Al-Rafidain J Med Sci. 2024;6(2):201-208.
DOI: https://doi.org/10.54133/ajms.v6i2.835

AJMS
Online ISSN (2789-3219)

Research Article

Synthesis, Characterization, Molecular Docking, ADMET Study, and Antimicrobial Evaluation of New Mannich Bases of Isatin–Thiazole Imine Bases

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Received: 18 April 2024; Revised: 17 June 2024; Accepted: 21 June 2024

Abstract

Background: The isatin molecule is present in many natural substances, including plants and animals, and is used to prepare compounds with various biological activities. ***Objectives***: To synthesize a new series of isatin derivatives with the expectation that they will have antimicrobial activity. ***Methods***: Thiazole Schiff bases were synthesized from various Mannich bases of isatin to evaluate their antimicrobial properties. Initially, Mannich bases (**2a–e**) were synthesized by reacting isatin with formaldehyde and different secondary amines. Subsequently, they were treated with 2-aminothiazole to yield the final compounds (**3a–e**). Spectroscopic characterization was done via FT-IR and ^{1}H-NMR. The antimicrobial screening was conducted on all derivatives. Molecular docking and ADMET analysis were performed on the final compounds, comparing them with standard drugs (ciprofloxacin and fluconazole). ***Results***: The antimicrobial activity was assessed on two Gram-positive bacteria, *Staphylococcus aureus* and *Bacillus licheniformis*; two Gram-negative bacteria, *Escherichia coli* and *Acinetobacter baumannii*; and one fungus species, *Candida albicans*. Molecular docking has recorded higher docking scores for **3d** and **3e** compared to ciprofloxacin and fluconazole. The virtually active molecules showed an adequate drug-like profile and desired pharmacokinetic properties in the ADMET analysis. ***Conclusions***: Most derivatives displayed significant antimicrobial activity, with compound **3e** being the most active, followed by compound **3b**. Molecular docking revealed higher scores for compound **3e** compared to fluconazole and for compounds **3d** and **3e** compared to ciprofloxacin. ADMET analysis of compound **3e** showed excellent absorption, consistent with its strong GIT absorption.

Keywords: 2-aminothiazole, Antimicrobial activity, Isatin, Mannich bases.

التوليف والتوصيف والالتحام الجزيئي ودراسة ADMET والتقييم المضاد للميكروبات لقواعد مانيخ الجديدة لقواعد أمين إيساتين-ثيازول

الخلاصة

الخلفية: يوجد جزيء الايساتين في العديد من المواد الطبيعية، بما في ذلك النباتات، ويستخدم لإعداد المركبات ذات الأنشطة البيولوجية المختلفة. **الأهداف**: توليف سلسلة جديدة من مشتقات الإيساتين مع توقع أن يكون لها نشاط مضاد للميكروبات. **الطرق**: تم تصنيع قواعد ثيازول شيف من قواعد مانيخ مختلفة من الإيساتين لتقييم خصائصها المضادة للميكروبات. في البداية، تم تصنيع قواعد مانيش (2 أ - ه) عن طريق تفاعل الإيساتين مع الفورمالديهايد والأمينات الثانوية المختلفة. بعد ذلك، عولجوا ب 2-أمينوثيازول لإنتاج المركبات النهائية (3 أ - ه). تم إجراء التوصيف الطيفي عبر FT-IR و1H-NMR. تم إجراء فحص مضادات الميكروبات على جميع المشتقات. تم إجراء الالتحام الجزيئي وتحليل ADMET على المركبات النهائية، ومقارنتها بالأدوية القياسية (سيبروفلوكساسين وفلوكونازول). **النتائج**: تم تقييم النشاط المضاد للميكروبات على اثنين من البكتيريا إيجابية الجرام، المكورات العنقودية الذهبية و Bacillus licheniformis.اثنين من البكتيريا سالبة الجرام ، الإشريكية القولونية والراكدة baumannii ؛ ونوع واحد من الفطريات، المبيضات البيض. سجل الالتحام الجزيئي درجات إرساء أعلى ل 3 Dو 3 eمقارنة بالسيبروفلوكساسين والفلوكونازول. أظهرت الجزيئات النشطة فعليا مظهرا مناسبا يشبه الدواء وخصائص حركية دوائية مرغوبة في تحليلADMET. **الاستنتاجات**: أظهرت معظم المشتقات نشاطا كبيرا مضادا للميكروبات، حيث كان المركب 3 eهو الأكثر نشاطا، يليه المركب 3b. كشف الالتحام الجزيئي عن درجات أعلى للمركب 3 eمقارنة بالفلوكونازول وللمركبات 3d و 3e مقارنة بالسيبروفلوكساسين. أظهر تحليل ADMET للمركب 3e امتصاصا ممتازا، بما يتفق مع امتصاصه القوي للجهاز الهضمي.

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Article citation: Al-Musawi MH, Al-Mudhafar MMJ. Synthesis, Characterization, Molecular Docking, ADMET Study, and Antimicrobial Evaluation of New Mannich Bases of Isatin–Thiazole Imine Bases. *Al-Rafidain J Med Sci*. 2024;6(2):201-208. doi: https://doi.org/10.54133/ajms.v6i2.835

INTRODUCTION

Among different nitrogen-containing heterocycles, isatin attracts significant attention from scientists due to its wide range of useful properties. Isatin, a naturally occurring compound, is an indole derivative identified in 1988 and has been noted for its extensive range of biological activities, as well as isatin analogs, which are significant chemical groups with considerable applications in medicinal chemistry [1,2]. The high activity of the nitrogen atom, as well as the carbonyl group at position 3 of the γ-lactam ring and the fused benzene ring, contribute to the isatin's chemical properties. Isatin derivatives have many well-known biological effects, including their ability to kill microbes [3], fight cancer, tuberculosis, and seizures [4], act as antioxidants, and fight Alzheimer's disease, diabetes, and arthritis [5]. Imine, also named Schiff based on the honor of Hugo Schiff, was first prepared in the 9th century [6]. An aldehyde or ketone and a primary amine are combined to create a Schiff base; this is achieved by substituting an imine group for the aldehyde or ketone's carbonyl group [7-9]. Azomethine's nitrogen atom has the potential to interfere with regular cell functions by forming hydrogen bonds with the active sites of cell components [10]. Mannich reactions are three-component condensation reactions that involve compounds such as formaldehyde, a primary or secondary amine, and a carbonyl compound. Mannich bases have many activities like anticancer, anti-inflammatory, analgesic, antifungal, antibacterial, antiviral, *antiepileptic*, antituberculosis, and antihistamine effects [11,12]. It has been suggested that parent amines and amides' lipophilicity can be increased by the Mannich base functional group, enhancing absorption across biomembranes. Additionally, Mannich bases can pass through fungal and bacterial membranes due to their lipophilicity [13]. Also, isatin derivatives can take part in the N-alkylation and N-acylation reactions of Mannich and Michael through the amide group [14–16]. *N*-aminomethylisatins, also referred to as Mannich *N*-bases, have the potential to be useful therapeutic agents for the treatment of malaria, tuberculosis (TB), cancer, and infections caused by both Gram-positive and Gram-negative bacteria, as well as certain fungi [4]. Thiazoles are cyclic organic compounds with heteroatoms of sulfur and nitrogen. In pharmaceutical chemistry, the pharmacophore 2-aminothiazole is a useful building block that can be used to synthesize a wide variety of heterocyclic compounds with a wide range of biological activities [17], such as anti-bacterial [18], antifungal [19], and anti-cancer [20]. Because of their structural characteristic, which is hydrophobic, the five-membered ring helps them pass through biological membranes more easily. Over the past decade, there has been growing interest in the Schiff's and Mannich bases of isatins due to their easy chemical synthesis and diverse pharmacological properties, such as antibacterial and antifungal [1], antiprotozoal [21], anthelminthic [22], antiviral, and anti-HIV [23]. Many Schiff's and Mannich bases of isatin have been reported to demonstrate broad-spectrum biological activities. Pandeya *et. al.* [24] described the synthesis of isatin's Schiff bases and its Mannich base derivatives with 4-(4'-chlorophenyl)-6-(4"-methyl phenyl)-2-aminopyrimidine. These derivatives have strong antibacterial and antifungal properties as well as an inhibitory effect on HIV-1 replication in human MT-4 cells. In another study, Karki *et al.* [25] synthesized a series of isatin-β-thiosemicarbazones; these compounds were then exposed to various influenza viruses; the majority of these derivatives exhibited a moderate level of effect toward HEL cell cultures in contrast to acyclovir (the standard reference). A new class of Schiff and Mannich base derivatives of 5-fluoroisatin was synthesized. The *N*, *N*-(dimethylamino) methyl-substituted Mannich base demonstrated potent antibacterial, anti-inflammatory, and analgesic action with the lowest ulcer index of all the derivatives examined [26]. Recently, Mukhlif and his coworker demonstrated the synthesis of various monomers of Mannich and bis-Mannich bases of 3-(2-phenylhydrazineylidene) indolin-2-one (Schiff base). Most of the synthesized derivatives show activity against *S. aureus* and *E. coli* pathogenic bacteria and moderate activity against *C. albicans* fungi [3]. Antifungals inhibit CYP51, an enzyme involved in ergosterol synthesis that binds heterocyclic nitrogen to heme iron. The CYP51 ligand-binding active site comprises four parts: An H-bond with water, a narrow region that repels water, a region that does not repel H_2O, and a coordination bond with iron in the heme group [27]. There are four classes of bacterial topoisomerases: I–IV. Topoisomerases II and IV are attractive antibacterial targets for a variety of reasons. These proteins duplicate bacterial DNA and are required for survival. Typically, inhibiting bacterial function kills them. These enzymes differ structurally from human enzymes when used in bacterial analyses. Researchers discovered numerous enzyme target areas, particularly for quinolones, which are potent antimicrobials. Drug-resistant bacteria emerge as a result of changes in DNA gyrase/topoisomerase IV, membrane permeability, drug efflux upregulation, and the widespread use of quinolone inhibitors [28]. The goal of this study is to synthesize different *N*-Mannich bases of isatin conjugated to the thiazole ring via imine linkage and predict their antimicrobial activity.

METHODS

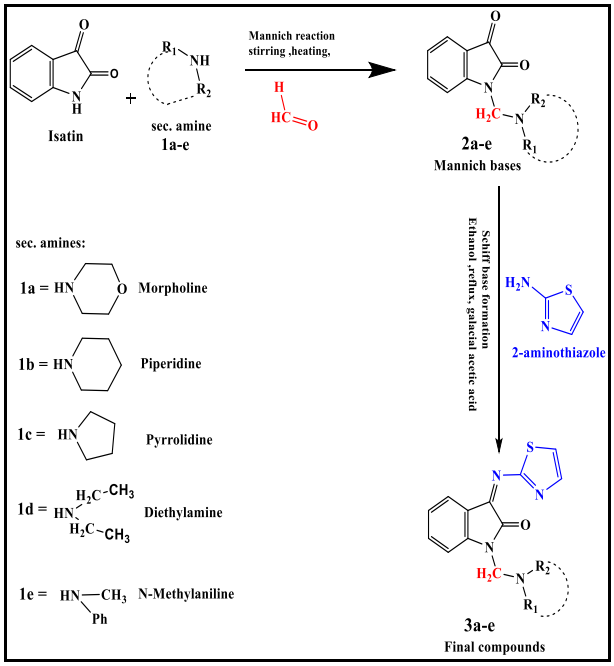
Materials and instruments

Hyper-chem (China) provided all of the substances—isatin, 2-aminothiazole, morpholine, piperidine, and pyrrolidine—used in the synthesis. Thin-layer chromatography (TLC) with Silica Gel GF254 (type 60) pre-coated aluminum sheets from Germany by Merck Company was utilized to monitor the completion of reactions and the compounds' purity. UV-254 light was employed to visualize the spots. The solvent systems employed were ethyl acetate, ethanol, and hexane (4:2:4) and ethyl acetate and hexane (3:7). Melting points were measured using Stuart's (SMP3) melting point apparatus and were uncorrected. The

Fourier transform infrared (υ, cm^{-1}), (Shimadzu, WQF-520/Japan) at the College of Pharmacy/University of Baghdad was used to determine the infrared spectrum. An NMR ultra-shield spectrophotometer of 400 MHz, BRUKER (Iraq/at the University of Basra), was used to record the proton nuclear magnetic resonance (^{1}H-NMR) spectra. For sample analysis, the applied solvent was DMSO-d_6. The molecular docking studies were performed utilizing a licensed Schrodinger suite of Glide, Desmond, and Qikprop applications at the College of Pharmacy/University of Baghdad.

Synthesis of N-Mannich bases of isatin (Compounds 2a-e)

After dissolving equimolar (0.002 moles) of isatin in 20 ml of THF (tetrahydrofuran), 0.002 moles of each secondary amine were added separately, followed by 2 ml of a 37% aqueous formaldehyde solution (Scheme 1). The mixture was allowed to reflux for 3 hours following an hour of standing at room temperature. Then, it was left in the refrigerator for 48 hours. Chloroform/petroleum ether 40–60 was used to recrystallize the resultant compounds (**2a–e**).



Scheme 1: The final compounds (3a-e) and their intermediates' steps of synthesis.

Analytical data for intermediate compounds (2a-e)

1-(morpholino ethyl) indoline-2,3-dione (2a)

Chemical formula: ($C_{13}H_{14}N_2O_3$), orange powder with a 60% yield, m.p: (125-128˚C). FT-IR (υ, cm^{-1}): 3035 (C-H aromatic stretching), 2954, 2858 (CH_2 Asymmetric and symmetric stretching), 1728 (C=O ketone stretching), 1608 (C=O ketone stretching), 1465-1436 (C=C aromatic stretching), 995 (stretching of C-O-C), 856 (stretching of C-N), 763 (out of the plane C-H bending of aromatic ring), and 671 (C=C of aromatic-ring bending).

1-(piperidin-1-ylmethyl) indoline-2,3-dione (2b)

Chemical formula: (C14H16N2O2), orange powder with a 59% yield, m.p: (130-135˚C), FT-IR (υ, cm^{-1}): 3047 (C-H Aromatic stretching), 2935, 2850 (CH_2 Asymmetric and symmetric stretching), 1728 (C=O amide stretching), 1608 (C=O ketone stretching), 1469 and 1442 (C=C aromatic stretching), 759 (out of the plane C-H bending of aromatic ring), 694 (C=C of aromatic-ring bending).

1-(pyrrolidin-1-ylmethyl) indoline-2,3-dione (2c)

Chemical formula: ($C_{13}H_{14}N_2O_2$), yellow powder with a 66% yield, m.p: (135-138˚C). FT-IR (υ, cm^{-1}): 3062 (C-H aromatic stretching), 2958, 2877 (CH_2 Asymmetric and symmetric stretching), 1708 (C=O ketone stretching), 1612 (C=O amide stretching), 1469 (C=C aromatic stretching), 752 (out of the plane (C-H) bending of aromatic ring), 678 (C=C of aromatic-ring bending).

1-((diethylamino)methyl)indoline-2,3-dione (2d)

Chemical formula: ($C_{13}H_{16}N_2O_2$), orange powder with a 50% yield, m.p: (126-128˚C). FT-IR (υ, cm^{-1}): 3039 (C-H aromatic stretching), 2962, 2835 (CH_2 Asymmetric and symmetric stretching), 1728 (C=O ketone stretching), 1604 (C=O amide stretching), 1465, 1411 (C=C aromatic stretching).

1-((methyl(phenyl)amino)methyl)indoline-2,3-dione (2e)

Chemical formula: ($C_{16}H_{14}N_2O_2$), orange powder with a 70% yield, m.p: (120-124˚C). FT-IR (υ, cm^{-1}): 3066 (C-H aromatic stretching), 2951, 2893 (CH_2 Asymmetric and symmetric stretching), 1732 (C=O ketone stretching), 1600 (C=O amide stretching), 1469, 1423 (C=C aromatic stretching).

Synthesis of Schiff base final compounds (3a-e)

In the 100-ml round-bottom flask, 10 ml of absolute ethanol was employed, and Mannich base derivatives **2(a–e)** of about 0.001 moles were added separately. After adding five drops of glacial acetic acid and stirring it with heat, we added 0.0012 moles of 2-aminothiazole and refluxed the mixture for 10 hours. After the reaction was monitored by TLC, the mixture was left overnight. After the solvent was vacuum-pressured and evaporated, the residue was washed many times with ethanol to remove any unwanted material, then recrystallized by ethanol-chloroform.

Analytical data for final compounds (3a-e)

1-(morpholinomethyl)-3-(thiazol-2-ylimino)indolin-2-one (3a)

Chemical formula: ($C_{16}H_{16}N_4O_2S$), red powder with a yield of 50%, m.p: (160-166˚C). FT-IR (υ, cm^{-1}): 3035 (C-H aromatic stretching), 2954,2897 (CH2

Asymmetric and symmetric stretching), 1693 (C=O amide stretching), 1616 (C=N imine stretching), 1543 (C=N of thiazole stretching), 1462 (C=C aromatic stretching), 1002 (stretching of C-O-C), 860 (stretching of C-N), 748 (out of plane C-H bending of aromatic ring), 678 (bending C=C of aromatic-ring). ^{1}H-NMR δ ppm: 7.66-6.90 (6H, m, Aromatic-H), 4.66 (2H, s, CH_2), 3.58-3.56 (4H, t, CH_2), 2.42-2.39 (4H, t, CH_2).

1-(piperidin-1-ylmethyl)-3-(thiazol-2-ylimino)indolin-2-one (3b)

Chemical formula: ($C_{17}H_{18}N_4OS$), red powder with a yield of 55%, m.p: (187-189˚C). FT-IR (υ, cm^{-1}): 3028 (C-H aromatic stretching), 2939, 2858 (CH_2 Asymmetric and symmetric stretching), 1690 (C=O amide stretching), 1612 (C=N of imine stretching), 1543 (C=N of thiazole stretching), 1516-1465 (C=C aromatic stretching), 887 (stretching of C-N), 748 (out of the plane (C-H) bending of aromatic ring), 678 (C=C of aromatic-ring bending). The ^{1}H-^{1}H-NMR (δ=ppm): 7.97-6.92 (6H, m, Aromatic-H), 4.08 (2H, s, CH_2), 2.28-2.24 (4H, t, CH_2), 1.59-1.53 (4H, t, CH_2), 1.26-1.21 (2H, m, CH_2).

1-(pyrrolidin-1-ylmethyl)-3-(thiazol-2-ylimino) indolin-2-one (3c)

Chemical formula: ($C_{16}H_{16}N_4OS$), dark red powder with a yield of 74%, m.p: (164-168˚C) FT-IR (υ, cm^{-1}): 3066 (C-H aromatic stretching), 2958, 2873 (CH_2 Asymmetric and symmetric stretching), 1658 (C=O amide stretching), 1612 (C=N imine stretching), 1512 (C=N thiazole stretching), 1500, 1469 (C=C aromatic stretching), 864 (stretching of C-N), 752 (out of the plane (C-H) bending of aromatic ring), 678 (C=C of aromatic-ring bending). ^{1}H-NMR (δ=ppm): 7.96 -6.67 (6H, m, Aromatic-H), 4.72 (2H, s, CH_2), 1.91-1.90 (4H, t, CH_2), 1.26-1.22 (4H, t, CH_3).

1-((diethylamino)methyl)-3-(thiazol-2-ylimino) indolin-2-one (3d)

Chemical formula: ($C_{16}H_{18}NOS$), brown powder with a yield of 75%, m.p:(164-168˚C), FT-IR (υ, cm^{-1}): 3069(C-H aromatic stretching), 2978, 2877(CH2 Asymmetric and symmetric stretching), 1660(C=O amide stretching), 1616 (C=N imine stretching), 1512 (C=N thiazole stretching), 1469 (C=C aromatic stretching), 860 (stretching of C-N), 752 (out of the plane (C-H) bending of aromatic ring), 678 (C=C of aromatic-ring bending).^{1}H-NMR (δ=ppm): 7.39 -6.54 (6H, m, Aromatic-H), 4.42 (2H, s, CH_2), 2.74-2.53 (4H, q, CH_2), 1.11-1.06(6H, t, CH_3).

1-((methyl(phenyl)amino)methyl)-3-(thiazol-2-ylimino) indolin-2-one (3e)

Chemical formula: ($C_{19}H_{16}N_4O_S$), red powder with a yield of 74%, m.p: (244-246˚C). FT-IR (υ, cm^{-1}): 3066(C-H aromatic stretching), 2978, 2877(CH_2 Asymmetric and symmetric stretching), 1655(C=O amide stretching), 1612 (C=N imine stretching), 1508 (C=N thiazole stretching), 1469(C=C aromatic stretching). ^{1}H-NMR (δ=ppm): 7.61 -6.60 (11H, m, Aromatic-H), 4.48 (2H, s, CH_2), 2.82-2.75 (3H, s, CH_3).

Antimicrobial activity

The researchers measured the antimicrobial activity of the resultant compounds using the well-diffusion method [29]. We conducted in vitro tests to assess the antimicrobial efficacy of the synthetic compounds against three distinct types of microorganisms: fungi (*Candida albicans*), Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus licheniformis*), and Gram-negative bacteria (*Acinetobacter baumannii and Escherichia coli*). We experimentally stimulated these microbes and kept them on nutritional agar for antimicrobial examination. Fluconazole is a typical medicine with antifungal properties. We measured the inhibition zone (IZ) in millimeters and dissolved all synthesized compounds and standard drugs in Dimethyl sulfoxide (DMSO) to achieve a concentration of 10 mg/mL.

Molecular docking

We conducted molecular docking studies using the Glide tool in the Maestro platform 13.0.135, 2021-4 of Schrodinger Suite, LLC, New York, NY, 2021, to evaluate the binding affinity of synthesized isatin derivatives into the binding sites of CYP-450 14-alpha sterol demethylase and topoisomerase IV enzymes [30,31]. The suggested fragments' chemical structure was created using the Ligprep module.

Protein preparation and grid generation

We downloaded the crystal structure for [CYP-450 14 alpha-sterol demethylase (CYP51)] (pdb code: [1EA1]) and [Topoisomerase IV (TopoIV)] (pdb code: [5EIX]) proteins from the Protein Data Bank and prepared them using the protein preparation tool [32]. Proteins were processed using the protein preparation wizard in Schrodinger (New York, NY, in 2021) to remove water molecules and non-essential atoms, after which the missing atoms from the protein residue were added, hydrogen was added, and OLPS 2005 was used to optimize the hydrogen bonds [30,31]. All proteins conserve NAD as a cofactor in their cores, especially when co-crystallized with a ligand. We created the receptor grid by docking the prepared ligands in the protein binding site, using the co-crystallized ligand as the center for the boundary box. We used a boundary box with a 12 A0 dimension [33]. We utilized the OPLS_2005 force field to minimize the energy of the protein [34]. We generated the grid boxes using the co-crystallized bound ligands as references.

Ligand separation

All ligands were sketched in ChemDraw version 18.0.0.231 (4029) and entered as input files into the prepare ligand module. The structures were optimized for the lowest energy after the force fields were applied

to the ligands using LigPrep [30]. Flexible ligand docking was implicated under the OPLS_2005 force field. A standard precision docking mode was exploited to generate 10 poses per ligand.

ADMET study

The drug-like properties of the top-ranked compounds were expected by Lipinski's rule of five, and the ADMET descriptors were calculated using Schrodinger Maestro's Qikprobe software. Many molecular properties are taken into consideration by Lipinski's rule of five, including the total number of hydrogen bond donors and acceptors [35]. The main goal of the study is to identify the most important pharmacokinetic characteristics of the compounds, including their intestinal absorption, systemic distribution, hepatotoxicity, excretion, metabolism, and aqueous solubility, as well as other ADMET descriptors.

RESULTS

This pertains to the chemical synthesis of intermediate compounds, specifically Mannich bases (**2a–e**). For each prepared Mannich base, it showed the disappearance of the N-H stretching band of isatin, which is about 3200 cm^{-1}. Also, in Synthesis of Schiff of Mannich base final compounds **(3a–e)**, the production of these compounds was confirmed by the appearance of new IR bands at 1612–1689 cm^{-1}(C=N imine) stretching and at 1512-1612cm^{-1} (C=N) stretching of the thiazole ring. They also found two peak ranges in the 1H-NMR spectrum (µ=ppm): 4.48 (2H, s, CH2) or 3.03 (2H, s, CH2) of CH2 between the N-terminus of an amine and the N-terminus of isatin. In the antimicrobial evaluation, in light of the results given in Table 1, the compounds (**3b**, **3d,** and **3e**) display broad-spectrum activity at 10 mg/mL against all examined bacteria: gram-positive (*Staphylococcus aureus*, *Bacillus licheniformis*), gram-negative *(Escherichia coli*, *Acinetobacter baumannii)*, except compound (**3c**), which has no activity compared to ciprofloxacin with the same concentration.

Table 1: Antimicrobial activity of final compounds (**3a-e**) (10 mg/mL)

Compounds	Gram-positive		Gram-negative		Fungi
	S. aureus	*B. licheniformis*	*E. coli*	*A. baumannii*	*C. albicans*
	Zone of inhibition (mm)				
3a	17	-	17	5	15
3b	15	15	17	16	21
3c	-	-	-	-	-
3d	15	10	17	12	20
3e	20	17	18	15	27
Ciprofloxacin	35	50	40	13	-
Fluconazole	-	-	-	-	20

(-) = without activity, slightly active (zone of inhibition within 5 to 10 mm), moderately active (inhibition zone within 10 and 20 mm), and strongly active (zone of inhibition more than 20 mm).

For the antifungal activity, the compounds (**3a, 3b, 3d,** and **3e**) display moderate to strong activity against *Candida albicans*, while **3c** shows no effect (Figure 1).

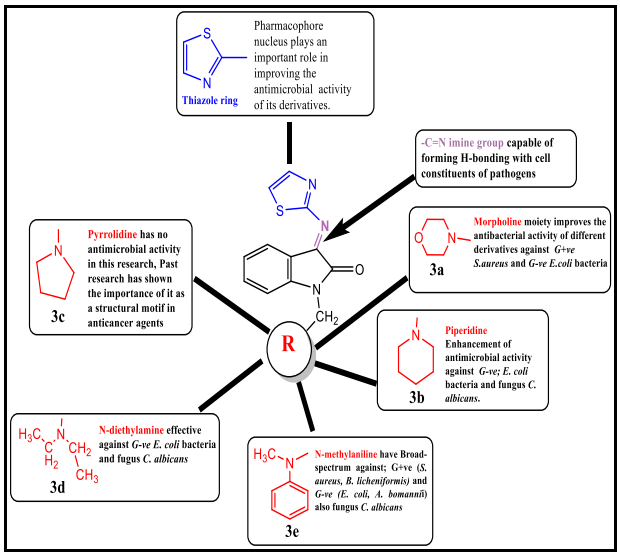


Figure 1: A summary of the antimicrobial activity of different isatin–thiazole Mannich bases derivatives.

In the docking study, the results presented in Table 2 offer valuable insights into the binding affinities of two compounds, 3e and 3b, in comparison to the reference compound fluconazole and the other two compounds, **3d** and **3e**, in comparison to the standard compound ciprofloxacin.

Table 2: The binding energy of the final compounds and the ligands in kcal/mol

Compounds	Docking Score
Fluconazole (1ea1- minimized)	-7.032
3a	-7.029
3b	-7.076
3c	-6.715
3d	-6.415
3e	-7.375
Ciprofloxacin (5eix-minimized)	-4.728
3a	-3.727
3b	-3.576
3c	-3.408
3d	-6.535
3e	-5.488

The purpose of this study was to determine whether these compounds, as inhibitors of CYP-450 14 alpha-sterol demethylase and topoisomerase, have the highest binding affinity, with docking scores of -6.535 and -5.488, respectively, compared to ciprofloxacin (-4.728). Figure 2 Explain how they bind and interact with the active site. Firstly, the two compounds demonstrated superior docking scores compared to fluconazole. Compound **(3e)** displayed the highest binding affinity with a docking score of (-7.3)**,** followed closely by compound (**3b)** with a score of (-7.07**)**. This means that these chemical compounds have

a high likelihood of binding efficiently to the **(1ea1)** binding pocket. The other two compounds (**3d**, **3e**) have the highest binding affinity, with docking scores of -6.535 and -5.488, respectively, compared to ciprofloxacin (-4.728). The drug-likeness properties (ADMET) include absorption, distribution, metabolism, excretion, and toxicity characteristics. The compounds were assessed using ADMET studies and Lipinski's rule of five. We analyzed the hit compounds' various physicochemical properties, which are necessary for drug development, in order to determine how drug-like they were. When compared to standard drugs, all compounds meet the acceptable range of the Lipinski rule. Furthermore, every compound mentioned showed an optimal profile for the percentage of human oral absorption. Compound (**3e**) exhibits no CNS penetration compared to other compounds, indicating that it does not cause any CNS side effects (Table 3).

Table 3: Drug likeness properties of promising compounds

Comp. ID	Rule of 5	Rule of 3	% Oral absorption	CNS
Ciprofloxacin	0	1	82.104	0
Fluconazole	0	0	48.865	0
3a	0	0	76.216	1
3b	0	0	84.522	1
3c	0	0	82.945	1
3d	0	0	83.223	1
3e	0	0	100.000	0

Rule of Five Number of violations of Lipinski's rule of five. The rules are mol_MW < 500, donor HB ≤ 5, acceptor HB ≤ 10, and QPlogPo/w < 5. Compounds that satisfy these rules are considered drug-like. The three rules are QPlogS > -5.7, QP PCaco > 22 nm/s, Primary Metabolites < 7. Compounds with fewer (and preferably no) violations of these rules are more likely to be orally available. Maximum is 100 percent of Human Oral Absorption Predicted qualitative percent human oral absorption: 48, 76, or 100 for low, medium, or high, respectively. CNS Predicted central nervous system activity on a –2 (inactive) to +2 (active) scale.

DISCUSSION

It was possible to make intermediate compounds Mannich bases (**2a–e**) by reacting the acidic hydrogen of -NH- of isatin with various secondary amines while 37% formaldehyde was present. In this reaction, the nitrogen of the secondary amine attacked the formyl carbonyl's carbon atom in a nucleophilic reaction, then water was eliminated and this, in turn, was reacted with the isatin molecule. The ultimate Schiff bases **(3a–e)** are obtained by condensation of Mannich bases separately with 2-aminothiazole. The first step in the acid-catalyzed, reversible process of Schiff base formation is the nucleophilic addition reaction. This is when the nitrogen atom of 2-aminothiazole attacks the partially positive carbon atom of isatin's ketone group, and water is then removed [36]. The antimicrobial evaluation revealed that compound **3e**, which has a phenyl group connected to the amide group of isatin through the methylene group, is the most potent among all derivatives against all tested microorganisms [37]. Additionally, **3e** has greater efficacy against fungi (*C. albicans*). In the docking study, we see that these compounds (**3e**, **3b**, and **3d**) share structural similarities with indole, thiazole, and cyclic secondary amines (N-methyl aniline, piperidine, and diethyl amine, respectively). This may be a big reason why they bind more strongly. These structural components differ from ciprofloxacin and fluconazole, which contain fluoroquinolone and triazole groups, respectively. These differences mean that the presence of indole, thiazole, and secondary amines in these compounds may contribute to their increased binding affinity. This implies that they require less energy to bind with 1ea1 and 5eix. There are some structural differences that might make the interaction with important residues in the 1ea1 and 5eix binding pockets better (Figure 2).

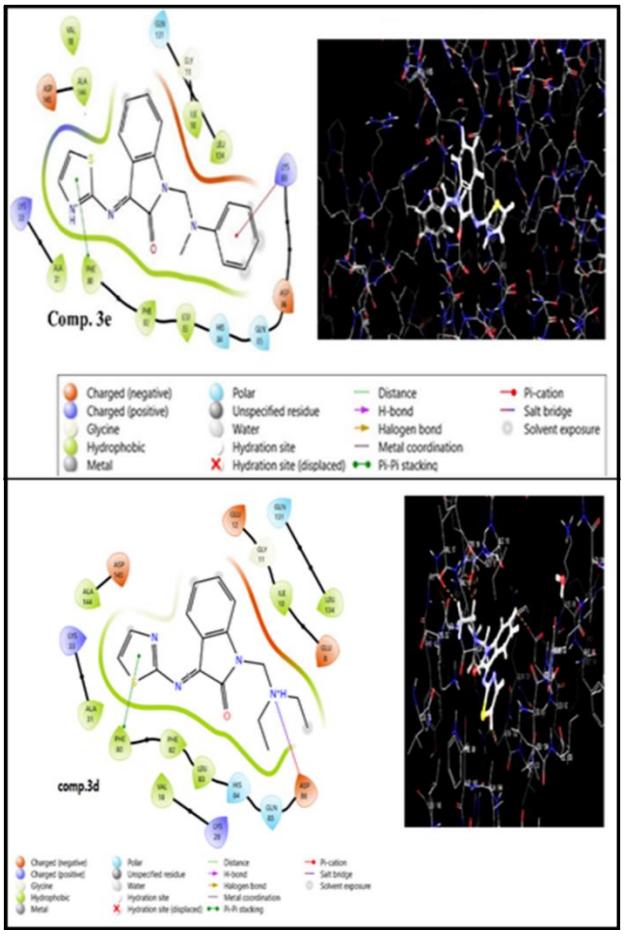


Figure 2: Top docked ligands with 1ea1 and 5eix enzymes.

Secondary amines, like N-methylaniline, have heteroatoms (N) that make it more likely that they will form an H-bond with the protein's active site [1–1]. Also, the secondary amine (N-methylaniline) in the designed compound (**3e**) could be placed into the hydrophobic pocket formed by PHE 81, 82, and LEU 83. Additionally, the phenyl ring in this secondary amine forms a pi-cation interaction with LYS 89, while the thiazole ring forms a pi-pi stacking interaction with PHE 80 and a polar bond with HIS84, GLN85. While compound **3d** could be placed into the hydrophobic pocket formed by TYR15, GLY16, VAL17, VAL18, PHE 80, GLU 81, PHE 82, and LEU83. This chemical alteration will allow newer Mannich bases and Schiff bases of the 2-aminothiazole ring to interact by many bonds with the active site of proteins, so we may expect these promising compounds to enhance activities. The drug-likeness properties (ADMET) determine whether the drug succeeds or fails, and these characteristics are often rate-limiting during the drug

development process. Consequently, it is critical to understand the anticipated ADMET attributes of the most promising leads in order to minimize the likelihood of late-stage attrition.

Conclusion

A first test in the lab using new Schiff's Mannich bases of isatin derivatives (**3a–e**) showed that 10 mg/mL of these compounds was effective against all the bacteria and fungi that were tested, except for (**3c**). Comparing compound **(3e)** with other derivatives, the former demonstrated strong, broad-ranging antibacterial and antifungal activity. The docking results presented in this study demonstrate the binding affinities of two compounds, namely (**3e**), in comparison to the reference compound fluconazole. Compound **(3e)** displayed the highest binding affinity with a docking score of (-7.3), as did compound **(3d),** which displayed the highest docking score of (-6.535) compared with ciprofloxacin. The ADMET study shows that these compounds are drug-like molecules and have an acceptable pharmacokinetic profile. Compound **3e** showed excellent absorption, consistent with its strong GIT absorption.

Conflict of interests

No conflict of interests was declared by the authors.

Funding source

The authors did not receive any source of fund.

Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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