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Synthesis, antibacterial and anticancer activity of novel bis-azetidinones

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ABSTRACT

*A novel series of 4,4'-(1,4-phenylene)bis(3-chloro-1-azetidin-2-one) (**11-20**) and 4,4'-(1,3-phenylene)bis(4-methoxyphenylazetidin-2-one) (**23**, **26**) along with azetidinone-Schiff base hybrids (**22**, **25**) were synthesized from the Staudinger ketene-imine cyclo addition reaction of the Schiff base dimers and the acetylchlorides. All the prepared compounds were screened for their antibacterial activity against nine different strains and anticancer activity against three cell lines including HeLa, MDA-MB-231 and ACHN. Many compounds showed good antibacterial activity. Particularly azetidinone-Schiff base hybrids showed remarkable antibacterial activity against six strains. (3S, 4S)-3-chloro-4-(4-((2R, 3R)-3-chloro-4-oxo-1-p-toylazetidin-2-yl)phenyl)-1-p-toylazetidin-2-one (**12**) and (3S, 4S)-3-chloro-4-(3-((2R, 3R)-3-chloro-4-oxo-1-p-toylazetidin-2-yl)phenyl)-1-p-toylazetidin-2-one (**17**) exhibited potent anticancer activity withthe IC_{50} values of (HeLa, 0.41; MDA-MB-231 0.42; ACHN, 0.45) and (HeLa, 0.46; MDA-MB-231, 0.40; ACHN, 0.48) respectively.*

Keywords: Schiff base dimers, 3-chloroazetidinone dimers, 3-(4-methoxyphenyl)azetidinone dimers, antibacterial activity, anticancer activity.

INTRODUCTION

The rationale behind the development of dimeric compounds as a drug candidate stems from their potential to bind two distinct individual binding sites on a single receptor or a defined site on two separate monomers of a dimeric protein. For example, the receptor tyrosine kinases VEGF and PDGF are important cellular growth factors activated as homodimers by ligand binding [1,2]. Certain cytokines, including human growth hormone and erythropoietin, have been shown to bind simultaneously to two receptors and create a receptor–ligand–receptor complex [3,4]. The estrogen receptor isoforms ERα and ERβ form homo- or heterodimers upon ligand binding [5–7]. The existence of such dimeric proteins as therapeutic targets suggests that dimeric small molecules can be appropriate building blocks for interaction/disruption of these and related protein complexes. Hence, there are many natural and synthetic dimers that are more active as anti-cancer [8], anti-HIV [9], anti-malarial [10], anti-bacterial [11] as well as opioid antagonists [12,13] than their monomers.

β-Lactam ring containing compounds are unique scaffolds and have various applications in the area of drug discovery (Fig. 1). *β*-Lactam antibiotics (e.g. penicillin, cephalosporin) functions to inhibit the synthesis of bacterial cell wall [14]. In addition to the bactericidal action of antibiotics, it has been discovered that many antibiotics are capable of inhibiting tumor cell growth [15]. There are currently many antitumor antibiotics approved for cancer therapy [16], and are currently used to treat cancer, such as the anthracyclines, bleomycin, mitomycin C,

dactinomycin, and mithramycin. [17] Several novel classes of β -lactams have been shown to act as tubulin-targeting agents [18], cholesterol absorption inhibitors [19], and anti-inflammatory agents [20]. Especially, large number of monocyclic β-lactams act as powerful anticancer agents which can induce DNA damage and inhibit DNA replication in Jurkat T cells (Fig 1). They also function as enzyme inhibitors and are effective on the central nervous system [21].

With the privilege of the β -lactam structure and the importance of dimer molecules as rationale approach for drug discovery, we direct our attention towards the synthesis of dimeric azetidinones (β-lactams) as novel antibacterial and anticancer agents.

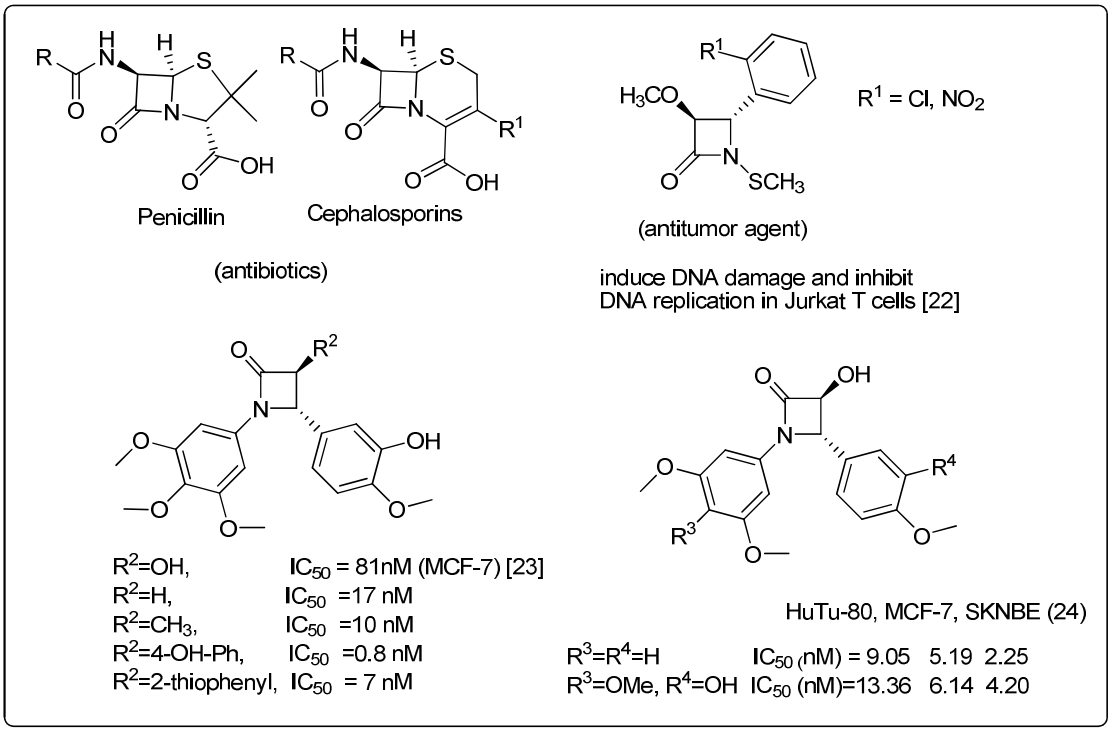


Fig 1.β-lactam scaffolds and their important biological applications

EXPERIMENTAL SECTION

2.1 Chemistry

Melting points (m.p.) were determined on Mettler FP 51apparatus (Mettler Instruments, Switzerland) and are uncorrected. They are expressed in degree centigrade (C). The IR spectra (in KBrpellets) were recorded on a Shimadzu FT-IR 157 spectrophotometer.^{1}H NMR spectra were recorded using $CDCl_3$ as solvent and TMS as an internal standard on a Bruker 300 MHz/400 MHz NMR spectrometer. Chemical shift values are given in δ(ppm scale). Micro analyses were performed on a Vario EL III model CHNS analyser (Vario, Germany) at the Department of Chemistry, Bharathiar University. The purity of the products was tested by TLC with plates coated with silica gel-G with petroleum ether, ethyl acetate and methanol as developing solvents. The purity of the compounds was checked by thin-layer chromatography(TLC) on silica gel plate using petroleum ether and ethyl acetate.

General procedure for the preparation of Schiff base (1-10)

To a solution of corresponding dialdehyde (10.00 mmol) in ethanol (10 mL), corresponding amines (20.00 mmol) were added and refluxed for 6 h. The obtained precipitate was collected by filtration and recrystallized from ethanol.

Synthesis of 4,4'-(1,3-phenylene)bis(3-chloro-1-azetidin-2-one)(11-20):

To a cooled solution of Schiff bases (**1-10**) (2.50 mmol) in methylene chloride (10 mL) at 0 °C, triethylamine (5.00 mmol) was added and stirred. To that reaction mixture, chloroacetyl chloride (5.10 mmol) was slowly added for a

period of 30 mins and further stirred at RT for 10 h. The reaction was monitored by TLC. After the completion of the reaction, water was added to the reaction mixture and extracted using methylene chloride. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The obtained crude residue was purified by column chromatography to get compounds **11-20**.

(3*S*, 4*S*)-3-chloro-4-(4-((2*R*, 3*R*)-3-chloro-4-oxo-1-phenylazetidin-2-yl)phenyl)-1-phenylazetidin-2-one (**11**)
Yield: 65%. White crystals, m.p.218-220 °C. IR (KBr): 2990, 2928, 1731 (C=O), 1535, 1021, 938. ^{1}H NMR ($CDCl_3$): 4.60 (d, *J* = 2.40 Hz, 2H) 5.04 (d, *J* = 2.40 Hz, 2H), 7.08-7.15 (m, 2H), 7.26–7.29 (m, 8H), 7.43(s, 4H). ^{13}CNMR ($CDCl_3$): 63.10, 65.42, 117.62, 125.27, 127.35, 129.50, 136.62, 136.65, 160.62. Anal.calc.for: $C_{24}H_{18}Cl_2N_2O_2$ (436.07): C 65.91 H 4.15 N 6.41 Found 65.88 H 4.13 N 6.42.

(3*S*, 4*S*)-3-chloro-4-(4-((2*R*, 3*R*)-3-chloro-4-oxo-1-*p*-toylazetidin-2-yl)phenyl)-1-p-toylazetidin-2-one (**12**)
Yield: 67%. White crystals, m.p.235-277 °C. IR (KBr): 2991, 2932, 1732 (C=O), 1536, 1024, 937. ^{1}H NMR ($CDCl_3$): 2.28 (s, 6H), 4.57 (d, *J* = 2.40 Hz, 2H) 5.01 (d, *J* = 2.40 Hz, 2H), 7.08 (d, *J* = 8.00 Hz, 4H), 7.15 (d, *J* = 8.00 Hz, 4H), 7.41 (s, 4H). C^{13} NMR ($CDCl_3$): 20.87, 62.99, 65.25, 117.45, 127.21, 129.86, 134.02, 134.06, 136.53, 160.22 Anal.calc.for: $C_{26}H_{22}Cl_2N_2O_2$ (464.10): C, 67.10; H, 4.76; N, 6.02. Found C, 65.88; H, 4.13; N, 6.42.

(3*S*, 4*S*)-3-chloro-4-(4-((2*R*, 3*R*)-3-chloro-1-(4-methoxyphenyl)-4-oxoazetidin-2-yl)phenyl)-1-(4-methoxyphenyl) azetidin-2-one (**13**)
Yield: 62%. White crystals, m.p. 238- 240 °C. IR (KBr): 2992, 2930, 1730 (C=O), 1533, 1087, 1023, 937. ^{1}H NMR ($CDCl_3$): 3.83 (s, 6H), 4.58 (d, *J* = 2.40 Hz, 2H) 5.07 (d, *J* = 2.40 Hz, 2H), 7.06 (d, *J* = 8.00 Hz, 4H), 7.38 (d, *J* = 7.80 Hz, 4H), 7.40 (s, 4H). ^{13}CNMR ($CDCl_3$): 55.86, 62.87, 66.13, 117.42, 127.19, 129.77, 134.12, 134.08, 151.48, 160.25. Anal.calc.for: $C_{26}H_{22}Cl_2N_2O_4$ (496.09): C, 62.79; H, 4.46; N, 5.63. found C, 62.88; H, 4.43; N, 5.59.

(3*S*, 4*S*)-3-chloro-4-(4-((2*R*, 3*R*)-3-chloro-1-(4-chlorophenyl)-4-oxoazetidin-2-yl)phenyl)-1-(4-chlorophenyl) azetidin-2-one (**14**)
Yield: 65%. White crystals, m.p. 243-245 °C. IR (KBr): 2989, 2932, 1730 (C=O), 1537, 1022, 936 .^{1}H NMR ($CDCl_3$): 4.60 (d, *J* = 2.40 Hz, 2H) 5.02 (d, *J* = 2.40 Hz, 2H), 7.24 (d, *J* = 8.00 Hz, 4H), 7.45 (s, 4H), 7.52 (d, *J* = 7.80 Hz, 4H). ^{13}CNMR ($CDCl_3$): 62.87, 66.13, 117.42, 127.19, 129.77, 134.12, 134.08, 141.34, 160.25 Anal.calc.for: $C_{24}H_{16}Cl_4N_2O_4$ (503.99): C, 56.94; H, 3.19; N, 5.53. Found C, 56.89; H, 3.24; N, 5.59.

(3*S*, 4*S*)-3-chloro-4-(4-((2*R*, 3*R*)-3-chloro-1cyclohexyl-4-oxoazetidin-2-yl)phenyl)-1-cyclohexyl azetidin-2-one (**15**)
Yield: 63%. White crystals, m.p. 202-204 °C. IR (KBr): 2929, 1733 (C=O), 1536, 1020, 937, 838. ^{1}H NMR ($CDCl_3$): 1.12-1.40 (m, 6H), 1.51-1.75 (m, 14H), 3.52 (m, 2H), 4.60 (d, *J* = 2.40 Hz, 2H), 5.01 (d, *J* = 2.40 Hz, 2H), 7.40 (s, 4H). ^{13} CNMR ($CDCl_3$): 24.80, 25.63, 30.23, 62.89, 66.15, 69.52, 127.20, 136.45, 160.24. Anal.calc.for: $C_{24}H_{30}Cl_2N_2O_2$ (448.16): C, 64.14; H, 6.73; N, 6.23. Found C, 64.19; H, 6.74; N, 6.24.

(3*S*, 4*S*)-3-chloro-4-(3-((2*R*, 3*R*)-3-chloro-4-oxo-1-phenylazetidin-2-yl)phenyl)-1-phenylazetidin-2-one (**16**)
Yield: 68%. White crystals, m.p. 142-144 °C. IR (KBr): 2993, 2926, 1734 (C=O), 1534, 1022, 936. ^{1}H NMR ($CDCl_3$): 4.60 (d, *J* = 2.40 Hz, 2H), 5.02 (d, *J* = 2.40 Hz, 2H), 7.13 (d, *J* =7.80 Hz, 2H), 7.19-7.22 (m, 8H), 7.42 (d, *J* = 7.80 Hz, 2H), 7.46 (s, 1H), 7.49 (t, J = 8.0 Hz, 1H). ^{13}CNMR ($CDCl_3$): 62.93, 65.34, 117.39, 125.14, 127.14, 129.32, 129.35, 136.57, 136.65, 160.43. Anal.calc.for: $C_{24}H_{18}Cl_2N_2O_2$ (436.07): C 65.91 H 4.15 N 6.41. Found 65.90 H 4.14 N 6.40.

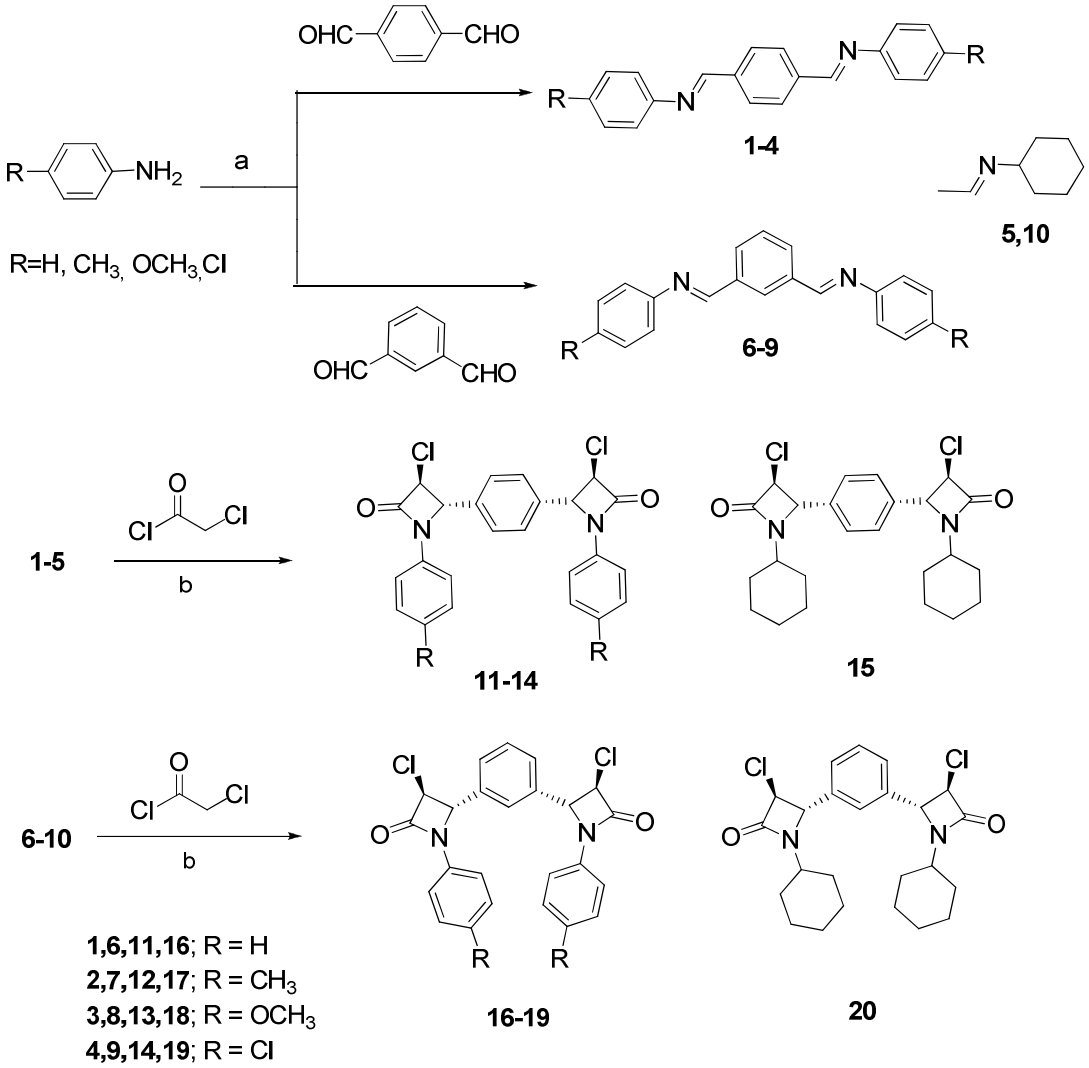
(3*S*, 4*S*)-3-chloro-4-(3-((2*R*, 3*R*)-3-chloro-4-oxo-1-*p*-toylazetidin-2-yl)phenyl)-1-*p*-toylazetidin-2-one (**17**)
Yield: 66%. White crystals, m.p.157-159 °C. IR (KBr): 2994, 2931, 1729 (C=O), 1532, 1025, 936. ^{1}H NMR ($CDCl_3$): 2.30 (s, 6H), 4.61 (d, *J* = 2.40 Hz, 2H), 4.98 (d, *J* = 2.40 Hz, 2H), 7.01- 7.11 (m, 7H), 7.39 (d, *J* = 8.00 Hz, 4H), 7.39 (t, *J* = 7.80 Hz, 1H). ^{13}CNMR ($CDCl_3$): 20.91, 62.90, 65.39, 117.42, 127.09, 129.74, 129.82, 130.03, 134.89, 136.62, 160.21 Anal.calc.for: $C_{26}H_{22}Cl_2N_2O_2$ (464.10): C, 67.10; H, 4.76; N, 6.02. Found C, 67.18; H, 4.73; N, 6.04.

(3*S*, 4*S*)-3-chloro-4-(3-((2*R*, 3*R*)-3-chloro-1-(4-methoxyphenyl)-4-oxoazetidin-2-yl)phenyl)-1-(4-methoxyphenyl) azetidin-2-one (**18**)
Yield: 63%. White crystals, m.p. 162-164°C. IR (KBr): 2992, 2927, 1736 (C=O), 1533, 1024, 1086, 935. ^{1}H NMR ($CDCl_3$): 3.85 (s, 6H), 4.59 (d, *J* = 2.40 Hz, 2H) 5.03 (d, *J* = 2.40 Hz, 2H), 7.02 (d, *J* = 8.00 Hz, 4H), 7.11 (d, *J* = 8.00 Hz, 2H), 7.23 (s, 1H), 7.35 (d, *J* = 7.80 Hz, 4H) 7.45 (t, *J* = 7.80 Hz, 1H). ^{13}CNMR ($CDCl_3$): 55.86, 62.87,

66.13, 117.49, 127.12, 129.76, 130.12, 134.82, 154.48, 160.25. Anal.calc.for: $C_{26}H_{22}Cl_2N_2O_4$ (496.09): C, 62.79; H, 4.46; N, 5.63. Found C, 62.88; H, 4.43; N, 5.59.

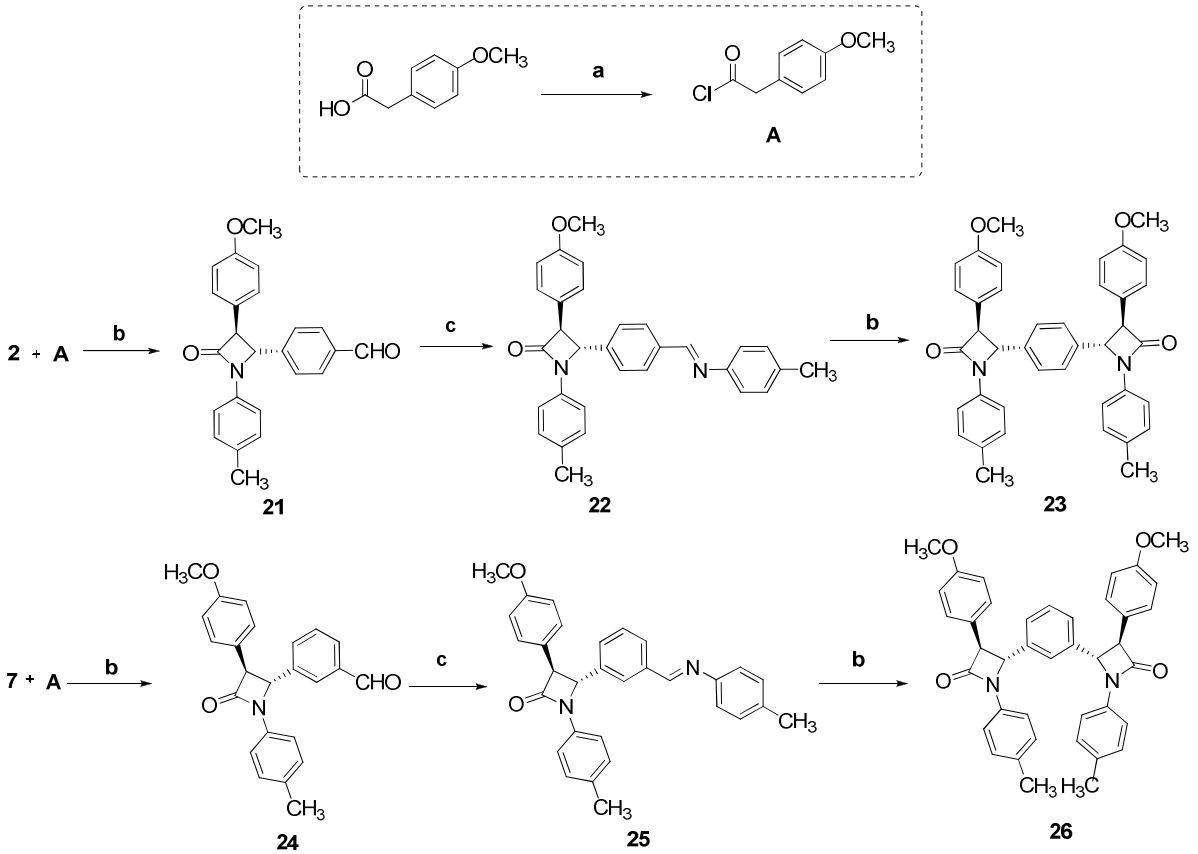
(3*S*, 4*S*)-3-chloro-4-(3-((2*R*, 3*R*)-3-chloro-1-(4-chlorophenyl)-4-oxoazetidin-2-yl)phenyl)-1-(4-chlorophenyl) azetidin-2-one (**19**)
Yield: 65%. White crystals, m.p.167-169 °C. IR (KBr): 2988, 2926, 1733 (C=O), 1532, 1024, 936. ^{1}H NMR ($CDCl_3$): 4.55 (d, *J* = 2.40 Hz, 2H) 5.07 (d, *J* = 2.40 Hz, 2H), 7.13 (d, *J* = 8.00Hz, 2H), 7.22 (s, 1H), 7.31 (d, *J* = 8.00 Hz, 4H), 7.45 (t, *J* = 7.80 Hz, 1H), 7.53 (d, = 8.00 Hz, 4H). ^{13}CNMR ($CDCl_3$): 62.87, 66.13, 117.42, 127.12, 129.76, 130.12, 133.82, 136.48, 160.25 Anal.calc.for: $C_{24}H_{16}Cl_4N_2O_4$ (503.99): C, 56.94; H, 3.19; N, 5.53. Found C, 56.96; H, 3.14; N, 5.52.

(3*S*, 4*S*)-3-chloro-4-(3-((2*R*, 3*R*)-3-chloro-1cyclohexyl-4-oxoazetidin-2-yl)phenyl)-1-cyclohexylazetidin-2-one (**20**)
Yield: 63 %. White crystals, m.p. 122-124°C. IR (KBr): 2929, 1732 (C=O), 1537, 1023, 936, 834. ^{1}H NMR ($CDCl_3$):1.14-1.42 (m, 6H), 1.50-1.73 (m, 14H), 3.55 (m, 2H), 4.61 (d, *J* = 2.40 Hz, 2H) 5.07 (d, *J* = 2.40 Hz, 2H), 7.25 (s, 1H), 7.37 (d, *J* = 8.00Hz, 2H), 7.43(t, *J* = 7.80Hz, 1H). ^{13}CNMR ($CDCl_3$): 62.89, 66.15, 117.43, 127.20, 129.75, 134.02, 134.12, 136.45, 141.36, 160.24 Anal.calc.for: $C_{24}H_{30}Cl_2N_2O_2$ (448.16): C, 64.14; H, 6.73; N, 6.23. found C, 64.19; H, 6.74; N, 6.24.



Scheme 1. Preparation of 3-chloro azetidinone (11-20)

*Reagents and conditions: a) Ethanol, reflux, 3 h. b) triethylamine, methylene chloride, 0 °C to RT, 10 h. Note: Compounds **5** and **10** were derived from cyclohexylamine.*



Scheme 2. Preparation of azetidinone (21-26)

Reagents and conditions: a) $SOCl_2$, methylene chloride, reflux, 3h b) triethylamine, methylene chloride, 0 °C to RT, 10 h c) Ethanol, reflux, 3 h.

Synthesis of 4-((2*R*, 3*S*)-3-(4-methoxyphenyl)-4-oxo-1-*p*-tolylazetidin-2-yl)benzaldehyde (21)

Preparation of 4-methoxyphenylacetyl chloride (**A**):
To a solution of 4-methoxyphenyl acetic acid (5.10 mmol) in methylene chloride (15 mL), thionyl chloride (6.12 mmol) was added and refluxed for 3 h. The resulting mixture was evaporated to remove excess thionyl chloride to afford 4-methoxyphenylacetyl chloride as colourless liquid.

To a cooled solution of (*N*,*N'E*,*N*,*N'E*)-*N*,*N*'-(-1,4-phenylenebis(methan-1-yl-1-ylidene))bis(4-methylaniline) (**2**, 2.50 mmol) in methylene chloride (10 mL) at 0°C, triethylamine (5.00 mmol) was added and stirred. To this reaction mixture, 4-methoxyphenylacetyl chloride (5.10 mmol) was slowly added for a period of 30 mins and further stirred at RT for 10 h. The reaction was monitored by TLC. After the completion of the reaction, water was added to the reaction mixture and extracted using methylene chloride. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The obtained crude residue was purified by column chromatography to get compound 4-((2*R*,3*S*)-3-(4-methoxyphenyl)-4-oxo-1-*p*-tolylazetidin-2-yl)benzaldehyde **(21)**.

4-((2*R*,3*S*)-3-(4-methoxyphenyl)-4-oxo-1-*p*-tolylazetidin-2-yl)benzaldehyde **(21)**
Yield: 70%. White crystals, m.p.92-94 °C. IR (KBr): 2992, 2932, 1730 (C=O), 1620, 1534, 1022, 941. ^{1}H NMR ($CDCl_3$): 2.29 (s, 3H), 3.82 (s, 3H), 4.21 (d, *J* = 2.40 Hz, 1H), 4.95 (d, *J* = 2.40 Hz, 1H), 6.92 (d, *J* = 7.80 Hz, 2H), 7.09 (d, *J* = 8.00Hz, 2H), 7.24 -7.34 (m, 4H), 7.54 (d, *J* = 8.00Hz, 2H), 7.92(d, *J* = 8.00 Hz, 2H), 10.03 (s, 1H). ^{13}CNMR ($CDCl_3$): 20.82, 55.32, 63.58, 64.89, 114.60, 117.07, 126.29, 126.51, 128.73, 129.79, 130.75, 130.76, 134.13, 134.79, 144.58, 159.57, 165.29, 191.58. Anal.calc.for: $C_{24}H_{21}NO_3$ (377.15): C, 77.61; H, 5.70; N, 3.77. found C, 77.64; H, 5.73; N, 3.74.

Synthesis of (3*S*, 4*R*)-3-(4-methoxyphenyl)-1-*p*-tolyl-4-(4-(9*E*)-(*p*- tolylimino)methyl)phenylazetidin-2-one (**22**)
To a solution of 4-((2*R*,3*S*)-3-(4-methoxyphenyl)-4-oxo-1-*p*-tolylazetidin-2-yl)benzaldehyde **(21)**in ethanol, *p*-toluidine was added and refluxed for 6 h. The obtained precipitate was collected by filtration and recrystallized from ethanol to get (*3S, 4R*)-3-(4-methoxyphenyl)-1-*p*-tolyl-4-(4-(9*E*)-(*p*-tolylimino)methyl)phenylazetidin-2-one (**22**)
(*3S, 4R*)-3-(4-methoxyphenyl)-1-*p*-tolyl-4-(4-((*E*)-(*p*-tolylimino)methyl)phenylazetidin-2-one (**22**)

Yield: 85%. White crystals, m.p. 198-200 ° C. IR (KBr): 2992, 2927, 1734 (C=O), 1534, 1623, 1019, 939. ^{1}H NMR ($CDCl_3$): 2.28 (s, 3H), 2.38 (s, 3H), 3.82 (s, 3H), 4.22 (d, *J* = 2.40 Hz, 1H), 4.92 (d, *J* = 2.40 Hz, 1H), 6.64 (d, *J* = 8.00Hz, 2H), 6.92 (d, *J* = 7.80 Hz, 2H), 6.97 (d, *J* = 8.00Hz, 2H), 7.08 (d, *J* = 8.02Hz, 2H), 7.14-7.27 (m, 2H), 7.23 (d, *J* = 8.00 Hz, 2H), 7.48 (d, *J* = 8.00 Hz, 2H), 7.92 (d, *J* = 7.80Hz, 2H), 8.47 (s, 1H). ^{13}CNMR ($CDCl_3$): 20.34, 20.82, 55.32, 63.79, 64.63, 115.52, 117.17, 120.85, 126.29, 126.67, 128.73, 129.67, 129.86, 133.92, 135.01, 136.18, 136.82, 140.90, 143.37, 149.26, 158.67, 159.48, 165.62. Anal.calc.for: $C_{31}H_{28}N_2O_2$ (460.21): C, 80.84; H, 6.13; N, 6.08. Found C, 80.82; H, 6.15; N, 6.04.

Synthesis of (*3S, 4R*)-3-(4-methoxyphenyl)-1-*p*-tolyl-4-(3-((*E*)-(*p*-tolylimino)methyl)phenylazetidin-2-one (**23**)
To a cooled solution of (*3S, 4R*)-3-(4-methoxyphenyl)-1-*p*-tolyl-4-(4-((*E*)-(*p*-tolylimino)methyl)phenylazetidin-2-one (**22**, 2.50 mmol) in methylene chloride (10 mL) at 0 °C, triethylamine (5.00 mmol) was added and stirred. To this mixture, 4-methoxyphenylacetyl chloride (5.10 mmol) was slowly added for a period of 30 mins and further stirred at RT for 10 h. The reaction was monitored by TLC. After the completion of the reaction, water was added to the reaction mixture and extracted using methylene chloride. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The obtained crude residue was purified by column chromatography to get compound **(23)**.
(*3S, 4R*)-3-(4-methoxyphenyl)-1-*p*-tolyl-4-(3-((*E*)-(*p*-tolylimino)methyl)phenylazetidin-2-one (**23**)

Yield: 72%. White crystals, m.p.245-247 °C. IR (KBr): 2990, 2928, 1731 (C=O), 1535, 1021, 938. ^{1}H NMR ($CDCl_3$): 2.28 (s, 6H), 3.85 (s, 6H), 4.24 (d, *J* = 2.40 Hz, 2H), 4.99 (d, *J* = 2.40 Hz, 2H), 6.91 (d, *J* = 7.80 Hz, 4H), 7.09 (d, *J* = 8.00Hz, 4H), 7.21-7.27 (m, 8H), 7.51 (s, 4H). ^{13} C NMR ($CDCl_3$): 20.85, 55.37, 63.61, 64.92, 114.61, 117.12, 126.25, 126.51, 129.87, 130.74, 130.76, 134.19, 134.89, 159.60, 165.22. Anal.calc.for: $C_{40}H_{36}N_2O_4$ (608.26): C, 78.92; H, 5.96; N, 4.60. Found C, 78.94; H, 5.93; N, 4.64.

Synthesis of 3-((2*R*,3*S*)-3-(4-methoxyphenyl)-4-oxo-1-*p*-tolylazetidin-2-yl)benzaldehyde (**24**).
To a cooled solution of (*N*,*N'E*,*N*,*N'E*)-*N*,*N'*-(-1,3-phenylenebis{methan-1-yl-1-ylidene})bis(4-methylaniline) (**7**,2.50 mmol) in methylene chloride (10 mL) at 0 °C, 4-methoxyphenylacetyl chloride (5.10 mmol) was slowly added for a period of 30 mins and further stirred at RT for 10 h. The reaction was monitored by TLC. After the completion of the reaction, water was added to the reaction mixture and extracted using methylene chloride. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The obtained crude residue was purified by column chromatography to get compounds 3-((2*R*,3*S*)-3-(4-methoxyphenyl)-4-oxo-1-*p*-tolylazetidin-2-yl)benzaldehyde **(24)**.

3-((2*R*,3*S*)-3-(4-methoxyphenyl)-4-oxo-1-*p*-tolylazetidin-2-yl)benzaldehyde **(24)**.
Yield: 70%. White crystals, m.p. 85-87° C. IR (KBr): 2995, 2930, 1729 (C=O), 1536, 1027, 1623, 940. ^{1}H NMR ($CDCl_3$): 2.25 (s, 3H), 3.83 (s, 3H), 4.26 (d, *J* = 2.40 Hz, 1H), 5.01 (d, *J* = 2.40 Hz, 1H), 6.89 (d, *J* = 7.80 Hz, 2H), 7.11 (d, *J* = 8.00Hz, 2H), 7.19-7.28 (m, 4H), 7.45 (t, *J* = 8.00Hz, 1H), 7.69- 7.71(m, 3H), 10.05 (s, 1H). ^{13}CNMR ($CDCl_3$): 20.80, 55.37, 63.54, 64.83, 114.60, 117.07, 126.29, 126.51, 128.15, 128.73, 129.78, 130.75, 130.92, 131.20, 131.46, 132.72, 134.13, 134.79, 144.61, 159.54, 165.32, 191.55. Anal.calc.for: $C_{24}H_{21}NO_3$ (377.15): C, 77.61; H, 5.70; N, 3.77. Found C, 77.64; H, 5.73; N, 3.74.

Synthesis of (*3S, 4R*)-3-(4-methoxyphenyl)-1-*p*-tolyl-4-(3-((*E*)-(*p*-tolylimino)methyl)phenylazetidin-2-one (**25**)
To a solution of 3-((2*R*,3*S*)-3-(4-methoxyphenyl)-4-oxo-1-*p*-tolylazetidin-2-yl)benzaldehyde **(24)** in ethanol, *p*-toluidine was added and refluxed for 6 h. The obtained precipitate was collected by filtration and recrystallized from ethanol to get (*3S, 4R*)-3-(4-methoxyphenyl)-1-*p*-tolyl-4-(3-((*E*)-(*p*-tolylimino)methyl)phenylazetidin-2-one (**25**).
(*3S, 4R*)-3-(4-methoxyphenyl)-1-*p*-tolyl-4-(3-((*E*)-(*p*-tolylimino)methyl)phenylazetidin-2-one (**25**)

Yield: 88%. White crystals, m.p. 122-124° C. IR (KBr): 2992, 2927, 1733 (C=O), 1534, 1622, 1021, 939. ^{1}H NMR ($CDCl_3$): 2.29 (s, 3H), 2.37 (s, 3H), 3.84 (s, 3H), 4.21 (d, *J* = 2.40 Hz, 1H), 4.96 (d, *J* = 2.40 Hz, 1H), 6.64 (d, *J* = 8.00Hz, 2H), 6.87 (d, *J* = 7.80 Hz, 2H), 6.92 (d, *J* = 8.00Hz, 2H), 7.09 (d, *J* = 8.02Hz, 2H), 7.15 (d, *J* = 8.00Hz, 2H), 7.25 (d, *J* = 8.00 Hz, 2H), 7.45 (t, *J* = 8.00 Hz, 1H), 7.67-7.71 (m, 3H), 8.44 (s, 1H). ^{13}CNMR ($CDCl_3$): 20.34,

20.82, 55.32, 63.79, 64.63, 117.17, 127.43, 128.73, 129.67, 129.86, 132.06, 135.01, 136.18, 140.90, 143.37, 149.26, 158.67, 159.48, 166.62. Anal.calc.for: $C_{31}H_{28}N_2O_2$ (460.21): C, 80.84; H, 6.13; N, 6.08. Found C, 80.82; H, 6.15; N, 6.04.

Synthesis of (3*R*,4*S*)-3-4-methoxyphenyl)-4-(3-((2*R*,3*S*)-3-(4-methoxyphenyl)-4-oxo-1-*p*-tolylazetidin-2-yl)phenyl)-1-p-tolylazetidin-2-yl)benzaldehyde (**26**)

To a cooled solution of (*3S, 4R*)-3-(4-methoxyphenyl)-1-*p*-tolyl-4-(3-((*E*)-(*p*-tolylimino)methyl)phenylazetidin-2-one (**23**,2.50 mmol) in methylene chloride (10 mL) at 0 °C, triethylamine (5.00 mmol) was added and stirred. To this reaction mixture, 4-methoxyphenylacetyl chloride (5.10 mmol) was slowly added for a period of 30 mins and further stirred at RT for 10 h. The reaction was monitored by TLC. After the completion of the reaction, water was added to the reaction mixture and extracted using methylene chloride. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The obtained crude residue was purified by column chromatography to get compounds (3*R*,4*S*)-3-4-methoxyphenyl)-4-(3-((2*R*,3*S*)-3-(4-methoxyphenyl)-4-oxo-1-*p*-tolylazetidin-2-yl)phenyl)-1-p-tolylazetidin-2-yl) benzaldehyde **(26)**.

(3*R*,4*S*)-3-4-methoxyphenyl)-4-(3-((2*R*,3*S*)-3-(4-methoxyphenyl)-4-oxo-1-*p*-tolylazetidin-2-yl)phenyl)-1-*p*-tolyl azetidin-2-yl)phenyl-1-*p*-tolylazetidin-2-one **(26)**.

Yield: 70%. White crystals, m.p.190-192 °C. IR (KBr): 2996, 2929, 1733 (C=O), 1536, 1023, 937. ^{1}H NMR ($CDCl_3$): 2.29 (s, 6H), 3.84 (s, 6H), 4.29 (d, *J* = 2.40 Hz, 2H), 4.98 (d, *J* = 2.40 Hz, 2H), 6.91 (d, *J* = 7.80 Hz, 4H), 7.13 (d, *J* = 8.00Hz, 4H), 7.17-7.26 (m, 8H), 7.24- 7.26(m, 3H), 7.45 (t, *J* = 8.00Hz, 1H). ^{13}CNMR ($CDCl_3$): 20.82, 55.35, 63.56, 64.83, 117.09, 126.29, 126.51, 126.97, 128.19, 128.73, 129.78, 130.23, 130.75, 130.94, 131.13, 134.54, 132.27, 134.76, 159.56, 165.32. Anal.calc.for: $C_{40}H_{36}N_2O_4$ (608.26): C, 78.92; H, 5.96; N, 4.60. Found C, 78.91; H, 5.95; N, 4.62.

2.2. Biology

2.2.1. Antibacterial activity

The agar well diffusion method was employed for the determination of anti-bacterial activities of the compounds [25]. All the tests were performed in duplicate and repeated twice. Modal values were selected. The compounds wereweighed and dissolved in DMSO (1mg/mL) andwere filter sterilized using a 0.45 µm membrane filter. Each pathogenic microorganism was suspended in sterile saline and diluted to ca. 106 colony forming units (cfu/mL). They were swabbed on to the surface of Mueller–Hinton Agar (MHA). The wells (8 mm in diameter) were cut from the agar and 0.1 mL of DMSO solution with compounds was delivered into them. After incubation for 24 h at 37° C, all plates were examined for zones of growth inhibition, and the diameter of these zones were measured inmillimeters. Amoxicillin and Ceftriaxone (1 mg/1 mL in DMSO) were used as standards. The solvent DMSO was used as negative control.

2.2.2. Anti-cancer activity

The anticancer screening was performed by SRB assay [26,27]. The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 µM L-glutamine. For present screening experiment, cells were inoculated into 96 well microtiter plates in 90 µL at 5000 cells per well. After cell inoculation, the micro titer plates were incubated at 37°C, 5%CO_2, 95% air and 100 % relative humidity for 24 h prior to addition of experimental drugs. Experimental drugs were solubilized in appropriate solvent to prepare stock of 10^{-2} concentration. At the time of experiment four 10-fold serial dilutions were made using complete medium. Aliquots of 10 µl of these different drug dilutions were added to the appropriate micro-titer wells already containing 90 µl of medium, resulting in the required final drug concentrations.

After compound addition, plates were incubated at standard conditions for 48 hours and assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 µl of cold 30 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 µl) at 0.4 % (w/v) in 1 % acetic acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1 % acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mMtrizma base, and the absorbance was read on an Elisa plate reader at a wavelength of 540 nm with 690 nm reference wavelength.

RESULTS AND DISCUSSION

3.1 Chemistry

The 1,4-isomers of dimeric Schiff bases (**1-5**) were prepared from terephalaldehyde and variety of amines that diverge the electronic effects of the aromatic ring. The next series of Schiff bases (**6-10**) were derived from isophthalaldehyde and different amines (Scheme 1). Azetidinones (**11-20**) were synthesized from the dimeric Schiff base (**1-10**) by Staudinger ketene-imine cyclo addition reaction, which involves the reaction of imine (**1-10**) with chloroacetylchloride in presence of triethylamine as a base (Scheme 1). We intended to replace the 3-chloro group in the lactam ring with aromatic ring. Hence the reaction of 4-methoxyphenylacetyl chloride (**A**) with the Schiff base **2** and **7** under the same Staudinger ketene-imine cyclo addition resulted in the azetidinone **21** and **24**respectively instead of the dimer **23** and **26**. The monomer **21** and **24** were then reacted with the amines to afford the azetidinone-Schiff base hybrids **22** and **25**.Finally **22** and **25** were reacted with 4-methoxyphenylacetyl chloride(**A**) to get the dimer **23** and **26** (Scheme 2). 4-Methoxy phenylacetyl chloride (**A**) was prepared from 4-methoxy phenylacetic acid by the treatment of thionyl chloride.

3.2 Biology

3.2.1 Antibacterial activity

The agar well diffusion method was employed for the determination of anti-bacterial activity of the compounds. Nine different strains (six Gram-negative and three Gram-positive) used were *Escherichia coli, Proteus mirabilis, Klebsiella pneumonia, Proteus vulgaris, Morganella morgana, Salmonella typhi, Staphylococcus aureus,* Methicillin-resistant *Staphylococcus aureus* and *Enterococci faecalis.* Results of anti-bacterial activity (zone of inhibition in millimeter) of the compounds (**11-26**) and standards were summarized in Table 1.

Table 1. Anti-bacterial activity (zone of inhibition in millimeter) of the synthesized compounds against nine different strains

Com. No	Gram-Negative						Gram-Positive		
	E.coli	***Proteus mirabilis***	***Klebsiellap neumonia***	***Proteus vulgaris***	***Morganella morgana***	***Salmonella typhi***	***Staphylococcus aureus***	**Methicillin-resistant *Staphylococcus aureus***	***Enterococci faecalis***
11	10	0	16	20	14	16	22	12	0
12	12	15	0	18	16	18	20	0	0
13	18	20	10	18	0	16	18	10	0
14	12	18	15	22	16	24	22	14	10
15	0	10	0	15	0	18	14	10	0
16	0	15	10	12	16	18	0	18	0
17	12	20	16	26	24	26	26	14	0
18	12	26	15	25	0	26	20	22	0
19	0	0	11	25	0	16	22	0	0
20	11	0	16	23	0	24	23	20	0
21	10	0	14	12	15	12	0	0	0
22	14	25	12	28	26	24	28	26	18
23	10	20	10	18	20	22	24	22	0
24	0	15	11	0	15	0	12	0	0
25	18	26	14	26	28	25	26	20	22
26	12	20	0	22	20	18	18	18	0
Amoxy cyllin	30	30	30	30	30	30	30	30	30
Ceftria xone	30	30	30	30	30	30	30	30	30

The anti-bacterial activity for the prepared azetidinone derivatives was screened against nine strains (Table 1). The 1,4-positional isomer of the prepared azetidinone dimers, **11-13** showed moderate antibacterial activity. The 4-chloro derivative **14**showed good activity against*P. mirabilis, P. vulgaris, S. typhi and S. aureus* (18, 22, 24, 22 mm). Among the 1,3-positional isomers of the azetidinone dimers, compound **17** with 4-methyl group showed a good antibacterial activity against *P. mirabilis, P. vulgaris, M. morgana, S. typhi and S. aureus* (20, 26, 24, 26, 26 mm). The azetidinone -Schiff base hybrid derivatives, **22** and **25** showed the best antibacterial activity against *P. mirabilis, P. vulgaris, M. morgana, S. typhi, S. aureus* and Methicillin-resistant *S. aureus.* (25, 28, 26, 24, 28, 26 mm and 26, 26, 28, 25, 26, 20 respectively). The dimer **23** showed a good result against *P. mirabilis, M. morgana, S. typhi, S. aureus,* Methicillin-resistant *S. aureus* (20, 20, 22 ,24, 22) when compared to its momomer derivatives **21** and **24**.

3.2.2 Anti-cancer activity

All the synthesized compounds **11-26** were evaluated for their *in vitro* inhibitory activities against three human cancer cell lines (cervical HeLa, breast MDA-MB-231 and renal ACHN) using SRB assay. The results as $IC_{50}$are mentioned in the Table 2.

Table 2. *In Vitro* anticancer activity of the synthesized compounds against three cell lines

Compound No.	HeLa IC_{50} (µM)	MDA- MB-231 IC_{50} (µM)	ACHN IC_{50} (µM)
11	0.56	0.67	0.52
12	0.41	0.42	0.45
13	1.24	0.98	1.59
14	6.89	>10	>10
15	4.56	1.27	3.45
16	0.61	0.53	0.60
17	0.46	0.40	0.48
18	0.96	1.44	1.01
19	5.45	9.01	7.77
20	>10	4.99	>10
21	>10	>10	>10
22	>10	>10	>10
23	0.99	1.10	0.86
24	>10	>10	>10
25	>10	>10	8.35
26	1.38	0.95	2.35
Adriamycin	0.52	0.51	0.58

The initially prepared 1,4-positional isomers of 3-chloroazetidinone derivative **11** showed a good anticancer activity (HeLa; 0.56, MDA- MB-231, 0.67, ACHN, 0.52) which was equivalent to the standard used (Adriamycin, HeLa; 0.52, MDA- MB-231, 0.51, ACHN, 0.58). The compound **12** with electron donating group at the *para* position increased the activity (0.41; 0.42; 0.45). The stronger electron donating 4-methoxy analog**13**, however decreased the activity (1.24; 0.98; 1.59). The electron withdrawing 4-chloro analog **14** and the bulky hydrophobic cyclohexyl derivative **15**did not fetch good inhibitory activity.

While analyzing the 1,3-isomers of the 3-chloroazetidinones, unsubstituted compound **16** showed a good activity (0.61; 0.53; 0.60). The 4-methyl derivative **17** showed potent cytotoxic activity (0.46; 0.40; 0.48)which was more than Adriamycin. The stronger electron donating 4-methoxy analog **18** did not exhibit stronger activity (0.96; 1.44; 1.01). The electron withdrawing 4-chloro analog **19** and the hydrophobic cyclohexyl derivative **20** was not effective for their inhibitory activity.

Encouraged by these results, the 3-chloro group of the azetidinone was changed to aromatic ring (**23** and **26**) by the reaction of 4-methoxy phenylacetyl chloride and the Schiff bases **2** and **7**. The reaction initially yielded the monomer **21** and **24** which was reacted with the amines to result in the azetidinone-Schiff base hybrid analogs **22** and **25**. Finally they were converted to the azetidinone dimers **23** and **26**. The momomers **21** and **24** did not fetch good cytotoxicity results. The azetidinone-Schiff base hybrid molecules **22** and **25**were ineffective to exhibit reasonable anticancer activity. However, the dimer molecules **23** and **26** showed a good activity. The azetidinone dimer of the 1,4-positional isomer, **23** showed (0.99; 1.10; 0.86) while the 1,3-positional dimer molecule **26** exhibited (1.38; 0.95; 2.35).

CONCLUSION

A novel series of dimers of 3-chloroazetidinone (**11-20**) were synthesized from the Staudinger ketene-imine cyclo addition reaction of the prepared Schiff base dimers (**1-10**) and chloro acetylchloride. They were screened for their antibacterial activity against nine different strains and anticancer activity against three cell lines including HeLa, MDA-MB-231 and ACHN. The compounds **14** and **17** showed good antibacterial activity against different strains. The 1,4-positional isomers**11** and **12** showed excellent anticancer activity with the IC_{50} values of (0.56; 0.67; 0.52) and (0.41; 0.42; 0.45) respectively. Similarly the 1,3-positional isomers of 3-chloroazetidinones **16** and **17** exhibited strong anticancer activity with the IC_{50}values of(0.61; 0.53; 0.60) and(0.46; 0.40; 0.48) respectively. The azetidinone-Schiff base hybrids **22** and **25** showed excellent antibacterial activity against six strains. The bis-

azetidinone **23** and **26** showed reasonable anticancer activity against the three cell line tested. It is noteworthy to mention that the monomer derivatives **21** and **24** were not effective in both antibacterial and anticancer screenings. Among the series, the dimer of 3-chloro-1-azetidin-2-one derivatives **11**,**12,16**,**17** were the most potent compounds.

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