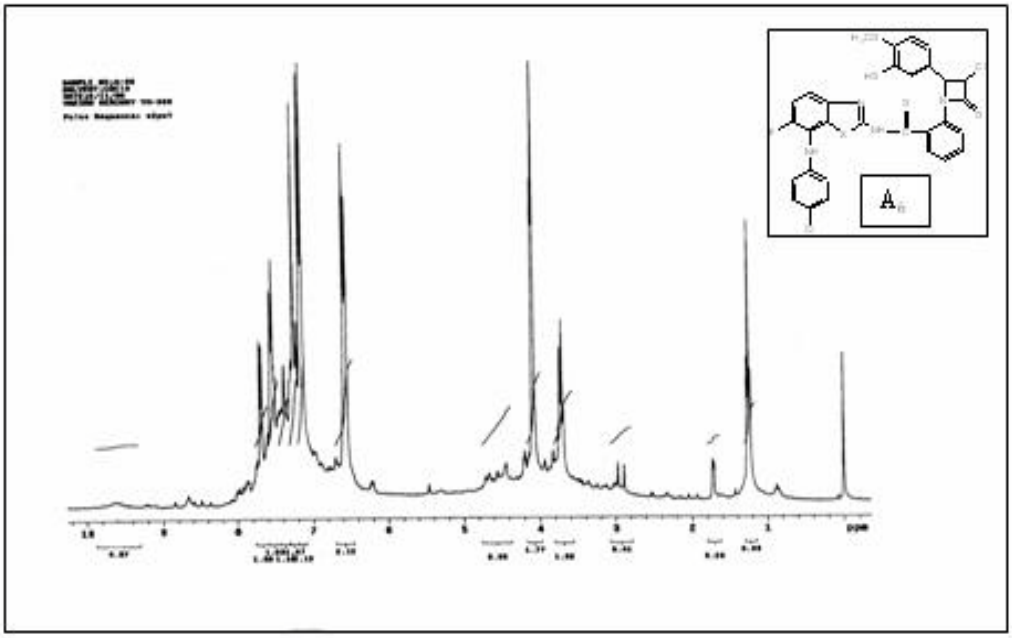


Fig 1. NMR Spectra - A_6

Table - 5. Mass spectra

Sl no	Compound Code	Calc. Mol. weight	Mol Formula	Fragmentation	m/z
1	A_3	634.03	$C_{30}H_{21}O_6SN_5FCl$	$M^{+1}(CH_3O, Cl, NO_2)$ $M^{+2}(C_6H_3, C_3NO)$ $M^{+3}\{(C_6H_4)_2O\}$	517.5 379.4 201.3
2	A_6	623.48	$C_{30}H_{21}O_4SN_4FCl_2$	$M^{+1}(-CH_3)$ $M^{+2}\{C_6H_3(OH)\text{-}O, Cl, O\}$ M^{+3} (N-C-C-C_9Cl) $M^{+4}\{(C_6H_4)_2\}$	613.9 454.7 369.8 203.2

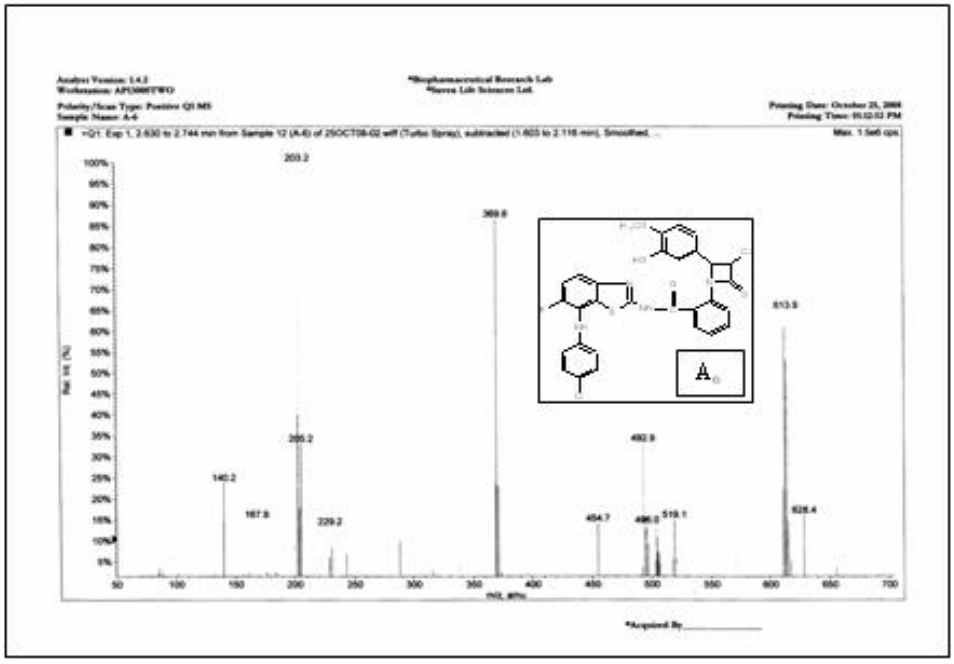
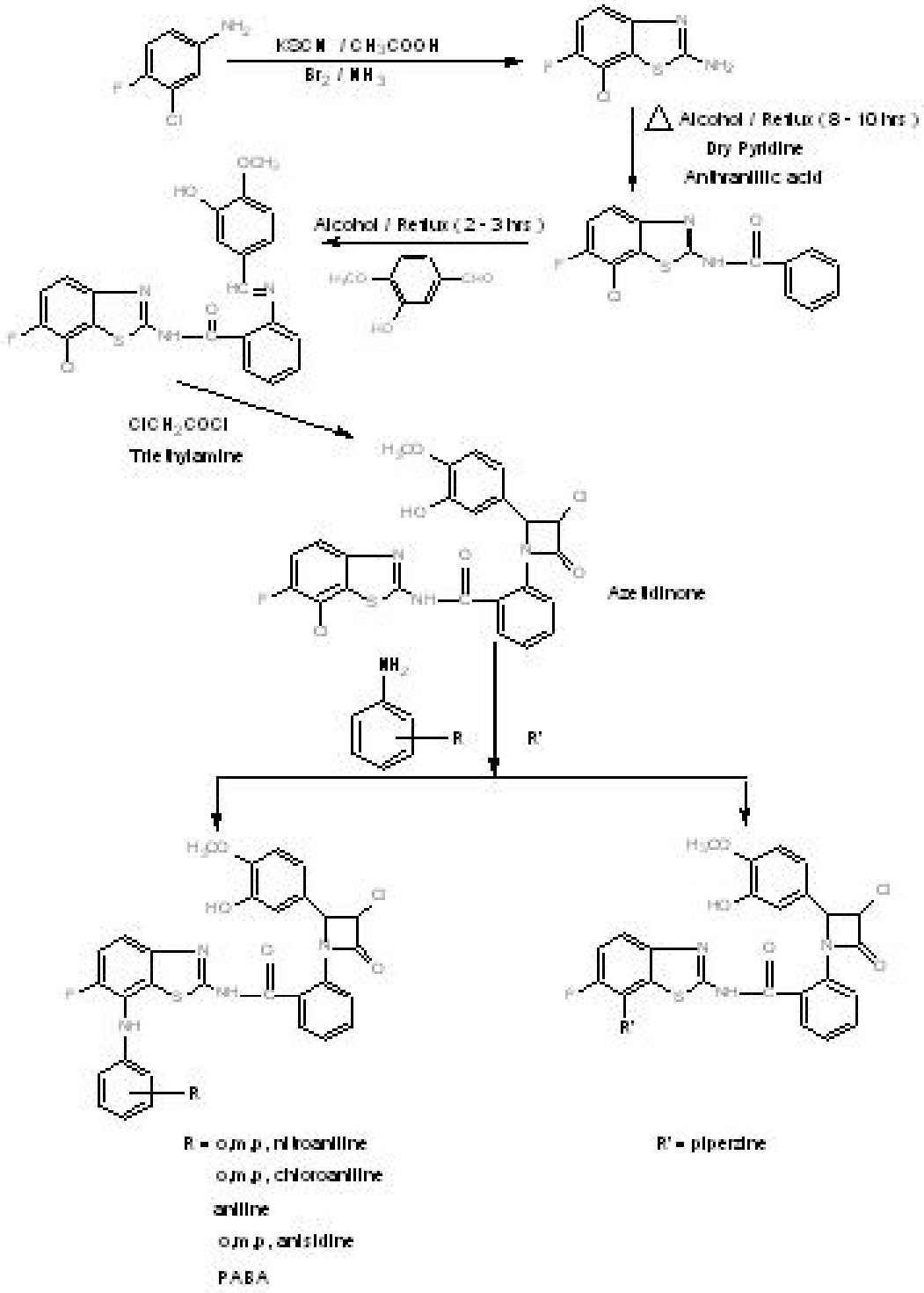


Fig 2. Mass Spectra - A_6

Scheme


KSCN / CH3COOH
Br2 / NH3
Alcohol / Reflux (8 - 10 hrs)
Dry Pyridine
Anthranilic acid
Alcohol / Reflux (2 - 3 hrs)
ClCH2COCl
Triethylamine
Azetidinone
R
R'
R = o,m,p, nitroaniline
o,m,p, chloroaniline
aniline
o,m,p, anisidine
PABA
R' = piperazine

RESULTS AND DISCUSSION

a) Anti-bacterial activity :

Synthesis and pharmacological screening of 4(m-hydroxy-p-methoxy phenyl)-1[(6'-fluoro-7'-substituted (1,3)-benzothiazol-2'-yl) amido-2-phenyl] 3-chloro azetidin–2–one were tested for the antibacterial activity against following bacteri;

a) 1] *S.aureus,* ii] *B.subtilis* (gram +ve) and
b) iii] *E.coli,* iv] *Pseudomonas* (gram –ve).

The test compounds A_3, A_4, A_5, A_7 and A_8 showed moderate antibacterial activity against *S.aureus* (gram +ve) compare to standard drug procaine penicillin.

Compounds A_3, A_4, A_9 and A_{10} showed promising antibacterial activity against, *E.coli* (gram –ve) compared to standard drugs and streptomycin.

Compounds A_6, A_8, A_{11} and A_{12} showed antibacterial activity against, gram +ve (*B.subtillis*) at lower concentration (50 μg/ml).

Compounds A_1, A_2, A_4, A_5 and A_6 showed moderate activity against gm –ve (*pseudomonas*) at both lower and higher concentration compare to standard drug streptomycin.

b) Anti-fungal activity:

Synthesized compounds were tested for antifungal activity against *Candida albicans* and *Aspergillus niger*. Among the compounds tested; A_1, A_9 and A_{10} showed good activity against *Candida albicans* at both concentration compare to standard Griseofulvin.

A_1, A_5, A_6 and A_7 showed significant activity against *Aspergillus niger* compared to standard Griseofulvin.

CONCLUSION

Result of present study demonstrate that, a new class of different aromatic aniline, anisidine, PABA, piperzine, encompassing azetidinone derivatives were synthesized and evaluated as antibacterial agents. The newly synthesized heterocyclics exhibited promising antimicrobial activity against *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa* and *Escherichia coli.* The antifungal studies against *Candida albicans* and *Asperagillus niger* showed significant activity at low and high concentration compared to standard. It can be concluded that this class of compounds certainly holds great promise towards good active leads in medicinal chemistry. A further study to acquire more information concerning pharmacological activity is in progress.

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