Indian Journal of Chemistry
Vol. 63, April 2024, pp. 400-406
DOI: 10.56042/ijc.v63i4.7109

Efficient synthesis, molecular docking and biological evaluation of novel isatine linked spiroheterocycle through multi-component reaction

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Received 8 December 2023; accepted (revised) 22 March 2024

In this work, twelve compounds have been newly synthesized by one pot N-Mannich base multicomponent reaction. The synthesized compounds have been characterized by using FT-IR, ^{1}H and ^{13}C NMR and mass spectrometry. The final compounds have been evaluated for their *in vitro* antibacterial and antifungal activity using agar-well diffusion method. The tested compounds show poor to good activity with inhibition zone value between 8 to 25 mm, when compared to reference Ciprofloxacin and Flocanazole. Among them compounds **6b**, **6c**, **6d** and **6g** show most promoting activity against *E.coli*, *B. cereus*, *B. substilis*, *A. niger* and *R. nigricans*.

Keywords: N-Mannich base, Isatine, Gabbapentin-lactum, Antibacterial activity, Antifungal activity

Time is needed for the synthesis of a newer class of anti-bacterial and anti-fungal agents, particularly against drug-resistant gram-positive and gram-negative strains of bacteria and fungi that are in charge of several serious infections in hospital acute and long-term care units. A review of the literature found that isatin has a variety of chemotherapeutic properties, including antibacterial, antifungal, antiviral, anti-HIV, anti-mycobacterial, anticancer, and anticonvulsant properties. A review of the literature showed that isatin derivatives, including hydrazine, Mannich bases, Schiff bases, and spiro indolinones, have a wide spectrum of biological functions such as actions of Isatine- gabbapentin lactum have been observed, including antibacterial, antifungal, antiviral, antimycobacterial, anticancer, anti-inflammatory, and anticonvulsant properties[1–15]. According to the analysis of the aforementioned pharmacophores, the union of these two substances may boost their antibacterial activity.

A significant family of chemical molecules with strong biological and pharmacological properties are heterocyclic compounds[16,17]. These heterocyclic moieties, either individually or in fused form, are continually employed by researchers for the creation of novel medications due to their extensive biological profile[18]. Isatin (1 H - indole-2,3-dione), also known as indole quinine and indenedione, is a nitrogen-containing heterocyclic molecule that stands out as a privileged moiety with a broad spectrum of biological activity and is thus regarded as a bio-active heterocyclic moiety[19,20]. Isatin structurally combines a nitrogen-containing five-membered ring with a six-membered benzene ring. One ring is aromatic and the other is anti-aromatic, but both are on the same plane[20,21].

MCRs are a useful synthesis method for quickly and often environmentally friendly production of novel drugs like oxindole derivatives. We have covered the most recent advancements in isatin-based MCRs and their corresponding biological activities in this study. It is interesting that, in contrast to target-based screening, phenotypic tests are the most popular method for biological activity screening of novel oxindole derivatives produced by MCRs. The primary causes of this are the extensive scaffold variety achieved by MCRs and the oxindole core's favoured position as a significant general pharmacophore[22-24]. We can see from the examples provided in this study that MCRs are a valuable tool to boost the output of novel drug-like compounds, enabling hit-to-lead optimisation and quicker hit discovery in a range of diseases[25,26].

In view of biological importance of these two moieties, A novel set of isatins incorporating gabapentin spiro lactum were to be created, and their anti-microbial properties would be tested.We created gabapentin-isatine derivatives here. For the synthesis

of Gabbapentin-isatine derivatives, many approaches have been described. However, we have changed one potent synthetic compound utilised to make gabapentin-isatine.

Experimental Section

Every chemical utilised was of the analytical grade. On a Fisher-Johns Melting Point device, melting points were calculated. The chemicals' purity was examined using TLC on silica gel plates together with UV and iodine chamber visualisation. Model FTIR 8400S was used to carry out the FTIR analysis, and the frequency was measured in cm-1 units. Using Bruker Avance II spectrometer equipment, 1H NMR spectra were acquired at 400 MHz and 13C-NMR spectra were recorded at 100 MHz. DMSO-d_6 was used as the solvent, and chemical shifts were reported in parts per million downfield from TMS. On the LC-MS, mass spectra were captured. Compound structures and nomenclatures were developed using Perkin Elmer ChemBio Office Ultra.

General procedure for the synthesis of 5-substituted-1-((3-oxo-2-azaspiro [4.5] decan-2-yl) methyl) indoline-2, 3-dione analogs, 6a-l

N-Mannic based was prepared by a solution of substituted isatine in 10 mL ethanol, Gabbapentin lactum and formaldehyde reaction reflux for 8 h. On cooling, the desired prouduct formed. It was filtered, dried and purified by recrystallization from DMF. All the derivatives **6a-l** were synthesized by this method. Physical characterization data of the compounds **6a-l** are shown in Table 1.

Representative Spectral Data

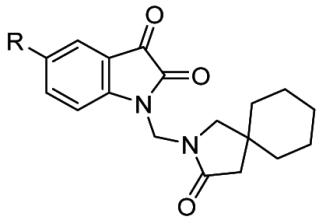
1-((3-Oxo-2-azaspiro [4.5] decan-2-yl) methyl) indoline-2, 3-dione, 6a

Orange. m.p.110°C. $C_{18}H_{20}N_2O_3$. IR (KBr): 2900 (sp^3 -CH stretching), 1200-1300 (C-N), 1725 (carbonyl), 1600 (benzene), 1480(CH2-), 1695 cm^{-1} (Amide); ^{1}H NMR: δ 73-7.63m (3H, J = 7.6Hz), 7.24t (1H, J=7.6), 5.72 S (2H), 3.50S (2H), 2.06S (2H), 1.53-1.69m (10H); ^{13}CNMR: δ 184 (C=O), 173, 160 (Amide), 114, 134, 132, 131, 118, 114 (aromatic benzene carbon), 59 (-CH_2), 56, (-CH2), 38 (-40C), 47 (-CH_2), 35, 25, 20 (Cyclohexane); LC-MS: *m/z* (%) 312.15 (M^+).

5-Chloro-1-((3-oxo-2-azaspiro [4, 5] decan-2-yl) methyl) indoline-2, 3-dione, 6b

Yellow. m.p.88°C. $C_{18}H_{19}ClN_2O_3$. IR (KBr): 850-560 (C-X), 2903 (sp^3-CH stretching), 1200-1300 (C-N), 1728 (carbonyl), 1603 (benzene), 1480 (CH_2-), 1697 cm^{-1} (Amide); ^{1}H NMR: δ 8.90s (1H, CH), 7.24d (1H, CH), 7.44d (1H, CH), 5.72S (2H), 3.50S (2H) 2.06S (2H), 1.53-1.69m (10H); ^{13}CNMR: δ 181, 171, 163, 144, 132, 131, 126, 114, 59, 56, 38, 47, 35, 25, 20; LC-MS: *m/z* (%) 346.11 (M^+).

Table 1 — Physical characterization data of 5-substituted-1-((3-oxo-2-azaspiro [4.5] decan-2-yl)methyl)indoline-2,3-dione analogs **6a-l**



Compd	R	Yield (%)	m.p. (°C) (±5°)	Mol. Formula
6a	H	72	110	$C_{18}H_{20}N_2O_3$
6b	Cl	79	88	$C_{18}H_{19}ClN_2O_3$
6c	Br	65	131	$C_{18}H_{19}BrN_2O_3$
6d	NO_2	50	170	C18H19N3O
6e	CH_3	78	126	$C_{19}H_{22}N_2O_3$
6f	F	75	124	$C_{18}H_{19}FN_2O_3$
6g	CN	76	172	$C_{19}H_{19}N_3O_3$
6h	OCH_3	57	135	$C_{19}H_{22}N_2O_4$
6i	I	84	168	$C_{18}H_{19}IN_2O_3$
6j	OCF_3	81	176	$C_{19}H_{19}F_3N_2O_4$
6k	OAc	47	101	$C_{20}H_{22}N_2O_5$
6l	CF_3	55	160	$C_{19}H_{19}F_3N_2O_3$

2-((5-Nitro-2, 3-dioxo-2, 3-dihydro-1*H*-3l4-benzo[d]imidazol-1-yl) methyl)-2-azaspiro [4,5] decan-3-one, 6d

Light Yellow. m.p.170°C. $C_{18}H_{19}N_3O$. IR (KBr):1380 NO_2, 2905 (sp^3 -CH stretching), 1200-1305(C-N), 1730 (carbonyl), 1605 (benzene), 1483(CH_2-), 1699 cm^{-1} (Amide); ^{1}H NMR: δ 8.91s (1H, CH), 7.45d (1H, CH), 7.25d (1H, CH), 5.73S (2H), 3.51s (2H), 2.07S (2H), 1.53-1.70m (10H); ^{13}CNMR: δ 182, 172, 164, 145, 133, 132, 127, 115, 60, 57, 39, 48, 36, 26, 21; LC-MS: *m/z* (%) 359.14 (M^+).

5-Bromo-1-((3-oxo-2-azaspiro [4.5] decan-2-yl) methyl) indoline-2, 3-dione, 6c

Yellowish white. m.p.131°C. $C_{18}H_{19}BrN_2O_3$. IR (KBr): 840-540 (C-Br), 2903 (sp^3-CH stretching), 1200-1300 (C-N), 1728 (carbonyl), 1603 (benzene), 1480(CH_2-), 1697 cm^{-1} (Amide); ^{1}H NMR: δ 8.80s (1H, CH), 7.14d (1H, CH), 7.34d (1H, CH), 5.62S (2H), 3.40 S (2H) 1.96.S (2H), 1.43-1.59m (10H); ^{13}CNMR: δ 119.2, 133.6, 145.3, 117.5, 147.1, 119.9, 179.9, 160.4, 69.6, 57.5, 37.2, 20.7, 37.2, 42.2,173.7; LC-MS: *m/z* (%) 390.01 (M^+).

5-Methyl-1-((3-oxo-2-azaspiro [4.5] decan-2-yl) methyl) indoline-2, 3-dione, 6e

White: m.p.126°C. $C_{19}H_{22}N_2O_3$. IR (KBr): 2900 (C-CH_3), 2908 (sp^3-CH stretching), 1208-1308 (C-N), 1735 (carbonyl), 1608 (benzene), 1488(CH_2-), 1699 cm^{-1} (Amide); ^{1}H NMR: δ 8.70s (1H, CH), 7.04d (1H, CH), 7.24d (1H, CH), 5.52S (2H), 3.20 S (2H) 1.86.S (2H), 1.33-1.49m (10H); ^{13}CNMR: δ 137.4, 21.3, 133.6, 145.3, 117.5, 147.1, 119.9, 179.9, 160.4, 69.6, 57.5, 37.2, 20.7, 37.2, 42.2, 173.7; LC-MS: *m/z* (%) 326.16 (M^+).

5-Fluoro-1-((3-oxo-2-azaspiro [4, 5] decan-2-yl) methyl) indoline-2,3-dione, 6f

Yellow. m.p.124°C. $C_{18}H_{19}FN_2O_3$. IR (KBr): 869-580 (C-X), 2909 (sp^3-CH stretching), 1210-1310 (C-N), 1738 (carbonyl), 1609 (benzene), 1490(CH_2-), 1698 cm^{-1} (Amide); ^{1}H NMR: δ 8.95s (1H, CH), 7.29d (1H, CH), 7.49d (1H, CH), 5.77S (2H), 3.55S (2H) 2.11S (2H), 1.58-1.74m (10H); ^{13}CNMR: δ 164.5, 133.6, 145.3, 117.5, 147.1, 119.9, 179.9, 160.4, 69.6, 57.5, 37.2, 20.7, 37.2, 42.2, 173.7; LC-MS: *m/z* (%) 330.14 (M^+).

2,3-Dioxo-1-((3-oxo-2-azaspiro [4.5] decan-2-yl) methyl) indoline-5-carbonitrile, 6g

White. m.p.172°C. $C_{19}H_{19}N_3O_3$. IR (KBr): 2250(C-N), 2910 (sp^3-CH stretching), 1211-1311 (C-N), 1739 (carbonyl), 1610(benzene), 1491(CH_2-), 1699 cm^{-1} (Amide); ^{1}H NMR: δ 8.96s (1H, CH), 7.30d (1H, CH), 7.50 d (1H, CH), 5.78S (2H), 3.56S (2H) 2.12S (2H), 1.58-1.75 m (10H); ^{13}CNMR: δ 118.6,108.7,133.6,145.3,117.5,147.1,119.9,179.9,160.4,69.6,57.5,37.2,20.7,37.2,42.2,173.7; LC-MS: *m/z* (%) 337.14 (M^+)

5-Methoxy-1-((3-oxo-2-azaspiro [4.5] decan-2-yl) methyl) indoline-2, 3-dione, 6h

White. m.p.135°C. $C_{19}H_{22}N_2O_4$. IR (KBr): 1200 (-O-), 2908 (sp^3-CH stretching), 1208-1308 (C-N), 1735 (carbonyl), 1608 (benzene), 1488(CH_2-), 1699 cm^{-1} (Amide); ^{1}H NMR: δ 8.68s (1H, CH), 7.00d (1H, CH), 7.20d (1H, CH), 5.50S (2H), 3.18 S (2H) 1.82.S (2H), 1.31-1.47m (10H), 3-5 S (3H); ^{13}CNMR: δ 160.9, 55.8, 133.6, 145.3, 117.5, 147.1, 119.9, 179.9, 160.4, 69.6, 57.5, 37.2, 20.7, 37.2, 42.2, 173.7; LC-MS: *m/z* (%) 342.17 (M^+).

5-Iodo-1-((3-oxo-2-azaspiro [4.5] decan-2-yl) methyl) indoline-2, 3-dione, 6i

Blues-Violet. m.p.168°C. $C_{18}H_{19}IN_2O_3$. IR (KBr): 800-850 (C-I), 2900 (sp^3-CH stretching), 1190-1300 (C-N), 1724 (carbonyl), 1600 (benzene), 1470(CH_2-), 1692 cm^{-1} (Amide); ^{1}H NMR: δ 8.70s (1H, CH), 7.04d (1H, CH), 7.24d (1H, CH), 5.52S (2H), 3.30 S (2H) 1.86.S (2H), 1.33-1.49m (10H); ^{13}C NMR: δ 92.2, 119.2, 133.6, 145.3, 117.5, 147.1, 119.9, 179.9, 160.4, 69.6, 57.5, 37.2, 20.7, 37.2, 42.2, 73.7; LC-MS: *m/z* (%) 338.06 (M^+).

1-((3-Oxo-2-azaspiro [4.5] decan-2-yl) methyl)-5-(trifluoromethoxy) indoline-2, 3-dione, 6j

Violet. m.p.176°C. $C_{19}H_{19}F3N_2O_4$. IR (KBr): 870-581 (C-X), 2910 (sp^3-CH stretching), 1211-1310 (C-N), 1738 (carbonyl), 1609 (benzene), 1491(CH_2-), 1698 cm^{-1} (Amide); ^{1}H NMR: δ 8.96s (1H, CH), 7.30d (1H, CH), 7.50d (1H, CH), 5.78S (2H), 3.56S (2H) 2.12 S (2H), 1.59-1.75m (10H); ^{13}C NMR: δ 156.7, 129.7, 133.6, 145.3, 117.5, 147.1, 119.9, 179.9, 160.4, 69.6, 57.5, 37.2, 20.7, 37.2, 42.2, 173.7; LC-MS: *m/z* (%) 396.14 (M^+).

2,3-Dioxo-1-((3-oxo-2-azaspiro [4.5] decan-2-yl) methyl) indolin-5-yl acetate, 6k

White. m.p.101°C. $C_{20}H_{22}N_2O_5$. IR (KBr): 1100-1180 (-OAC), 2908 (sp^3-CH stretching), 1208-1308 (C-N), 1735 (carbonyl), 1608 (benzene), 1488(CH_2-), 1699 cm^{-1} (Amide); ^{1}H NMR: δ 8.68s (1H, CH), 7.00d (1H, CH), 7.20d (1H, CH), 5.50S (2H), 3.18 S (2H) 1.82.S (2H), 1.31-1.47m (10H),3-5 S (3H);

^{13}C NMR: δ 153, 169, 133.6, 145.3, 117.5, 147.1, 119.9, 179.9, 160.4, 69.6, 57.5, 37.2, 20.7, 37.2, 42.2,173.7; LC-MS: *m/z* (%) 317.25 (M^+).

1-((3-Oxo-2-azaspiro [4.5] decan-2-yl) methyl)-5-(trifluoromethyl) indoline-2, 3-dione, 6l

White: m.p.126°C. $C_{19}H_{22}N_2O_3$. IR (KBr): 980-690 (C-CF_3), 2912 (sp^3-CH stretching), 1208-1320 (C-N), 1740 (carbonyl), 1618 (benzene), 1498 (CH_2-), 1700 cm^{-1} (Amide); ^{1}H NMR: δ 8.80s (1H, CH), 7.14d (1H, CH), 7.34d (1H, CH), 5.62S (2H), 3.30 S (2H) 1.96.S (2H), 1.43-1.59m (10H); ^{13}C NMR: δ 127.1, 124.1, 133.6, 145.3, 117.5, 147.1, 119.9, 179.9, 160.4, 69.6, 57.5, 37.2, 20.7, 37.2, 42.2, 173.7; LC-MS: *m/z* (%) 380.20 (M^+).

Results and Discussion

Chemistry

Given the widespread use of Mannich bases in pharmaceuticals and other areas of life, this research was done to synthesise some major isatine Mannich Bases and test their anti-mycobacterial efficacy. The IR spectra of Mannich bases showed perfect agreement with the expected structures. Mannich base **6a** exhibited a distinct absorption peak at 1695 cm^{-1}, likely originating from the amide stretching vibrations of secondary aromatic amines. The absorption band of the NH-functional group vanished, while new absorption bands in the range of 1300 to 1200 cm^{-1}, attributed to -C-N stretching vibrations, enabled the identification of the -N-CH_2-N group in Mannich base **6a**. H^1-NMR-5.7 ppm Singlet for (-CH_2-), C^{13}-NMR-59 ppm for -CH_2- carbon indaicate that formation of N-Mannich base (Scheme 1).

Antimicrobial activity and antifungal activity

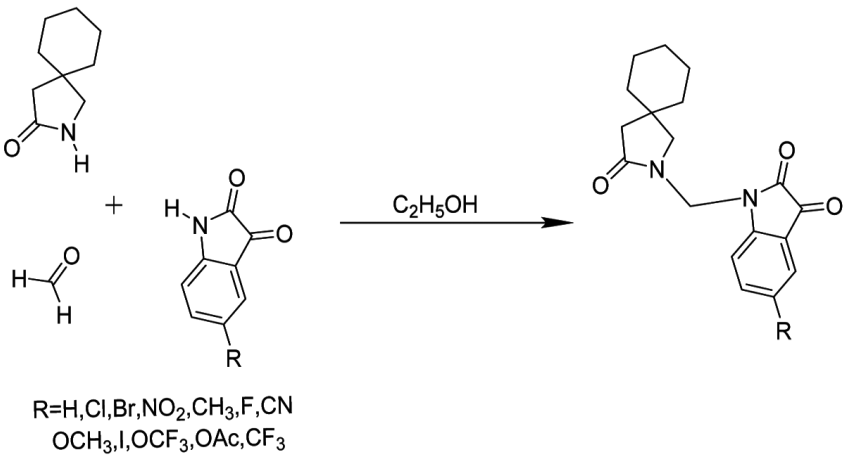
Synthesized molecules were evaluated for their antibacterial activity against Gram-positive organisms *B. substilis (MTCC-441)* as well as two Gram-negative organisms *viz E. coli (MTCC-443)*, *B. cereus (MTCC-492)* and antifungal activity against three fungal species *A. niger* (*MTCC282*) and *R. nigricans (MTCC 9277)*. Microbial strains used were procured from the Microbial Type Culture Collection (MTCC). Antimicrobial activities were determined by the Agar-well Diffusion Method[27-29]. Ciprofloxacin is a common antibacterial medication. Fluconazole is a common antifungal medication. According to results shown in Table 2, the investigated substances had varying inhibitory effects on the development of bacterial strains of both Gram-positive and Gram-negative morphology. The compounds active against several antibacterial species **6b**, **6c**, **6d**, and **6g** among those examined have excellent efficacy. The antibacterial efficacy of the compounds with an electron-withdrawing substitution was encouraging.

In vitro antimicrobial activity

Computational study

In silico *molecular docking analysis*

The 3D structures of *E. coli* DNA gyrase domain (PDB ID: 1KZN) were obtained from the Protein Data Bank (https://www.rcsb.org/). These structures were then converted into SDF files using Chemdraw. Subsequently, we refined the structures using the DFT basis set 6-311G (d,p) with Gaussian 09W and converted the optimized structures into PDB format using Gauss View 5.0. Before conducting further analysis, we prepared the protein by removing water



Scheme 1 — Synthesis of N-Mannich base

Table 2 — Antimicrobial and antifungal activity of **6a-l**

Compd	Antibacterial Activity Inhibition zone (mm) Gram negative		Gram positive	Compd	Antifungal Activity Inhibition zone (mm)	
	E.coli	*B.cereus*	*B.substilis*		*A.Niger*	*R.Nigricans*
6a	9	10	8	**6a**	14	–
6b	21	23	22	**6b**	14	15
6c	22	21	23	**6c**	16	17
6d	18	18	19	**6d**	13	–
6e	8	9	–	**6e**	8	9
6f	19	18	19	**6f**	14	–
6g	21	22	21	**6g**	15	16
6h	12	8	–	**6h**	9	–
6i	17	15	14	**6i**	15	14
6j	16	14	16	**6j**	14	–
6k	9	8	–	**6k**	9	8
6l	18	21	19	**6l**	15	13
Ciprofloxacin	22	25	24	–	–	–
Fluconazole	–	–	–	–	17	21

molecules, adding polar hydrogen atoms, validating torsion angles, and assigning Kollman charges using the Autodock Tools. AutoDock was employed to assess the binding energies and interactions of the synthesized compounds with the α-amylase protein. To facilitate this analysis, both the compounds and protein PDB files were converted to PDBQT format using the Autodock Tools. (https://doi.org/10.1002/0471250953.BI0814S24).

For the site-specific docking within the active site, we defined a grid box with dimensions of 60 Å × 60 Å × 60 Å. Molecular docking investigations were carried out using the Lamarckian genetic algorithm (LGA), considering ten unique conformers. The conformers with the highest binding affinity values were selected for further investigation. The resulting output files were then transformed into protein-ligand complexes and evaluated in Biovia Discovery Studio V21.1.0.20298. As part of the validation process, the DNA gyrase inhibitor was manually separated from the protein and saved as an inhibitor in PDB file format. The same docking technique, including grid settings, was applied to ensure that the inhibitors precisely targeted the active site cleft and caused minimal deviation from the co-crystallized complex. Additionally, Biovia Discovery Studio and the Computed Atlas for Surface Topography of Proteins were utilized to identify the amino acids present in the active sites (https://doi.org/10.1007/s11030-023-10683-x).

We employed molecular docking techniques to assess the *in silico* binding interactions and inhibitory capacities of synthesized hybrids. To facilitate this analysis, we acquired the crystal structure of *E. coli* DNA gyrase (PDB ID: 1KZN) from the PDB server.

Table 3 — Binding affinity of synthesized compounds against *E. Coli* DNA gyrase inhibitor (PDB: 1KZN)

Compd	Binding affinity (kcal/mol)
6a	–7.1
6b	–7.9
6c	–8
6d	8.4
6e	8.4
6f	–8.5
6g	–8.2
6h	7.9
6i	–8.1
6j	–8.9
6k	–8.4
6l	–8.7
Ciprofloxacin	–7.2

Our docking studies revealed that all the synthesized hybrids effectively bound to the active site of DNA gyrase, yielding docking score values ranging from –7.1 to –8.9, as detailed in Table 3. Notably, compound **6j** displayed a commendable docking score of –8.9 kcal/mol among the hybrids tested. In contrast, the co-crystallized inhibitor, Ciprofloxacin, exhibited a docking score of –7.2 kcal/mol.

As illustrated in Fig. 1, representative compounds **6j** and Ciprofloxacin established promising binding interactionsat the enzyme's active site. Compound **6j** formed hydrogen bonds with GLY:77 and ALA:47,

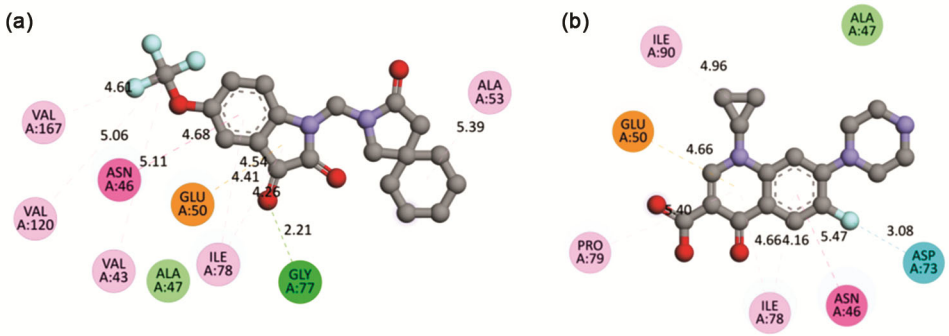


Fig. 1 — (A) 2D representation of **6j** interaction with 1KZN, and (B) 2D representation of Ciprofloxacin interaction with 1KZN

while non polar interactions with amino acids VAL:167, VAL:120, ASN:46, VAL:43, ILE:78 and ALA:53 at the active site of the 1KZN protein. Moreover, as seen in Fig. 1, common interactions between **6j** and Ciprofloxacin were found with proteins ASN:46, GLU:50, ALA:47, ILE:78. Which indicates the potential inhibitory interaction of our synthesized compound **6j** with respective of standard drug Ciprofloxacin.

Conclusion

The synthesis and the characterization of a novel Mannich base three-component condensation reaction involving active hydrogen containing compounds isatine, formaldehyde and gabapentin lactum has been achieved. *In vitro* assessment of their antibacterial activity and antifungal activity has been carried out. The different substitution of isatine showed significant biological activity against different microbes using Agar well diffusion activity. For all new compounds a better antimicrobial activity and antifungal activity was reported.

Acknowledgments

Authors are thankful to Department of Chemistry, Veer Naemad South Gujarat University, Surat, India for providing laboratory facilities. They are also thankful to SVNIT Surat for spectral analysis as well as to the Microbiology Laboratory, GSC, Vankal for Biological studies. One of the authors Kumar G. Gamit is thankful to all those have helped.

Conflict of interest

The authors have no conflict of interest to declare.

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