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Synthesis, Molecular Docking and Pharmacological Evaluation of Some New Schiff and Mannich Bases of 5-Methylisatin Derivatives

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Two novel series of Schiff and Mannich based 5-methylisatin were synthesized by Mannich reactions and evaluated for anthelmintic and antibacterial activities. The Mannich bases were synthesized from the Schiff base using various secondary amines and formaldehyde along with the derivatives of piperazinyl groups. N-Methyl piperazinyl (**ICP-2B** and **ICM-2B**) and piperidinyl derivatives (**ICP-2C** and **ICM-2C**) were found to have a highly significant anthelmintic potential. For compound **ICP-2B**, the paralysis time was observed at 4.936 ± 0.12 min at low concentration (0.1 % w/v) and 1.95 ± 0.10 min at high concentration (1% w/v). At low concentration (0.1%), compound **ICM-2B**, the paralysis time is 4.43 ± 0.17 min; at high concentration (1% w/v), it is 1.675 ± 0.08 min. These data indicated that compounds **ICP-2B** and **ICM-2B** has immense potential as anthelminthic and antimicrobial drug in future. Moreover, the *in vitro* study was confirmed by molecular docking studies, indicating both compounds **ICP-2B** and **ICM-2B** can find its use as novel drug against various pathogenic helminthes and bacteria.

Keywords: 5-Methylisatin, Schiff base, Mannich base, Anthelmintic activity, Antibacterial activity, Molecular docking.

INTRODUCTION

Schiff bases has gained importance in the medical and pharmaceutical industries due to its vast spectrum of biological functions [1-4]. A Schiff base like isatin, also known as 1*H*-indole-2,3-dione, has become a high priority due to its multitude of applications in the field of chemical and pharmaceutical sciences. According to a thorough literature review [5], isatin and substituted isatins, were found to have major relevance due to their multipurpose pharmacological properties including antifungal [6], antibacterial [7], anticonvulsant [8], antidepressant [9], anti-inflammatory [10], anti-HIV [11] activities. Antibacterial, anti-inflammatory and analgesic properties have also been documented for thiazole and its derivatives [12]. Anthelmintic action of piperazinyl and replacement piperazinyl compounds are well known [13,14].

Apart from exploring untapped targets, a different approach involves combining many pharmacophores into one molecule. This suggests that those specific diseases may respond well to a single molecule that contains multiple pharmacophores, each of which has a unique mechanism of action. A review of the literature revealed that no such compounds have been synthesized using piperazinyl and substituted piperazinyl Mannich bases with 5-methyl isatin and investigated any pharmacological studies [15]. So, the present work was designed to synthesize some novel series of molecules of Schiff and Mannich bases to investigate the anthelmintic and antibacterial activity and with an enormous potential for exploration of anticancer, antiviral, anti-inflammatory activities, *etc*. using molecular modelling as well as *in vitro* studies. As a consequence of the above statements, in this present investigation, a novel series of Schiff and Mannich based on 5-methyl isatin was synthesized with substituted 2-amino thiazole and tested for anti-anthelmintic potential on *Pheretima posthuma* and antibacterial activity using the procedure previously described [16]. The molecular docking study further strengthens the effectiveness of the prepared compounds as antibacterial and anthelmintic activities [14].

EXPERIMENTAL

The required chemicals were obtained from Merck, India. 5-Methylisatin (99.56%, AR grade), 4-phenyl aminothiazole

(99.32%, AR grade), ethanol (99%, AR grade), glacial acetic acid (99.25%, AR grade) and all secondary amines (99.56%) AR grade were utilized. Using a Kofelar melting point instrument, the melting points of the synthesized compounds were recorded. To obtain IR spectra (KBr), a Shimadzu FTIR-8300 spectrophotometer (Japan) was employed. ^{1}H NMR spectra were obtained using a Bruker Avance-500 MHz spectrometer and ^{13}C NMR spectra were obtained from the same instruments at 124 MHz. Elements were analyzed using a Perkin-Elmer 240C Micro analyzer. The purity of ccompounds was checked using thin-layer chromatographic monitoring. The micro mass 7070E spectrometer Shimadzu Qp-2010 was used to obtain the mass spectra.

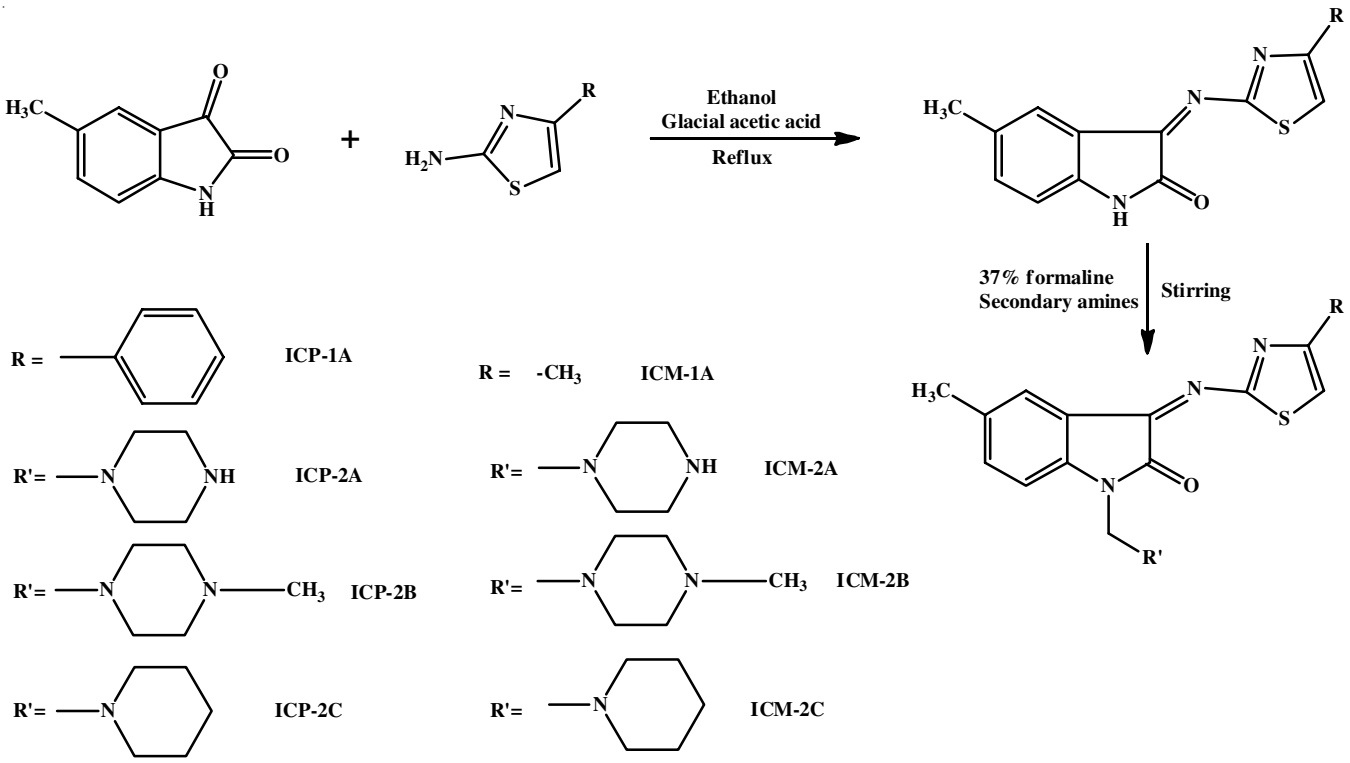
Synthesis of 5-methyl-3-((4-phenylthiazol-2-yl)imino)-indolin-2-one (ICP-1A): To a equimolar mixture of 5-methyl-isatin (0.5 mol) and 4-phenyl-thiazole-2-amine (0.5 mol) few drops of ethanol and 2-3 drops of glacial acetic acid were added to completely dissolve the mixture. The reaction mixture was refluxed at 90 ºC for 4.5 h and then poured in to 50 mL ice-cold water for 40 min. The solid compound (**ICP-1A**) was obtained by filtration was air-dried primarily overnight followed by heating for 6 h in hot air oven at 50 ºC. The compound was then recrystallized using ethanol and confirmed using TLC (acetone and toluene, a mobile phase in a 3:1 volume ratio).

Synthesis of 5-methyl-3-((4-methylthiazol-2-yl)imino)-indolin-2-one (ICM-1A): Compound 5-methyl-3-((4-methyl-thiazol-2-yl)imino)indolin-2-one (**ICM-1A**) was synthesized similarly by the reaction with equimolar quantity of 5-methyl isatin (0.5 mol) and 4-methyl thiazole-2- amine (0.5 mol), followed by the reflux for 4.5 h at 90 ºC.

Synthesis of 5-methyl-3-((4-phenylthiazol-2-yl)-imino)-1-(piperazin-1-ylmethyl)indolin-2-one (ICP-2A): The synthesized compound **ICP-1A** (0.005 mol) along with ethanol (5 mL) and 37 % w/v formalin were combined and concentrated to make a slurry (2 mL). Piperazine (0.005 mol) was gently added to this slurry mixture, stirring constantly with a magnetic stirrer. With intermittent shaking, the reaction mixture was cooled using an ice bath and allowed to stand at room temperature for 1 h. It was then maintained warm for 15 min in a steam bath at 70 ºC. After cooling at room temperature, the product was recovered and dried air primarily overnight followed by heating in hot air oven at 60 ºC. The recrystallization was conducted by dissolving the product in a 50:50 mixture of methanol and chloroform, utilizing a heated plate to improve solubility. The solution was filtered using a glass funnel and filtrate was kept in a China dish for 48 h. The grown crystals were collected still with some solvents present in the dish to avoid dissolved impurities. The obtained recrystallized product was stored in desiccator. The precoated TLC plates were utilized to monitor the reaction by sampling the reaction mixture using capillary tubes. Ethyl acetate:methanol:hexane = (3:1:1) used as a mobile phase, to examine the R_f value of reactants and the reaction mixture every 30 min in intervals to optimize the time and condition of reaction.

Similarly, compounds 5-methyl-1-((4-methylpiperazin-1-yl)methyl)-3-((4-methylthiazol-2-yl)imino)indolin-2-one (**ICP-2B**) and 5-methyl-3-((4-phenylthiazol-2-yl)imino)-1-(piperidin-4-ylmethyl)indolin-2-one (**ICP-2C**) were also synthesized by following the same procedure. The secondary amine N-methyl piperazine and piperidine were used for the synthesis of compounds **ICP-2B** and **ICP-2C**, respectively (**Scheme-I**).

Synthesis of 5-methyl-3-((4-methylthiazol-2-yl)imino)-1-(piperazin-1-ylmethyl)indolin-2-one (ICM-2A): Ethanol



Scheme-I: Reaction scheme of the isatin derivatized compound

(5 mL) and 37% formalin (2 mL) were combined along with 0.05 mol of synthesized **ICM-1A**, to form a slurry, in a clean, dry beaker. 0.005 mol of piperazine was gradually added while being stirred continuously using a magnetic stirrer. After cooling it to 4 ºC, the reaction mixture was occasionally shaken at room temperature for 1 h. It was then maintained warm for 15 min using a steam bath. After cooling, the product(**ICM-2A**) was filtered and recrystallized using absolute ethanol and water in the volume ratio of 75:25 v/v.

Compounds 5-methyl-1-((4-methylpiperazin-1-yl)-methyl)-3-((4-methylthiazol-2-yl)imino)indolin-2-one (**ICM-2B**) and 5-methyl-3-((4-methylthiazol-2-yl)imino)-1-(piperidin-4-yl-methyl)indolin-2-one (**ICM-2C**) were also synthesized using the similar procedure. In this case, secondary amine N-methyl piperazine and piperidine were used for the synthesis of compounds **ICM-2B** and **ICM-2C**, respectively (**Scheme-I**).

5-Methyl-3-((4-phenylthiazol-2-yl)imino)indolin-2-one (ICP-1A): Yield: 67.22%, m.p.: 136-138 ºC; m.w.: 319.38; Elemental anal. of $C_{18}H_{13}N_3OS$, calcd. (found) %: C, 67.24 (67.69); H, 4.08 (4.10); N, 13.27 (13.16). FTIR (KBr, ν_{max}, cm^{-1}): 3726 (-NH), 1722 (-C=O), 2911 (-C-H, alkyl), 1642 (-C=N), 1503 (CH=CH arom.), 1104 (-C-S-C), 1501 (-CH=CH arom.), 1023 (-C-N), 775-694 (-C=C, arom.); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 9.77 (s, 1H, NH), 7.83 (CH of thiazole) 7.29 (s, 1H), 7.19 (d, *J* = 7.6 Hz, 1H), 7.11-7.07 (m, ArH), 5.15 (s, 1H, methine), 2.33 (d, *J* = 0.7 Hz, 3H of methyl). ^{13}C NMR (126 MHz, DMSO-d_6, δ ppm): 175.77 (C of 2-thiazole), 167.75 (C of 2-thiazole), 153.72 (C of 1-amide), 142.55, 139.81 (C of 1-benzene), 137.92 (C of 1-benzene), 132.29 (C of 1-benzene), 129.44 (CH of 1-benzene), 127.94 (CH of 1-benzene), 126.16 (CH of 1-benzene), 120.75 (CH of 1-benzene), 119.90 (CH of 2-thiazole), 48.76, 20.48 (CH_3 of aliphatic). Mass *m/z*: 319.38 $[M+H]^+$.

5-Methyl-3-((4-phenylthiazol-2-yl)imino)-1-(piperazine-1-ylmethyl)indolin-2-one (ICP-2A): Yield: 69.74%, m.p.: 234-235 ºC; m.w.: 417.53; Elemental anal. of $C_{23}H_{23}N_5OS$, calcd. (found) %: C, 68.04 (68.29); H, 5.23 (5.55); N, 13.38 (13.85). FTIR (KBr, ν_{max}, cm^{-1}): 3725 (-NH pyrazine), 2956 (-C-H, alkyl), 1720 (-C=O), 1640 (-C=N), 1323 (-CH_2), 1150 (-C-S-C), 1020 (-C-N), 785-764 (-C=C, arom.); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 9.77 (s, 1H, NH), 7.82 (CH of thiazole) 7.29 (s, 1H), 7.50-7.43 (m, ArH), 4.47 (s, 1H, methine), 2.61 (d, *J* = 3.3 Hz, 3H of methyl). 2.33 (s, 1H), 1.09 (s, 1H of amine); ^{13}C NMR (126 MHz, DMSO-d_6, δ ppm): 172.67 (C of 2-thiazole), 168.65 (C of 1-amide), 163.21 (C of 1-imine)153.72 (C of 2-thiazole), 142.55 (C of 1-benzene), 139.12 (CH of 1-benzene), 135.35 (CH of 1-benzene), 134.82 (CH of 1 benzene), 132.29 (CH of 1-benzene), 128.92 (C of 1-benzene), 127.40 (CH of 1-benzene), 126.00 (CH of 1-benzene), 120.67 (CH of 2-thiazole), 68.91 (CH_2 of cyclohexane), 53.36 (CH_2 of cyclohexane), 45.69 (CH_2 of cyclohexane), 20.41 (CH_2 of cyclohexane). Mass *m/z*: 417.53 $[M+H]^+$.

5-Methyl-1-((4-methylpiperazin-1-yl)methyl)-3-((4-phenylthiazol-2-yl)imino)indolin-2-one (ICP-2B): Yield: 72.37%, m.p.: 242-244 ºC; m.w.: 431.56; Elemental anal. of $C_{23}H_{23}N_5OS$, calcd. (found) %: C, 66.23 (66.80); H, 5.27 (5.84); N, 16.01 (16.23). FTIR (KBr, ν_{max}, cm^{-1}): 2925 (-C-H, alkyl), 1732 (-C=O), 1639 (-C=N), 1506 (-C-C), 1035 (-C-S-C), 1011 (-C-N), 724, 665 (-C=C, arom.); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 7.63 (CH of thiazole) 7.56 (tt, *J* =1.9, 1.0 Hz 1H), 7.44 (t, *J* = 7.9 Hz, 1H) 5.19 (s, 1H, methine), 4.53 (s, 3H), 2.61 (d, *J* = 3.3 Hz, 3H of methyl), 2.34 (s, 1H, methylene); ^{13}C NMR (126 MHz, DMSO-d_6, δ ppm): 172.67 (C of 2-thiazole), 168.65 (C of 1-amide), 163.72 (C of 1-imine), 152 (C of 2-thiazole) 142.55 (CH of 1- benzene), 139.12 (CH of 1-benzene), 135.35 (CH of 1- benzene), 132.29 (CH of 1-benzene), 128.92 (CH of 1-benzene), 126.00 (CH of 1-benzene), 124.60 (CH of 1-benzene), 110.21 (CH of 2-thiazole), 68.92 (CH_2 of aliph.), 45.48 (CH_3), 20.41 (CH_3); Mass *m/z*: 431.56 $[M+H]^+$.

5-Methyl-3-((4-phenylthiazol-2-yl)imino)-1-(piperidine-4-ylmethyl)indolin-2-one (ICP-2C): Yield: 71.91%, m.p.: 196-198 ºC; m.w.: 416.54; Elemental anal. of $C_{24}H_{24}N_4OS$, calcd. (found) %: C, 69.11 (69.20); H, 5.34 (5.81); N, 13.23 (13.45). FTIR (KBr, ν_{max}, cm^{-1}): 2936 (-C-H, alkyl), 1735 (-C=O), 1648 (-C=N), 1521 (-C-C), 1102 (-C-S-C), 1083 (-C-N), 734.41 (-C=C, arom.). ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 7.83 (CH of thiazole), 7.38-7.29 (m 3H), 4.93, 4.87 (d, *J* = 0.7 Hz, 1H) (s, 1H, methine), 4.03 (d, *J* = 5.1Hz, 2H, methylene), 3.12 (ddd *J* = 2.7), 2.87-2.69 (m, 3H); ^{13}C NMR (126 MHz, DMSO-d_6, δ ppm): 176.66 (C of 2-thiazole), 168.45 (C of 1-amide), 153.72 (C of 2-thiazole), 142.55, 139.88 (CH of 1-benzene), 135.2 (C of 1-benzene), 134.82 (C of 1-benzene), 132.29 (CH of 1-benzene), 128.92 (CH of 1-benzene), 127.40 (CH of 1-benzene), 124.74 (CH of 1-benzene), 110.3 (CH of 2-thiazole), 75.19 (CH_2 aliphatic), 47.45, 34.36, 30.39, 20.41 (CH_2 piperidine). Mass *m/z*: 416.54 $[M+H]^+$.

5-Methyl-3-((4-methyl thiazole-2-yl)imino)indolin-2-one (ICM-1A): Yield: 65.88%, m.p.: 210-212 ºC; m.w.: 257.31; Elemental anal. of $C_{13}H_{11}N_3OS$, calcd. (found) %: C, 60.37 (60.68); H, 4.16 (4.31); N, 16.07 (16.33). FTIR (KBr, ν_{max}, cm^{-1}): 3759 (-NH), 2998 (-C-H, alkyl), 1758 (-C=O), 1626 (C=N), 1512 (-C-C), 1359 (-CH_2), 1146 (-C-S-C), 1083 (-C-N), 794, 786 (-C=C, arom.); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 9.31 (s, 1H, NH), 7.20 (d, *J* = 7.9 Hz, 1H), 7.12-7.06 (m, 1H), 5.15 (s, 1H, methine), 2.32-2.31 (m, 4H); ^{13}C NMR (126 MHz, DMSO-d_6, δ ppm): 176.10 (C of 2-thiazole), 143.84 (C of 2-thiazole), 150.33 (C of 1-amide), 141.74 (C of 2-thiazole), 139.81, 137.35 (C of 1-benzene), 129.93 (C of 1-benzene), 131.22 (CH of 1-benzene), 129.40 (C of 1-benzene), 125.53 (CH of 1-benzene), 20.48 (CH_3 aliph.), 18.15 (CH_3 aliph.); Mass *m/z*: 257.31 $[M+H]^+$.

5-Methyl-3-((4-methylthiazol-2-yl)imino)-1-(piperazin-1-ylmethyl)indolin-2-one (ICM2A): Yield: 64.83%, m.p.: 224-225 ºC; m.w.: 355.46; Elemental anal. of $C_{18}H_{21}N_5OS$, calcd. (found) %: C, 60.48 (60.82); H, 5.21 (5.46); N, 19.26 (19.70). FTIR (KBr, ν_{max}, cm^{-1}): 3659 (-NH), 2856 (-C-H, alkyl), 1724 (-C=O), 1648 (-C=N), 1004 (-C-C), 1329 (-CH_2), 1123 (-C-S-C), 1083 (-C-N), 784, 711 (-C=C, arom.); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 8.83-8.29 (m, 1H), 7.84 (d, *J* = 8.4 Hz, 1H), 7.08 (ddd, *J* = 7.8, 2.0, 1.0 Hz), 5.00 (s 1H, CH, methine), 4.51 (s 2H), 2.82-2.71 (m, 1H), 1.10- 1.04 (m, 1H, NH); ^{13}C NMR (126 MHz, DMSO-d_6, δ ppm): 171.82 (C of 2-thiazole), 153.23 (C of 1-amide), 144.20 (C of 2-thiazole), 130.27 (CH of 1-benzene), 127.25 (CH of 1-benzene), 125.34

(CH of 1-benzene), 120.44 (C of 1-benzene), 101.42 (CH of 2-thiazole), 68.85 (CH_2 aliph.), 53.37 (CH_2 of cyclohexane), 45.69 (CH_2 of cyclohexane), 20.48 (CH_3 aliph.), 18.12 (CH_3 aliphatic); Mass *m/z*: 355.46 $[M+H]^+$.

5-Methyl-1-((4-methyl piperazine-1-yl)methyl)-3-((4-methyl thiazole-2-yl)imino)indolin-2-one (ICM-2B): Yield: 70.64%, m.p.: 229-231 ºC; m.w.: 369.49; Elemental anal. of $C_{19}H_{23}N_5OS$, calcd. (found) %: C, 61.27 (61.76); H, 6.10 (6.27); N, 18.66 (18.95). FTIR (KBr, ν_{max}, cm^{-1}): 2918 (-C-H, alkyl), 1746 (-C=O), 1635 (-C=N), 1321 (-CH_2), 1142 (-C-S- C), 1022 (-C-N), 893, 753. (-C=C, arom.); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 8.20 (s, 1H), 7.01 (d, *J* = 1.3 Hz, 1H), 5.03 (s, 1H), 4.54 (s, 2H), 2.60-2.55 (m, 5H), 2.39, (s, 3H), 2.51-2.46 (m, 8H), 2.34 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6, δ ppm): 172.10 (C of 2-thiazole), 152.06, 148.26 (C of 2-thiazole), 141.11 (C of 1-benzene), 148.81 (C of 1-benzene), 131.14, 128.86 (CH of 1-benzene), 127.16 (CH of 1-benzene), 120.44 (CH of 1-benzene), 116.73 (CH of 1-benzene), 106.21 (CH of 2-thiazole), 68.86 (CH_2 of cyclohexane), 55.08 (CH_2 of cyclohexane), 51.79 (CH_2 of cyclohexane), 20.48 (CH_3 aliph.), 19.38 (CH_3 aliph.); Mass *m/z*: 369.49 $[M+H]^+$.

5-Methyl-3-((4-methylthiazol-2-yl)imino)-1-(piperidin-4-ylmethyl)indolin-2-one (ICM-2C): Yield: 62.55%, m.p.: 158-160 ºC; m.w.: 354.47; Elemental anal. of $C_{19}H_{22}N_4OS$, calcd. (found) %: C, 64.21 (64.38); H, 6.17 (6.26); N, 15.43 (15.81). FTIR (KBr, ν_{max}, cm^{-1}): 2944 (-C-H, alkyl), 1782 (-C=O), 1682 (-C=N), 1334 (-CH_2), 1125 (-C-S- C), 1097 (-C-N), 784 (-C=C, arom.); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 7.82 (d, *J* = 8.4Hz, 1H), 7.25-7.20 (m, 1H), 7.11-7.05 (m, 1H), 5.07 (p, *J* = 3.9, Hz, 1H), 4.34 (s, 1H), 4.04 (d, *J* = 5.3 Hz, 2H), 3.35 (d, *J* = 1.0 Hz, 1H), 2.41 (d, *J* = 0.7Hz, 3H), 2.33 (d, *J* = 1.3, 2H); ^{13}C NMR (126 MHz, DMSO-d_6, δ ppm): 175.53 (CH of 2-thiazole), 139.57 (CH of 1-benzene), 133.69 (C of 1-benzene), 126.52 (CH of 1-benzene), 116.18 (C of 1-benzene), 106.23 (C of 2-thiazole), 47.19 (CH_2 of piperidine), 44.69 (CH_2 of piperidine), 33.43 (CH_2 of piperidine), 31.33 (CH_2 of piperidine), 21.73 (CH_3 of aliph.), 19.30 (CH_3 of aliph.); Mass *m/z*: 354.47 $[M+H]^+$.

Anthelmintic activity: Due to the morphological and physiological similarities to the intestinal parasite of humans, Indian earthworm (*Pheretima posthuma*) was selected for the experiment to determine the anthelminthic efficacy of the synthesized compounds [17]. Earthworms were removed from moist soil and cleaned to get rid of any foreign matter or faeces. Earthworms with dimensions of 0.1 to 0.2 cm in width and 3-5 cm in length were used in all testing methods. *P. posthuma* of nearly equal size (6 cm ± 0.1) was randomly selected for this study. The worms were accustomed to the laboratory setting before the experiment. Four sets of six earthworms each were created from the earthworms. Albendazole concentrations of 0.1 % w/v, 0.2 % w/v, 0.5 % w/v and 1 % w/v were used as benchmarks and placed in petri dishes. A modest amount of DMSO was used to synthesize the compounds (**ICP-1A**, **2A**, **2B**, **2C** and **ICM-1A**, **2A**, **2B**, **2C**), which were then diluted to four different concentrations: 0.1% w/v, 0.2 % w/v, 0.5 % w/v and 1 % w/v for each molecule. Regular saline solution was used as a control. For each concentration, six earthworms of nearly the same size (6.2 cm ± 0.1) were collected, placed in petri plates and kept at room temperature. It was timed how long it took for death and total paralysis to occur. For each sample, the average paralysis and death times were measured (triplicate readings were taken). The length of time it took the worms to become motionless was noted and each worm was subjected to cues from the environment that, if they were still alive, stimulated and produced movement in the earthworms [18]. Six worms from each group were used to calculate the mean SEM of the results.

Antibacterial activity: By using the cup plate method, *in vitro* antibacterial activity [16] was performed against 24 h old cultures of four bacteria. The study was performed for the novel synthesized compounds against the bacteria *Bacillus subtilis* (ATCC-1086), *Pseudomonas auroginosa* (ATCC-1232), *Escherichia coli* (ATCC-3273), *Proteus mirabilis* (ATCC-224) and *Staphylococcus aureus* (ATCC-449). The petri dishes were properly cleaned and sterilized in a hot air oven at 160 °C temperature for 1h to conduct antibacterial experiments. The Muller Hinton agar media was prepared as per the instruction of the manufacturer (Hi-media). Ampicillin trihydrate was used as the standard drug in sterile dimethyl formamide (DMF) test solutions at concentrations of 25, 50 and 100 µg/mL. The test organism's broth culture was injected into the sterile agar plates within 24 h. A sterile borer was used to create consistent 6mm holes in the agar plate and each bore was individually filled with 0.2 mL of standard drug and the blank DMF under aseptic conditions or inside a laminar airflow. The plates were then left at room temperature for 2 h to allow the solutions to diffuse into the agar media, after 48 h of incubation at 37 ºC (Hicon Pvt. Ltd., Delhi India). The zone of inhibition was measured in mm and compared against the standard. All results were measured in triplicates.

Molecular docking studies

Ligands preparation and optimization: For this, ligands (**ICM 1A**, **ICM 2A**, **ICM 2B**, **ICM 2C**, **ICP 1A**, **ICP 2A**, **ICP 2B**, **ICM 2C**) were drawn in ChemDraw Professional 15.0 and 3D structures of the ligands were produced in Open babel and saved as SDF format for further preparation and molecular docking analysis [14].

Receptor preparation and optimization: The crystallographic structures of oligosaccharide substrate binding in *E. coli* proteins (PDB ID: 1ahp) and Novel X-ray structure of Na-ASP-2, a PR-1 protein from the nematode parasite *Necator americanus* (PDB ID: 1u53), were obtained from the protein data bank. To prepare the protein for molecular docking, the water molecules were removed and then hydrogen atoms were supplied to the protein using the BIOVIA Discovery Studio 2021 Client program to correct the ionization of the amino acid residues [15].

Molecular docking: The proteins were saved in pdb format and then loaded into the PyRx program for molecular docking, which was done with the Autodock Vina tool. Fig. 1 depicts the complex structures of bacterial and helminthic proteins, both of which were imported in .pdb format from the ChemDraw 3D application. To find the most stable conformer,

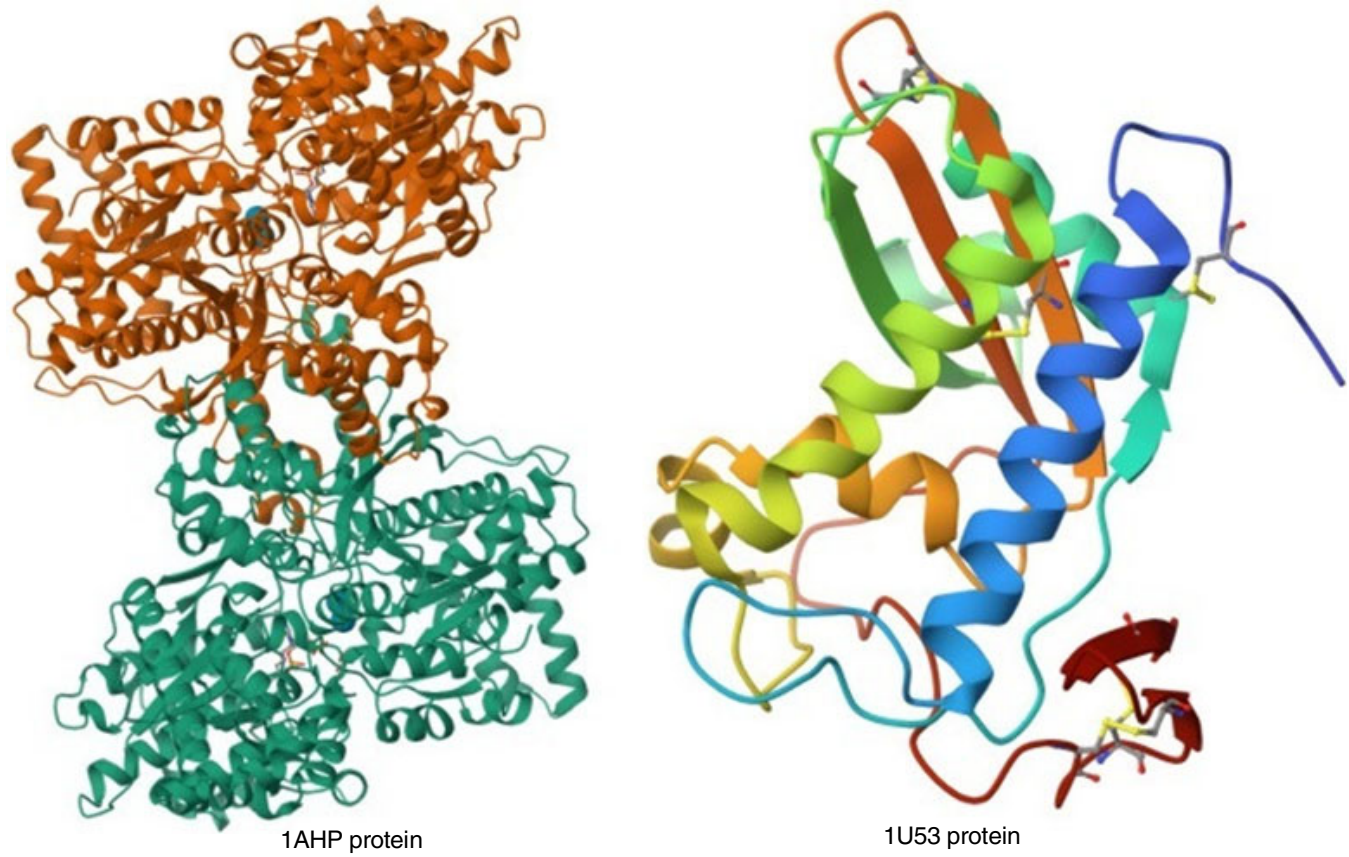


Fig. 1. Crystal structure of 1AHP and 1U53 proteins

the PyPx program was utilized. Using Discovery Studio 2021 Client software, the intermolecular interactions between synthetic compounds and active residues of proteins were determined and depicted [19-21].

RESULTS AND DISCUSSION

The present study utilized a docking study to develop an approach for synthesizing novel isatin derivatives, evaluate them for anthelmintic and antibacterial properties; and construct a SAR. The study was initiated by synthesis of a novel Schiff and Mannich bases of isatin compounds. The analytical and spectrophotometric results useful for structural elucidation were in good agreement with all the predicted data. The novel isatin compounds were characterized using a general spectroscopic methods (FT-IR, $^{1}H/^{13}C$ NMR and LC-MS).

The structure of the synthesized compounds as indicated in **Scheme-I** was confirmed based on the spectrophotometric data for structural elucidation of the synthesized compounds. For the anthelminthic activity, among the synthesized compounds, **ICP-2B** shows paralysis time 4.936 ± 0.12 min (0.1% w/v), 1.953 ± 0.1 min (1% w/v), compound **ICP-2C** shows paralysis time 4.221 ± 0.08 min (0.1% w/v), 1.846 ± 0.08 min (1% w/v), compound **ICM-2B** shows paralysis time 4.432 ± 0.17 min (0.1% w/v), 1.675 ± 0.08 min (1% w/v), **ICM-2C** shows paralysis time 4.685 ± 0.24 min (0.1% w/v), 2.104 ± 0.03 min (1% w/v), when compared with the standard albendazole which shows paralysis time 3.923 ± 0.03 min (0.1% w/v), 1.124 ± 0.1 min (1% w/v). The details of the other compounds are shown in Table-1.

The antibacterial activity of each synthesized compound which was measured in terms of the zone of inhibition against different Gram-positive and Gram-negative bacteria demonstrates the potential of each compound at three different concentration levels. Compounds **ICP-2B**, **ICP-2C**, **ICM-2B** and **ICM-2C** have demonstrated significant antibacterial activity among these synthetic derivatives. Compound **ICP-2B** showed a 20.37 mm clear zone of inhibition at 100 µg/mL against *B. subtilis* compared to a 23.34 mm zone for standard ampicillin at the same concentration. Additionally, this compound also demonstrated an inhibitory zone of 18.28 mm against Gram-negative *E. coli* and a zone of 15.95 mm *versus* 17.64 mm of ampicillin against *P. aeruginosa*. Similar results were obtained with the molecule **ICM-2C**, against the microorganisms *B. subtilis* and *P. aeruginosa*, which showed 20.16 mm and 15.73 mm distinct zones of inhibition at 100 µg/mL, respectively. Additionally, compared to ampicillin, the inhibitory zone was found to be 25.29 mm for *S. aureus* (26.82 mm). At a concentration of 100 µg/mL, compound **ICM-2B** exhibits a 20.46 mm inhibition zone against *B. subtilis,* 15.87 mm against *P. aeruginosa,* 19.32 mm against *E. coli*, 22.78 mm against *P. mirabilis* and 25.72 mm against *S. aureus* (Table-2). Based on these results, it can be concluded that the compounds' zones of inhibition (**ICP-2B**, **ICM-2B**, **ICP-2C** and **ICM-2C**) were highly similar to those of conventional ampicillin and showed strong antibacterial capability that was dosage dependent.

TABLE-1
ANTHELMINTIC POTENTIAL OF THE NEWLY SYNTHESIZED COMPOUNDS

Compounds	Paralysis time# (Mean±SEM) (min)				Death time# (Mean±SEM) (min)			
	0.1% w/v	0.2% w/v	0.5% w/v	1.0% w/v	0.1% w/v	0.2% w/v	0.5% w/v	1.0% w/v
ICP-1A	5.231±0.05*	4.856±0.21*	4.270±0.08*	3.423±0.03	6.431±0.05^{ns}	5.084±0.07*	4.327±0.1	3.045±0.06*
ICP-2A	5.013±0.31*	4.725±0.24*	4.488±0.12	3.107±0.2	6.215±0.31^{ns}	5.341±0.05*	4.846±0.07	3.143±0.02*
ICP-2B	4.936±0.12	3.741±0.05**	2.536±0.07**	1.953±0.1**	5.827±0.12*	4.727±0.03**	3.845±0.03*	1.837±0.17***
ICP-2C	4.221±0.08***	3.120±0.13***	2.143±0.14***	1.846±0.08**	5.721±0.08*	4.655±0.08**	3.681±0.23*	1.508±0.21***
ICM-1A	5.347±0.07	5.018±0.18	4.458±0.21	4.103±0.02	6.867±0.07^{ns}	5.482±0.14	4.876±0.21	3.273±0.13
ICM-2A	5.673±0.12	4.675±0.13	4.132±0.01	3.726±0.11	6.351±0.12^{c}	5.187±0.15	4.375±0.03	3.828±0.03
ICM-2B	4.432±0.17***	2.845±0.12***	2.345±0.18**	1.675±0.08***	5.548±0.17	4.833±0.34*	3.844±0.14**	1.756±0.04***
ICM-2C	4.685±0.24**	3.931±0.08**	2.732±0.07**	2.104±0.03*	5.920±0.24*	5.210±0.24*	4.191±0.13	2.014±0.16**
Albendazole	3.923±0.16	2.072±0.15	1.372±0.09	1.124±0.1	5.327±0.16	4.118±0.03	3.215±0.08	1.348±0.04
Control (saline solution)	No paralysis observed				No death observed			

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

TABLE-2
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)		
	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
Ampicillin	11.14±2.34	19.62±0.74	23.04±0.34	17.64±0.94	21.42±0.33	26.67±0.83	13.89±0.91	18.54±0.57	21.79±1.33
ICP-1A	08.21±0.84	10.76±0.65	12.53±1.77	10.44±1.12	13.23±1.10	16.76±0.35	08.32±1.38	11.10±0.27	15.43±1.87
ICP-2A	09.32±0.46	13.21±0.91	20.53±3.55	13.84±1.78	17.21±1.15	24.93±1.25	09.53±0.35	15.83±2.82	19.83±1.67
ICP-2B	10.54±1.84	16.36±3.33	20.37±1.92	15.95±4.27	18.04±2.61	19.44±1.34	11.27±1.81	16.12±2.83	18.28±2.04
ICP-2C	10.76±1.08	14.65±0.55	19.96±2.43	14.21±1.54	18.90±0.87	25.04±1.03	12.19±1.73	15.83±2.31	19.37±0.21
ICM-1A	10.40±2.82	14.35±2.54	21.27±1.21	15.53±2.54	17.38±1.65	21.67±0.44	11.52±0.28	15.90±1.21	20.62±1.68
ICM-2A	09.68±1.24	13.21±0.26	17.11±0.55	12.97±0.65	19.72±0.27	20.43±1.63	11.43±1.33	13.63±0.89	16.83±1.72
ICM-2B	10.45±0.56	17.32±1.24	20.46±0.54	15.87±1.87	18.43±2.82	24.53±1.98	12.88±1.21	16.43±1.87	19.32±3.28
ICM-2C	10.45±1.90	17.32±0.27	20.16±0.28	16.73±0.58	19.43±0.36	24.65±3.64	12.21±0.28	16.93±0.28	20.84±0.24

Compounds	*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)
DMF	00	00	00	00	00	00
Ampicillin	15.96±0.25	20.33±1.65	24.28±0.27	17.24±0.69	21.32±1.38	26.82±2.25
ICP-1A	09.88±1.35	12.51±1.67	17.64±1.82	07.34±1.64	12.21±1.22	14.68±1.41
ICP-2A	11.80±1.85	16.21±2.77	20.17±1.57	13.53±0.57	18.41±1.36	22.92±1.83
ICP-2B	09.73±1.51	13.43±1.46	17.54±1.35	14.93±1.37	14.64±1.85	22.33±1.52
ICP-2C	09.22±0.53	15.45±1.82	20.41±0.73	14.69±2.02	19.72±0.86	23.65±0.83
ICM-1A	12.32±3.41	17.35±1.42	21.74±1.33	14.28±1.84	18.78±2.84	25.32±3.53
ICM-2A	14.64±1.24	17.64±1.41	17.38±1.27	10.10±2.16	19.10±1.44	25.10±2.63
ICM-2B	14.21±1.67	18.78±1.28	22.78±1.45	14.53±1.26	19.43±1.35	25.29±2.77
ICM-2C	14.44±2.84	18.94±1.76	23.64±0.24	14.87±2.24	20.32±1.75	25.72±1.87

*Average of three readings

When compared to the conventional ampicillin, the remaining three compounds (**ICP-2A**, **ICP-1A** and **ICM-1A**, **ICM-2A**) were also effective against all of the microorganisms under study.

Structure-activity relationship (SAR) study: The features of recently produced isatin analogues and the synthesis methods have been the focus of chemists around the world to optimize these molecules for synthesis of new analogues. These drug like analogues may be used to investigate a variety of pharmacokinetic and pharmacodynamic activities, such as enhancing potency, lowering toxicity and producing adequate bioavailability [22]. Based on the above observation and possible structure activity relation study, these prepared compounds containing piperazinyl, N-methyl piperazinyl and piperidinyl groups can be potential in effectively block the muscular response to acetylcholine, causing worm paralysis and resulting in the gut wall detachment and worms are ejected through the faeces. The piperazine nucleus contains two -N atoms, providing high pK_a [23], thereby improving the pharmacokinetic properties of therapeutic candidates. These N-sites provide a significant increase in the water solubility of drug-like compounds, which is important for bioavailability thereby maintaining a balance between the pharmacodynamic and pharmacokinetic profiles of drug-like molecules, which is critical in the design and development of new drugs. Due to the characteristics of the piperazine template, this molecular component is an important and strategically

placed system in the rational design of drugs for this purpose [24]. The antibacterial as well as anthelminthic potential, were similarly influenced by substituents on the phenyl ring of the isatin molecule. Substituted analogues of the C-5 position of the isatin molecule were more powerful than analogues at the C-7 position, also the substituents in the nitrogen of the isatin ring had a significant impact on both the activity [25]. The antibacterial capabilities of electron-donating group substituted derivatives were shown to be superior to those of electron withdrawing compounds [26].

The presence of an electron-donating methyl (-CH_3) group in piperazinyl ring of the compounds **ICP-2B** and **ICM-2B** can be thought to make them more powerful anthelmintic. Fig. 2 illustrates the particulars of the paralysis and death time depending on the examined concentrations of the synthesized substituted isatinoid Mannich bases. For antibacterial activity, it was established that piperazine targets the cytoplasmic membrane of the bacteria resulting in the leakage of intercellular components leading to cell death [26] and the presence of methyl group with a positive inductive effect makes the compounds more potent antibacterial agents (**ICP-2B** and **ICM-2B**) in comparison to only piperazine substitution for this present series of compounds.

The presence of Mannich base with a piperidine ring in the present synthesized compounds is considered an important component for the inhibition of bacterial translation and growth [27,28]. Ismail *et al.* [29] reported that phenyl piperazine acetyl indole derivatives where piperazine nucleus when combined with isatin molecule displayed anti-Alzheimer's potential through the inhibition of acetylcholine esterase enzyme inhibition, based on the structural modification of donepezil. Similarly in the present scaffold where the isatin Schiff bases have been fused with piperazine and substituted piperazine as a Mannich base much expected to have anti-Alzheimer's potential [30], therefore, the possible neurotoxicity issues of piperazine ring have been minimized with isatin conjugation. Based on the evidence from the antibacterial results the compounds **ICP-2C** and **ICM-2C** was also considered more effective against the studied bacterial inhibition (Fig. 3).

The study of the structure-activity relationship further reinforces the connection between the chemical structure of the newly synthesized isatin molecules and their evaluated anti-

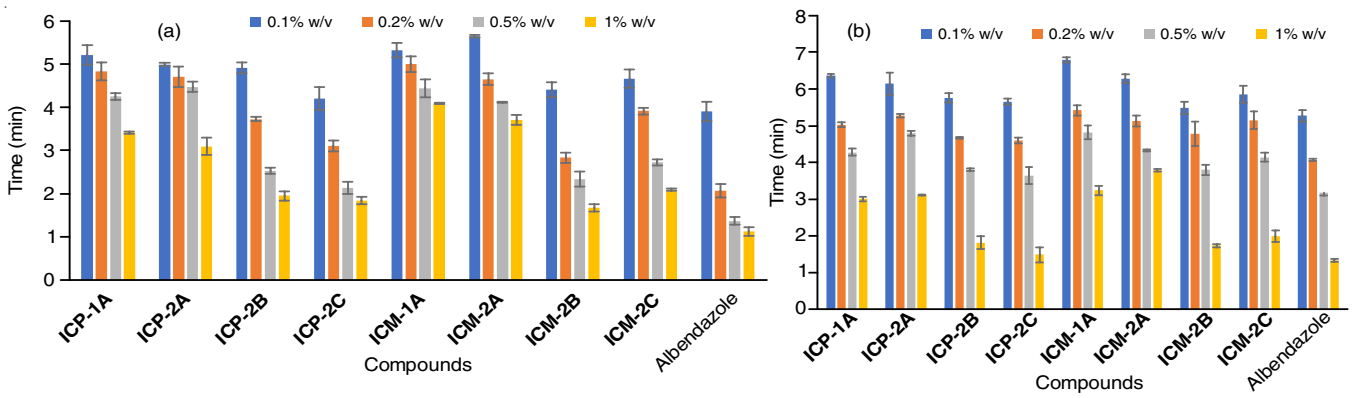


Fig. 2. Anthelmintic activity of paralysis time (a) and death time (b) of the synthesized compounds

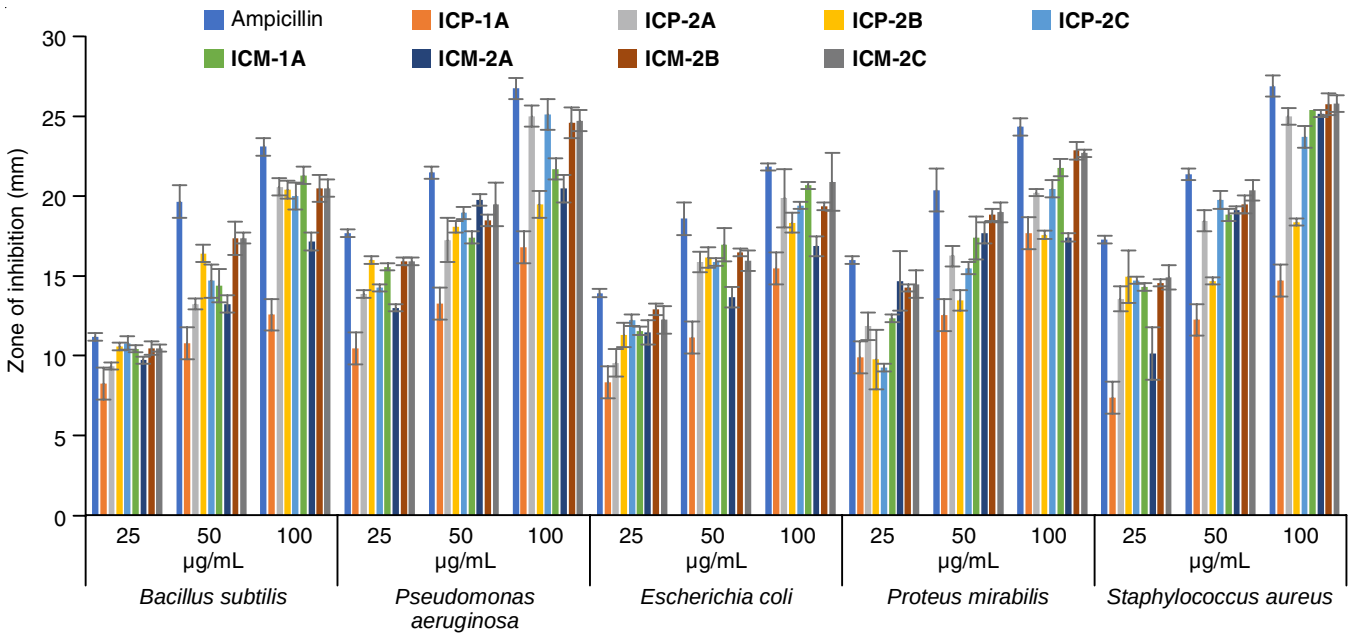


Fig. 3. Antibacterial activity of the synthesized compound

bacterial and anthelminthic potential. The SAR analysis also provides a clear path for future research into the anticholinesterase inhibition property for the development of anti-Alzheimer's drugs and the neurotoxicity of piperazine to determine safe levels of the synthesized compounds.

Docking results: PyRx docking was used to determine binding affinities and key interactions between the bacterial and helminthic proteins with the novel synthetic ligands. The binding affinities were compared to the antibacterial ampicillin and anthelminthic albendazole. Table-3 shows the binding affinity obtained from ligands and proteins. The binding affinity values for ligands ranged from -6.8 to -8.7 kcal/mol in case of 1ahp and -5.7 to -7.3 kcal/mol in case of 1U53 proteins. The molecular interactions between the active site of proteins and the most active ligands were visualized using the Discovery Studio 2021 Client software (Figs. 4 and 5) with 1ahp and 1u53. These samples revealed the predicted interactions with the amino acids in the active region of the protein, implying strong antagonistic capabilities against parasitic helminth proteins. For 1AHP bacterial protein, compounds **ICP 2C** and **ICM 2C** had the highest binding affinity with a value of -8.3 and -8.7 kcal/mol respectively as compared to ampicillin (-7.6 kcal/mol). While for 1u53 helminthic protein, **ICP 2B** (-7.2 kcal/mol) and **ICM 2B** (-7.3 kcal/mol) showed significantly higher values of binding energy compared to standard albendazole (-6.9 kcal/mol). Thus, these results can predict the antibacterial properties of compounds **ICP 2C** and **ICM 2C** while the prediction indicated compounds **ICP 2B** and **ICM 2B** having anthelminthic properties [31]. Thus, this results confirms the activity of the compounds as shown in *in vitro* studies [32-34].

TABLE-3
RESULTS OF COMPOUND DOCKING STUDY

Ligands	Binding affinity (ΔG, kcal/mol)	
	1AHP	1U53
ICP-1A	-7.1	-5.7
ICP-2A	-7.4	-6.4
ICP-2B	-7.2	-7.2
ICP-2C	-8.7	-6.3
ICM-1A	-7.3	-6.4
ICM-2A	-7.3	-6.6
ICM-2B	-6.8	-7.3
ICM-2C	-8.3	-6.1
Albendazole	–	-6.9
Ampicillin	-7.6	–

Conclusion

In this study, eight new Schiff and Mannich developed using 5-methyl indole 2,3-dione derivatives containing piperazine and substituted piperazine derivatives. Several spectroscopic approaches as well as magnetic and conductance measurements, were used to characterize the structure of the synthesized compounds. The *in vitro* anthelmintic potential of almost all compounds was found satisfactory, but compounds **ICP-2B** and **ICM-2B** exhibit very significant anthelminthic potential. The SAR study further confirms the correlation between the chemical structure of the newly synthesized isatin molecules and their tested activities. *In silico* molecular docking studies against the oligosaccharide substrate binding in *Escherichia coli* proteins and a PR-1 protein from the nematode parasite *Necator americanus* were carried out. Compounds **ICP 2B** and **ICM 2B** showed stronger protein inhibition in case of helminthic protein while compounds **ICP 2C** and **ICM 2C**

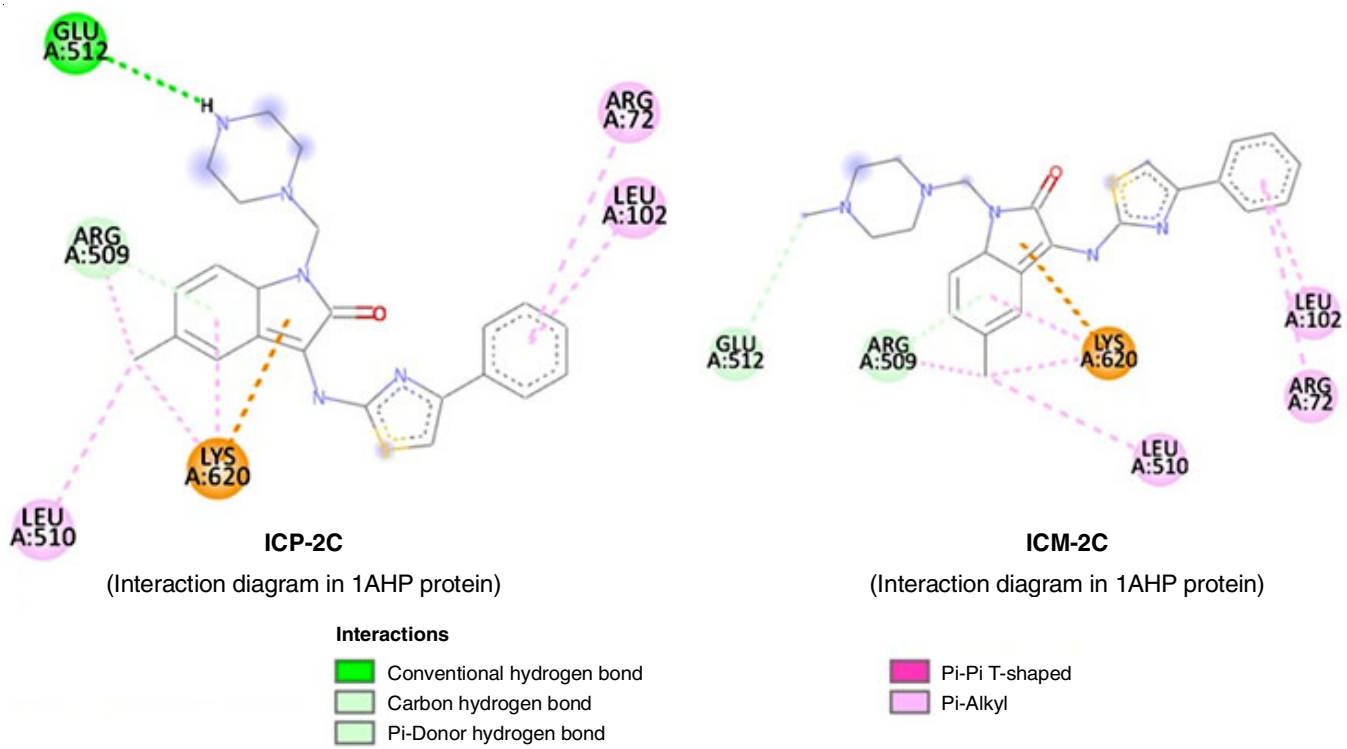


Fig. 4. Interaction of compounds (**ICP-2C** and **ICM-2C**) with 1AHP protein

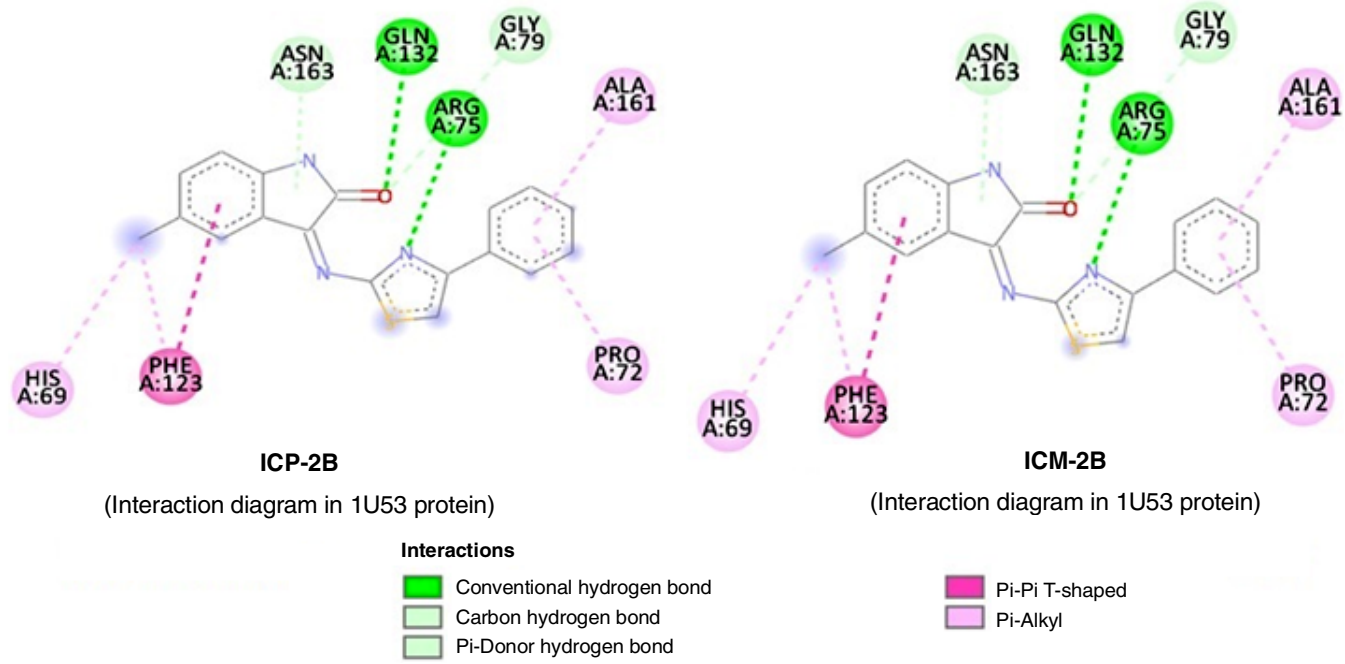


Fig. 5. Interaction of compounds (**ICP-2C** and **ICM-2C**) with 1U53 protein

showed the maximum antibacterial capacity agreeing with *in vitro* studies. Thus, these new series of Schiff and Mannich bases could provide a novel scaffold for the development of anthelminthic and antimicrobial agents.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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