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Synthesis and biological evaluation of some novel optically active 3-chloro-1-[4-({4-[(S)-(4-chlorophenyl)(phenyl)methyl]-1-piperazinyl} acetyl)phenyl]-4-aryl-2-azetidinone derivatives

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Abstract

Various novel optically active substituted aryl 2-Azetidinone **4a-j** have been prepared from corresponding Schiff's bases **3a-j** and chloroacetyl chloride in presence of glacial acetic acid in benzene using Dien and Stark apparatus. Structure of the synthesized compound was confirmed by Spectral data (IR, ^{1}H NMR) and elemental analysis. All the newly synthesized compounds were evaluated for their antimicrobial activities. Investigation of antimicrobial activities of compounds was done by Broth dilution method used for the determination of minimum inhibitory concentration. The synthesize compounds were screened for antibacterial against gram-positive bacteria [*Staphylococcus aureus* (MTCC96), *Streptococcus pyogenes* (MTCC442)] and gram-negative bacteria [*Escherichia coli* (MTCC443), *Pseudomonas aeruginosa* (MTCC424)] and antifungal against *Candida albicans* (MTCC227), *Aspergillus niger* (MTCC282) and *Aspergillus clavatus* (MTCC1323).The compounds showed good antibacterial activity but less active against fungal strain used.

Keywords: Schiff's base, 2-azetidinone, specific optical rotation, spectral studies, antibacterial and antifungal activity.

Introduction

β-lactam antibiotics have been successfully used in the treatment of infectious diseases for many years [1]. Despite the large number of compounds containing a β-lactam moiety that have already been synthesized and tested, there is still a need for new compounds of this kind [2] due to the increasing resistance of bacterial strains to certain types of antibiotics [3]. A class of β-

lactams, known as the monocyclic β-lactams, which includes compounds such as the nocardicins, aztreonam and carumonam, has been described for their chemotherapeutic importance as antibiotics [4-7]. On the other hand, during the past few years carbohydrates have received increasing attention as stereo differentiating auxiliaries in stereoselective syntheses [8]. Also, several recent studies of β - lactams have uncovered novel therapeutic activity such as cholesterol lowering ability and serine protease inhibition [9]. Additionally, β-lactams serve as important chiral building blocks in organic chemistry (e.g. taxol semi synthesis) [10]. As a result of the long standing interest of β-lactams in medicine, biology and chemistry, many approaches to their stereoselective synthesis have been developed [11]. A large number of β-lactams possess powerful antibacterial, antifungal, anti-inflammatory, anticonvulsant and antitubercular activity [12-16]. Hence, with a view to further assess the pharmacological profile of this class of compounds, it was thought worthwhile to synthesize some new optically active azetidinone moieties.

Results and Discussion

New series of compounds namely aryl 2-azetidinone (**4a-j**) have been synthesized by using experimental protocol as shown in **Scheme I**. All the derivatives were supported by spectral data. The Schiff's base (**3a-j**) was easily prepared in good yield (65-80%) by refluxing two equivalents of 1-(4-Amino phenyl)-2-{4-[(*S*)-(4-chlorophenyl)(phenyl)methyl]-1-piperazinyl} ethanone (**2**) with substituted benzaldehyde in presence of glacial acetic acid in benzene. The water formed during the reaction was removed azeotropically by Dean and Stark apparatus. Complete condensation of primary amino groups is confirmed by the lack of N-H stretching bands in the 3150-3450cm^{-1} IR region and the presence of strong (C=N) stretching bonds in the 1690-1550cm^{-1}. (C-H) stretching vibration band in the 3000-2850 cm^{-1} region indicated the presence of piperazine moiety in the compound. ^{1}H NMR spectrum displayed signals for the presence of one imine proton (CH=N-) at 10.03 ppm (1H, s), one keton group (-CO-CH_2) at 2.61 ppm (2H, s), eight protons (4H+4H, m) of piperazine ring at (2.54-4.20) ppm, which also confirms the condensation of reactants. The structures of substituted aryl azetidinone **4a-j** were prepared by refluxing aryl Schiff's base with chloroacetyl chloride in presence of glacial acetic acid in benzene. The water formed during the reaction was removed azeotropically by Dean and Stark apparatus. The compound was confirmed by elemental analysis and IR spectra, showed (C=O) stretching absorption band at 1725-1660cm^{-1}, (-Cl) stretching absorption band at 800-600 cm^{-1} and ^{1}H NMR spectrum displayed signals for the presence of one proton of azetidinone ring (-CH-Cl) at 4.30 ppm (1H, d), one proton of azetidinone ring (-CH-N) at 6.60ppm (1H, d), one keton group (-CO-CH_2) at 2.65 ppm (2H, s) and aromatic ring proton (Ar-H) at 6.80-8.29 ppm (m).

Antibacterial activities

Antibacterial activities of all the compounds were studied against gram-positive bacteria [*Staphylococcus aureus* (MTCC96), *Streptococcus pyogenes* (MTCC442)] and gram-negative bacteria [*Escherichia coli* (MTCC443), *Pseudomonas aeruginosa* (MTCC424)] by the broth dilution method. Stock solutions of the series of compounds were prepared in DMSO. Each synthesized drug was diluted obtaining 2000 microgram/ml concentration, as a stock solution. Serial dilutions were prepared in primary and secondary screening. In primary screening 500 micro/ml, 250 micro/ml, and 125 micro/ml concentrations of the synthesized drugs were taken.

The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms.

Table-3: Antimicrobial activity of compounds (3a-j) and (4a-j)

Comp.	Antibacterial activity				Antifungal activity		
	Minimum bactericidal concentration µg/ml				Minimum fungicidal concentration µg/ml		
	S.a[a]	S.p[b]	E.c[c]	P.a[d]	C.a[g]	A.n[e]	A.c[f]
3a	50	250	25	250	200	500	500
3b	250	500	500	500	250	500	250
3c	62.5	500	50	250	500	1000	500
3d	100	62.5	25	50	125	500	1000
3e	100	50	62.5	125	250	500	1000
3f	250	500	100	125	200	250	1000
3g	50	500	50	250	250	500	100
3h	500	50	500	250	100	500	1000
3i	25	50	12.5	125	500	250	1000
3j	100	125	500	250	>1000	1000	>1000
4a	50	100	50	125	250	>1000	500
4b	50	100	100	50	125	500	1000
4c	250	125	250	500	200	500	1000
4d	50	100	62.5	100	100	200	500
4e	50	62.5	50	250	125	500	500
4f	50	50	25	100	250	1000	>1000
4g	100	125	125	250	500	250	500
4h	125	250	500	250	125	500	1000
4i	25	25	100	125	500	>1000	>1000
4j	50	250	100	250	100	500	500
Gentamycin	0.05	1	0.25	0.5	-	-	-
Ampicillin	100	100	250	100	-	-	-
Chloramphenicol	50	50	50	50	-	-	-
Ciprofloxacin	25	25	50	50	-	-	-
Norfloxacin	10	10	10	10	-	-	-
Nystatin	-	-	-	-	100	100	100
Greseofulvin	-	-	-	-	500	100	100

S.a[a] - *Staphylococcus aureus*(MTCC96); S.p[b]- *Streptococcus pyogenes*(MTCC442); .c[c]- *Escherichia coli*(MTCC443), P.a[d]- *Pseudomonas aeruginosa*(MTCC441); A.n[e]- *Aspergillus niger*(MTCC282), A.c[f]- *Aspergillus clavatus*(MTCC1323); C.a[g]- *Candida albicans*(MTCC227).

The drugs found active in primary screening were similarly diluted to obtain 100 micro/ml, 50 micro/ml, 25 micro/ml, 12.5 micro/ml, 6.250 micro/ml, 3.125 micro/ml and 1.5625 micro/ml

concentrations. Under similar condition using Gentamycin, Ampicillin, Chloramphenicol, Ciprofloxacin and Norfloxacin as a standard for comparison control experiment was carried out.

Antifungal activities

The compounds **3a-j** and **4a-j** were also screened for their antifungal activity against *Candida albicans* (MTCC227), *Aspergillus niger* (MTCC282) and *Aspergillus clavatus* (MTCC1323) at 2000µg/ml concentration using agar cup plate method. The antifungal activity was compared with the known standard drugs Greseofulvin, Nystatin.

Materials and Methods

All melting points were taken in open capillary tubes and are uncorrected. Purity of compound was checked by thin layer chromatography, performed on precoated TLC plates with silica gel (Merck 60 F_{254}) and detection was done by UV lamp (254 nm). Specific optical rotations (SOR) were taken in Jasco digital polarimeter. The IR spectra were obtained on a Perkin-Elmer BX series FTIR-5000 spectrophotometer using KBr pellets. The ^{1}H NMR spectra in DMSO-d_6 or $CDCl_3$ were recorded on Bruker WM 400FT MHz spectrometer and chemical shift were reported as parts per million (δppm) down field using TMS as internal standard. The antimicrobial activities were carried out at Microcare Laboratory, Surat.

Experimental:

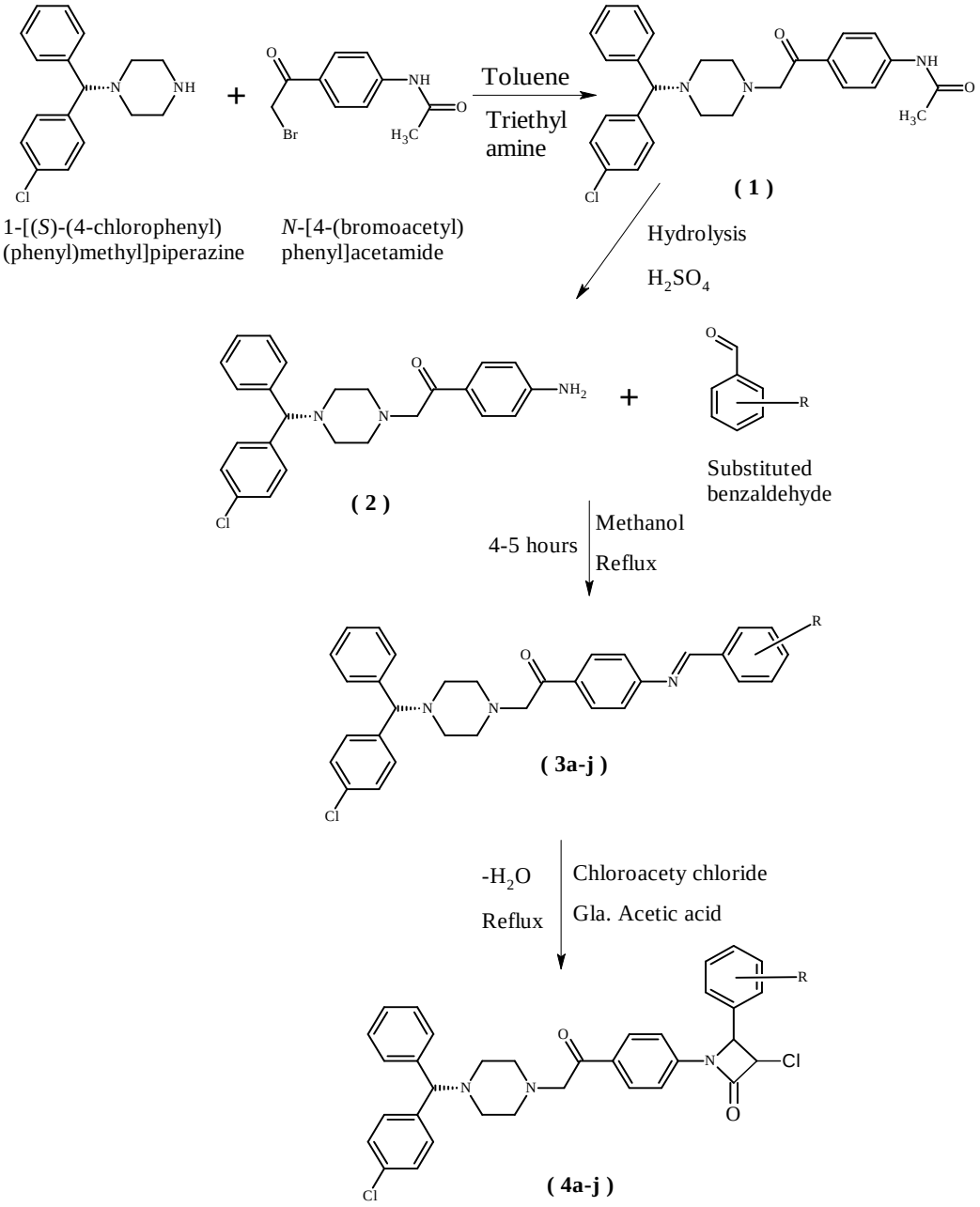
Synthesis of N-[4-(2-{4-[(S)-(4-Chlorophenyl)(phenyl)methyl]-1-piperazinyl}acetyl)phenyl] acetamide (1).

A mixture of 1-[(*S*)-(4-Chlorophenyl)(phenyl)methyl] piperazine (0.01 mol) [SOR: -20.18° (c = 1 in toluene)] and N-[4-(2-bromoacetyl)phenyl]acetamide (0.01 mol) was taken in methylene dichloride (50 ml). Triethylamine (0.01 mol) was added drop wise below 20°C. After the completion of addition, the mixture was refluxed for 4-5 hours. After completion of reaction, it was cooled and washed with water. The organic mass was dry over sodium sulphate and solvent was removed. The product was recrystalised from methanol. Yield: 86% MP: 75-76 °C SOR $[\alpha]_d^{28}$: -8.25° (c = 1, methanol). IR [ν, cm^{-1}, KBr]: 1672 (C=O), 3284 (NH), 1248 (C-N), 756 (C-Cl). ^{1}H NMR [400MHz, δ, ppm, DMSO]: 2.12 (3H, s, –$COCH_3$), 3.86 (2H, s, –$COCH_2$), 8.81 (1H, s, –NH), 5.59 (1H, s, –CH-N), 2.59-4.12 (8H, m, CH_2 piperazine),7.05-7.94 (13H, m, Ar-H).

Synthesis of 1-(4-Aminophenyl)-2-{4-[(S)-(4-chlorophenyl)(phenyl)methyl]-1-piperazinyl} ethanone (2).

N-[4-(2-{4-[(*S*)-(4-chlorophenyl)(phenyl)methyl]-1- piperazinyl}acetyl)phenyl]acetamide (0.01 mol) was dissolve in methanol (40ml). Sulfuric acid (0.025 mol) was added drop wise by maintaining temperature below 15°C with constant stirring. The reaction mixture was refluxed for 3 hours. After the completion of reaction, solvent was removed and added water (300 ml), made alkaline with concentrated ammonium hydroxide. The solid was separated and recrystalised from methanol. Yield: 78% MP: 110-112 °C. SOR $[\alpha]_d^{28}$: -6.95° (c = 1, methanol) IR [ν, cm^{-1}, KBr]: 1682 (C=O), 3358 (NH_2), 1253 (C-N), 745 (C-Cl). ^{1}H NMR [400MHz, δ, ppm, DMSO]: 3.89 (2H, s, –$COCH_2$), 5.10 (2H, s, –NH_2), 5.51 (1H, s, –CH-N), 2.56-4.16 (8H, m, CH_2 piperazine), 6.70-8.22 (13H, m, Ar-H).

Scheme-I: Synthetic scheme for the title compounds



Synthesis of 2-{4-[(S)-(4-Chlorophenyl)(phenyl)methyl]-1-piperazinyl}-1-(4-{[arylmethylidene] amino}phenyl)ethanone (3a-j).

A mixture of 1-(4-Amino phenyl)-2-{4-[(*S*)-(4-chlorophenyl)(phenyl)methyl]-1-piperazinyl} ethanone (0.01 mol) and substituted benzaldehyde (0.01 mol) in presence of glacial acetic acid (0.5 ml) in benzene (25 ml) was refluxed for 4-5 hours. The water formed during the reaction was removed azeotropically by Dean and Stark apparatus. The solvent was then removed and the product was isolated and recrystalised in methanol. The remaining compounds **3a-j** was

synthesized by using substituted benzaldehyde similarly. Their characterization data were recorded in **Table-1**.

Table 1: The physical and analytical data (3a-j)

Comp. No.	R	Molecular Formula (M. Wt.)	MP °C	SOR $[\alpha]_d^{28}$ °	Yield %	Elementary Analysis % Found (Calculated)		
						% C	% H	% N
3a	H	$C_{32}H_{30}ClN_3O$ (508)	152-153	- 2.26	74	75.64 (75.65)	5.89 (5.95)	8.21 (8.27)
3b	2 - Cl	$C_{32}H_{29}Cl_2N_3O$ (542)	204-205	-2.15	69	70.83 (70.85)	5.32 (5.39)	7.84 (7.75)
3c	4 - Cl	$C_{32}H_{29}Cl_2N_3O$ (542)	125-126	-1.90	73	70.81 (70.85)	5.38 (5.39)	7.80 (7.75)
3d	2 -OCH_3	$C_{33}H_{32}ClN_3O_2$ (538)	179-180	-2.08	79	73.69 (73.66)	5.95 (5.99)	7.85 (7.81)
3e	4 -OCH_3	$C_{33}H_{32}ClN_3O_2$ (538)	148-150	-2.19	76	73.65 (73.66)	5.98 (5.99)	7.80 (7.81)
3f	4 - CH_3	$C_{33}H_{32}ClN_3O$ (522)	141-142	-3.10	75	75.90 (75.92)	5.17 (6.18)	8.01 (8.05)
3g	3 - NO_2	$C_{32}H_{29}ClN_4O_3$ (553)	146-147	-3.58	72	69.41 (69.49)	5.25 (5.29)	10.09 (10.13)
3h	2 - NO_2	$C_{32}H_{29}ClN_4O_3$ (553)	171-172	-3.27	68	69.47 (69.49)	5.22 (5.29)	10.15 (10.13)
3i	2 - OH	$C_{32}H_{30}ClN_3O_2$ (524)	133-134	-1.99	65	73.31 (73.34)	5.78 (5.77)	8.00 (8.02)
3j	4 - OH 3 - Br	$C_{32}H_{29}BrClN_3O_2$ (603)	155-156	-2.45	80	60.33 (60.32)	4.63 (4.62)	6.19 (6.21)

Note: (c = 1 in methanol)

2-{4-[(S)-(4-chlorophenyl)(phenyl)methyl]-1-piperazinyl}-1-(4-{[phenylmethylidene]amino} phenyl)ethanone ***(3a).***

IR [ν, cm^{-1}, KBr]: 1672 (C=O), 1593 (–C=N), 757 (C-Cl). ^{1}H NMR [400MHz, δ, ppm, DMSO]: 3.31 (2H, s, $–COCH_2$), 8.71 (1H, s, –CH=N), 5.48 (1H, s, –CH-N), 2.54-4.11 (8H, m, CH_2 piperazine), 7.04-7.89 (18H, m, Ar-H).

1-(4-{[(2-chlorophenyl)methylidene]amino}phenyl)-2-{4-[(S)-(4-chlorophenyl)(phenyl)methyl]-1-piperazinyl}ethanone ***(3b).***

IR [ν, cm^{-1}, KBr]: 1681 (C=O), 1595 (–C=N), 774 (C-Cl). ^{1}H NMR [400MHz, δ, ppm, DMSO]: 3.33 (2H, s, $–COCH_2$), 8.80 (1H, s, –CH=N), 5.42 (1H, s, –CH-N), 2.58-4.17 (8H, m, CH_2 piperazine), 7.05-7.85 (17H, m, Ar-H).

1-(4-{[(4-chlorophenyl)methylidene]amino}phenyl)-2-{4-[(S)-(4-chlorophenyl)(phenyl)]methyl]-1- piperazinyl}ethanone ***(3c).***

IR [ν, cm^{-1}, KBr]: 1678 (C=O), 1597 (–C=N), 768 (C-Cl). ^{1}H NMR [400MHz, δ, ppm, DMSO]: 3.38 (2H, s, $–COCH_2$), 8.78 (1H, –CH=N), 5.44 (1H, s, –CH-N), 2.59-4.18 (8H, m, CH_2 piperazine), 7.00-7.82 (17H, m, Ar-H).

2-{4-[(S)-(4-chlorophenyl)(phenyl)methyl]-1-piperazinyl}-1-(4-{[(2-methoxyphenyl)methylidene amino}phenyl)ethanone ***(3d).***
IR [ν, cm^{-1}, KBr]: 1667 (C=O), 1594 (–C=N), 2827 (Ar-OCH_3), 756 (C-Cl). ^{1}H NMR [400MHz, δ, ppm, DMSO]: 3.70 (3H, s, $–OCH_3$), 3.35 (2H, s, $–COCH_2$), 8.68 (1H, –CH=N), 5.51 (1H, s,–CH-N), 2.57-4.16 (8H, m, CH_2 piperazine), 6.96-7.85 (17H, m, Ar-H).

2-{4-[(S)-(4-chlorophenyl)(phenyl)methyl]-1-piperazinyl}-1-(4-{[(4-methoxyphenyl) methylidene]amino}phenyl)ethanone ***(3e).***
IR [ν, cm^{-1}, KBr]: 1675 (C=O), 1588 (–C=N), 2822 (Ar-OCH_3), 755 (C-Cl). ^{1}H NMR [400MHz, δ, ppm, DMSO]: 3.74 (3H, s, $–OCH_3$), 3.32 (2H, s, $–COCH_2$), 8.79 (1H, s, –CH=N), 5.49 (1H, s, –CH-N), 2.57-4.10 (8H, m, CH_2 piperazine), 6.90-7.87 (17H, m, Ar-H).

2-{4-[(S)-(4-chlorophenyl)(phenyl)methyl]-1-piperazinyl}-1-(4-{[(4-methylphenyl) methylidene] amino}phenyl)ethanone ***(3f).***
IR [ν, cm^{-1}, KBr]: 1672 (C=O), 1596 (–C=N), 1332 (Ar-CH_3), 757 (C-Cl). ^{1}H NMR [400MHz, δ, ppm, DMSO]: 2.25 (3H, s, $–CH_3$), 3.34 (2H, s,–$COCH_2$), 5.56 (1H, s,–CH-N), 8.71 (1H, s, –CH=N), 2.55-4.13 (8H, m, CH_2 piperazine), 7.01-7.85 (17H, m, Ar-H).

2-{4-[(S)-(4-chlorophenyl)(phenyl)methyl]-1-piperazinyl}-1-(4-{[(3-nitrophenyl) methylidene] amino}phenyl)ethanone ***(3g).***
IR [ν, cm^{-1}, KBr]: 1692 (C=O), 1587 (–C=N), 1536 (Ar-NO_2), 751 (C-Cl). ^{1}H NMR [400MHz, δ, ppm, DMSO]: 3.36 (2H, s, $–COCH_2$), 8.64 (1H, s, –CH=N), 5.52 (1H, s, –CH-N), 2.54-4.20 (8H, m, CH_2 piperazine), 7.04-7.98 (17H, m, Ar-H).

2-{4-[(S)-(4-chlorophenyl)(phenyl)methyl]-1-piperazinyl}-1-(4-{[(2-nitrophenyl) methylidene] amino}phenyl)ethanone ***(3h).***
IR [ν, cm^{-1}, KBr]: 1695 (C=O), 1583 (–CH=N), 1549 (Ar-NO_2), 754 (C-Cl). ^{1}H NMR [400MHz, δ, ppm, DMSO]: 3.37 (2H, s, $–COCH_2$), 8.68 (1H, s, –CH=N), 5.53 (1H, s, –CH-N), 2.56-4.17 (8H, m, CH_2 piperazine), 7.02-8.08 (17H, m, Ar-H).

1-(4-{[(2-hydroxyphenyl)methylidene]amino}phenyl)-2-{4-[(S)-(4-chlorophenyl)(phenyl) methyl]-1-piperazinyl}ethanone ***(3i).***
IR [ν, cm^{-1}, KBr]: 1681 (C=O), 1595 (–C=N), 3374 (-OH), 754 (C-Cl). ^{1}H NMR [400MHz, δ, ppm, DMSO]: 3.35 (2H, s, $–COCH_2$), 4.50 (1H, s, -OH), 8.86 (1H, s, –CH=N), 5.50 (1H, s, –CH-N), 2.59-4.15 (8H, m, CH_2 piperazine), 6.92-7.84 (17H, m, Ar-H).

1-(4-{[(3-bromo-4-hydroxyphenyl)methylidene]amino}phenyl)-2-{4-[(S)-(4-chlorophenyl) (phenyl)methyl]-1-piperazinyl}ethanone ***(3j).***
IR [ν, cm^{-1}, KBr]: 1678 (C=O), 1591 (–C=N), 3364 (-OH), 763 (C-Cl), 687 (C-Br). ^{1}H NMR [400MHz, δ, ppm, DMSO]: 3.41 (2H, s, $–COCH_2$), 4.46 (1H, s, -OH), 8.69 (1H, s, –CH=N), 5.40 (1H, s, –CH-N), 2.59-4.15 (8H, m, CH_2 piperazine), 6.95-7.98 (16H, m, Ar-H).

General procedure for the synthesis of 3-chloro-1-[4-({4-[(S)-(4-chlorophenyl) (phenyl) methyl]-1-piperazinyl} acetyl)phenyl]-4-aryl-2-azetidinone 4a-j.

A solution of 2-{4-[(*S*)-(4-Chlorophenyl)(phenyl)methyl]-1-piperazinyl}-1-(4-{[arylmethylidine]amino}phenyl)ethanone (0.01 mol) in benzene (30ml) was taken and chloroacetyl chloride (0.01 mol) was added drop wise with constant stirring below 15 °C temperature. Triethylamine (0.01mol) was added and the reaction mixture was refluxed for 18 hours. After complies the reaction, solvent was removed and the mixture was treated with water. The product was isolated by filtration and recrystalised from methylene dichloride. The remaining compounds 4a-j was synthesized by using substituted aryl Schiff's base similarly. Their characterization data were recorded in Table-2.

Table 2: The physical and analytical data of (4a-j)

Comp. No.	R	Molecular Formula (M.wt.)	MR °C	SOR $[\alpha]_d^{28}$ °	Yield %	Elementary Analysis % Found (Calculated)		
						% C	% H	% N
4a	H	$C_{34}H_{31}Cl_2N_3O_2$ (508)	188-190	-1.77	68	69.80 (69.86)	5.30 (5.35)	7.12 (7.19)
4b	2 - Cl	$C_{34}H_{30}Cl_3N_3O_2$ (542)	206-208	-0.79	64	65.90 (65.97)	4.48 (4.89)	6.76 (6.79)
4c	4 - Cl	$C_{34}H_{30}Cl_3N_3O_2$ (542)	154-155	-1.28	62	65.78 (65.97)	4.59 (4.89)	6.70 (6.79)
4d	2 - OCH_3	$C_{35}H_{33}Cl_2N_3O_3$ (538)	161-162	-1.09	71	68.32 (68.40)	5.33 (5.41)	6.88 (6.84)
4e	4 - OCH_3	$C_{35}H_{33}Cl_2N_3O_3$ (538)	220-221	-1.92	76	68.37 (68.40)	5.38 (5.41)	6.80 (6.84)
4f	4 - CH_3	$C_{35}H_{33}Cl_2N_3O_2$ (522)	146-148	-1.42	75	70.21 (70.23)	5.45 (5.56)	7.10 (7.02)
4g	3 - NO_2	$C_{34}H_{30}Cl_2N_4O_4$ (553)	151-152	-1.68	79	64.81 (64.87)	4.85 (4.80)	8.87 (8.90)
4h	2 - NO_2	$C_{34}H_{30}Cl_2N_4O_4$ (553)	178-179	-1.86	70	64.92 (64.87)	4.78 (4.80)	8.93 (8.92)
4i	2 - OH	$C_{34}H_{31}Cl_2N_3O_3$ (524)	193-194	-1.96	74	68.07 (68.00)	5.24 (5.20)	7.31 (7.00)
4j	4 - OH 3 - Br	C35H32BrCl2N3O4 (633)	181-182	-1.88	68	59.19 (59.25)	4.48 (4.55)	5.87 (5.92)

Note: (c=1.5 in Methylene chloride)

3-chloro-1-[4-({4-[(S)-(4-chlorophenyl)(phenyl)methyl]-1-piperazinyl}acetyl)phenyl]-4-phenyl-2-azetidinone ***4a.***

IR [ν, cm^{-1}, KBr]: 1710(C=O), 802 (C-Cl). ^{1}H NMR [400MHz, δ, ppm, DMSO]: 3.30 (2H, s, $–COCH_2$), 4.30 (1H, d, -CH-Cl of azetidinone), 6.60 (1H, d, –CH-N of azetidinone), 5.50 (1H, s, –CH-N), 2.59-4.15 (8H, m, CH_2 piperazine), 6.82-8.24 (18H, m, Ar-H).

3-chloro-4-(2-chlorophenyl)-1-[4-({4-[(S)-(4-chlorophenyl)(phenyl)methyl]-1-piperazinyl} acetyl)phenyl]-2-azetidinone ***4b.***
IR [ν, cm^{-1}, KBr]: 1717(C=O), 796 (C-Cl). ^{1}H NMR [400MHz, δ, ppm, DMSO]: 3.35 (2H, s, $–COCH_2$), 4.26 (1H, d, -CH-Cl of azetidinone), 6.58 (1H, d, –CH-N of azetidinone), 5.35 (1H, s, –CH-N), 2.61-4.04 (8H, m, CH_2 piperazine), 6.78-8.18 (17H, m, Ar-H).

3-chloro-4-(4-chlorophenyl)-1-[4-({4-[(S)-(4-chlorophenyl)(phenyl)methyl]-1-piperazinyl} acetyl)phenyl]-2-azetidinone ***4c.***
IR [ν, cm^{-1}, KBr]: 1712(C=O), 791 (C-Cl). ^{1}H NMR [400MHz, δ, ppm, DMSO]: 3.22 (2H, s, $–COCH_2$), 4.29 (1H, d, -CH-Cl of azetidinone), 6.63 (1H, d, –CH-N of azetidinone), 5.55 (1H, s, –CH-N), 2.54-4.18 (8H, m, CH_2 piperazine), 6.89-8.17 (17H, m, Ar-H).

3-chloro-1-[4-({4-[(S)-(4-chlorophenyl)(phenyl)methyl]-1-piperazinyl}acetyl)phenyl]-4-(2-methoxyphenyl)-2-azetidinone ***4d.***
IR [ν, cm^{-1}, KBr]: 1724(C=O), 2843 (Ar-OCH_3), 801 (C-Cl). ^{1}H NMR [400MHz, δ, ppm, DMSO]: 3.88 (3H, s, $–OCH_3$), 3.28 (2H, s, $–COCH_2$), 4.46 (1H, d, -CH-Cl of azetidinone), 6.47 (1H, d, –CH-N of azetidinone), 5.39 (1H, s, –CH-N), 2.59-4.02 (8H, m, CH_2 piperazine), 6.95-8.11 (17H, m, Ar-H).

3-chloro-1-[4-({4-[(S)-(4-chlorophenyl)(phenyl)methyl]-1-piperazinyl}acetyl)phenyl]-4-(4-methoxyphenyl)-2-azetidinone ***4e.***
IR [ν, cm^{-1}, KBr]: 1718 (C=O), 2829 (Ar-OCH_3), 791 (C-Cl). ^{1}H NMR [400MHz, δ, ppm, DMSO]: 3.81 (3H, s, $–OCH_3$), 3.38 (2H, s, $–COCH_2$), 4.66 (1H, d, –CH-Cl of azetidinone), 6.44 (1H, d, –CH-N of azetidinone), 5.54 (1H, s, –CH-N), 2.68-4.18 (8H, m, CH_2 piperazine), 6.84-8.25 (17H, m, Ar-H).

3-chloro-1-[4-({4-[(S)-(4-chlorophenyl)(phenyl)methyl]-1-piperazinyl}acetyl)phenyl]-4-(4-methylphenyl)-2-azetidinone ***4f.***
IR [ν, cm^{-1}, KBr]: 1729 (C=O), 1339 (Ar-CH_3), 797 (C-Cl). ^{1}H NMR [400MHz, δ, ppm, DMSO]: 2.38 (3H, s, $–CH_3$), 3.45 (2H, s, $–COCH_2$), 4.46 (1H, d, –CH-Cl of azetidinone), 6.71 (1H, d, –CH-N of azetidinone), 5.58 (1H, s, –CH-N), 2.52-4.02 (8H, m, CH_2 piperazine), 6.91-8.21 (17H, m, Ar-H).

3-chloro-1-[4-({4-[(S)-(4-chlorophenyl)(phenyl)methyl]-1-piperazinyl}acetyl)phenyl]-4-(3-nitrophenyl)-2-azetidinone ***4g.***
IR [ν, cm^{-1}, KBr]: 1709 (C=O), 1557 (Ar-NO_2), 785 (C-Cl). ^{1}H NMR [400MHz, δ, ppm, DMSO]: 3.32 (2H, s, $–COCH_2$), 4.39 (1H, d, –CH-Cl of azetidinone), 6.59 (1H, d, –CH-N of azetidinone), 5.28 (1H, s, –CH-N), 2.59-4.12 (8H, m, CH_2 piperazine), 6.82-8.16 (17H, m, Ar-H).
3-chloro-1-[4-({4-[(S)-(4-chlorophenyl)(phenyl)methyl]-1-piperazinyl}acetyl)phenyl]-4-(2-nitrophenyl)-2-azetidinone ***4h.***
IR [ν, cm^{-1}, KBr]: 1713 (C=O), 1562 (Ar-NO_2), 796 (C-Cl). ^{1}H NMR [400MHz, δ, ppm, DMSO]: 3.41 (2H, s, $–COCH_2$), 4.44 (1H, d, –CH-Cl of azetidinone), 6.48 (1H, d, –CH-N of azetidinone), 5.46 (1H, s, –CH-N), 2.62-4.22 (8H, m, CH_2 piperazine), 6.75-8.12 (17H, m, Ar-H).

3-chloro-1-[4-({4-[(S)-(4-chlorophenyl)(phenyl)methyl]-1-piperazinyl}acetyl)phenyl]-4-(2-hydroxyphenyl)-2-azetidinone ***4i.***
IR [ν, cm^{-1}, KBr]: 1724 (C=O), 3381 (-OH), 808 (C-Cl). ^{1}H NMR [400MHz, δ, ppm, DMSO]: 3.37 (2H, s, $–COCH_2$), 4.72 (1H, s, –OH), 4.38 (1H, d, –CH-Cl of azetidinone), 6.53 (1H, d, –CH-N of azetidinone), 5.67 (1H, s, –CH-N), 2.53-4.01 (8H, m, CH_2 piperazine), 6.82-7.92 (17H, m, Ar-H).

4-(3-bromo-4-hydroxyphenyl)-3-chloro-1-[4-({4-[(S)-(4-chlorophenyl)(phenyl)methyl]-1-piperazinyl}acetyl)phenyl]-2-azetidinone ***4j.***
IR [ν, cm^{-1}, KBr]: 1711(C=O), 3389 (-OH), 708 (C-Br), 812 (C-Cl). ^{1}H NMR [400MHz, δ, ppm, DMSO]: 3.42 (2H, s, $–COCH_2$), 4.76 (1H, s, –OH), 4.34 (1H, d, –CH-Cl of azetidinone), 6.61 (1H, d, –CH-N of azetidinone), 5.45 (1H, s, –CH-N), 2.49-4.08 (8H, m, CH_2 piperazine), 6.91-8.05 (16H, m, Ar-H).

Conclusion

The screening data revealed that most of the tested compounds showed good bacterial inhibition. The compounds 3d, 3e, 3i, 4a, 4b, 4d, 4f and 4i were highly active against all four organisms employed. The compounds 3a, 3c, 3d, 3g, 3i, 4a, 4b, 4d, 4e, 4f, 4i and 4j were highly active against *Escherichia coli* (MTCC443) and *Staphylococcus aureus* (MTCC96). Some of the compounds had good antifungal activity against *Candida albicans* but these compounds were less active against *Aspergillus niger* and *Aspergillus clavatus.* Results were presented in**Table-3**.

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