Synthesizing, Studying Molecular Docking, Characterizing, and Preliminary Evaluating Anti-Bacterial Effects of Derivatives of Serotonin Contain Imidazolidine Ring

Marwa Abdullah Saleh*, Karima Fadhil Ali **, Basma M. Abd Razik**
***AL-Dour health care sector, Salah Al-Deen Directorate of Health, Ministry of Health, 34007, Salah Al-Deen, Iraq**
****Department of Pharmaceutical Chemistry, College of Pharmacy, Mustansiriyah University, 10001, Baghdad, Iraq**

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Corresponding Author email:
basma-m.abd-razik@uomustansiriyah.edu.iq
orcid: https://orcid.org/ 0000-0002-2658-9349

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Abstract:

This study included synthesis of new serotonin derivatives in which imidazolidine rings are present in their structures. The final imidazolidine derivatives compounds were synthesized by reaction of synthesized Schiff bases derivatives of serotonin with the glycine (NH2-CH2COOH) in presence of tetrahydrofuran (THF) as a solvent. The imidazolidine derivatives were identified by physical characteristics, FT-IR spectroscopy and 1H- NMR spectroscopy. Biological activities against two Gram negative (Klebsiella and E. coli) and two Gram positive (Streptococcus pyogenes and Staphylococcus aureus) bacteria were also distinguished. All the synthesized compounds III(a-d) exhibit moderate activities on four types of bacteria comparing with the activity of standard drug (Trimethoprim) but the highest activities of these compounds occur on Streptococcus pyogenes and their least activities occur on E. coli. The synthesized compounds were studied for the molecular docking to know the interaction and affinity of binding between them and bacteria

Key words: Serotonin; Imidazolidine; Molecular docking.

تخليق، دراسة النمذجة الجزيئية، تشخيص وتقييم أولي للتأثيرات المضادة للبكتيريا لمشتقات السيروتونين المحتوية على حلقة الأيميدازوليدين

مروة عبد الله صالح*، كريمة فاضل علي، بسمة منجد عبد الرزاق****
**** قطاع الدور للرعاية الصحية، دائرة صحة صلاح الدين، وزارة الصحة, 34007، صلاح الدين، العراق***
*****فرع الكيمياء الصيدلانية، كلية الصيدلة، الجامعة المستنصرية, 10001، بغداد، العراق***

الخلاصة:

تشمل هذه الدراسة تصنيع مشتقات سيروتونين جديدة التي تحتوي على حلقات الأيميدازوليدين .تم تصنيع مركبات مشتقات الأيميدازوليدين النهائية بواسطة تفاعل مشتقات قواعد شيف للسيروتونين المصنعة مع الجلايسين (NH2-CH2-COOH) بوجود رباعي هيدرو الفوران(THF) كمذيب. تم تميز مشتقات الأيميدازوليدين بواسطة الخصائص الفيزيائية، كقياس طيف الأشعة تحت الحمراء وطيف الرنين المغناطيسي.كذلك تم قياس الفعالية الحيوية ضد اثنان من البكتيريا السالبة لصبغة غرام (الكلبسيلة الرئوية و الأشريكية القولونية) وأثنان من البكتيريا الموجبة لصبغة غرام (المكورات العنقودية الذهبية والمكورات العقدية المقيحة).أظهرت جميع المركبات المصنعة أظهرت فعاليات معتدلة على الأنواع الأربعة للبكتريا مقارنتا بفعالية الدواء القياسي(التريميثوبريم) ولكن أعلى فعالية لهذه المركبات ظهرت على المكورات

العقدية المقيحة وأقل فعالية لها ظهرت على الأشريكية القولونية وكذلك تم دراسة النمذجة الجزيئية للمركبات المصنعة لمعرفة التداخل وميل الارتباط بينها وبين البكتيريا.

الكلمات المفتاحية: السيروتونين;الأيميدازوليدين;النمذجة الجزيئية.

Introduction

Heterocyclic compounds are one of the most important organic compounds, which represent more than 85% of biologically active chemical compounds. Heterocyclic defined as an organic compound that contain one or more hetero atoms arranged as a cycle; these heteroatoms are nitrogen, oxygen and sulphur, but other atoms are also present [1]. The common biological uses of heterocyclic compounds are antibacterial [2], anti-inflammatory [3], antifungal [4], anticonvulsant [3], antioxidants [5], anti-allergic [3], anticancer [6], and herbicidal activities [3].

Serotonin is one of an ancient discovered natural occurring substance in mammalian's body and even plants. It was known when firstly isolated from the enterochromaffin cells in the 1930's from mammalian gut but was really investigated in the 1940's [7]. Moreover, indoles have been increasingly studied because of their interesting biological activities [8, 9] such as anti-inflammatory [10] antibacterial. [11], antiviral [12,13], anti- tuberculosis [14], and antitumor effects. [15]

On the other hand, Schiff bases are one of the valuable organic compounds which were firstly discovered by the German chemist, Nobel Prize winner, Hugo Schiff in 1864[16]. It is formed when an aldehyde or ketone is reacting by condensation reaction with primary amines [17]. As structure, Schiff base contain an imine or azomethine group (–N=CH) [16]. The main uses of Schiff base in medicine and pharmacy include antitumor, antiviral, antifungal, and antibacterial [18].

Imidazolidines (tetrahydroimidazoles) are active heterocyclic compounds which are considered (with their fused derivatives) as an influential building block in biologically active compounds with both of natural and synthetic sources, like biotin and hydantoin [19,20,21]. The development of imidazolidines and their derivatives (as an efficient and less toxic products) was improved as a result of increasing in infectious diseases which caused by bacteria and fungi and their resistance to antimicrobial drugs due to antibiotic misuse [22,23,24]. The main uses of imidazolidines and their derivatives include antifungal [25], antibacterial, anti-inflammatory [26], anti-hypoglycemia [27], hypolipidemic, anticancer. In addition, it was used as antiviral for a variety of viruses such as herpes virus, HSV, HIV and tuberculosis. Also, it is used as antimicrobial and as anti-ulcer [28].

Furthermore, boronic acid has many therapeutic uses. Such as: anti-cancer [29], anti-bacterial [30] and anti-viral activities [31]). Some boronic acids derivatives are used as sensors and in drug delivery systems [32].

Molecular docking is a technique utilized to know the kind of interaction that occur between proteins and small molecules at an atomic level. It also allows to recognize the attitude of small molecules when they connect with targeting protein. Also, allow us to explain the essential biochemical processes [33]. Procedures of docking includes two important steps: prognostication of conformation, position and orientation of the ligand within binding sites (known as pose) and evaluation the affinities for binding (34). Docking studies include: rigid ligand and rigid receptor docking [35], flexible ligand and rigid receptor docking [36,37], and flexible ligand and flexible receptor docking [38]. The major applications of molecular docking are DNA-drug interaction [39], optimization of the lead [40] and identifications of the Hit [41].

Materials and Methods

Chemicals and Instrumentation

Aldehydes of boronic acid and Serotonin HCL were bought from China / the Zhejiang Medicine Co.Ltd., Xinchang pharmaceutical Factory. The rest of utilized substances were bought from companies of chemicals in Germany including Merck, Fluka and Alfa also in United Kingdom including BDH. The utilized instruments for identifying the compounds that we synthesized were: Stuart (SMP30) apparatus to determine the melting point of each compound ,6100 Type A Shimadzu spectrometer in potassium bromide discs to determine Fourier-transform infrared spectrum, and Bruker (Varian) 500 MHz instrument using deuterated dimethyl sulfoxide solvent and tetramethylsilane as an internal standard for analyzing proton nuclear magnetic resonance spectra.

Table (1): Physical characteristics include molecular formula, molecular weight and melting point of intermediates and final products

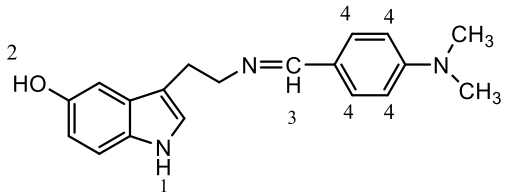
Compound Code	Molecular formula	Molecular weight	Melting Point (˚C)
Ia	$C_{19}H_{21}N_3O$	307.40	200-204
Ib	$C_{17}H_{15}ClN_2O$	298.77	254-256
Ic	$C_{17}H_{16}BFN_2O_3$	326.13	240-243
Id	$C_{18}H_{19}BN_2O_4$	338.17	220-223
IIIa	$C_{21}H_{24}N_4O_2$	364.45	160-162
IIIb	$C_{19}H_{18}ClN_3O_2$	355.82	235-237
IIIc	$C_{19}H_{19}BFN_3O_4$	383.19	252-255
IIId	$C_{20}H_{22}BN_3O_5$	395.22	240-242

Methods

General procedure for Schiff-base I (a-d) synthesizing:

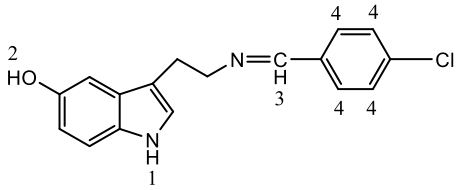
Derivatives of Schiff-base I(a-d) were synthesized through the addition of Serotonin HCL solution (1.0634g, 0.005mol) which dissolved in 30 milliliter of an absolute ethanol by degrees to (0.005mol) solution of derivatives of aldehydes [(0.745g) of 4-dimethylamino benzaldehyde, (0.702g) of 4-chlorobenzaldehyde, (0.839g) of 2-fluoro-3-formylphenyl boronic acid and (0.899g) of 2-formyl-4-methoxyphenyl boronic acid) which dissolved in 20 milliliter of an absolute ethanol. Addition of seven drops from the glacial acetic acid catalyst and refluxing the mixture for twenty hours at 80°C was done then the resulted precipitate from the filtration of refluxed mixture were collected also washed with a cold water and lastly recrystallized from ethanol.

3-(2-((4-(dimethyl amino) benzylidene) amino) ethyl)-1H-indol-5-ol (Ia):



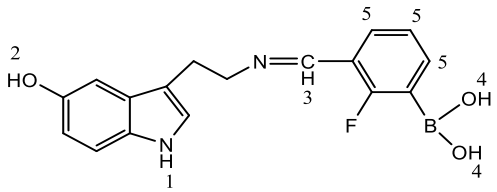
This compound has orange color solid (1.0759g); m.p.200-204°C;70 percent of yield; FT-IR (potassium bromide) *v*max (cm^{-1}): 3259 (stretching of NH), 3221(stretching of hydroxyl), 2829 (C-H sp3 stretching), 1651(stretching of imine). ^{1}H-NMR (500 MHz, DMSO-*d*6): ppm10.65(s,1H, N-H),10.58(s,1H, hydroxyl), 8.67(s,1H, H-C=N), 7.05-7.67 (m, 7H, C-H of aromatic ring).

3-(2-((4-chlorobenzylidene) amino) ethyl)-1H-indol-5-ol (Ib):



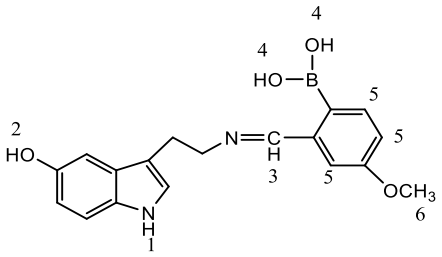
This compound has off white color solid (1.1203g); m.p.254-256℃;75 percent of yield; FT-IR (potassium bromide) *vmax*(cm^{-1}): 3435(stretching of NH), 3217(stretching of hydroxyl), 1626(stretching of imine).^{1}H-NMR (500 MHz, DMSO-*d*6): ppm10.54(s,1H, N-H), 9.70(s,1H, hydroxyl), 8.82(s,1H, H-C=N), 6.64-7.55(m,7H, C-H of aromatic ring).

(2-fluoro-3-(((2-(5-hydroxy-1H-indol-3-yl) ethyl) imino) methyl) phenyl) boronic acid (Ic):



This compound has off white color solid (1.3045g), m.p.240-243℃,80 percent of yield;FT-IR(potassium bromide)*v*max(cm^{-}1): 3342(stretching of N-H), 3309(stretching of hydroxyl), 1606 stretching of imine), 1342(B-O stretching), 1271(C-F stretching). ^{1}H-NMR (500 MHz, DMSO-*d*6): ppm10.61(s,1H, N-H),10.61(s,1H, hydroxyl),7.74(s,1H, H-C=N), 7.73(s,2H, hydroxyl of boronic acid), 6.65-7.21(m,6H, C-H of aromatic ring).

(2-(((2-(5-hydroxy-1H-indol-3-yl) ethyl) imino) methyl)-4-methoxyphenyl) boronic acid (Id):

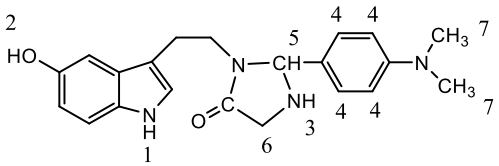


This compound has dark green color solid (1.35268g), m.p.220-223℃ ,80 percent of yield; FT-IR (potassium bromide) vmax(cm^{-}1):3429(stretching of NH),3369(stretching of hydroxyl), 1606(stretching of imine), 1365(stretching of B-O). ^{1}H-NMR (500 MHz, DMSO-*d*6): ppm10.26(s,1H, hydroxyl),8.35(s,1H, H-C=N),7.94(s,2H, hydroxyl of boronic acid), 6.75-7.40(m,6H, C-H of aromatic ring), 3.83(s,3H, CH3).

General procedure for the synthesis of imidazolidine compounds:

Schiff bases I (a-d) (0.001mol) were dissolved in (15mL) of tetrahydrofuran (THF) and mixed with the solution of glycine (0.002mol, 0.154g) which dissolved in (15mL) of tetrahydrofuran (THF). After that refluxing of mixture was taken place for (35 hours) at 66℃.The solution was, cooled down and resulting products were collected.

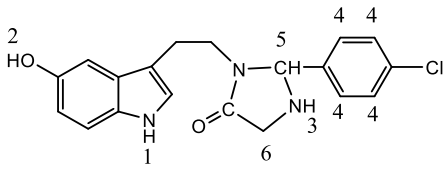
2-(4-(dimethyl amino) phenyl)-3-(2-(5-hydroxy-1H-indol-3-yl) ethyl) imidazolidin-4-one (IIIa):



This compound has yellow color solid(0.13282g),m.p.160-162℃ ,81percent of yield;FT-IR (potassium bromide) *v*max(cm^{-}1):3394(stretching of N-

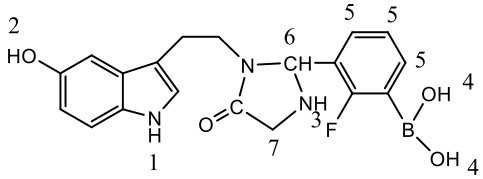
H),3207(stretching of hydroxyl),2918(C-H sp3 stretching),1645(C=O stretching).[1]H-NMR(500 MHz, DMSO-*d*6):ppm9.66 (s,1H,hydroxyl),8.20(s,1H,N-H),6.60-7.70(m,7H,C-H of aromatic),6.59(d,1H,HC-N),3.77-3.80(d d,2H,C-H sp^2),3.02(s,6H,2xCH3).

2-(4-chlorophenyl)-3-(2-(5-hydroxy-1H-indol-3-yl) ethyl) imidazolidin-4-one (IIIb):



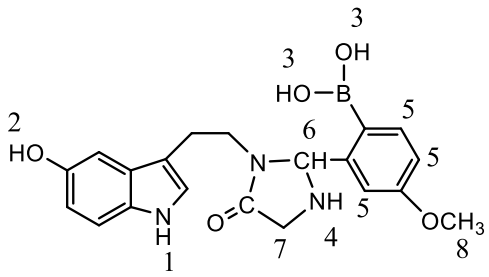
This compound has Pale yellow color solid (0.30244g), m.p.235-237℃,85 percent of yield;FT-IR(potassium bromide) *v*max(cm^{-1}): 3394(stretching of N-H), 3350(stretching of hydroxyl), 1745(C=O stretching).[1]H-NMR(500 MHz, DMSO-*d*6):ppm9.71(s,1H,hydroxyl), 8.82(s,1H,N-H), 6.64-7.54(m,7H,C-H of aromatic ring), 5.85(d,1H,HC-N), 3.41-3.60(d of d,2H,C-H sp^2).

(2-fluoro-3-(1-(2-(5-hydroxy-1H-indol-3-yl) ethyl)-5-oxoimidazolidin-2-yl) phenyl) boronic acid (IIIc):

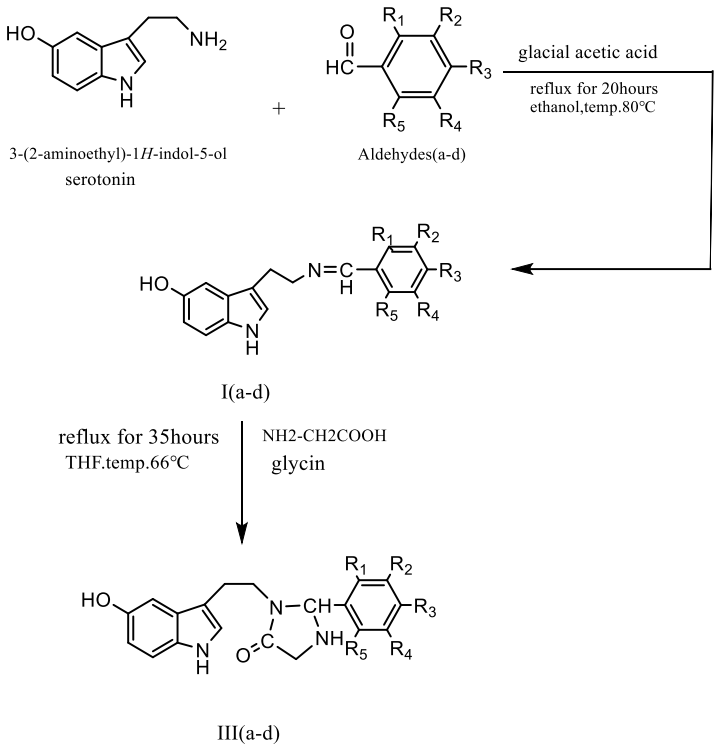


This compound has Pale yellow color solid (0.31453g), m.p.252-255℃ ,81 percent of yield;FTIR (potassium bromide) *v*max (cm^{-1}): 3427(N-H stretching), 3369(stretching of hydroxyl), 1737(C=O stretching), 1392(B-O stretching), 1273(C-F stretching). [1]H-NMR (500 MHz, DMSO-*d*6): ppm10.57(s,1H, hydroxyl),7.15(s,1H, N-H), 7.13(s,2H, hydroxyl), 6.65-7.09(m,5H, C-H of aromatic ring), 6.01(d,1H, HC-N), 3.51-3.55(d d,2H, C-H sp2).

(2-(1-(2-(5-hydroxy-1H-indol-3-yl) ethyl)-5-oxoimidazolidin-2-yl)-4-methoxyphenyl) boronic acid (IIId):



This compound has dark green color solid (0.31617g), m.p.240-242℃,80 percent of yield; FT-IR (potassium bromide) *v*max (cm^{-1}): 3495(stretching of N-H), 3414(stretching of hydroxyl), 1597(C=O stretching), 1396 (B-O stretching).[1]H-NMR (500 MHz, DMSO-*d*6): ppm7.92(s,2H, hydroxyl), 6.98(s,1H. N-H), 6.53-6.95(m,6H, C-H of aromatic), 6.51(d,1H,HC-N), 3.52-3.55(d d,2H,C-H sp^2).



a: R_1 =H R_2=H R_3=$N(CH_3)_2$ R_4=H R_5=H
b: R_1=H R_2=H R_3=Cl R_4=H R_5=H
c: R_1=H R_2=H R_3=H R_4=B(OH)2 R_5=F
d: R_1=$B(OH)_2$ R_2=H R_3=H R_4=O-CH3 R_5=H

Scheme (1): Synthesis of intermediates and imidazolidin compounds

Antibacterial screening of t synthesized compounds III(a-d)

Antibacterial activity of the final compounds which we synthesized were tested in Mustansiriyah University-College of Pharmacy- Department of Clinical Laboratory Science. The method used for the preliminary antibacterial was well diffusion method [42,43]. The antibacterial activity of the synthesized compounds was studied in vitro against two-gram negative (*Klebsiella* and *E. coli*) and two-gram positive *(Streptococcus* and *Staphylococcus aureus*) bacteria on nutrient agar medium. Trimethoprim was used as standard drug for antibacterial activity.

Procedures of ADME

Through utilizing Chem.Draw S ketch (v. 19) a ligand which involved III (a-d) were drew then transformed to SMILE name by Swiss ADME tool to predict pharmacokinetic properties and physicochemical properties. By utilizing BOILED EGG polarity and lipophilicity of the small molecules was determined.

Studies of molecular docking

For studying the molecular docking, we used Glide™, (version 5.7, Schrödinger, LLC, New York, NY, 2011.On an efficacious site of four kinds of bacteria in which two of them are Gram positive and the others are Gram negative and they derived from crystal structures of enzymes in complex with the anti-bacterial drug Trimethoprim (Protein Data Bank identifier :3G7E,4HL2,2W9S,2XCT and 4RKX we docked compounds with the highest activity. Enzymes were removed from water & hetero atoms beyond a radius of five Å from a reference ligand (Trimethoprim) then a resulted structures of proteins were refined & minimized by Protein Preparation wizard™ utilizing OPLS - 2005 force field. Receptor Grid Generation program was utilized to

prepare a grid of two Gram positive and two Gram negative bacteria (Streptococcus Pyogenes, Staphylococcus aureus, Klebsiella Pneumoniae and +Escherichia Coli) respectively, and through Lig Prep™ ligands were optimized and energy state of ligands were minimized by utilizing OPLS-2005 force field. five poses were docked for each ligand and one with high score was displayed for each ligand.

Results and Discussion

Interpretation of synthesis results

The mixing of serotonin with the four different types of aldehydes in presence of ethanol as a solvent and glacial acetic acid as a catalyst lead to form the four series of Schiff bases derivatives of serotonin which have RHC=N-R general formula. Schiff base compounds were identified by physical properties (color and melting point). Structure of Schiff base compounds was assured by their FT-IR spectroscopy. The data of IR shows distinctive band of absorption at (3259.81-3435.34 cm^{-1}) is for υ NH stretching of serotonin and stretching band of υ C=N at (1606.76 -1651.12) cm^{-1}. The data of ^{1}H-NMR of I(a-d) compounds shows a signal at (7.74-8.82 ppm) for proton of imine (CH=N) group also show signal at (10.26-10.65) for proton of NH of serotonin. The imidazolidine compounds III(a-d) were resulted from mixing a mixture of Schiff base compounds I(a-d) with glycine in tetrahydrofuran (THF) under refluxing for 35 hours as illustrated in the scheme (1). The structure of compounds III(a-d) was identified by physical properties (color and melting point). The imidazolidine compound's structure was assured by their FT-IR spectroscopy. The data of IR shows the disappearance of the band of stretching of (C=N) group of Schiff base at (1606.76-1651) cm^{-1} and appearance of absorption band at (1597.10-1745.64) cm^{-1} due to the C=O of imidazolidine ring. The ^{1}H-NMR data for the compounds III (a-d) shows appearance of N-H, N-CH and CH2 of imidazolidine ring signals at (6.98-8.82) ppm, (5.85-6.59) ppm and (3.41-3.80) ppm respectively.

Results from studying anti-bacterial effects

Compounds that we synthesized including IIIa-IIId were studied to detect their activities on bacteria by using trimethoprim as a reference drug and dimethyl sulfoxide as control in a pure state. These compounds tested against four types of bacteria, two of them were gram positive bacteria and the other were two-gram negative bacteria at four different concentrations (62.5,125, 250 and 500 µg/mL). All the inhibition zone of the tested compounds at all concentrations are present in table (1). From the table, it can be noticed that the lowest activity was against *Staphylococcus aureus* and *Escherichia coli* and the moderate activity was against *Streptococcus pyogenes* and *Klebsiella pneumoniae.*According to the standard drug (Trimethoprim), the results of antibacterial activity of the synthesized compounds are considered acceptable.

Table (2): The antibacterial activity of synthesized compounds III(a-d) and Trimethoprim on tested bacteria.

Compounds	Concentration (µg/mL)	Inhibition zone(mm)			
		Gram positive		Gram negative	
		Staphylococcus aureus	*Streptococcus Pyogenes*	*Klebsiella Pneumoniae*	*Escherichia Coli*
Trimethoprim	5	25	25	20	13
DMSO	Pure	0	0	0	0
IIIa	500	0	14	10	0
	250	0	14	0	0
	125	0	10	8	0
	62.5	0	14	0	0
IIIb	500	12	16	14	8
	250	10	16	14	12
	125	0	14	10	0
	62.5	0	16	10	0
IIIc	500	0	16	10	8
	250	0	14	10	8
	125	0	14	8	0
	62.5	0	14	12	0
IIId	500	14	18	12	0
	250	8	16	10	0
	125	10	14	14	0
	62.5	0	12	10	0

Interpretation of ADME results

By utilizing Lipinski's rule of five also known as (RO5) Pfizer's rule of five we calculated the drug-like properties of all final compounds III(a-d). This method was extensively applied as a filter for the compounds that will be more used in drug design programs as a lead. To take drug by oral way it must have several characteristics according to the RO5 [44]:

• Donors to hydrogen bond must be equal or little than five

• Acceptors to hydrogen bond must be equal or little than ten

•The molecular weight should be equal to or little than five hundred g/mol

•Log p (Partition coefficient) should be equal to or little than five

Moreover, calculation of (TPSA) which mean Topological polar Surface Area was accomplished. It regarded as a character of high importance in molecules' bioavailability. A low bioavailability is expected for absorbed compounds when the value of TPSA is higher than 140 A^{o} [45]. As shown in table (2) which comprise important data of ADME prediction, it is clear that all the final compounds III(a-d)

which represent ligands were within the range of accepted values. From the table (71.60,68.36,108.82,118.05) were the TPSA values of final compounds III(a-d) respectively, all these values were below 140 A^o and bioavailability values of them were 0.55 this interpreted as that the synthesized compounds have the ability to extend to a systemic circulation. From the Lipinski's rule of five (RO5), all compounds have no violation and fulfilled the topological descriptors and fingerprints of molecular drug-likeness structure keys as LogP and Log S.A Score of GI absorption is known as the absorption rate of the compounds by the intestine when they took by the oral way. The absorption always occurs if the results are high therefore all our compounds were expected to have good absorption rate by the intestine.

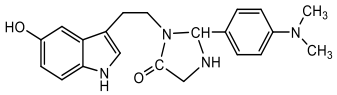
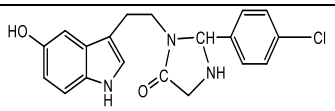
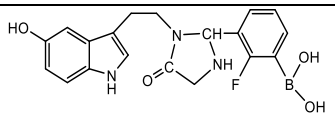
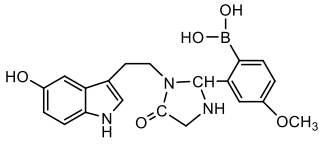
Table (3): Data of compounds from the tool of ADME

Compounds	Hydrogen-bond acceptor	Hydrogen-bond donor	Molar refractivity	TPSA (A^o)	GI absorption	BBB permeant	Bioavailability	Lipinski violation
IIIa	3	3	114.82	71.60	High	yes	0.55	0 violation
IIIb	3	3	105.63	68.36	High	yes	0.55	0 violation
IIIc	6	5	110.40	108.82	High	No	0.55	0 violation
IIId	6	5	116.93	118.05	High	No	0.55	0 violation

Results of docking interpretation

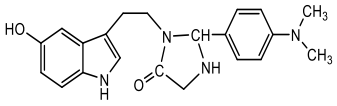
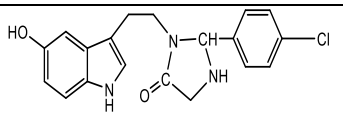
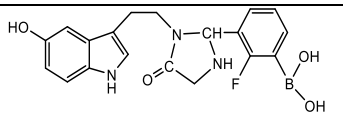
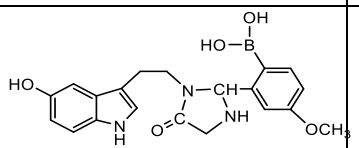
In virtual screening all the synthesized compounds were docked to predict their orientations, interactions and binding affinities to the active sites of receptors of four types of bacteria. All the synthesis compounds with their orientations, interactions and binding affinities scores are present in the table (3) and table (4). From these tables it is clear that there are different types of interactions between synthesized compounds(ligands) and four types of bacteria(receptor) which include H-bond, Pi-cation, Pi-Pi stacking as well as hydrophobic interactions, these interactions strength the binding affinities and give good orientations of the compounds to the active sites of receptors.As seen from tables(3) and (4) the highest docking scores on two gram positive(*Streptococcus Pyogenes* and *Staphylococcus aureus*) were -9.014 and -9.33 respectively and on two gram negative(*Escherichia coli* and *Klebsiella*) were -7.383 and -7.502 respectively was given by compound(IIIc).The docking scores of trimethoprim on the four types of gram positive and gram negative bacteria(Streptococcus Pyogenes, Staphylococcus aureus, Escherichia coli and Klebsiella) were -9.168,-9.486,-5.971and -5.43 respectively .These results of docking gave accepted range in compared with the results of experiments of the synthesis.

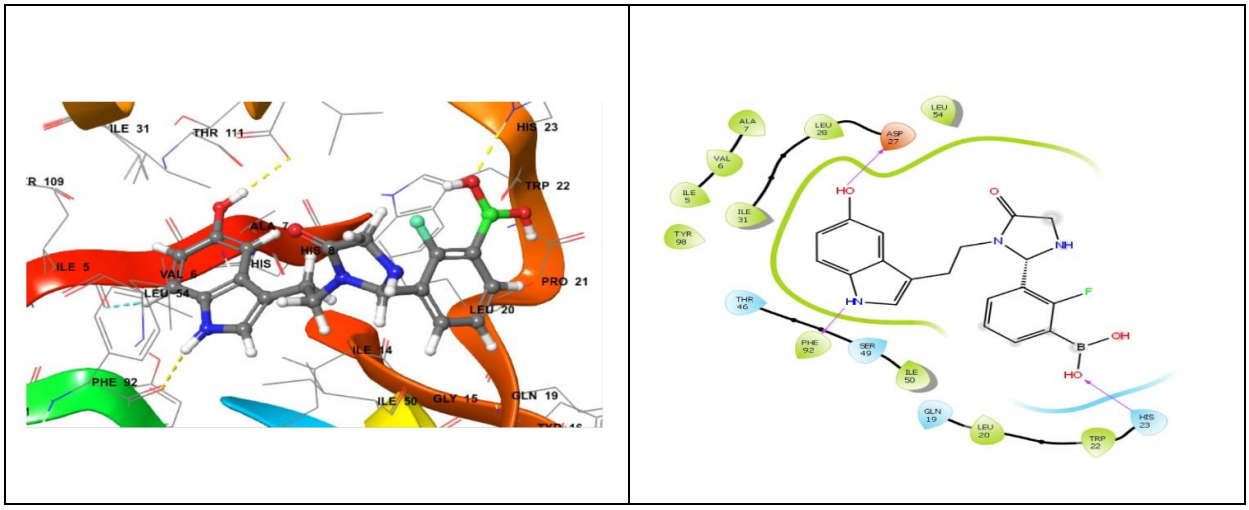
Table (4): Scores of binding and major interactions between Gram positive bacteria and synthesized compounds

Compounds	Chemical structure	Scores of docking on Staphylococcus aureus	Kind of interactions of compound 's ligand with Staphylococcus aureus	Scores of docking on Streptococcus pyogenes	kind of interactions of compound's ligand with Streptococcus pyogenes
IIIa		-7.775	The ASP27 amino acid interact with the hydroxyl group of the ring of indole by hydrogen bond also PHE92 amino acid interact with NH group of the ring of indole by hydrogen bond in addition to the hydrophobic interactions that are : ILE50,LEU20,THR46,TYR98,LEU28,ILE31,LYS32,ILE5,VAL6,ALA7,LEU54, ARG57	-4.992	H-bond interaction between SER282 & Oxygen atom of C=O group of imidazolidine ring and the VAL193 amino acid interact with Oxygen atom of C=O group of imidazolidine ring by hydrogen bond through a molecule of H_2O also the SER280 and GLN162 amino acids interact with the hydroxyl group of the ring of indole by hydrogen bond through a molecule of H_2O also the SER280 amino acid interact with GLN162 amino acid and with VAL193 amino acid by hydrogen bond through a molecule of H2O in addition to the hydrophobic interactions that are : TYR389,GLY284,ALA283,GLY281,SER279,CYS192,GLY191,VAL334,GLY333,GLN332,TYR330, ALA341,HIE340,GLY339
IIIb		-8.103	The ASP27 amino acid interact with hydroxyl group of the ring of indole by hydrogen bond and the PHE92 amino acid interact with NH group of the ring of indole by hydrogen bond also the ARG57 amino acid interact with chlorine atom by Halogen bond in addition to the hydrophobic interactions that are : LEU20,ILE50,LEU54,PRO55,LYS32,ILE31,LEU28,ILE5, VAL6,ALA7,THR46 & TYR98	-5.855	The SER282 amino acid interact with hydroxyl group of the ring of the indole by hydrogen bond in addition to the hydrophobic interactions that are : TYR389,VAL334,GLY333,GLN332,GLY284,ALA283,GLY281,ALA341, HIE340,GLY339 & GLY338
IIIc		-9.33	The HIS23 amino acid interact with one of hydroxyl groups of boronic acid by hydrogen bond and the PHE92 amino acid interact with NH group of the ring of indole by hydrogen bond also the ASP27 amino acid interact with the hydroxyl group of the ring of indole by hydrogen bond in addition to the hydrophobic interactions that are : TRP22,LEU20,GLN19,ILE50,SER49,THR46,ILE31, LEU28,TYR98,ILE5,VAL6, ALA7 & LEU54	-9.014	The two TYR389 and SER286 amino acids interact with hydroxyl group of the ring of indole by hydrogen bond through a molecule of H2O and the SER282 amino acid interact with NH group of the ring of imidazolidine by hydrogen bond also the SER280 amino acid interact with one of hydroxyl groups of boronic acid through a molecule of H2O and directly with the other hydroxyl group of same boronic acid by hydrogen bond also the CYS192 amino acid interact with one of hydroxyl groups of boronic acid by hydrogen bond and the SER286 amino acid interact with ALA283 amino acid through a molecule of H2O by hydrogen bond in addition to the hydrophobic interactions that are : SER279,GLY281,GLY284,GLN304,TYR330,GLN332,GLY333,VAL334,VAL193,GLY191,ALA341,HIE340 & GLY339
IIId		-7.369	The SER49 amino acid interact with the two hydroxyl groups of boronic acid by hydrogen bond and the LEU28 amino acid interact with the NH group of the ring of indole by hydrogen bond also the ARG57 amino acid interact with the benzene ring by Pi-cation interaction in	-7.079	The TYR389 amino acid interact with hydroxyl group of the ring of indole by hydrogen bond through a molecule of H2O and the SER282 amino acid interact with the one of hydroxyl groups of boronic acid by hydrogen bond also the VAL193 amino acid interact with the same hydroxyl group of boronic acid by hydrogen bond through a molecule of H2O in addition to the hydrophobic

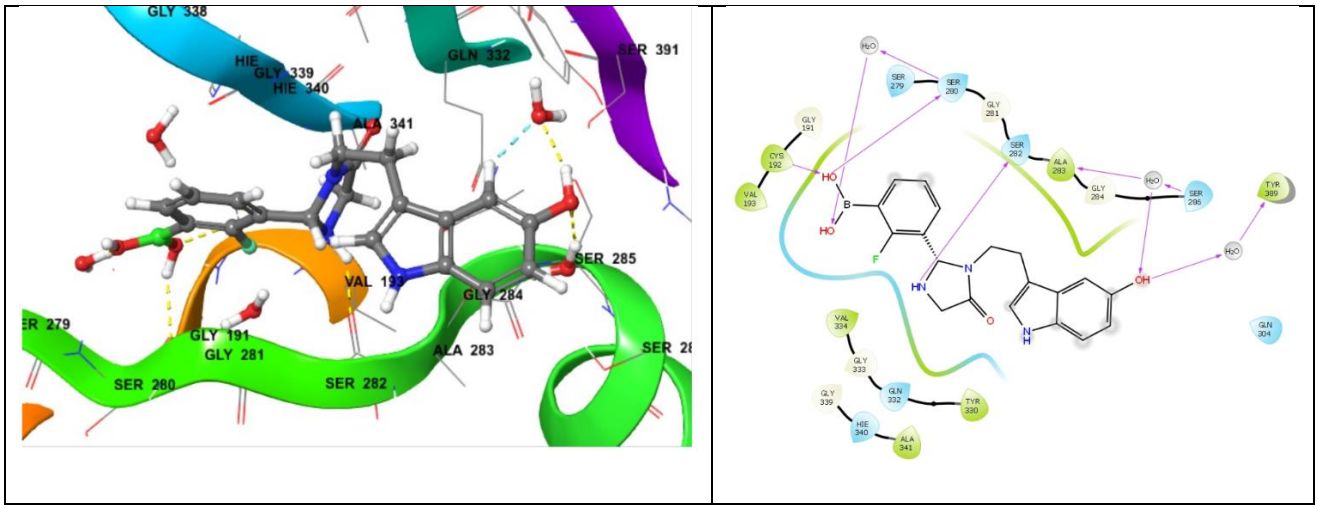
			addition to the hydrophobic interactions that are : LEU20,GLN19,PHE92,ILE5, VAL6,THR46,ILE50,LYS52, LEU54,PRO55,LYS32, ILE31		interactions that are : GLN304,SER286,GLY284,ALA283,GLY281,ALA341,HIE340,GLY339,GLY338,CYS192,VAL334,GLY333, GLN332 & TYR330

Table (5): Scores of binding and major interactions between Gram negative bacteria and synthesized compounds

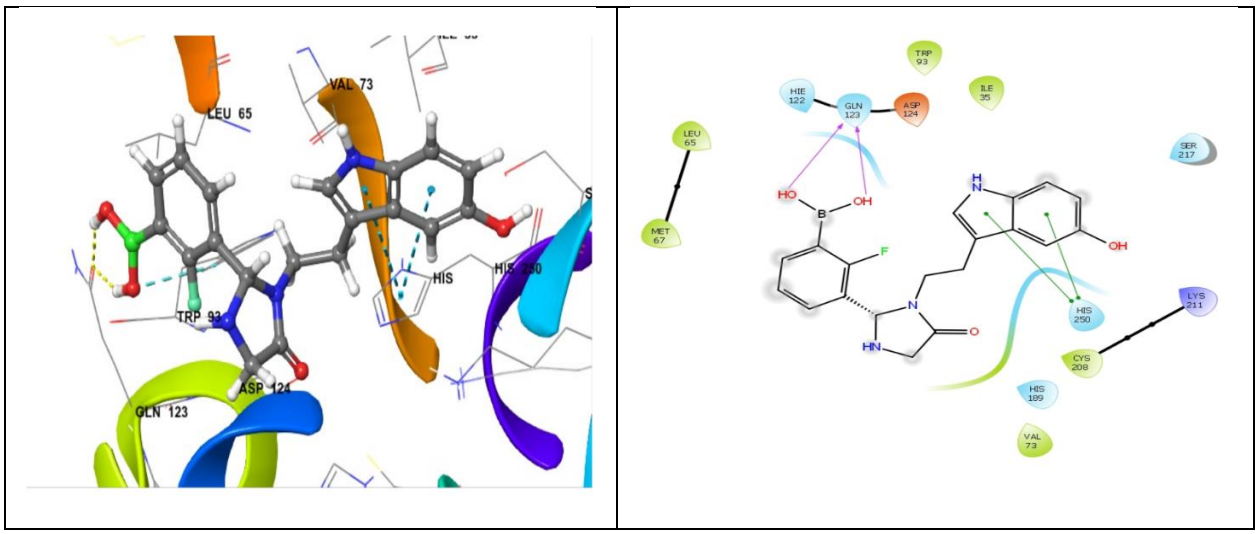
Compounds	Chemical structure	Scores of dockings on Klebsiella	Kind of interactions of compound 's ligand with Klebsiella	Scores of dockings on Escherichia coli	Kind of interactions of compound's ligand with Escherichia coli
IIIa		-6.652	The HIS250 amino acid interact with benzene ring by Pi-Pi stacking interaction in addition to the hydrophobic interactions that are: ALA215, LYS216, SER217, GLY36, ILE35, SER251, HIS189, VAL73, HIE122, LEU65, MET67, LYS211, CYS208 & TRP93	-5.969	The ASN46 amino acid interact with the hydroxyl group of the ring of indole by hydrogen bond also the ASP73 amino acid interact with NH group of the ring of indole by hydrogen bond in addition to the hydrophobic interactions that are: ARG136, VAL120, ARG76, HIS116, GLY101, GLY102, LYS103, PHE104, ASP105, VAL167, THR165, ALA90 and ILE94
IIIb		-5.51	The TRP93 amino acid interact with the ring of pyrrole by Pi-Pi stacking interaction in addition to the hydrophobic interactions that are: LEU65, MET67, VAL73, ILE35, HIS250, CYS208, LYS211, GLY222, ASP223, GLU152, MET154, HIE122 & HIS189	-6.667	The ASP73 amino acid interact with & NH group of the ring of indole by hydrogen bond and the GLY102 amino acid interact with NH group of the ring of imidazolidine in addition to the hydrophobic interactions that are: ILE94, ASN46, VAL120, VAL71, ARG76, ASP105, PHE104, LYS103, GLY101, VAL167, THR165 and ALA90
IIIc		-7.502	The GLN123 amino acid interact with the two hydroxyl groups of boronic acid by hydrogen bond also the HIS250 amino acid interact with the both benzene ring & pyrrole ring of the indole by Pi-Pi stacking interaction in addition to the hydrophobic interactions that are: TRP93, ILE35, SER217, LYS211, CYS208, HIS189, MET67, LEU65, HIE122, ASP124 & VAL73	-7.383	The VAL71 and ASP73 amino acids interact with the one and same hydroxyl group of the boronic acid by hydrogen bond through a molecule of H2O and the PHE104 amino acid interact with NH group of the ring of indole by hydrogen bond also the ASP49 amino acid interact with the hydroxyl group of the ring of indole by hydrogen bond and the ARG76 amino acid interact with both benzene & pyrrole rings of indole by pi-cation interaction in addition to the hydrophobic interactions that are : VAL120,ILE94,GLY101,GLY102,LYS103,ASP105,ALA53,LEU52,GLU50,ALA47,ASN46,VAL167, THR165 andVAL111
IIId		-7.191	The GLU152 amino acid interact with the hydroxyl group of the ring of indole by hydrogen bond in addition to the hydrophobic interactions that are: ILE35, VAL73, LYS211, HIS250, HIS189, HIS120ALA121, HIE122, MET154, LEU65, MET67 & TRP93	-7.175	H -bond interactions between PHE104 and oxygen atom of C=O of imidazolidine ring in one of direction & with OH group of indole ring in the other direction in addition to the hydrophobic interactions that are: HIS116, VAL120, ASN46, ALA47, THR165, ALA90, ASP73, ARG76, ARG136, ASP105, LYS103, GLY102, GLY101,ALA53 & ILE94



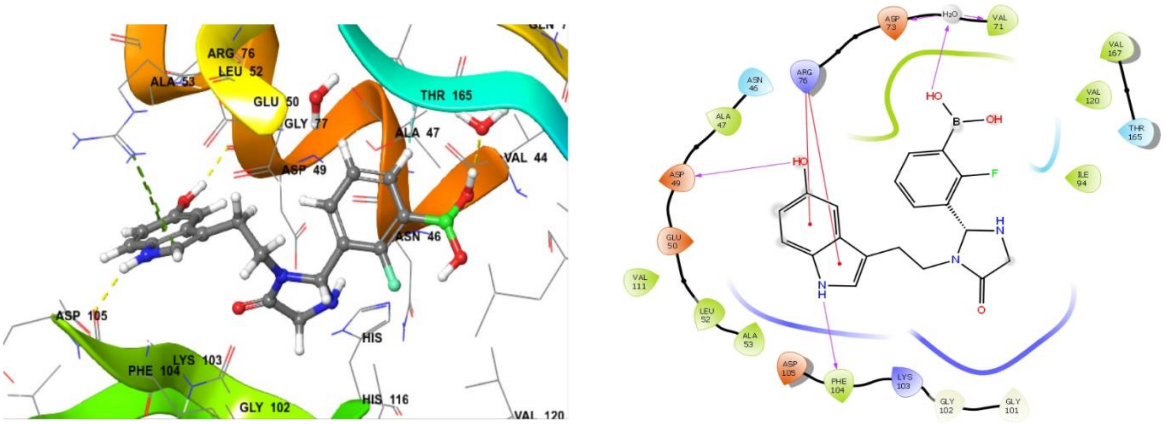
Compound IIIc inside *Staphylococcus aureus* active site



Compound IIIc inside *Streptococcus Pyogenes* active sites



Compound IIIc inside *Klebsiella* active sites



Compound IIIc inside *Escherichia coli* active sites

Conclusion

Synthesizing of final compounds were accomplished auspiciously and target products were obtained. Synthesized compounds were identified and characterized by physical properties, FT-IR spectroscopy & 1H-NMR spectroscopy. All the synthesized compounds exhibit moderate activity in comparing with the standard drug (Trimethoprim), but their highest activity occur on Streptococcus pyogenes bacteria and their lowest activity occur on the Escherichia coli bacteria. The high docking scores were given by the compounds IIIc for both gram positive (*Staphylococcus aureus* and *Streptococcus pyogenes*) and gram negative (*Klebsiella* and *Escherichia coli*). According to the ADME studies entire of synthesized compounds were fulfilled Lipinski's rule.

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