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Synthesis, Characterization, Molecular Docking, and Antimicrobial Evaluation of New Acetylenic Mannich Bases of Isatin–Thiazole Derivatives

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Abstract

The isatin molecule is present in many natural substances, like plants and animals, and it is utilized to prepare compounds with various biological activities. A series of Schiff and Mannich bases derived from isatin were synthesized in this research. First, acetylenic Mannich bases (**IIa-e**) were prepared by a reaction of isatin with propargyl bromide and different secondary amines (morpholine, piperidine, pyrrolidine, dimethylamine and diphenylamine) separately, cuprous chloride (CuCl) was used as a catalyst. Second, these Mannich bases were treated with 2-aminothiazole to obtain the desired final compounds (**AM1-AM5**). The synthesized compounds were characterized on the basis of their spectral (FT-IR, ^{1}H- NMR) data. All these derivatives had been screened for their antimicrobial activity against two Gram-positive bacteria; *"Staphylococcus aureus* and *Bacillus licheniformis"*, two Gram-negative bacteria; *"Escherichia coli* and *Acinetobacter baumannii"*, one fungus species, "*Candida albicans*". Most of the compounds were found to exhibit a significant antimicrobial activity and the most active one is compound **AM1**. The molecular docking was performed for the final synthesized compounds and then compared with the standard drugs (Ciprofloxacin and Fluconazole). Molecular docking has recorded higher docking scores of two final derivatives (**AM1** and **AM2**) in comparison with fluconazole.

Keywords: Acetylenic Mannich base, 2-aminothiazole, Antimicrobial activity, Propargyl bromide, Isatin

Introduction

Many diseases caused by different microbes as bacteria, fungi and other yeast are on the rise in combination with the rapid growth of the population of immunocompromised patients. The medical field faces difficulties with infections produced by these microbes. These microorganisms pose a serious challenge and highlight the necessity of new, highly effective and more selective antimicrobial medications. The incidence of bacterial diseases has significantly increased in recent years [(1)]. The importance of drug resistance by microorganisms is becoming more and more important in both human and animal health [(2)]. Since aromatic heterocycles represent the basis for the side groups of the most widespread and vital components of living cells, heterocyclic compounds play a significant role in biological processes. As the field of organic synthesis has developed, researchers have continued to be interested in heterocyclic compounds that contain sulphur- and/ or nitrogen. These compounds include fused thiazoles, thiadiazoles, triazoles, and oxadiazoles, which are structural elements of numerous biologically active compounds [(3)].

One of the indole derivatives is regarded as a fundamental class of heterocyclic compounds: isatin (2, 3-dioxindole). Derivatives of isatin are significant synthetic substrates that can be used as building blocks for the synthesis of a wide range of heterocyclic compounds, including medications. Because of their effective biological and pharmacological activities, isatin derivatives have drawn a lot of attention in organic and pharmaceutical chemistry [(4)]. They possess many biological characteristics such as antibacterial, antifungal [(5-10)], anti-HIV [(11, 12)], antiviral [(13)], anti-tubercular [(14)], and anti-inflammatory [(15)].

A five-membered heterocyclic aromatic compound known as thiazole was first prepared in 1888 by Hantzsch [(16)]. This ring is one of the important pharmacophores in the process of developing new medicinal drugs. Many derivatives of thiazole moiety cover a wide range of biological activities, including antifungal, antibacterial, antitumor, anti-allergic, antipyretic, anti-inflammatory and anti-HIV [(17-19)]. Many novel medications were approved clinically for healing certain diseases having an aminothiazole nucleus; Famotidine is used for treating peptic ulcers and

gastroesophageal reflux. Abafungin is a drug applied for dermatomycoses and a third-generation cephalosporin known as Cefdinir, which is a broad-spectrum semi-synthetic antibiotic [19]. New approaches to drug design aim to introduce certain functional groups that are physiologically inert but can be activated when desired. Alkynes satisfy these requirements by being reasonably stable but having the ability to become extremely reactive when a relevant trigger is present, providing exciting opportunities. In addition to acting as a location for the complicated functionalization of biomolecules, this functions as an important pharmacophoric key unit for various classes of antibiotics [20]. Accordingly, a study revealed that acetylenic Mannich bases bearing biphenyl-4,4-dithiol moiety were properly synthesized and their antimicrobial activity was evaluated on different microorganisms and most of them were effective against *Staphylococcus aureus* and *Candida albicans* [21].

There is an obvious relationship between lipophilicity and activity. Adding acetylenic groups into bioactive compounds, such as isatin and its analogs, could improve their therapeutic efficacy and minimize their toxicity [22]. This effect may arise from greater cell membrane penetration [22]. Numerous biological functions, including cytotoxic, antiviral and caspase-inhibiting properties, have been revealed by *N*-acetylenic isatins. [23]. Singh *et. al* [20] study the antimicrobial activity of different acetylenic isatin derivatives and it was reported that acetylenic isatin of fluoro-substituted Schiff bases had the most potent antibacterial activity against *E. coli*, while the bis-isatin assembly was the most effective compound against *C. albicans*.

Mannich reaction has been used in the organic synthesis of natural substances such as, nucleotides, alkaloids (tropinone), and peptides [24]. It is also used in the synthesis of many medicinal drugs that have strong anti-inflammatory, anticancer, antibacterial, antifungal, anticonvulsant, antitubercular, analgesic, antiviral and antihistamine activities [25-27]. *In vitro* study revealed that various *N*-Mannich bases of isatin-imino ciprofloxacin derivatives showed enhancement in the lipophilicity and were more effective against mycobacteria than ciprofloxacin itself [28].

Conventional bases known as Schiff bases were discovered by German scientist Hugo Schiff (1864 –1915) [29]. Under specific situations, a primary amine reacts with carbonyl compounds (ketones or aldehydes) to obtain those bases [30]. The functional group of Schiff bases (azomethine) is (>C=N–) particularly those associated with heterocyclic moiety, exhibits a wide range of pharmacological properties including antibacterial, antifungal, antioxidant, anticancer, anticonvulsant, and anti-inflammatory properties. [31, 32]. Furthermore, due to Schiff bases' structural similarity to naturally occurring biological molecules, they represent an important family of compounds [33] and their medicinal effects may be explained by the azomethine group that forms H-bonding with the microorganisms' active region of the cell and interferes with the normal functioning of the cell [34]. It was demonstrated that isatin Schiff bases' derivatives possess numerous biological activities, including anticonvulsant, anti-inflammatory, antibacterial, anti-fungal, anti-HIV, and antidepressant effects [35]. Suaad *et. al.* [36] synthesized different acetylenic Mannich bases from Schiff base known as 3-(2, 3-dimethyl phenyl- imino)-1H-indole-2-one, it was found that morpholino Mannich base possesses potent activity against three different types of bacteria; *Staph. aureus*, *Staph. epidermidis*, and *Pseudomonas aeruginosa*. Azoles have a high therapeutic index, making them first-choice drugs for treating invasive fungal infections.

Most fungal infection treatments target either the end product or the process that leads to the biosynthesis of ergosterol. Lanosterol 14-α-demethylase (CYP51) is a member of the cytochrome P450 monooxygenase superfamily in fungi. It is responsible for the oxidative removal of lanosterol's 14-methyl group (C-32) to produce 14,15-desaturated intermediates in the biosynthesis of ergosterol [37].

The antifungal drugs function blocking CYP51, a necessary enzyme in the biosynthesis of ergosterol, which is responsible for binding of the heterocyclic nitrogen atom of compounds to the heme iron atom. Fungal resistance to pharmaceuticals in clinical use, particularly fluconazole, can also extend to new structurally related azoles like ravuconazole and voriconazole. Genetic mutations and increased use of antifungal medications are the causes of this resistance. Resistance reveals the need for novel, broad-spectrum antifungal drugs with better therapeutic indices than fluconazole. In general, The CYP51 ligand binding active site is consist of four subsites: the hydrophilic H-bonding region, the narrow hydrophobic region the hydrophobic region, , and a coordination bond with iron of the heme group [38].

Bacterial topoisomerases are classified into four main classes, I through IV. Type IA topoisomerases are bacterial topoisomerases I and III. Type IIA topoisomerases are bacterial topoisomerases II and IV. The type IIA topoisomerases have been the subject of the majority of research on antibacterial topoisomerase inhibitors.

For a number of reasons, topoisomerases II and IV make excellent antibacterial targets. (1) These are proteins involved in bacterial DNA replication that are necessary for the survival of bacteria. (2) they

are vital components of all bacteria, (3) any inhibition of their function in bacteria usually leads to a bactericidal (versus bacteriostatic) (4) To enable bacterial specificity, they differ structurally from their counterparts in mammalian enzymes. (5) multiple target sites have been identified within the enzymes. Quinolones are highly potent antimicrobial medications. However, the development of drug-resistant bacteria as a result of modifications in DNA gyrase/topoisomerase IV, or a reduction in intracellular drug levels brought on by modifications in membrane permeability or overexpression of drug efflux, poses a serious barrier to the widespread use of quinolone inhibitors [(39)].From the previous investigations, the N-alkylation and Mannich base formation of isatin Schiff bases will enhance the biological activity of isatin itself. The goal of the present study is to synthesize different Schiff bases of 2-aminothiazole and *N*-propargylated Mannich bases of isatin and predict their antimicrobial activity. Moreover, the molecular docking study prepared a set of isatin-thiazole derivatives (**AM1-AM5**) and found whether these derivatives have an inhibitory effect on (Cytochrome p450 14 alpha-sterol demethylase) and (topoisomerase IV).

Materials and Methods

General

Hyper-chem (China) provided all of the substances; isatin, propargyl bromide, 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 purity of compounds. UV-254 light was employed to visualize the spots. The solvent systems employed were methanol: hexane (8:2) and ethyl acetate: hexane (3:7). Melting points were measured by using Stuart (SMP3) melting point apparatus and are uncorrected. The infrared spectra were determined using a Fourier Transform Infrared (υ, cm^{-1}), (Shimadzu, WQF-520/ Japan) at the University of Baghdad/ College of Pharmacy. An NMR ultra-shield spectrophotometer of 600 MHz, BRUKER (Iran), was used to record the proton nuclear magnetic resonance (^{1}H-NMR) spectra. The solvent applied for sample analysis was DMSO-d_6. The molecular docking studies were performed utilizing a licensed Schrodinger suite of Glide, Desmond, and Qikprop applications, at the University of Baghdad/College of Pharmacy.

Chemical synthesis

Synthesis of N-propargylated isatin (Compound I) *(40)*

Procedure A*: Heating conditions supported by a microwave with a power of 100 W*

A solution of isatin (0.001 mole, 0.147 g) and anhydrous K_2CO_3 (0.0015 mole, 0.207 g) was dissolved in anhydrous DMF in a microwave vessel, followed by propargyl bromide (0.0015 mole, 0.167 mL). A TLC of solvent system ethyl acetate and toluene, 1:1 volume by volume, was used to monitor the reaction process. Iced water in the same amount was utilized to dissolve non-organic salts when the process was finished. Then after filtration, the precipitate was washed with cold water and dried. The product was recrystallized from 96% ethanol. The *N*-propargyl isatin of an orange-colored precipitate separated. The product, compound (**I**) yields 99%, M.P:152-154 °C, FT-IR (υ, cm^{-1}): (3263) for ≡C-H, (3066 and 2966) CH_2 stretching, (2121) C≡C stretching and (1465) CH_2 bending.

Procedure B: Under conventional heating conditions

To a solution of isatin (0.001 mole, 0.147 g) was added anhydrous K_2CO_3 (0.0015 mole, 0.207 g) in anhydrous DMF (10 mL) in a round bottom flask. Propargyl bromide (0.0015 mole, 0.167 mL) was then added drop by drop into the reaction mixture. After that, the reaction mixture was heated for 5 hours at 50 °C in a water bath using reflux and stirring. TLC was used to monitor the reaction process. The mixture was processed using the same technique as in **procedure A** after the reaction was finished. The separated solid product of *N*-propargyl isatin was filtered, rinsed with water, and recrystallized from 96% ethanol to obtain the title compound (**I**) was orange crystals, yield 99%, M.P:152-154 °C, FT-IR (υ, cm^{-1}): (3263) for ≡C-H, (3066 and 2966) CH_2 stretching, (2121) C≡C stretching and (1465) CH_2 bending.

Synthesis of Mannich bases

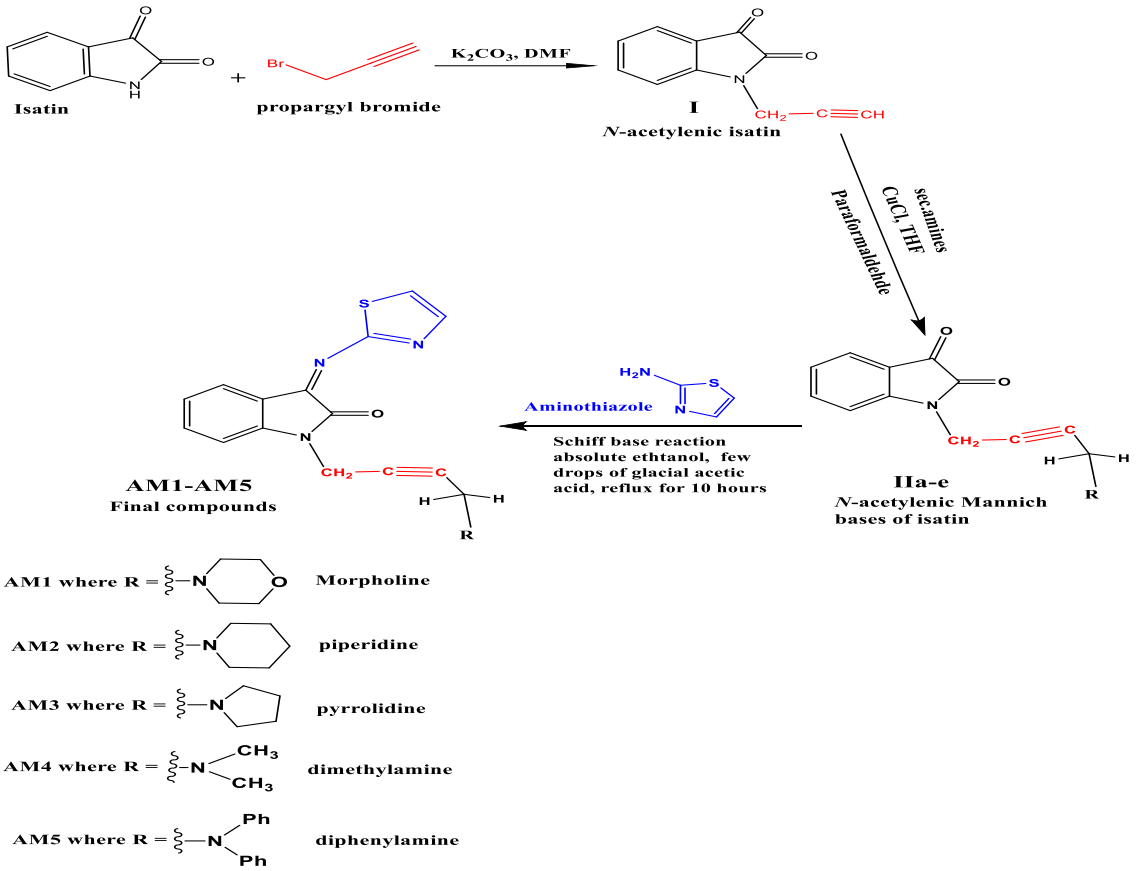
General procedure for the synthesis of N-propargylated isatin- Mannich bases (IIa-e):

Compound **I** (0.003 mole, 0.55 g) was dissolved in 10 mL of dry tetrahydrofuran, and then paraformaldehyde (0.003 mole, 0.09 g) was added. Next, cuprous chloride and 0.003 moles of each of the following secondary amines were added: morpholine, piperidine, pyrrolidine, dimethylamine, and diphenylamine individually. The resulting mixture was heated to 60°C for a period of four hours. After cooling the product mixture to ambient temperature, the filtrate was added to 10 milliliters of ice water mixture, and then recrystallized from 70 % ethanol.

1-(4-morpholinobut-2-yn-1-yl) indoline-2,3-dione (IIa)

Morpholine (0.003mole, 0.304g) and cuprous chloride were added to the solution of compound (**I**) as described previously in the general procedure and as shown in Scheme 1. The product compound (**IIa**) was yellow-colored powder, yielding 56%, M.P: 149-151ºC, FT-IR (υ, cm^{-1}): (3093) stretching of aromatic C-H, (2912 and 2831) stretching of C-

H (CH_2), (2360) stretching of C≡C, (1732) stretching of C=O of (C=O-C=O-N), (1660) stretching of C=O of amide, (1508 and 1469) stretching of C=C of Ar-ring and (1111) strong stretching of C-O-C of cyclic ether.



Scheme 1. Schematic synthesis of final compounds (AM1-AM5) and their intermediates.

1-(4-(piperidin-1-yl)but-2-yn-1-yl)indoline-2,3-dione (IIb):

Piperidine (0.003 mole, 0.29g) and cuprous chloride were added to the solution of compound (**I**) as described previously in the general procedure and as shown in scheme 1. The product compound (**IIb**) was yellow powder, yield 50%, M.P: 138-140ºC, FT-IR (υ, cm^{-1}): (2958 and 2880) stretching of C-H (CH_2), (2360) stretching of C≡C, (1735) stretching of C=O of (C=O-C=O-N), (1650) stretching of C=O of amide,(1508 and 1469) stretching of C=C of Ar-ring, (1435) bending of C-H(CH_2).

1-(4-(pyrrolidin-1-yl)but-2-yn-1-yl)indoline-2,3-dion (IIc):

Pyrrolidine (0.003 mole, 0.292g) and cuprous chloride were added to the solution of compound (**I**) as described previously in the general procedure and as shown in scheme 1. The product compound (**IIc**) was brown powder, yield 40%, M.P: decomposed 140 ºC, FT-IR(υ, cm^{-1}): (3055) stretching of CH of aromatic,(2908 and 2800) stretching of C-H (CH_2), (2160) stretching of C≡C, (1720) stretching of of (C=O-C=O-N), (1650) stretching of (C=O) of amide, (1485 and 1465) stretching of C=C of Ar-ring, (1427) bending of C-H(CH_2).

1-(4-(dimethylamino)but-2-yn-1-yl)indoline-2,3-dione(IId):

Dimethylamine (0.003mole, 0.135g) and cuprous chloride were added to the solution of compound (**I**) as described previously in the general procedure and as shown in scheme 1. The product compound (**11d**) was brown colored powder, yielding 56%, M.P: decomposed at 160 ºC.FT-IR (υ, cm^{-1}): (3032) stretching of CH of aromatic ring, (2974 and 2870) stretching of C-H (CH_2) and (CH_3), (2360) stretching of C≡C, (1732) stretching of C=O (C=O-C=O-N), (1665) stretching of C=O of amide, (1512 and 1465) stretching of C=C of Ar-ring, (1419) bending of C-H(CH_2).

1-(4-(diphenylamino)but-2-yn-1-yl)indoline-2,3-dione(IIe):

Diphenylamine (0.003 mole, 0.507g) and cuprous chloride were added to the solution of compound (**I**) as described previously in the general procedure and as shown in scheme 1. The product compound (**IIe**) was brown colored powder, yield 50%, M.P: decomposed 150ºC, FT-IR (υ, cm^{-1}):, (2924) stretching of C-H (CH_2), (2360) stretching of C≡C, (1735) stretching C=O of C=O-C=O-N, (1660) stretching of C=O of amide, (1508 and 1469)

stretching of C=C of Ar-ring, (1419) bending of C-H(CH_2).

General procedure for Schiff base synthesis (AM1-AM5):

Separately, (0.001 mole) of compounds (**IIa-e**) and three drops of glacial acetic acid were added to 15 mL absolute ethanol, followed by (0.0012 mole) of 2-aminothiazole. After refluxing the mixture for ten hours, it was left overnight. The residue was recrystallized from methanol after the solvent was evaporated in a vacuum.

1-(4-morpholinobut-2-yn-1-yl)-3-(thiazol-2-ylimino) indolin-2-one (AM1):

Dark-red crystals; R*f* =0.79, M.P: 252-254 ºC; yield 50%. FT-IR (υ, cm^{-1}): (2954 and 2854) CH stretching of CH_2, (1689) stretching of C=O of amide, (1608) stretching of C=N of thiazole, (1516 and 1465) stretching of C=C of an aromatic ring,(1002) stretching of C-O-C of cyclic ether, (860) stretching of C-N, (748)out of plane (C-H) bending of aromatic ring,(694) bending C=C of Ar-ring. The ^{1}H-NMR (δ=ppm):7.40-6.86 (6H, m, Ar-H), 4.54 (2H, s, CH_2-N of isatin), 3.53 (2H, s, CH_2-N of morpholine), 2.36 (4H, t, CH_2 of morpholine close to N), 0.86-0.83 (4H, t, CH_2 of morpholine close to O)

1-(4-(piperidin-1-yl)but-2-yn-1-yl)-3-(thiazol-2-ylimino)indolin-2-one (AM2):

Dark- red crystals; R*f* =0.6, M.P:252-254 ºC; yield 50%. FT-IR (υ, cm^{-1}): (2966 and 2866) CH stretching of aliphatic CH_2, (2106) stretching of C≡C, (1635) C=N stretching of imine, (1612) C=N stretching of thiazole, (1516) and 1465) stretching of C=C aromatic ring. (879) out of the plane (C-H) bending of aromatic ring ,(682) C=C of Ar-ring bending. The ^{1}H-NMR (δ=ppm): 7.84-6.75 (6H, m, Ar-H), 4.67 (2H, s, CH_2-N of isatin), 2.89 (2H, s, CH_2-N of piperidine), 2.48-2.46 (4H, t, CH_2 of piperidine close to N), 1.25-1.07 (6H, m, CH_2).

1-(4-(pyrrolidin-1-yl)but-2-yn-1-yl)-3-(thiazol-2-ylimino)indolin-2-one (AM3):

Red crystals; R*f* =0.7, M.P: decomposed 240 ºC; yield 40%. FT-IR (υ, cm^{-1}): (2966) C-H of CH_2, (2368) stretching of C≡C, (1680) C=O stretching of amide, (1658) C=N stretching of imine, (1608) C=N stretching of thiazole, (1512 and 1485) stretching of C=C) aromatic ring, (752) out of plane (C-H) bending of Ar-ring, (694) C=C of Ar-ring bending. The ^{1}H-NMR (δ=ppm):7.94-6.62 (6H, m, Ar-H), 4.53 (2H, s, CH_2-N of isatin), 3.09(2H, s, CH_2-N of pyrrolidine), 2.45-2.43(4H, t, CH_2 of pyrrolidine close to N), 1.60-1.56 (4H, t, CH_2 of pyrrolidine)

1-(4-(dimethylamino)but-2-yn-1-yl)-3-(thiazol-2-ylimino)indolin-2-one (AM4):

Brown crystals; R*f* =0.6, M.P decomposed 140 ºC; yield 40%. FT-IR (υ, cm^{-1}): (3059) stretching of aromatic ring, (2962 and 2881) C-H of CH_2, (1685) C=O stretching of amide, (1608) stretching of C=N of thiazole, (1512 and 1469) str. of C=C aromatic ring, (756) out of plane C-H bending of Ar-ring, (690) C=C of Ar-ring bending. The ^{1}H-NMR (δ=ppm): 7.91- 6.89 (6H, m, Ar-H), 4.50 (2H, s, CH_2-N of isatin), 3.49 (2H, s, CH_2-N of dimethylamine), 2.33 (6H, s, CH_3 of dimethylamine).

1-(4-(diphenylamino)but-2-yn-1-yl)-3-(thiazol-2-ylimino)indolin-2-one (AM5):

Brown crystals; R*f* =0.5, M.P: decomposed 200 ºC; yield 40%. FTIR (υ, cm^{-1}): (2924) stretching C-H of CH_2, (1680) C=O stretching of amide, (1651) stretching of C=N of imine, (1612) C=N stretching of thiazole, (1508 and 1469) stretching of C=C of Ar-ring, (752) out of plane C-H bending of Ar-ring, (694) C=C of Ar-ring bending. The ^{1}H-NMR (δ=ppm):7.76-7.14 (16H, m, Ar-H), 4.67 (2H, s, CH_2-N of isatin), 4.47 (2H, s, CH_2-N of diphenylamine).

Antimicrobial Activity test

Using the well-diffusion method, the antimicrobial activity of the resultant compounds was measured. *In vitro* tests were performed to determine the synthetic compounds' antibacterial efficacy against three distinct kinds of microorganisms: fungi (*Candida albicans*), Gram-positive bacteria (*Staphylococcus aureus*,*Bacillus licheniformis*) and Gram-negative bacteria (*Acinetobacter baumannii*, and *Escherichia coli*), these microbes were experimentally stimulated and kept on nutritional agar for antibacterial examination, The standard medication for both Gram-positive and Gram-negative pathogens was ciprofloxacin. Fluconazole was a typical medicine with antifungal properties.

Molecular Docking

The molecular docking studies were performed using Glide tool in the Maestro platform 13.0.135, 2021-4 of Schrodinger suite, LLC, New York, NY, 2021 to assess the binding affinity of the Synthesized derivatives of alkylated isatin into the binding site of Cytochrome p450 14 alpha sterol demethylase and Topoisomerase IV enzymes [41,42]. The suggested fragments' chemical structure was created using the Ligprep module.

Protein Preparation and Grid Generation The crystal structure for [Cytochrome P450 14 alpha-sterol demethylase **(CYP51)**] (**pdb code: [1EA1]**) and [Topoisomerase IV(**TopoIV)**] (**pdb code: [5EIX]**) proteins were downloaded from Protein Data Bank and prepared through the employing of the protein preparation tool [43].

Proteins processed using protein preparation wizard in Schrodinger, New York, NY, 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 [41, 42]. NAD is conserved in the core of all proteins as a

cofactor and when co-crystallized with a ligand. When the prepared ligands docked in the protein binding site, the receptor grid was created using the co-crystallized ligand as the center for the boundary box. The boundary box that was used had a 12 A_0. dimension. OPLS_2005 force field was utilized to minimize the energy of the protein [44]. The grid boxes were generated using the co-crystallized bound ligands as references.

Ligand Preparation

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 applied to the ligands using LigPrep [41]. The flexible ligand docking was implicated under OPLS_2005 force field. A standard precision docking mode was exploited to generate 5 pose per ligand.

Results and Discussion

Chemistry

Synthesis of compound (I)

Propargyl bromide was used to alkylate the potassium salt of isatin to produce the compound (**I**). The S_N2 mechanism was followed by the alkylation stage. The desired alkylated isatin derivative is produced by the anion's nucleophilic attack on propargyl bromide, which initiates the reaction. There was no allylic rearrangement noticed [45]. The propargylated derivative of isatin was obtained by alkylation of isatin using propargyl bromide and anhydrous K_2CO_3 as a base in polar aprotic solvent for S_N2 reaction, such as DMF, (Scheme 1). The strong band at $\bar{\upsilon}$ = 3286–3224 cm^{-1} that corresponded to the stretching vibration of the terminal alkyne C–H bond indicated the presence of the propargylic group; other weak bands in the region $\bar{\upsilon}$ = 2121–2117 cm^{-1} belonged to the (C≡C) triple bond of a propargylic group linked to isatin ring [40].

Synthesis of Mannich bases (IIa-e)

The Mannich reaction of propargylated isatin with paraformaldehyde, appropriate secondary amine and a catalytic amount of cuprous chloride in tetrahydrofuran was heated to 60 °C to yield the desired compounds [46]. A molecule having active hydrogen, a primary or secondary amine and formaldehyde condenses in the Mannich reaction, known a nucleophilic addition process. [25]. The presence of compounds as derivatives of Mannich alkylated isatin disappear of the strong band at $\bar{\upsilon}$ = 3286–3224 cm^{-1} belonged to the stretching vibration of terminal alkyne C–H bond.

Synthesis of final compounds (AM1-AM5)

A nucleophilic addition reaction is the first step in the reversible, acid-catalyzed mechanism of Schiff base formation. The production of Schiff bases (**AM1-AM5**) were confirmed by the appearance of new FT-IR bands at 1612-1689 cm^{-1}(C=N imine) stretching, and at1562-1612cm^{-1} (C=N) stretching of the thiazole ring. Additionally, two peak ranges may be seen in the ^{1}H-NMR spectrum data (δ=ppm): 4.48 (2H, s, CH_2), 3.03(2H, s, CH_2) of CH_2 close to NH of isatin moiety, and CH_2 close to N of secondary amine, respectively.

Antimicrobial evaluation

In the light of the results in (Table 1) on the anti-bacterial evaluation, all the compounds (**AM1-AM5**) display a moderate to strong activity at 100 mg/mL against both *Acinetobacter baumannii* and *E. coli* while ciprofloxacin has no activity at the same concentration and the most potent one is **AM1**. Furthermore, convergent results when comparing the antifungal reference compound (fluconazole) with the tested derivatives. Besides, the compounds **AM1**, **AM3**, and **AM4** have broad activity towards the tested microorganisms. Taking into consideration that the chemical structure of compound **AM1** has cyclic amine (morpholine moiety) of two heteroatoms N and O; improved hydrophilicity and thus increased absorption and good bioavailability, besides that acetylenic moiety, which considerably improves the molecule's lipophilicity, thus, its activity [22].

Table 1. Antimicrobial activity of final compounds (AM1- AM5) (100mg/mL)

Compounds	Gram-positive		Gram-negative		Fungi
	Staphylococcus aureus	*Bacillus licheniformis*	*Escherichia coli*	*Acinetobacter baumannii*	*Candida albicans*
	Zone of inhibition(mm)				
AM1	45	18	65	60	24
AM2	15	-	40	40	23
AM3	22	16	15	26	20
AM4	20	26	16	50	17
AM5	13	-	20	18	21
Ciprofloxacin	23	26	26	-	-
Fluconazole	-	-	-	-	22

(-) = without activity, mildly active (inhibition zone within 5 and 10 mm), moderately active (inhibition zone within10 and 20 mm), and particularly active (inhibition zone more than 20 mm) [47].

Docking Study

Docking Study regarding [1EA1]

The docking results presented in this research (table 2) provide beneficial observation into the binding affinities of two compounds, namely **AM1, AM2** compared to the reference compound fluconazole. The purpose of the study was to determine whether these compounds as inhibitors of (Cytochrome p450 14 alpha-sterol demethylase) explain the ways in which they bind and interact within the active site. The two compounds demonstrated superior docking scores compared to fluconazole. Compound **AM1** displayed the highest binding affinity with a docking score of **(-7.9),** followed closely by compound **AM2** with a score of **(-7.712**). This means that these chemical compounds have a high chance of binding efficiently to **[1EA1]** active site. Exceeding the fiuconazole, which had a docking score of **(-7.42).** The structural similarities between these compounds **AM1** and **AM2** which include indole, thiazole, cyclic secondary amine (morpholine, piperidine respectively), and acetylenic moiety, may play a significant role in their enhanced binding affinity. These structural components differ from fluconazole, which contain triazole groups. These differences mean that the presence of indole, thiazole, secondary amine, and acetylenic moieties groups in these compounds may contribute to their increased binding affinity. This means that they needed less energy to bind with **[1EA1**]. Secondary amine (morpholine) have 2 heteroatom (N,O) that increase chance to form H-bonding with active site. These structural differences may improve the interaction with crucial residues in the [1EA1] binding pockets. This chemical alteration will allow newer acetylenic Mannich bases having Schiff bases of 2-aminothiazole ring to enhance activities. As usual, the secondary amine morpholine group in the designed compound (AM1) could be placed into the hydrophobic pocket formed by TYR76, PHE78, MET79, PHE255, ALA256, MET422 and thiazole , Isatin group could be placed into the hydrophobic pocket formed by PRO219, PRO220, LEU221, LEU224, CYS264, PRO266, PHE267 would generate polar bonding interactions with the HIS259, THR260. PI-cation interaction with ARG 96.

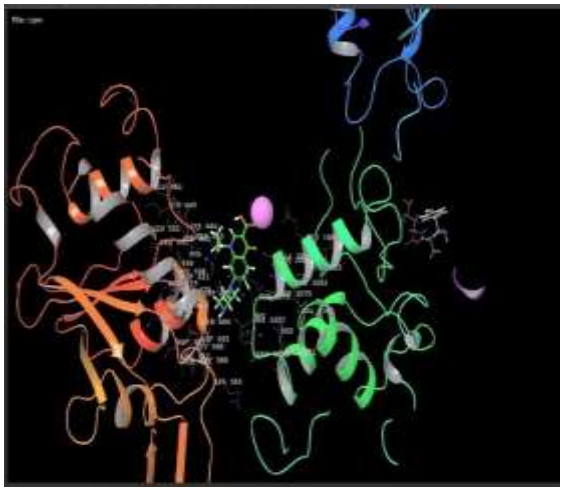
Docking Study regarding [5EIX]

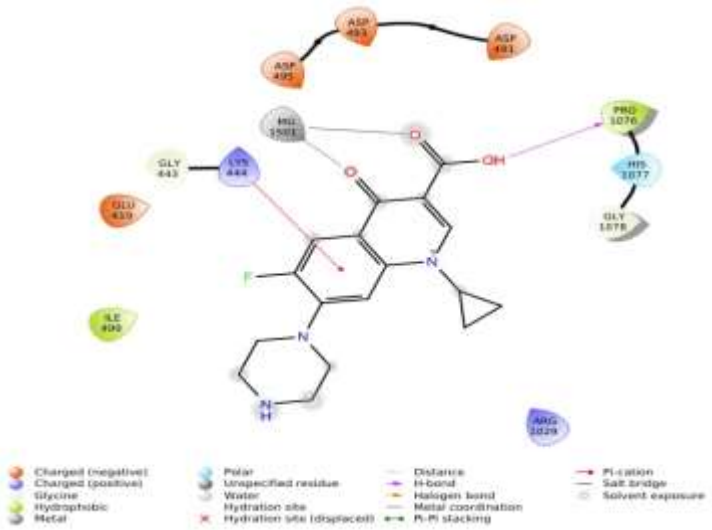
The docking results presented in this research (table 2) provided a beneficial observation into the binding affinities of two compounds, namely **AM3, AM4** compared to the reference compound Ciprofloxacin. The purpose of the study was to determine whether these compounds as inhibitors of (Topoisomerase IV) explain the ways in which they bind and interact within the active site. The **AM3** compounds demonstrated superior docking scores compared to Ciprofloxacin. Compound **AM3** displayed the highest binding affinity with a docking score of **(-4.512),** followed closely by compound **AM4** with a score of **(-4.072**). This means that these chemical compounds have a high chance of binding efficiently to **[5EIX]** active site. Exceeding the Ciprofloxacin, which had a docking score of **(--4.28).** The structural similarities between these compounds **AM3** and **AM4** which include indole, thiazole, cyclic secondary amine (pyrrolidine, dimethyl amine respectively), and acetylenic moiety, may play a significant role in their enhanced binding affinity. These structural components differ from Ciprofloxacin, which contain fluoroquinolone groups and piperazine. These differences mean that the presence of indole, thiazole, secondary amine, and acetylenic moieties groups in these compounds may contribute to their increased binding affinity. This means that they needed less energy to bind with **[5EIX**]. These structural differences may improve the interaction with crucial residues in the **[5EIX]** binding pockets. This chemical alteration will allow newer acetylenic Mannich bases having Schiff bases of 2-aminothiazole ring to enhance activities. As usual, the carbonyl of isatin in the designed compound (**AM3)** bind with MG1501Metal Coordination, pi-cation interaction with LYS565, pi-cation withGLU419, ASP421, ASP491, ASP493, GLU569, ASP1079. RMSD (root-mean-square deviation) values,are presented in Table **2**. Compounds **AM1**-**AM5** exhibited with low RMSD values.

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

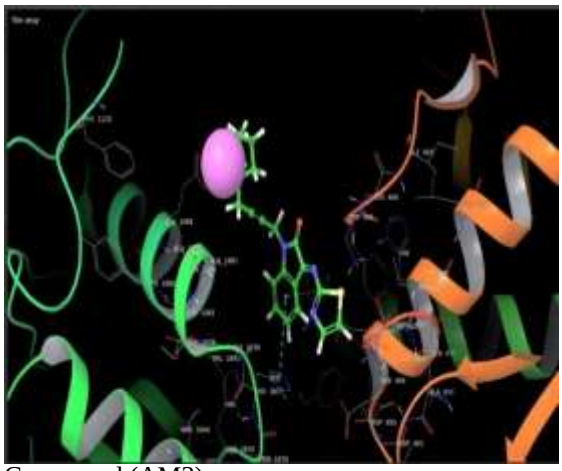
Compounds	Docking Score	RMSD
Fluconazole	-7.420	1.424
AM1	-7.900	0.0236
AM2	-7.712	0.0242
AM3	-7.016	0.0343
AM4	-6.158	0.0262
AM5	-6.102	0.0329

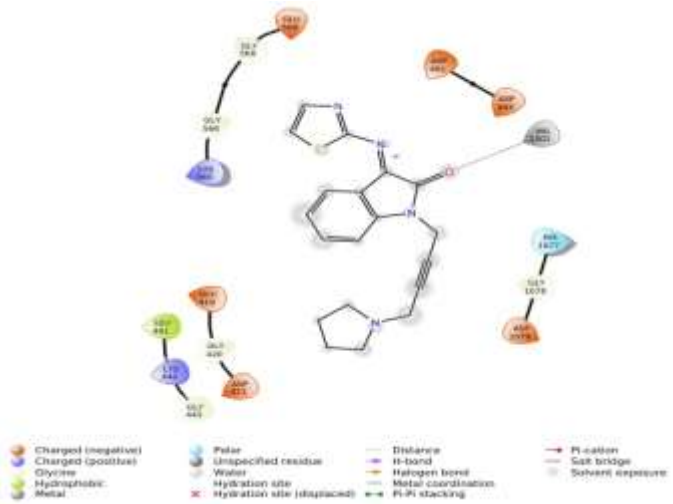
Ciprofloxacin	-4.728	0.840
AM1	-3.545	0.033
AM2	-3.754	0.047
AM3	-4.512	0.045
AM4	-4.072	0.029
AM5	-3.663	0.028



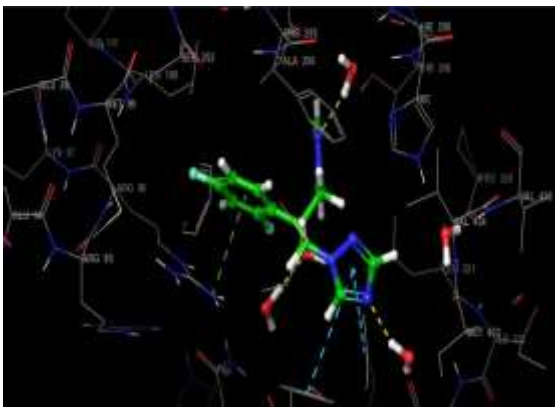


Ciprofloxacin(standard compound)

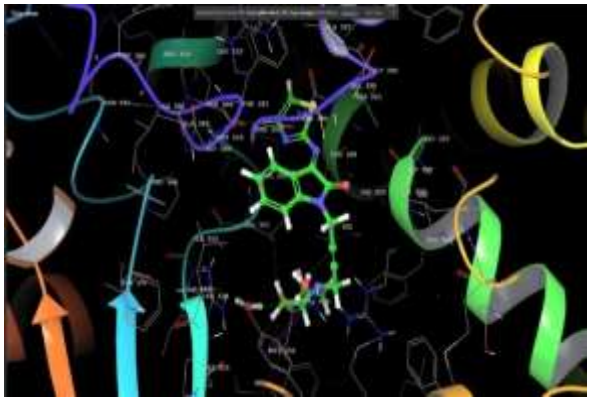




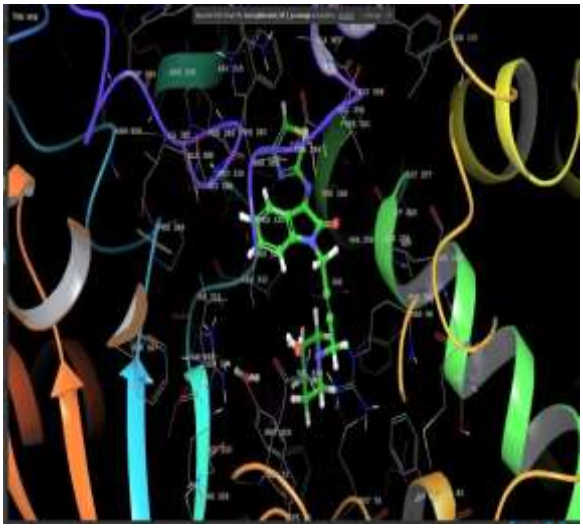
Compound (AM3)

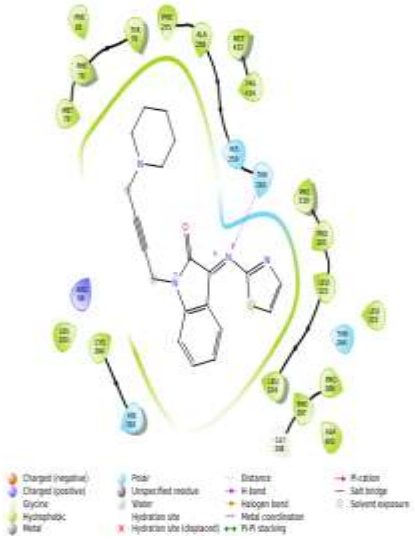


Fluconazole (standard compound)



Compound (AM1)





Compound (AM2)

Figure1.Top docked ligands with 1ea1 and 5eix enzymes.

Conclusion

Five derivatives of alkylated isatin' Mannich bases connecting to thiazole imine (Schiff base), were produced in reasonable yields and evaluated by FT-IR and ^{1}H-NMR spectroscopy analysis. A preliminary *in vitro* examination of new Schiff's and Mannich bases of acetylenic isatin derivatives (**AM1-AM5**) revealed moderate to potent antimicrobial efficacy against bacteria and fungus. Comparing compound **AM1** with other derivatives, the former demonstrated strong, broad-ranging antibacterial and antifungal activity. The docking results presented in this study show the binding affinities of two compounds, namely (**AM1** and **AM2)** compared to the reference compound fluconazole. Compound **AM1** displayed the highest binding affinity with a docking score of **(-7.9)**, followed closely by compound **AM2** with a score of **(-7.712**).

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Conflicts of Interest

The authors have declared there is no conflict of interest.

Funding

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Ethics Statements

The authors state that as this was an *in-vitro* investigation, no ethics committee permission was required for the synthesis and evaluation of new compounds.

Author Contribution

The first author; (Marwa) assisted with the synthesis of desired compounds; analysis of IR and ^{1}H-NMR data, discussion of the antimicrobial activity, manuscript's drafting; and critical revision. The second author; (May) contributed to the design of the study, analyzed the final results, authorized the manuscript in its final form, and participated in the study's design.

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التوليف والتوصيف والالتحام الجزيئي والتقييم المضاد للميكروبات لقواعد مانخ الأسيتيلينية الجديدة لمشتقات إيزاتين- ثيازول

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الخلاصة

يتواجد جزيء الإيزاتين في العديد من المواد الطبيعية، مثل النباتات والحيوانات، ويستخدم في تحضير المركبات ذات الأنشطة البيولوجية المختلفة. تم تصنيع سلسلة من قواعد شيف ومانخ المشتقة من الإيزاتين في هذا البحث. أولاً، تم تحضير قواعد مانخ الأسيتيلينية(**IIa-e**) من تفاعل الإيزاتين مع بروميد البروبارجيل وأمينات ثانوية مختلفة (مورفولين، بيبيريدين، بيروليدين، ثنائي ميثيل أمين، و ثنائي فينيل أمين) بشكل منفصل، وتم استخدام كلوريد النحاسوز (CuCl) كعامل محفز. ثانياً، عولجت قواعد مانخ هذه بـ ٢-أمينوثيازول للحصول على المركبات النهائية المطلوبة (**AM1-AM5**). تم تشخيص المركبات المحضرة على أساس بياناتها الطيفية (FT-IR, ^{1}H-NMR). وتم فحص جميع هذه المشتقات لنشاطها المضاد للميكروبات ضد نوعين من البكتيريا إيجابية الجرام؛ "المكورات العنقودية الذهبية والعصية الحزازية"، واثنين من البكتيريا سالبة الجرام؛ "الإشريكية القولونية والراكدة البومانية"، ونوع واحد من الفطريات "المبيضات البيضاء". وقد وجد أن معظم المركبات تظهر نشاطًا مضادًا للميكروبات، وأكثرها نشاطًا هو المركب **AM1**. ثم تم إجراء الالتحام الجزيئي للمركبات المصنعة النهائية وتمت مقارنتها مع الأدوية القياسية (سيبروفلوكساسين وفلوكونازول). سجل الالتحام الجزيئي درجات أعلى من الالتحام لمشتقين نهائيين (**AM1** و**AM2**) مقارنةً بالفلوكونازول.

الكلمات المفتاحية: ، قواعد مانخ الأسيتيلينية، ٢-امينوثيازول، الفعالية المضادة للميكروبات، بروبارجيل بروماید، ايزاتين