Current Chemistry Letters 11 (2022) 403–414

Contents lists available at GrowingScience

Current Chemistry Letters

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Synthesis, characterization and SAR studies of Novel Series of Spiro β-Lactam of 5-methyl-indole-2,3-dione derivatives as a potential antibacterial and anthelmintic agent

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C H R O N I C L E

Article history:
Received February 2, 2022
Received in revised form
March 8, 2022
Accepted April 27, 2022
Available online
April 27, 2022

Keywords:
Spiro β-Lactam
Synthesis
Anthelmintic activity
Antibacterial activity

A B S T R A C T

A novel series of spiro cyclo-indolyl β-lactam compounds of the 5-methyl-indole-2,3-dione derivatives has been synthesized from the new Schiff bases Isatin, using the Staudinger synthesis. FT-IR, 1H-NMR, 13C-NMR, mass spectroscopy, and elemental analyses were used to describe the produced chemicals. The anthelmintic potency of the produced compounds was evaluated using standard albendazole. The antibacterial activity also tested for the synthesized compounds by calculating the zone of inhibition using cup plate method and by comparison with the standard Ampicillin against the five different pathogens (*Bacillus subtilis* (ATCC-1086), *Pseudomonas auroginosa* (ATCC-1232), Escherichia coli, (ATCC-3273), *Proteus mirabilis* (ATCC-224), and *Staphylococcus aureus* (ATCC-449)). The result of anthelmintic potential, when compared to conventional albendazole (PT: 1.324±0.12, DT: 1.421±0.21), synthesized compounds ICP-3B (PT: 1.883±0.24, DT: 1.943±0.02) and ICM-3B (PT: 1.758±0.27, DT: 1.675±0.32) were shown significant activity in terms of paralysis and death time. In the antibacterial study, the compound ICP-3B has shown 20.53mm clear zone of inhibition at 100 µg/mL against the bacteria *Bacillus subtilis* in comparison to the standard ampicillin (23.04 mm zone). The compound ICM-3B, clearly shows the inhibition zone of 21.27 mm against *Bacillus subtilis,* 24.64 mm against *pseudomonas aeruginosa,* 20.62mm against *E. coli,* 20.41 mm against *Proteus mirabilis* and 23.65 mm against the bacteria *staphylococcus aureus* at the level of 100µg/mL. It was confirmed that the compounds were synthesized as expected in the reaction scheme based on the collected spectrum and elemental data. The obtained anthelmintic ant antibacterial results also affirm the potentiality of the synthesized compounds. The further compounds can be synthesised as well as other pharmacological activities can be tested for these compounds with the concept of molecular modelling.

1. Introduction

Spirocyclic compounds are characterized by having two rings sharing the same atom, the quaternary spiro carbon[1] This structural feature induces rigidity on a molecule and allows unique three-dimensional disposition of molecular functional groups. The inherent rigidity of spirocyclic compounds causes a decrease in conformational entropy penalty when it comes to an interaction between a potential bioactive spiro compound and its putative molecular target[2]. In this context, it is not surprising that several biologically active molecules possess a spirocyclic scaffold in its molecular structure. 2-Azetidinones, commonly known as β-lactams, are the key structural motifs in the most widely used class of antibiotics, i.e., β-lactam antibiotics such as penicillins, cephalosporins, carbapenems, etc. There is a huge importance of organic chemistry in applied science[3,4,5,6] The development of novel synthetic methodologies for the preparation of functionalized β-lactams and the screening of their biological activity has occupied a pivotal position in medicinal chemistry for almost a century

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doi: 10.5267/j.ccl.2022.5.001

now[7]. The [2+2] cycloaddition can proceed via stepwise mechanism with zwitterionic intermediate, or, in the case of low-polar nature of intermolecular interatcions - via stepwse mechanism with the biradical intermediate[8]. The beta lacum ring can alternatively be prepared by *Kinugasa* process (reactions between nitrons and alkynes catalysed by Cu(I) compounds)[9]. Some time ago, Padwa *et al.* described a new method of synthesis of β-lactams that used a simple thermal conversion of 2,3,4-trisubstituted-5-nitroisoxazolidines to execute[10]. Radomir Jasinski in 2015, postulate that the analysed reaction takes place according to a two-step mechanism, *via* an opening step of the isoxazolidine ring[11]

Based on the literature review, besides being antibacterial agents, various other biological activities, such as cholesterol absorption inhibition, inhibition of different kinds of enzymes, and antitubercular, hypoglycemic and anticancer activity, have been discovered to be associated with β-lactams[12, 13]. The spiro 2-azetidinones was also reported for its anti HIV & plasmodium[14], antimicrobial activities[15]. Isatin and its derivatives have a lot of medicinal chemistry perspective, hence their chemistry is fascinating. According to a thorough literature review, isatin and substituted isatin are particularly relevant compounds because of their multipurpose properties. Isatin derivatives with Schiff and Mannich bases have been reported to have a wide range of biological functions like antidepressant[16] antibacterial[17], anticonvulsant[18], antidepressant[19], anti-inflammatory[20], anti HIV[21], antifungal[22], activities. Antibacterial, anti-inflammatory, and analgesic properties have been documented for thiazole and its derivatives[23]. Anthelmintic action of piperazinyl and replacement piperazinyl compounds are well known[24,25]By considering the aforementioned reported activities of 2-azetidinone, Isatin, and mannich bases with piperazine derivatives, the present research work was planned to synthesized a novel series of spiro cycloxyindole β lactams with the piperazinyl mannich base, and to test for their anthelmintics and antibacterial activities. The increasing number of resistant pathogens and the discovery of β-lactamase inhibition properties of β-lactams gave further impetus to these present studies.

2. Results and discussion

The newly synthesized compounds have been prepared from the Schiff bases of 5 methyl isatin which occurs through a formal [2+2] cycloaddition (or cyclocondensation) between imines and ketenes, a two-step process involving zwitterionic intermediates. Here chloro acetyl chloride with triethylamine is used for the in-situ generation of ketenes. With the discovery and structural elucidation of the antibiotic penicillin, the Staudinger synthesis became of major importance in medicinal chemistry as this procedure allowed the synthesis of penicillin derivatives in the laboratory[26].The structures of the newly synthesized compounds were elucidated by utilizing FT-IR, 1H-NMR, 13C-NMR mass spectra and elemental analysis. The synthesis of the newly synthesized compounds was performed using the following reaction scheme as shown in **Fig. 1.**

2.1. Characterization of the synthesized individual compounds

2.1.1. 3-chloro-5'-methyl-1-(4-phenylthiazol-2-yl)-1'-(piperazin-1 ylmethyl)spiro[azetidine-2,3'-indoline]-2',4-dione (ICP-3A)

FT-IR (KBr cm^{-1}), 3559 (NH pyrazine), 2927(C-H, alkyl), 1722 (C = O), 1638 (C=N), 1353 (CH_2), 1192(C-S-C), 1020 (C-N), 865 (CH=CH, aromatic), 643 (C=O) ^{1}H-NMR (500MHz, DMSO-d_6, δ ppm), 5.70 (s,1H) of lactam, 7.84-7.78 (t, 2H), 7.42-7.35 d,CH), 7.50-7.43 (t, 3H, CH of thiazole),6.86 (d, J=8.2 Hz,1H), 4.53 (s,1H, methylene), 2.81-2.77 (Q, J=3.9, 4.2, 3.3, 2H piperazine), 2.63-2.60 (P, J=3.228, NH piperazine) ^{13}C-NMR (126 MHz, DMSO-$d_{6,;.}$ δ ppm), 170.13, 163.45, 154.00, 151.30, 141.05, 134.68, 133.17, 129.78, 128.11, 126.23, 125.98, 121.58, 112.80, 111.38, 72.55, 66.08, 58.30, 55.36, 45.69, 21.42. *m/z:* 494.01 $[M+H]^+$

2.1.2 3-chloro-5'-methyl-1'-((4-methylpiperazin-1-yl)methyl)-1-(4-phenylthiazol-2-yl) spiro[azetidine-2,3'-indoline]-2',4-dione (ICP-3B)

FT-IR (KBr cm^{-1}), 2935 (C-H, alkyl), 1758(C = O), 1629(C=N), 1318 (C-C), 1013(C-S-C), 1011 (C-N),782 (CH=CH, aromatic), 632 (C-Cl). ^{1}H-NMR (500MHz, DMSO-d_6, δ ppm), 7.84-7.78 (d, 1H, thiazole,) 7.50-7.43 (m, 1H), 7.42-7.35 (m,2H), 5.70 (s,1H, lactam), 4.52 (s,1H), 2.61-2.55 (m,4H), 2.34 (s, 3H methyl), ^{13}C-NMR (126 MHz, DMSO-$d_{6,;.}$ δ ppm), 170.12, 163.45, 154.12, 151.00, 141.03, 134.68, 129.78,129.82, 128.11, 126.23, 125.98, 121.58, 112.60, 111.38,72.55, 66.10, 58.20, 55.08, 51.83, 45.48, 21.43. *m/z:* 508.04 $[M+H]^+$

1.1.3. 3-chloro-5'-methyl-1-(4-methylthiazol-2-yl)-1'-(piperidin-1-ylmethyl) spiro [azetidine-2,3'-indoline]-2',4-dione (ICP-3C)

FT-IR (KBr cm^{-1}), 2875(C-H, alkyl), 1752(C = O), 1639(C=N), 1197(C-S-C), 1122 (C-N), 833 (CH=CH, aromatic), 612 (C-Cl). ^{1}H-NMR (500MHz, DMSO-d_6, δ ppm), 7.81-7.78 (m,1H), 7.46-7.43(m,1H), 7.42-7.35 (t, J=7.46Hz 1H), 7.15-7.06(t, J=10.46, 1H), 6.86 (d, J=8.2, CH), 5.70 (s, 1H), 4.54 (s,2H, methylene), 2.57(t, 2H, J=5.27, piperidine), 2.42-2.21 (m, 3H, methyl), 1.60(p, J=5.523, 2H piperidine). ^{13}C-NMR (126 MHz, DMSO-$d_{6,;.}$ δ ppm), 170.02, 163.45, 154.00, 151.30, 141.04, 134.66, 133.17, 129.77, 128.11, 126.38, 111.40, 72.53, 66.28, 58.32, 25.97, 24.87, 21.42. *m/z:* 493.02 $[M+H]^+$

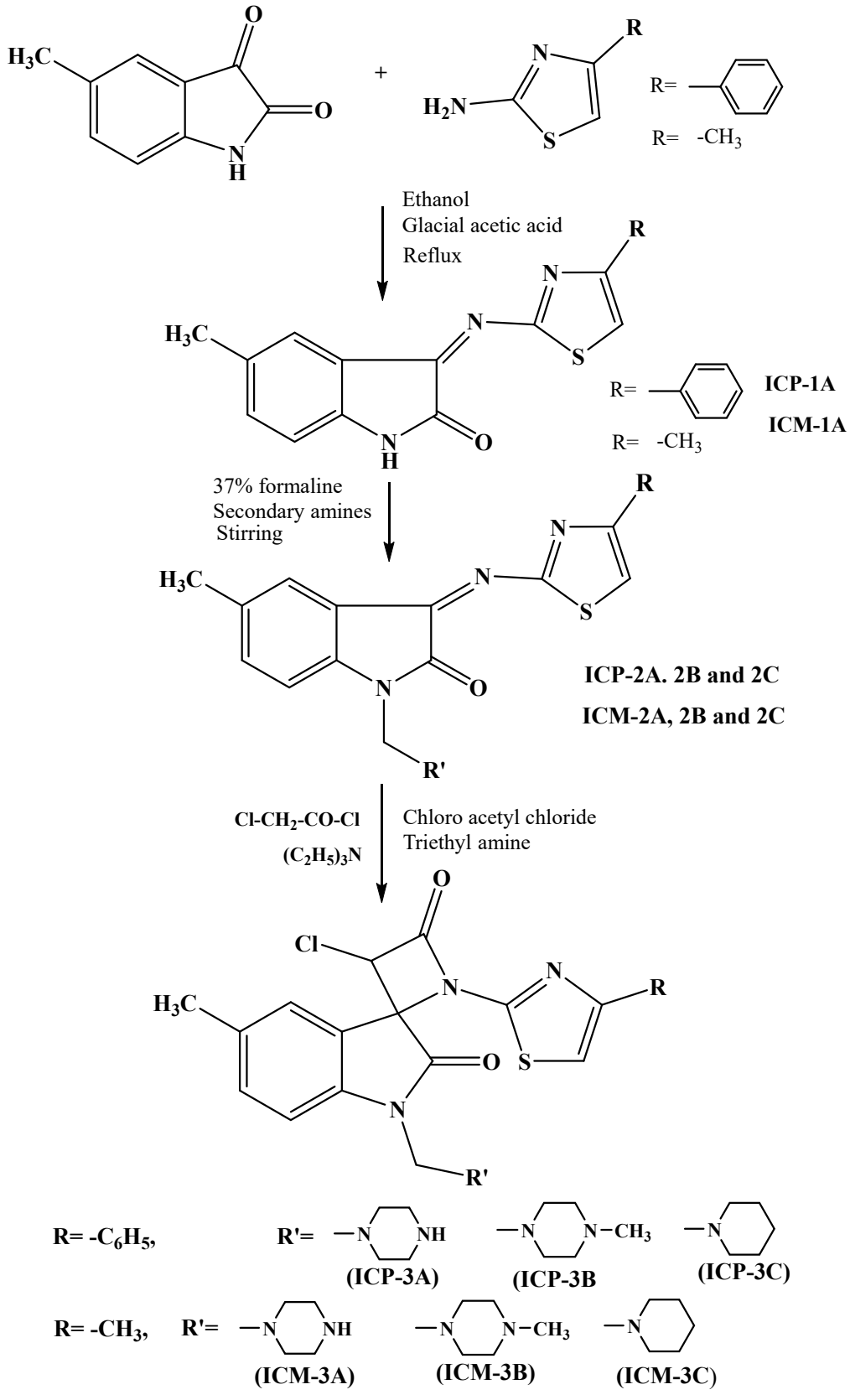


Fig. 1. Reaction scheme of the newly synthesized compounds

2.1.4. 3-chloro-5'-methyl-1-(4-methylthiazol-2-yl)-1'-(piperazin-1yl methyl) spiro [azetidine-2,3'-indoline] -2',4-dione (ICM-3A)

FT-IR (KBr cm^{-1}), 3661(NH), 2817(C-H, alkyl), 1729(C = O), 1646(C=N), 1512 (C-C), 1329(CH_2), 1191(C-S-C), 1019 (C-N), 865 (CH=CH, aromatic), 625 (C-Cl). ^{1}H-NMR (500MHz, DMSO-d_6, δ ppm), 6.86 (d, *J*=8.2, 1H Benzene), 6.66 (s, 1H thiazole), 7.09 (d, *J*=8.08 Hz, 1H Benzene), 7.1 (d, *J*=2.2Hz, 1H Benzene), 5.70 (s, 1H lactam ring), 4.53 (s,2H, methine), 2.61 (P, *J*=3.3 Hz, 1H, NH piperazine), 2.81-2.78, (Q, *J*=3.8 Hz, CH_2 of piperazine), 2.76 (t, *J*=1.2Hz, CH_2 of piperazine), 2.30 (s, 3H, methyl in thiazole), 2.27 (s, 3H, methyl in phenyl). ^{13}C-NMR (126 MHz, DMSO-$d_{6,}$ δ ppm), 170.13, 163.52, 152.92, 1455.96, 141.05, 134.68, 129.78, 125.98, 121.60, 111.38, 110.07, 72.54, 66.08, 59.39, 53.36, 45.59, 21.42, 16.42 *m/z:* 431.94 $[M+H]^+$

2.1.5. 3-chloro-5'-methyl-1'-((4-methylpiperazin-1-yl)methyl)-1-(4-methylthiazol-2-yl)spiro [azetidine-2,3'-indoline]-2',4-dione (ICM-3B)

FT-IR (KBr cm^{-1}), 2965C-H, alkyl), 1781(C = O), 1692(C=N), 1004 (C-C), 1391 (CH_2), 1149(C-S-C), 1095 (C-N), 892 (CH=CH, aromatic), 696 (C-Cl). ^{1}H-NMR (500MHz, DMSO-d_6, δ ppm), 7.12 (d, J=2.2Hz, 1H benzene), 7.09 (d, J=8.31 Hz, 1H benzene), 6.86 (dd, 8.2, 1H benzene)\, 6.66(s, 1H thiazole), 5.70 (s, 1H lactam), 4.52 (s, 2H, methine). 2.58 (m, 4H), 2.48 (m,4H). ^{13}C-NMR (126 MHz, DMSO-$d_{6,}$ δ ppm), 170.13, 163,12, 152.32, 145.96, 141.05, 134.68, 129.75, 125.99, 121.60, 111.38, 110.07, 72.53, 66.10, 59.39, 55.08, 51.83, 45.48, 21.40, 16.42. *m/z:* 445.13 $[M+H]^+$

2.1.6. 3-chloro-5'-methyl-1-(4-methylthiazol-2-yl)-1'-(piperidin-1-ylmethyl) spiro [azetidine-2,3'-indoline]-2',4-dione (ICM-3C)

FT-IR (KBr cm^{-1}), 2921(C-H, alkyl), 1791(C = O), 1698(C=N), 1348 (CH_2), 1181(C-S- C), 1052 (C-N), 892 (CH=CH, aromatic), 626 (C-Cl) . ^{1}H-NMR (500MHz, DMSO-d_6, δ ppm), 7.12 (dd, J=2.2, 0.6 Hz, 1H benzene), 7.09 (d,6.2 Hz 1H benzene), 6.84 (d, J=8.2 Hz, 1H, benzene),6.65 (d, J=0.92Hz,1H of thiazole), 5.70 (s 1H lactam), 4.54 (s,2H, methine), 2.68-2.65 (t, J=5.21Hz, 4H), 2.30 (s, 3H, methyl in thiazole), 2.27 (s. 3H methyl of phenyl),1.60 (p,J=6.4, 5.7, 3.8 Hz, 4H, piperidine), 1.37 (p, J=6.7, 4.9 Hz, 2H of piperidine). ^{13}C-NMR (126 MHz, DMSO-$d_{6,}$ δ ppm), 170.60, 163.52, 152.32, 145.96, 141.04, 234.68, 129.78, 125.98, 121.60, 111.40, 110.07, 72.54, 66.28, 59.39, 53.27, 25.97, 24.57, 21.42, 16.42. *m/z:*430.95 $[M+H]^+$

The chemical structures of the compounds as shown in the reaction scheme was satisfactorily confirmed based on the results of the spectrophotometric data for structural elucidation of the generated compounds. Table 1 contains the data of the elemental analysis as well as the yield percentage, were found satisfactory for all the synthesized compounds. The anthelmintic activity of the synthesised compounds demonstrates that all the compounds under study have shown good anthelmintic effect against Pheretima posthuma. When compared to conventional albendazole (PT: 1.324±0.12, DT: 1.421±0.21), synthesized compounds ICP-3B (PT: 1.883±0.24, DT: 1.943±0.02) and ICM-3B (PT: 1.758±0.27, DT: 1.675±0.32) were shown significant activity in terms of paralysis and death time. Table 2 which contains the details of the results also stated that compounds, ICM-3A and ICM-3C shows moderate anthelmintics potential. The synthesised spiro phenyl substituted β-Lactam compound with N-methyl piperazinyl (ICP-3B), and spiro methyl substituted β-Lactam compound with N-methyl piperazinyl ICM-3B) showed highly significant anthelmintic potential.

2.2. Structure activity relationship (SAR) for anthelmintic activity

Based on the above experimental observation and possible structure activity relation study, it can be remark that chemicals containing piperazinyl and methyl piperazinyl, binds directly and selectively to muscle membrane GABA receptors, presumably causing hyperpolarization of nerve endings, resulting in flaccid paralysis of the worm. While the worm is paralyzed, it is dislodged from the intestinal lumen and expelled live from the body by normal intestinal peristalsis [27]. The presence of an electron-donating methyl (-CH_3) group in the piperazinyl ring of the compounds ICP-3B and ICM-3B can be thought to make them more influential anthelmintic. **Fig. 2** and **Fig. 3** illustrate the specifics of the paralysis and death time depending on the examined concentrations of the synthesised compounds. The results of antibacterial activity by measuring the zone of inhibition of every synthesized compounds against the various gram positive and gram negative bacteria shows the potentially of all the compounds at three different concentration levels. Within these six compounds, the compound ICP-3B, ICM-3A and ICM-3B has shown significant antibacterial potential. The compound, ICP-3B has shown 20.53mm clear zone of inhibition at 100 µg/mL against the bacteria *Bacillus subtilis* in comparison to the standard ampicillin (23.04 mm zone) at the same level. This compound also shown the zone of 24.93 mm in comparison to ampicillin (26.20 mm) against *pseudomonas aeruginosa* and 19.83 mm zone of inhibition against the gram negative *E.coli*. In the same way the compound ICM-3A has shown 19.37 mm and 25.04 mm clear zones of inhibition at 100 µg/mL against the bacteria *Bacillus subtilis* and pseudomonas aeruginosa respectively. And for *staphylococcus aureus* it was 23.65 mm in comparison to ampicillin (27.88 mm). The compound ICM-3B, clearly shows the inhibition zone of 21.27 mm against *Bacillus subtilis,* 24.64 mm against *pseudomonas aeruginosa,* 20.62mm against *E. coli,* 20.41 mm against *Proteus mirabilis* and 23.65 mm against the bacteria *staphylococcus aureus* at the level of 100µg/mL. In the above all cases the obtained zones of inhibitions of the compounds (ICP-3B, ICM-3A and ICM-3B) were very close to the standard ampicillin and exhibits the dose dependent significant antibacterial potential. The remaining three compounds (ICP-3A, ICP-3C and ICM-3C) also found

active against the all studied bacteria when compared the standard ampicillin. The details of the results have been listed din the Table 3 of antibacterial activity.

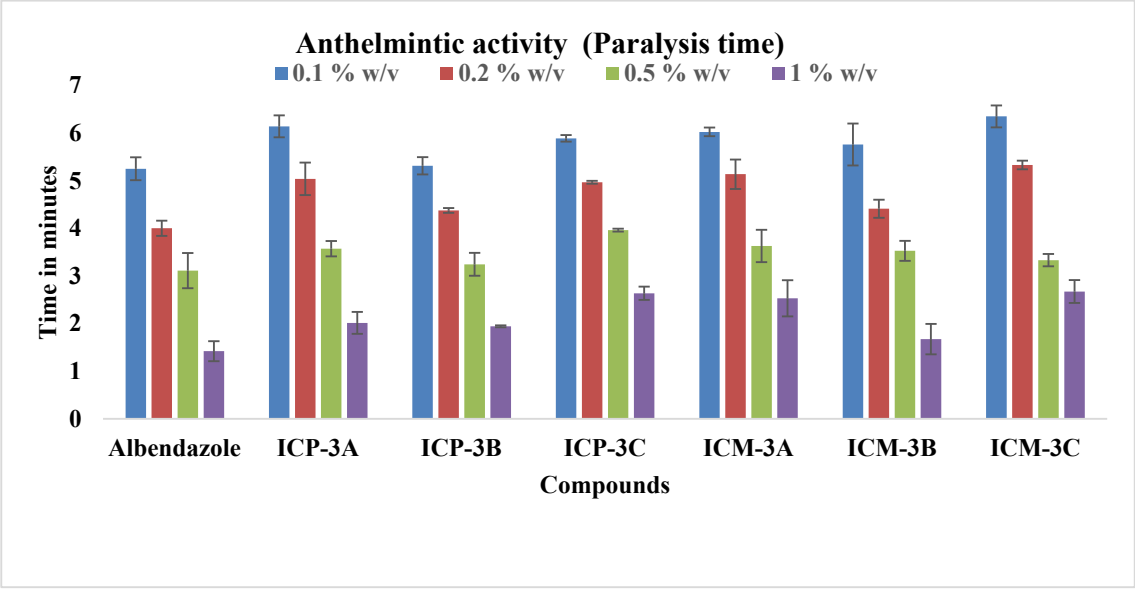


Fig. 2. Anthelmintic activity (Paralysis time) of the synthesized compounds

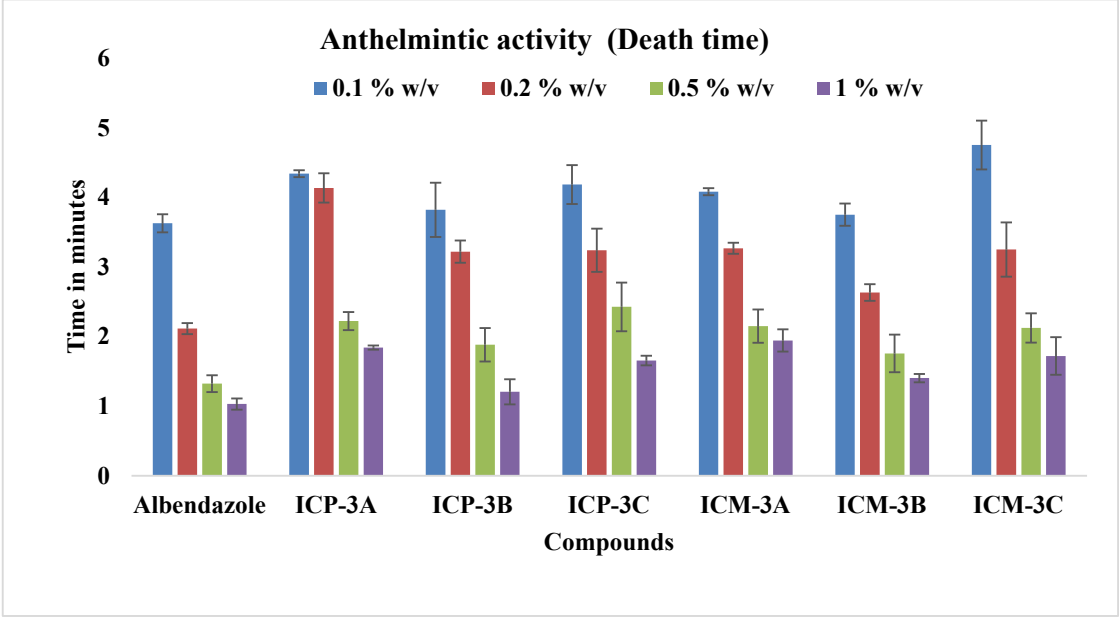
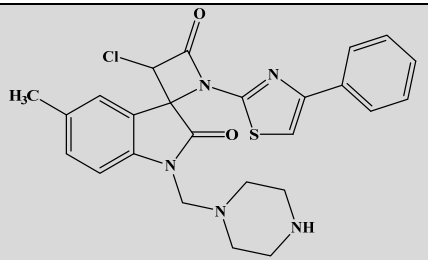
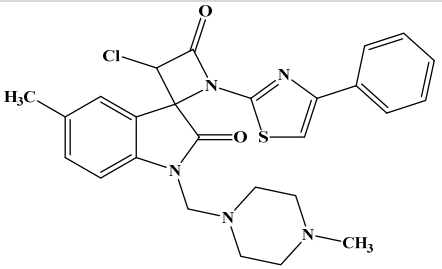
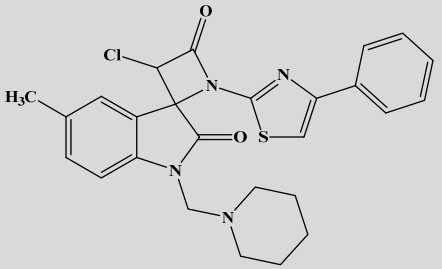


Fig. 3. Anthelmintic activity (Death time) of the synthesized compounds

2.3. Structure activity relationship (SAR) study for antibacterial activity

The current research revealed that most of the synthesized compounds exhibited significant anti-bacterial activities. The most potent antibacterial activity exhibited by the compounds ICP-3B, ICM-3A and ICM-3B. In the compound ICP-3B, it might be due to the presence of an electron donating methyl group in the piperazine ring, with the positive inductive effect of the methyl group making the compound lipophilic and more potent. In the compound ICM-3A, where the presence of methyl group in the thiazole erring and the compound, ICM-3B, where the presence of N-methyl group in the piperazine ring and C-methyl group in the thiazole ring, both in the same compound, might be make it a highly lipophilic, electron donating and more potent due to above said reasons. In the above said compounds due to the presence of electron donating lipophilic groups in the chemical structures helps the synthesized compounds to penetrate the bacterial cell wall and possibly to bind with the penicillin binding proteins[28]located inside the bacterial cell wall and inhibit the cell wall synthesis, and hence inhibit their growth.

Table 1. Characterization data of the synthesized compounds

Compounds	Formula	Molecular weight	Found (calculated)			IUPAC name	% Yield
 **ICP-3A**	$C_{25}H_{24}ClN_5O_2S$	494.01	**C** 60.78 (58.65)	**H** 4.90 (4.12)	**N** 14.18 (13.86)	3-chloro-5'-methyl-1-(4-phenylthiazol-2-yl)-1'-(piperazin-1-ylmethyl)spiro[azetidine-2,3'-indoline]-2',4-dione	68.55
 **ICP-3B**	$C_{26}H_{26}ClN_5O_2S$	508.04	60.31 (61.47)	5.01 (5.16)	13.56 (13.97)	3-chloro-5'-methyl-1'-((4-methylpiperazin-1-yl)methyl)-1-(4-phenylthiazol-2-yl) spiro[azetidine-2,3'-indoline]-2',4-dione	72.31
 **ICP-3C**	$C_{26}H_{25}ClN_4O_2S$	493.02	62.46 (63.34);	5.01 (5.11)	10.23 (11.36)	3-chloro-5'-methyl-1-(4-phenylthiazol-2-yl)-1'-(piperidin-1-ylmethyl)spiro[azetidine-2,3'-indoline]-2',4-dione	70.19

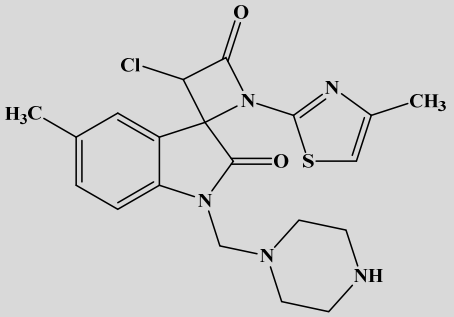
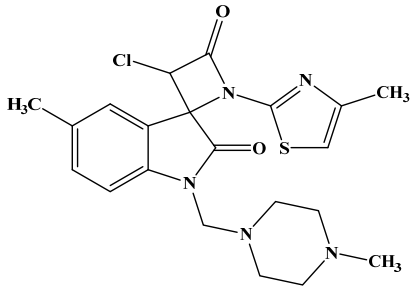
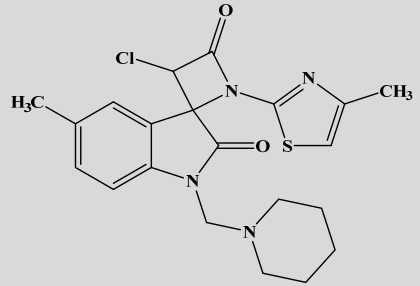
 **ICM-3A**	$C_{20}H_{22}ClN_5O_2S$	431.94	54.67 (55.61)	4.76 (5.13)	16.17 (16.21)	3-chloro-5'-methyl-1-(4-methylthiazol-2-yl)-1'-(piperazin-1 ylmethyl) spiro [azetidine-2,3'-indoline] -2',4-dione	68.67
 **ICM-3B**	$C_{21}H_{24}ClN_5O_2S$	445.13	55.37 (56.56)	5.07 (5.42)	14.23 (15.70)	3-chloro-5'-methyl-1'-((4-methylpiperazin-1-yl)methyl)-1-(4-methylthiazol-2-yl)spiro [azetidine-2,3'-indoline]-2',4-dione	74.49
 **ICM-3C**	$C_{21}H_{23}ClN_4O_2S$	430.95	57.23 (58.53)	5.01 (5.38)	12.34 (11.38)	3-chloro-5'-methyl-1-(4-methylthiazol-2-yl)-1'-(piperidin-1-ylmethyl) spiro [azetidine-2,3'-indoline]-2',4-dione	65.46

Table 2. Anthelmintic activity of the synthesized compounds

Compounds	(PT) Paralysis time# (Mean ± SEM) in minutes				(DT) Death time# (Mean ± SEM) in minutes			
	0.1% w/v	**0.2 % w/v**	**0.5 % w/v**	**1 % w/v**	**0.1 % w/v**	**0.2 % w/v**	**0.5 % w/v**	**1 % w/v**
Control (saline solution)	No paralysis observed				No death observed			
Albendazole	3.628±0.13	2.115±0.08	1.324±0.12	1.031 ±0.08	5.253±0.24	4.003±0.16	3.114±0.37	1.421±0.21
ICP-3A	4.342± 0.05*	4.137± 0.21^{ns}	2.224±0.13*	1.843±0.03*	6.143±0.23*	5.041±0.34*	3.574±0.16*	2.015±0.23**
ICP-3B	3.822±0.39***	3.222± 0.16	1.883±0.24***	1.207±0.18***	5.315±0.18***	4.378±0.05***	3.245±0.24***	1.943±0.02***
ICP-3C	4.185±0.28*	3.241± 0.31*	2.427±0.35*	1.656±0.07**	5.891±0.07**	4.968±0.03**	3.964±0.03*	2.637±0.14*
ICM-3A	4.083±0.05**	3.270±0.08**	2.151±0.24*	1.944±0.16*	6.027±0.09*	5.137±0.31*	3.631±0.34**	2.532±0.38*
ICM-3B	3.753±0.16***	2.634±0.12***	1.758±0.27***	1.404±0.06***	5.763±0.44***	4.414±0.19***	3.529±0.21*	1.675±0.32***
ICM-3C	4.753±0.35*	3.251±0.39**	2.124±0.21**	1.721±0.27*	6.351±0.23^{ns}	5.331±0.09*	3.334±0.13***	2.675±0.24*

#average of 3 replicates, SEM: Standard error mean. n = 3, Significant at P < 0.05*, 0.01** and 0.001***, ns = not significant

Table 3. Antibacterial activity of the synthesized compounds

Compounds	Zone of Inhibition* (mm) of the synthesized compounds														
	Bacillus subtilis (ATCC-1086)			*Pseudomonas aeruginosa* (ATCC-1232)			*Escherichia coli* (ATCC-3273)			*Proteus mirabilis* (ATCC-224)			Staphylococcus aureus (ATCC-449)		
	25 (µg/ml)	50 (µg/ml)	100 (µg/ml)	25 (µg/ml)	50 (µg/ml)	100 (µg/ml)	25 (µg/ml)	50 (µg/ml)	100 (µg/ml)	25 (µg/ml)	50 (µg/ml)	100 (µg/ml)	25 (µg/ml)	50 (µg/ml)	100 (µg/ml)
DMF	00	00	00	00	00	00	00	00	00	00	00	00	00	00	00
Ampicillin	12.12 ± 2.32	19.32 ±3.21	23.04 ±2.30	17.64 ±0.94	21.32 ± 1.32	26.20 ±0.92	13.64 ± 2.31	18.91 ± 2.42	21.07 ±1.43	15.13 ± 0.95	20.86 ± 1.43	24.91 ±0.87	16.21 ±0.54	21.32 ±2.38	27.88 ±2.31
ICP-3A	08.21 ±4.82	10.32 ±4.02	14.54 ± 3.29	09.44 ±3.12	13.23± 2.10	16.76 ±1.32	08.32 ±1.28	11.10 ±1.29	15.43 ± 0.89	06.88 ± 1.32	12.51 ±1.27	17.64 ±1.32	07.34 ±1.53	12.21 ±2.03	15.52 ±0.61
ICP-3B	09.32 ±2.72	13.21 ±2.93	20.53 ±3.93	13.85 ±1.98	17.21 ±1.18	24.93 ±1.16	09.53± 0.77	16.83 ± 0.82	19.83 ± 1.32	11.80 ±0.86	16.21 ±2.75	20.17 ±1.43	13.53 ±0.54	18.41 ±1.83	24.92 ±0.27
ICP-3C	10.54 ± 1.84	13.36 ± 3.08	16.37 ±3.92	11.95 ±1.86	14.04 ±1.67	19.44 ± 1.31	10.27 ± 2.81	14.12 ±1.82	17.28 ±2.36	09.73 ±1.54	13.43 ± 3.54	17.54 ±2.35	11.93 ±1.11	14.64 ±0.85	18.33 ±1.32
ICM-3A	09.76± 1.08	14.65± 4.55	19.37 ±2.43	14.21 ±1.54	18.90 ±0.87	25.04 ±1.03	11.19 ±1.73	15.83 ±2.31	19.37 ±2.21	09.22 ± 0.53	15.45± 1.82	20.41 ±0.73	12.69 ± 2.02	19.72 ±0.86	23.65 ±0.83
ICM-3B	10.40± 2.88	16.35 ± 2.43	21.27 ±1.32	15.53 ±2.63	19.38 ±1.32	24.67 ±0.87	12.52 ±0.32	16.90 ±1.43	20.62 ± 1.27	12.32 ±0.43	17.35 ±0.43	21.74 ±0.63	14.28 ±2.88	18.28 ±0.89	25.32 ±1.63
ICM-3C	09.47 ± 1.22	13.21 ±3.25	17.11 ±3.82	12.97 ±3.21	15.72 ± 1.43	18.43± 0.87	10.43 ±1.73	13.63 ±0.89	16.83 ± 0.78	10.64± 2.26	13.64 ±0.41	16.38± 1.87	10.10 ±2.19	14.10 ±1.43	19.10 ±0.63

*Average of three readings.

3. Conclusion

A novel series of spiro cyclo-indolyl β-lactam compounds of the 5-methyl indole 2,3 dione derivatives has been synthesized using the Staudinger synthesis with a satisfactory percentage of yield. The prepared spiro cyclo-indolyl β-lactam compounds derivatives have been tested for its anthelmintic potential using Prathima posthuma and antibacterial activity and found the potency. Almost all the compounds exhibit its anthelmintic potential, but compounds ICP-3B and ICM-3B show significant anthelmintic potential. The presence of N-methyl piperazine rings similar to the anthelmintic drug piperazine citrate make these compounds more potent. In case of antibacterial potency, the compounds ICP-3B, ICM-3A and ICM-3B were found significant. Therefore, it is envisaged to study the other biological activities using molecular modelling of these newly synthesized compounds.

4. Experimental

4.1. Chemicals and instruments

The required chemicals of synthetic grade for this work (5 methyl isatin, 4 phenyl aminothiazole, chloroacetyl chloride, triethylamine, ethanol, glacial acetic acid, and all secondary amine) were obtained from Merck in Mumbai, India. The uncorrected melting points of the synthesized compounds were recorded using a Kofelar melting point device. A SHIMADZU FTIR-8300 spectrophotometer (Japan) was used to record the IR spectra (KBr). A Bruker Avance-500 MHz spectrometer was used to record 1H-NMR spectra, while a 124MHz instrument was used to record ^{13}C NMR spectra. A Perkin-Elmer 240°C Micro analyzer was used to do the elemental analysis. The mass spectra were obtained from Shimadzu Qp-2010, Micro mass 7070E spectrometer. Thin layer chromatographic monitoring was used to ensure that the compounds were pure. Laminar air flow (Klenzaid and Biochem, Device (p) Ltd., Valsad, India. Autoclave from Unilab, Mumbai, India), Incubator from (Hicon Private Ltd., Delhi India).

4.2. Preparation of compounds

The compounds *5-methyl-3-((4-phenylthiazol-2-yl)imino)indolin-2-one (ICP-1A) and 5-methyl-3-((4-methylthiazol-2-yl) imino) indolin-2-one (ICM-1A)* has been synthesised as per the standard Schiff base preparation method described in our previous part of the work. In the same way the compounds*, 5-methyl-3-((4-phenylthiazol-2-yl)imino)-1-(piperazin-1-ylmethyl)indolin-2-one (ICP-2A),* the compound, 5-methyl-1-((4-methylpiperazin-1-yl)methyl)-3-((4-methylthiazol-2-yl)imino)indolin-2-one **(***ICP-2B***)** and the compound, 5-methyl-3-((4-phenylthiazol-2-yl)imino)-1-(piperidin-4-ylmethyl)indolin-2-one *(ICP-2C), has been prepared from the compound, ICP-1A.*

In the similar manner and the compound, *5-methyl-3-((4-methylthiazol-2-yl)imino)-1-(piperazin-1-ylmethyl) indolin-2-one (ICM-2A*), the compound, *5-methyl-1-((4-methylpiperazin-1-yl) methyl)-3-((4-methylthiazol-2-yl)imino)indolin-2-one (ICM-2B)* and the compound *5-methyl-3-((4-methylthiazol-2-yl)imino)-1-(piperidin-4-ylmethyl)indolin-2-one (ICM-2C)* has also been prepared from *ICM-1A*, described in our previous work, as shown in the present the reaction scheme to understand the sequence.

4.3. Preparation of 3-chloro-5'-methyl-1-(4-phenylthiazol-2-yl)-1'-(piperazin-1-ylmethyl)spiro[azetidine-2,3'-indoline]-2',4-dione (ICP-3A)

Previously synthesized compound, *5-methyl-3-((4-phenylthiazol-2-yl)imino)-1-(piperazin-1-ylmethyl)indolin-2-one (ICP-2A)* (0.1mol) was dissolved in 20 mL of 1-4 dioxan in a 100 ml beaker and trimethyl amine (0.01 mol) was added to it. The contents of the beaker were then stirred using a magnetic stirrer and chloroacetyl chloride (0.01 mol) was added slowly and continued stirring of the entire content. After the addition the mixture was refluxed for 9 hours. The resulting solution was poured into ice cold water, filtered off, dried and recrystallized from methanol and chloroform (1:1).

4.4. Preparation of 3-chloro-5'-methyl-1'-((4-methylpiperazin-1-yl)methyl)-1-(4-phenylthiazol-2-yl) spiro[azetidine-2,3'-indoline]-2',4-dione (ICP-3B)

For the preparation of **ICP 3B**, previously synthesized compound *5-methyl-1-((4-methylpiperazin-1-yl)methyl)-3-((4-phenylthiazol-2-yl)imino)indolin-2-one(ICP-2B)* (0.1 mol) was taken as a starting material and followed the similar procedure as described for the preparation of **ICP3A.** The prepared **ICP 3B** was recrystallised using diethyl ether. The percentage yield was calculated.

4.5. Preparation of 3-chloro-5'-methyl-1-(4-phenylthiazol-2-yl)-1'-(piperidin-1-ylmethyl) spiro [azetidine-2,3'-indoline]-2',4-dione (ICP-3C)

For the preparation of **ICP 3C**, previously synthesized compound*, 5-methyl-3-((4-phenylthiazol-2-yl)imino)-1-(piperidin-1-ylmethyl)indolin-2-one (ICP-2C)* (0.1 mol) was taken as a starting material and followed the similar procedure as described for the preparation of **ICP3A.** The prepared **ICP 3C** was recrystallised using diethyl ether. The percentage yield was calculated.

4.6. Preparation of 3-chloro-5'-methyl-1-(4-methylthiazol-2-yl)-1'-(piperazin-1yl methyl) spiro [azetidine-2,3'-indoline] -2',4-dione (ICM-3A)

For the preparation of **ICM-3A**, previously synthesized compound, 5-methyl-3-((4-methylthiazol-2-yl)imino)-1-(piperazin-1-ylmethyl)indolin-2-one *(ICM-2A)* (0.1 mol) was taken as a starting material and followed the similar procedure as described earlier**.** The prepared **ICM-3A** was recrystallised using diethyl ether. The percentage yield was calculated.

4.7. Preparation of 3-chloro-5'-methyl-1'-((4-methylpiperazin-1-yl)methyl)-1-(4-methylthiazol-2-yl)spiro [azetidine-2,3'-indoline]-2',4-dione (ICM-3B) or the preparation of **ICM-3B**, previously synthesized compound, 5-methyl-1-((4-methylpiperazin-1-yl)methyl)-3-((4-methylthiazol-2-yl)imino)indolin-2-one *(ICM-2B)* (0.1 mol) was taken as a starting material and followed the similar procedure as described earlier**.** The prepared **ICM-3B** was recrystallised using chloroform: methanol. The percentage yield was calculated.

4.8. Preparation of 3-chloro-5'-methyl-1-(4-methylthiazol-2-yl)-1'-(piperidin-1-ylmethyl) spiro [azetidine-2,3'-indoline]-2',4-dione (ICM-3C)

For the preparation of **ICM-3C**, previously synthesized compound, 5-methyl-3-((4-methylthiazol-2-yl)imino)-1-(piperidin-1-ylmethyl)indolin-2-one *(ICM-2C)* (0.1 mol) was taken as a starting material and followed the similar procedure as described earlier**.** The prepared **ICM-3C** was recrystallised using chloroform: methanol. The percentage yield was calculated.

4.9. Anthelmintic activity

Because of its morphological and physiological similarities to the intestinal earthworm parasite of humans, the anthelmintic activity[18] of the synthesized spiro cyclo-indolyl β-lactam compounds was tested on adult Indian earthworm Pheretima posthuma. The earthworms were dug up from damp soil and cleansed to remove any faeces. For all experimental protocols, earthworms measuring 3–5 cm in length and 0.1 to 0.1–2 cm in width were utilised. For this investigation, Pheretima posthuma of almost comparable size (6 cm 1) were chosen at random. Before the experiment, the worms were acclimatised to the laboratory environment. The earthworms were categorized into four groups, each with six earthworms. Albendazole was diluted with normal saline solution to obtain concentrations of 0.1 percent w/v, 0.2 percent w/v, 0.5 percent w/v, and 1 percent w/v, which were placed into Petri dishes as standards. The synthesised compounds (ICP-1A, 2A, 2B, 2C and ICM-1A, 2A, 2B, 2C) were produced in a small amount of DMSO and diluted to four different concentrations, 0.1 percent w/v, 0.2 percent w/v, 0.5 percent w/v, and 1 percent w/v for each molecule. Normal saline is used as a control. Nearly identical six earthworm sizes (6 cm ±1) were taken for every concentration and located in the Petri dishes and maintained room temperature. It was timed how long it took for total paralysis and death to occur. The paralysis time (mean) and fatal time (mean) for each sample were observed (triplicate readings were taken). To determine death, the time it took the worms to become immobile was recorded, and each worm was exposed to external stimuli that stimulated and caused movement in the earthworms, if they were still alive. The findings were compiled into a table and expressed as the mean SEM of six worms from each group.

4.10.Anti-bacterial activity

In vitro antibacterial activity[19] was carried out against 24-h old cultures of four bacteria by the cup plate method. The compounds (ICP-3A to ICP-3C) and (ICM-3A to ICM-3C) were tested against *Bacillus subtilis* (ATCC-1086), *Pseudomonas auroginosa* (ATCC-1232), Escherichia coli, (ATCC-3273), *Proteus mirabilis* (ATCC-224), and *Staphylococcus aureus* (ATCC-449). For antibacterial studies. The Petri dishes were thoroughly washed and sterilized in a hot air oven at 160^0C temperature for 1 hour. The medium was prepared as per the instructions of the manufacturer of dry Muller Hinton agar media (Hi-media). The test solutions of the compounds and the standard drug (Ampicillin trihydrate) was prepared in sterile dimethyl formamide (DMF) at the concentration of 25, 50 and 100 µg/ml. The sterile agar plates were inoculated within 24hours with the broth culture of the test organism. The uniform holes(6mm) were made in the agar plate by the help of a sterile borer and each bore was filled with tests and standard drug of 0.2ml and the blank DMF, separately under aseptic conditions i.e. inside the laminar air flow. Then the plates were kept in a room temperature for 2hr for the diffusion of the solutions in to the agar medium. Incubation was carried out at 37°C for 48 h (Hicon Private Ltd., Delhi India). The zone of inhibition was calculated in millimetres and compared with the standard. The results taken in triplicate.

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