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Synthesis, Characterization and *in Silico* Pharmacological Predictions Studies of Some Oxadiazole, Triazole, Pyrrole Based Fused Indole Derivatives as Potential Antimicrobial Agents

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Abstract

Indole moiety is one of the key heterocyclic rings presents in a large number of biological active molecules. Synthesis of 5-hydroxy derivatives has attracted considerable attention in view of therapeutic applications. In the present study, a series of 1-phenylethyl-2-methyl-3-ethoxycarbonyl-5-hydroxyindole derivatives have been synthesized. All the synthesized compounds have been characterized by using FT-IR, ^{1}H NMR, ^{13}CNMR spectroscopy and further supported by mass spectroscopy. All the synthesized compounds were screened for their antibacterial and antifungal activity by using well diffusion assays. Molecular docking investigations with Glucosamine 6-phosphate synthase (G6P), revealed lower binding energy values for lead compound (1-phenylethyl-2-methyl-3-ethoxycarbonyl-5-hydroxyindole) amongst all derivatives and Ciprofloxacin, indicating most favourable hydrogen-bond interactions with active site residues of G6P. The lead was able to interact with most active site residues Cys1, Trp74, Gly99 of G6P suggesting their strong inhibitory potential similar to Ciprofloxacin. Along with hydrogen bonds, the lead was able to make a π-π stacking with Arg26 of G6P that makes this interaction different than Ciprofloxacin. The lead molecule and its derivatives showed promising antimicrobial

activities comparable to standard drugs. Also, *in silico* analysis revealed that these compounds followed Lipinski's rule of five and showed no signs of carcinogenicity and lower risk of toxicity. Hence it may be developed as a potential pharmacological agent.

Keywords
Indole, Molecular docking, Antibacterial activity, Ciprofloxacin, *In silico* drug relevant properties.

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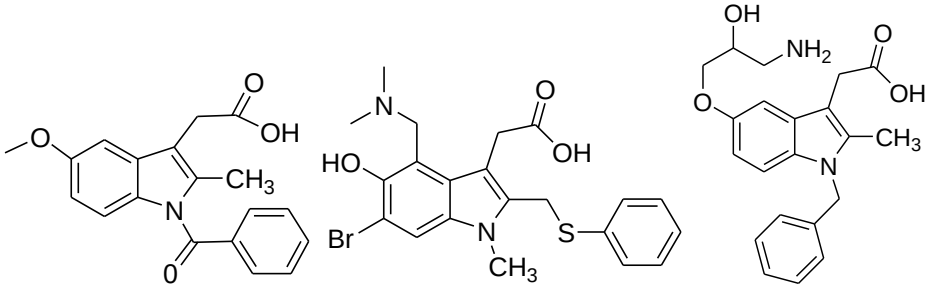
INTRODUCTION

Antimicrobial resistance is fast becoming global concern with rapid increase in multi-drug resistant bacteria and fungi. The rises of antimicrobial resistance, antimalarial resistance are complex and severe health issue. The increased rate of infection due to resistance of available drugs has reached at its alarming level [1]. Infectious disease due to gram-positive bacteria such as methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant *Enterococcus faecium* (VREF), and penicillin resistant *Streptococcus pneumoniae* are the leading cause of morbidity and mortality to the community today [2]. During last few years an increase of invasive microbial and fungal infection has been observed, particularly in immune suppressed patients, which are now the cause of morbidity and mortality as well. Therefore, there is urgent need to develop new antimicrobial agents [3]. A large number of heterocyclic compounds containing the indole ring are associated with diverse pharmacological properties such as analgesic, ant allergic, antibacterial, anticonvulsant, antifungal, antihistaminic, anti-inflammatory, anticancer, anthelmintic, anti-hypertensive and antioxidant [4]. Previous studies have shown that indole moiety has significant antimicrobial activity [5-11].

Computational biology and bioinformatics have the potential not only of speeding up the drug discovery process thus reducing the costs, but also of changing the way drugs are designed. Rational Drug Design (RDD) helps to facilitate and speedup the drug designing process, which involves variety of methods to identify novel compounds. One such method is the docking of the drug molecule with the target. Docking is the process by which two molecules fit together in 3D space [12].



Indomethacin Arbidol 2-(5-(3-amino-2-hydroxypropoxy)-1-benzyl-2-methyl-1H-indol-3-yl) acetic acid
Fig-1 Fig-2 Fig-3

This manuscripts presents the key structural features of molecule is the 1-phenylethyl-2-methyl-3-ethoxycarbonyl indole ring system bearing a substituent oxadiazole, pyrrole, triazole end linked with 5th position of indole ring system. As part of structure activity relationship study the above molecule such as Indomethacin, Arbidol and 2-(5-(3-amino-2-hydroxypropoxy)-1-benzyl-2-methyl-1H-indol-3-yl)acetic acid encourage us to choose N-substituted phenyl ethyl group, 2-substituted methyl group, 3-substituted ethoxcarbonyl group, 5-substituted hydroxyl indole derivatives to get better antimicrobial activity of fused indole derivatives.

Along with this, we have concentrated to prepare fused indole derivatives with oxadizole, triazole, pyrrole because Gadaginamath et al synthesized several bis-heterocyclic compounds containing indole and other heterocyclic ring system like oxadizole, triazole, pyrrole reported antimicrobial

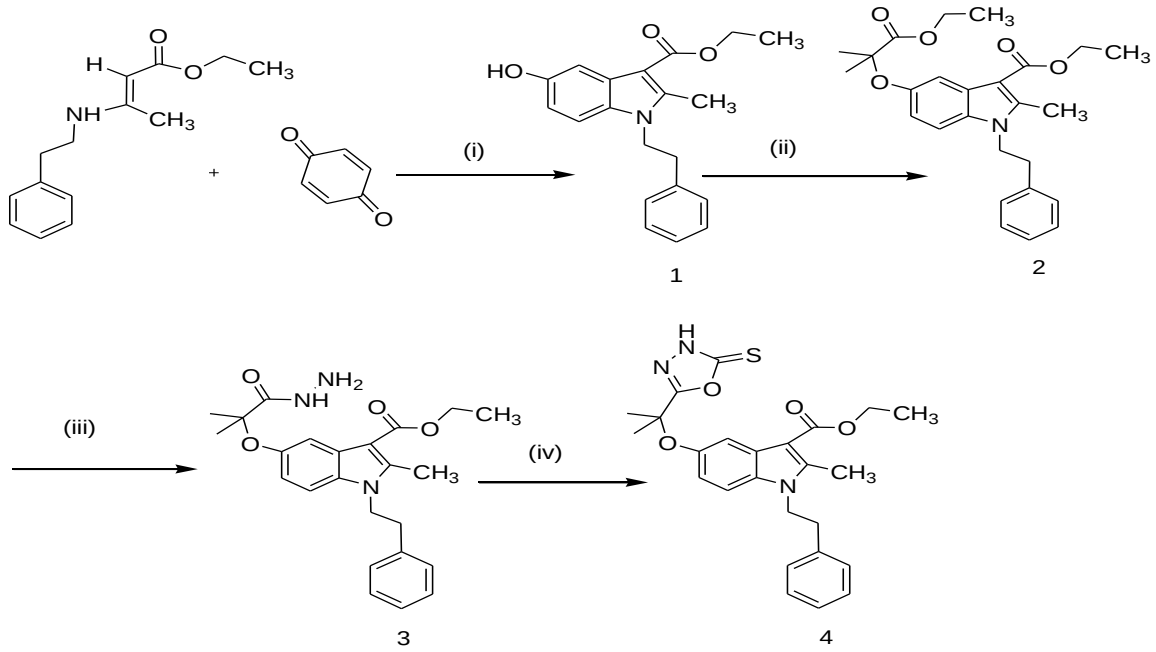
activity. This literature evidence encourages us to prepare fused indole derivatives.

Keeping in view of the biological importance of bis-heterocycles containing indole, it was felt worthwhile to synthesize the indole derived compounds as depicted in scheme-1-3. Here we aimed at generating chemo selective 5-hydroyindole derivatives (compound 1-9), perform their structural characterization, *in vitro* antimicrobial testing and *in silico* calculations of physicochemical and ADMET parameters. Our data suggests a therapeutic potential for these novel hydroxyindole derivatives as plausible antimicrobial agents.

MATERIALS AND METHODS:

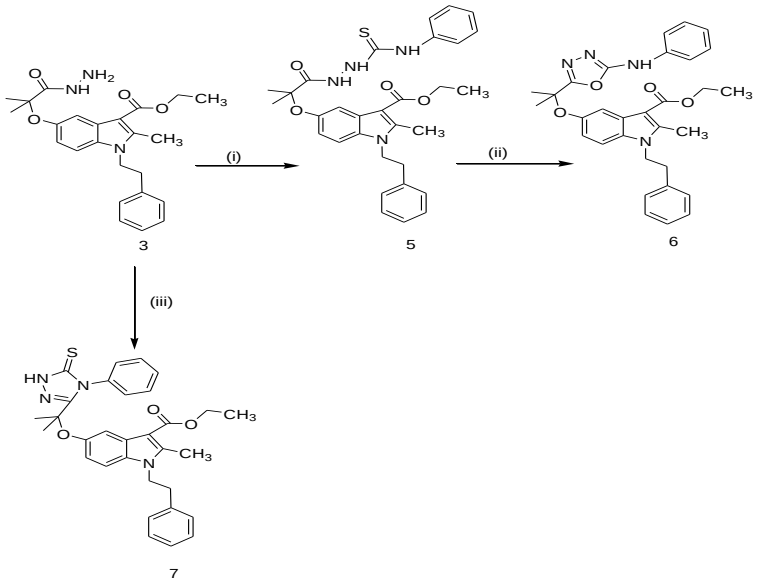
All the reagents and solvents purchased from Sigma–Aldrich, India and used without further purification. Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was checked by TLC on silica gel matrix layer thickness 200 µm and spots were located by iodine and UV light. All compounds were purified by recrystallization with suitable organic solvents. IR spectra were recorded on Bruker ALPHA FTIR instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using direct inlet probe technique. ^{1}HNMR and ^{13}CNMR was determined in $CDCl_3$ solution on a Bruker Avance 400 MHz spectrometer.

Scheme-1. Synthesis of compounds (1-4).



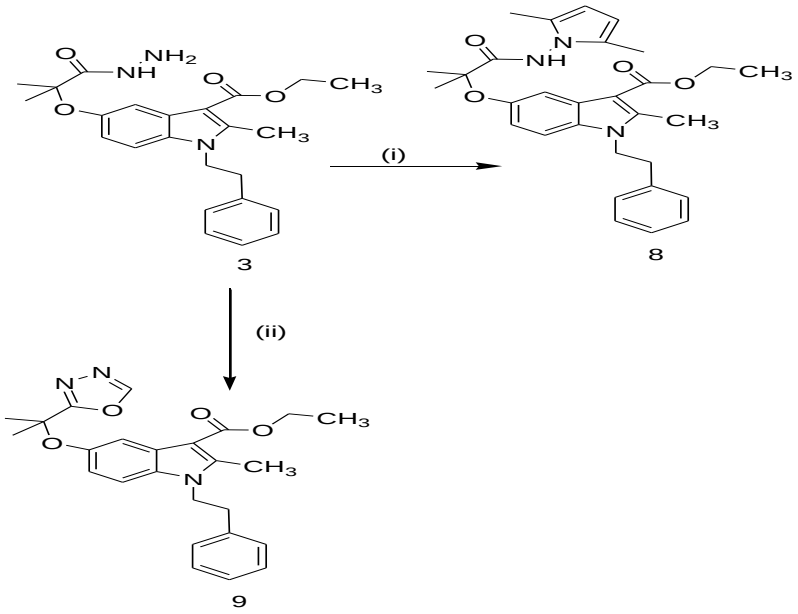
"Reagents and conditions: (i) Anhydrous acetone, Temp, 56°C reflux for 6-8 h. (ii) Anhydrous acetone, K_2CO_3, KI, Ethyl-bromoisobutyrate, Temp, 56°C reflux for 12-14 h. (iii) Hydrazine hydrate, Anhydrous ethanol, Temp, 75°C reflux for 7-8 h. (iv) Carbon disulphide, KOH, HCl, Anhydrous ethanol, Temp, 75°C reflux for 18-20 h.

Scheme-2. Synthesis of compounds (3-7).



"Reagents and conditions: (i) Phenylisothocynate, Anhydrous ethanol, Temp, 75°C reflux for 10-12 h. (ii) 5% NaOH, Anhydrous ethanol, 5% KI, Temp, 75°C reflux for 7-8 h. (iii) 4% NaOH, Temp, 85 °C reflux for 1-2 h.

Scheme-3. Synthesis of compounds (3-8-9).



"Reagents and conditions: (i) Acetyl acetone, Glacial acetic acid, Anhydrous ethanol, Temp, 75 °C reflux for 3-4 h. (ii) Anhydrous ethanol, triethylorthoformate, Temp, 75 °C reflux for 10-12 h

General procedure for the synthesis of Compounds (1-9) as per Scheme-1-3.

Compound 1: Synthesis of 1-phenylethyl-3-ethoxy carbonyl-5-hydroxy-2-methyl Indole:

Into the solution of p-benzoquinone (12 g, 0.11 mol) in anhydrous acetone (60mL), ethyl (E) 3-

(phenethylamino) but-2-enoate (28.4 g, 0.122 mol) was added at room temperature. The reaction mixture was allowed to stand at room temperature for about an hour. It was than heated to reflux for about 6 h. The completion of reaction was monitored by TLC using mobile phase (Ethyl acetate: Hexane; 1:4). After completion of reaction, half of the solvent was distilled out under reduced pressure. The reaction mass was further cooled to 5ºC and filtered. Washed with acetone and dried under vacuum for about 6 hrs and recrystallized from appropriate solvent. (Yield= 60-62%). Melting point is 177-178°C. Molecular formula: $C_{20}H_{21}NO_3$. IR (KBr) (vmax, cm^{-1}): 1657 (ester C=O), 3252 (OH). ^1H NMR (400 MHz, $CDCl_3$/TMS): δ ppm, 1.44(t, 3H), 2.43(s, 3H), 3.0(t, 2H), 4.29(t, 2H), 4.44(q, 2H), 5.36(s, 1H), 6.82(dd, 1H, J=8.44 Hz), 7.28(d, 1H, J=2.7 Hz), 7.65(d, 1H, J=2.44 Hz), 7.03(d, 1H, J=7.62 Hz), 7.16(d, 1H, J=8.54 Hz), 7.14(d, 1H, J=8.54 Hz), 7.25(dd, 1H, J=6.7 Hz), 7.23(dd, 1H, J=6.40 Hz). ^{13}C NMR (200 MHz, CDCl3): δ ppm, 11, 14, 35, 38, 40, 44, 58, 101, 105, 110, 111, 126, 126, 127, 128, 129, 138, 144, 152, 165. LCMS (m/z, % relative intensity): 346 (M+23) (10); 324 (M+1) (100); 278 (10).

Compound 2: 1-phenyl ethyl-3-ethoxy carbonyl-5-(1-ethoxy carbonyl-1-methyl ethoxy)-2-methyl indole.

Into the solution of 1-phenylethyl-3-ethoxy carbonyl-5-hydroxy-2-methyl Indole (20 g, 0.0619 mol) in anhydrous acetone (150 mL), Ethyl-bromoisobutyrate (14.48 g, 0.0742 mol) was added at room temperature. The reaction mixture was cooled to 10ºC. Then dried potassium carbonate (42.72 g, 0.309 mol) and a pinch of potassium iodide were added. The reaction mass was further heated to reflux for 12-14 h. The completion of reaction was monitored by TLC using mobile phase (Ethyl acetate: Hexane; 1:4). After completion of reaction, the reaction mass was cooled to room temperature and filtered. The clear filtrate containing product was distilled out under reduced pressure. The residue was collected and recrystallized in ethanol. The resultant residue was purified by silca column using 10% ethyl acetate in hexane. (Yield: 62%). Melting point is 74-77°C. Molecular formula: $C_{26}H_{31}NO_5$. IR (vmax, cm^{-1}): 1686 (C3-C=O), 1731 (C5 ester C=O) and absence of C5-OH group was observed. ^1H NMR (400 MHz, $CDCl_3$/TMS) δ ppm,1.28 (t, 3H), 2.48(s, 3H), 1.46(t, 3H), 1.62(s, 3H), 1.6(s, 3H), 3.03(t, 2H), 4.29(q, 2H,), 4.39(t, 2H), 6.86(dd, 1H, J=8.54 Hz), 7.28(d, 1H, J=2.7 Hz), 7.63(d, 1H, J=2.4 Hz), 7.05(dd, 1H, J=7.62 Hz), 7.15(d, 1H, J=8.85 Hz), 7.13(d, 1H, J=8.85 Hz), 7.27(dd, 1H, J=6.7 Hz), 7.24(dd, 1H, J=6.7 Hz)^{13}CNMR (200MHz, $CDCl_3$): δ ppm, 11, 14, 14, 25, 35, 44, 59, 61, 76, 77, 77, 79, 103, 109, 111, 116, 126, 126,128, 128, 131, 137, 145, 150, 165, 174). LCMS (m/z, % relative intensity); 438 (M^++1) (100); 392 (30); 324 (10).

Compound 3: 1-phenyl ethyl-3-ethoxycarbonyl-2-methylindole-5-(1-ethyl ethoxy acetic acid hydrazide.

Into the solution of 1-phenyl ethyl-3-ethoxy carbonyl-5-(1-ethoxy carbonyl-1-methyl ethoxy)-2-methyl indole. (18 g, 0.0411 mol) in anhydrous ethanol (90 mL) and 99% hydrazine hydrate was added. The reaction mass further heated to reflux for 7-8 h. The completion of reaction was monitored by TLC using mobile phase (Ethyl acetate: Hexane; 1:4). After completion of reaction, the reaction mass was cooled to 5ºC, filtered, washed with acetone and dried under vacuum for about 6 h.(Yield= 61%). Melting point is 142-145°C. Molecular formula: $C_{24}H_{29}N_3O_4$. IR (KBr) (vmax, cm^{-1}): 1674 (C3-ester, C=O), 1649 (C5-Amide, C=O), 3315-3375(NH/NH_2). ^1H NMR ($CDCl_3$/TMS);δ ppm, 1.45(t, 3H), 1.53(s, 6H), 2.48(s, 3H), 3.04(t, 2H), 4.32(t, 2H), 4.39(q, 2H), 6.84(dd, 1H, J=8.54 Hz), 7.29(d, 1H, J=2.7 Hz), 7.72(d, 1H, J=2.4 Hz), 7.04(dd, 1H, J=7.62 Hz), 7.16(d, 1H, J=8.85 Hz), 7.14(d, 1H, J=8.85 Hz), 7.26(dd, 1H), 7.23(dd, 1H), 8.12(s, 1H, Amide, Disappeared on D_2O exchange). ^{13}C NMR (200 MHz, $CDCl_3$): δ ppm, 11, 14, 24, 29, 35, 44, 59, 76, 76, 77, 81, 103, 109, 114, 117, 126, 127, 128, 132, 137, 145, 148, 165, 175. LCMS (m/z, % relative intensity) 446 (M^+ +23) (40); 424 (M^+ +1) (20); 378 (100).

Compound-4:1-phenylethyl-2-methyl-3-ethoxycarbonyl-5(5'-mercapto-1',3',4'-oxadiazol-2'-yl)-1-methyl ethoxy indole.

Into the solution of 1-phenyl ethyl-3-ethoxycarbonyl-2-methylindole-5-(1-ethyl ethoxy acetic acid hydrazide (10 g, 0.0243 mol) in anhydrous ethanol (90 mL), carbon disulphide (4.63 g, 0.060 mol), dried potassium hydroxide (2 g, 0.036 mol) was added. The reaction mass was refluxed until the evolution of H_2S ceased (approx 20h). The reaction mixture was cooled to room temperature and poured into ice-cold water. It was then neutralized with dil. HCl. The precipitated solid was filtered, washed with water and dried. (Yield= 68%). Melting point is 134-136°C. Molecular formula: $C_{25}H_{27}N_3O_4S$. IR (KBr) (vmax, cm^{-1}): 1655 (C3-ester, C=O), 3084(NH). ^1H NMR ($CDCl_3$/TMS); δ ppm, 1.30(t, 3H), 1.32(s, 6H), 2.48(s, 3H), 3.04(t, 2H), 4.32(t, 2H), 4.39(q, 2H), 6.84(dd, 1H, J=8.54 Hz), 7.29(d, 1H, J=2.7 Hz), 7.72(d, 1H, J=2.4

Hz), 7.04(dd, 1H, J=7.62 Hz), 7.16(d, 1H, J=8.85 Hz), 7.14(d, 1H, J=8.85 Hz), 7.26(dd, 1H), 7.23(dd, 1H), 14.5(s, NH of oxdiazole, disappeared on D_2O exchange), ^{13}C NMR (200MHz, $CDCl_3$): δ ppm, 11. 14. 25.29. 35.45. 59. 75. 76. 77. 77. 104. 109. 115. 117. 127. 127. 128. 132. 137. 146. 149. 165. 166. 178. LCMS (m/z, relative intensity); 488 (M +23) (15); 420 (100).

Compound-5:1-phenylethyl-3-ethoxycarbonyl-2-methyl-5-yl (1-methyl ethoxy carbothio semicarbazide.

Into the solution of 1-phenyl ethyl-3-ethoxycarbonyl-2-methylindole-5-(1-ethyl ethoxy acetic acid hydrazide. (10 g, 0.0243 mol) in anhydrous ethanol (90 mL), phenylisothiocynate (3.29 g, 0.0243 mol), the mixture was refluxed for 12h. The yellow solid that separated upon cooling to room temperature was filtered and recrystallized in ethanol. (Yield=69%). Melting point is 127-129°C. The molecular formula is $C_{31}H_{34}N_4O_4S$. IR (KBr) (vmax, cm^{-1}): 3222, 3310 (secondary amide NH). 1693 (C-5-ester C=O) and 1673 (C-3-ester C=O). ^1H NMR ($CDCl_3$/TMS): δ ppm, 1.45(t, 3H), 1.61(s, 6H), 2.48(s, 3H), 3.03(t, 2H), 4.39(t, 2H), 4.27(q, 2H), 7.78(d, 1H, J=2.19 Hz), 6.96(dd, 1H, J=8.79 Hz), 7.29(d, 1H, J=2.7 Hz), 7.2 to 7.4(m 10H, Aromatic H), 8.39, 9.1, 9.9(s 1H, Amide NH, Disappeared on D_2O exchange). ^{13}C NMR (200MHz, $CDCl_3$): 11, 14.25, 29, 45, 75, 76, 77, 77, 78, 103, 109, 114, 117, 121, 124, 125, 126, 126, 127, 128, 129, 129, 132, 137, 145, 148, 165,171, 188. LCMS (m/z, % relative intensity); 559 (M+1) (100); 513 (M -45) (60).

Compound-6:1-phenylethyl-3-ethoxycarbonyl-2-methyl-5(5'analino-1',3',4'-oxadiazol-2'-yl-1- ethyl ethoxy indole.

Into the solution of 1-phenylethyl-3-ethoxycarbonyl-2-methyl-5-yl(1-methyl ethoxy carbothio semicarbazide (5g, 0.0089 mol) in anhydrous ethanol (35 mL), 5mL each of 5% sodium hydroxide solution and 5% potassium iodide solution was added gradually with shaking till the colour of iodine persisted at room temperature. The contents were refluxed on a water bath for 7h. The solvent was removed under reduced pressure and residue was recrystallized from ethanol. (Yield=76%). Melting point is 202-205°C. Molecular formula $C_{31}H_{32}N_4O_4$.IR (KBr) (vmax, cm^{-1}):1674(C3-easterC=O), 3222 (NH). ^1H NMR ($CDCl_3$/TMS): δ ppm, 1.32(t, 3H), 1.6(s, 6H), 2.41(s, 3H), 3.01(t, 1H, J=7.32 Hz), 4.34(t, 2H), 4.26(q, 2H), 6.6-7.5(m, 13H, Aromatic H), 10.6(s, 1H of phenyl NH, disappeared on D_2O exchange). LCMS (m/z, % relative intensity): 541 (M +NH3) (100) 495; (M-45) (15).

Compound-7:1-phenylethyl-3-ethoxycarbonyl-2-methyl- 5(4'-phenyl-5'-mercapto-1',2',4'-trizole-3'-yl) 1-methylethoxyindole.

Into the solution of 1-phenyl ethyl-3-ethoxycarbonyl-2-methylindole-5-(1-ethyl ethoxy acetic acid hydrazide. (10 g, 0.0243 mol) in 4% sodium hydroxide (25 mL) solution was added and reaction mass refluxed for 1 h. The reaction mass cooled to room temperature then poured in to crushed ice and acidified carefully with dilute acetic acid. The precipitation thus obtained was filtered, washed with water, dried and recrystallized from suitable solvent. Yield=62%. Melting point is 218-220°C. Molecular formula $C_{31}H_{32}N_4O_3S$. ^1H NMR ($CDCl_3$/TMS): δ ppm, 1.32(t, 3H), 1.61(s, 6H), 2.48(s, 3H), 3.04(t, 2H), 4.32(t, 2H), 4.39(q, 2H), 6.6 - 7.7(m, 8H, Aromatic H). LCMS (m/z, % relative intensity): 541 (M +1) (100); 465 (M -45) (15).

Compound-8:1-phenylethyl-3-ethoxycarbonyl-5-(2-5-dimethylpyrrole-1-yl)-aminocarbonyl-1-methyl ethoxy-2- methyl indole.

Into the solution of 1-phenyl ethyl-3-ethoxycarbonyl-2-methylindole-5-(1-ethyl ethoxy acetic acid hydrazide. (10 g, 0.0243 mol) in absolute ethanol (75 mL), Acetyl acetone (2.44 g, 0.0243 mol) and glacial acetic acid (1 mL) were added. The reaction mass was refluxed on water bath for 3 h. The reaction was monitored by TLC, after completion of reaction, the reaction mixture was concentrated to half of its original volume and poured into ice cold water (20mL). The separated solid was collected by filtration, washed with water, dried and recrystallized from ethanol. (Yield= 73%). Melting point is 133-135°C. The Molecular formula $C_{30}H_{35}N_3O_4$. IR (KBr) (vmax, cm^{-1}): 1680(C3-ester C=O), 1698(C5- C=O); 3285(NH). ^1H NMR($CDCl_3$/TMS); δ ppm, 1.44(t, 3H), 2.13(s, 6H), 2.49(s, 3H), 3.05(t, 2H), 4.32(t, 2H), 4.39(q, 2H), 5.80(s, 2H), 8.98(s, 1H amide NH), 6.92(dd, 1H, J=8.54 Hz), 7.29(d, 1H, J=2.7 Hz), 7.72(d, 1H, J=2.4 Hz), 7.01(dd, 1H, J=7.62 Hz), 7.16(d, 1H, J= 8.85 Hz), 7.14(d, 1H, J= 8.85 Hz), 7.26(dd, 1H), 7.23(dd, 1H), 8.98(s, 1H of Amide NH). ^{13}C NMR (200MHz, $CDCl_3$): δ ppm, 11, 11, 14, 25, 29, 35, 45, 59, 76, 77, 77, 81, 103, 104, 105, 109, 114, 117, 126, 126, 127, 127, 128, 132., 137, 145, 148, 165, 165, 173. LCMS (m/z, % relative Intensity): 502 (M^+), (100).

Compound-9:1-phenylethyl-3-ethoxycarbonyl-2-methyl-5(1,3,4, oxadiazole-2'yl-1 methyl ethoxy indole.

Into the solution of 1-phenyl ethyl-3-ethoxycarbonyl-2-methylindole-5-(1-ethyl ethoxy acetic acid hydrazide. (10 g, 0.0243 mol) in absolute ethanol 75 mL, triethylorthoformate (20 mL) was added. The reaction mass was refluxed for 10-12 h. The excess of triethylorthoformate was removed under reduced pressure and the residue was triturated with petroleum ether. The resulting solid was filtered and recrystallized from ethanol. (Yield= 73%). Melting point is 124-260°C. Molecular formula is $C_{31}H_{32}N_4O_4$. IR (KBr) (vmax, cm^{-1}): 1657 (C3-ester C=O), 3173 secondary amide (NH). ^1H NMR ($CDCl_3$/TMS): δ ppm, 1.28(t, 3H), 1.6(s, 6H), 2.48(s, 3H), 3.94(t, 2H), 4.35(t, 2H), 4.24(q, 2H), 6.6-7.5(m, 13H, Aromatic H). LCMS (m/z, relative intensity): 541 (M +NH3) (100); 495 (M-45) (15).

***In vitro* antimicrobial assay:**

The bacterial and fungal pathogenic clinical isolates purchased from NCIM (Pune, India) were used as a source for antibacterial and antifungal activity studies. The test bacterial isolates comprised of gram negative bacterium- *Escherichia coli* and gram positive bacterium- *Bacillus cirroflagellosus*. The test fungal pathogens comprised of *Aspergillus niger* and *Candida albicans*. The antimicrobial activities of all the newly synthesized derivatives (1mg/ mL) were tested against these pathogens by using the agar well diffusion method. Ciprofloxacin and Griseofulvin (1mg/ mL each) were used a standard drugs during the assay.

Molecular docking and *in silco* pharmacological predictions:

Ligand Preparation

The derivatives were drawn using Maestro 2D Draw (Version 10.6). LigPrep (Schrödinger, LLC, USA) was used for the preparation of ligands for the docking upon optimization (force field OPLS 2005).

Protein preparation

The X-ray crystal structure of Glucosamine 6-Phosphate synthase (G6P) (PDB ID: 1XFF) with a resolution of 1.81 Å was obtained from the Protein Data Bank (http://www.rcsb.org/pdb). Protein preparation wizard (Schrödinger LLC, USA) was used to prepare the protein for docking [13]. All the heteroatoms and water molecules were removed. Polar hydrogens and Gasteiger charges were assigned after merging of non-polar hydrogen atoms. The grid map was generated placing the binding pocket involving Cys 1, Trp 74, Thr 76, His 77, Gly 99, and Asp 123 as previously reported [14].

Molecular docking

The docking was performed using GLIDE (Grid Based Ligand Docking with Energetics) module in Schrodinger suite. The compounds were subjected to flexible docking using the pre-computed grid files using XP (extra precision) mode [15]. The poses that have the potential G-Score (Glide Score) was saved and only the best scoring pose was analysed further.

Molecular parameters

The molecular parameters of the synthesized compounds were predicted using Mo inspiration online server (http://www.molinspiration.com). The estimated parameters included miLogP, Topological polar surface area (TPSA), number of hydrogen bond acceptors (HBA), hydrogen bond donors (HBD) and molecular flexibility (nrotb). The drug-likeness property of the synthesized compounds and standards were investigated as G-protein coupled receptor (GPCR) ligands, ion channel modulators, kinase inhibitors, nuclear receptor ligands and enzyme inhibitors.

Absorption-Distribution-Metabolism-Excretion and toxicity (ADME/Tox) predictions

The ADME properties of all the ligands were calculated by using admetSAR (Version 3.4) [16]. The admetSAR, is a comprehensive knowledge and tool for predicting Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) properties of drug candidates and environmental chemicals. The Simplified Molecular-Input Line-Entry System (SMILES) format of each ligand was submitted to admetSAR webserver for the prediction of ADMET properties.

RESULTS AND DISCUSSION:

In Scheme 1-3, 1- phenylethyl-2-methyl-3-ethoxycarbonyl-5-hydroxyindole (compound-1) was used as a lead molecule, which was prepared by adopting the Nenitzescu reaction [17]. All other compounds namely, 2-9 were prepared as described in the methodology section (Scheme-1) and recrystallized for attaining maximum purity. The structures of synthesized compounds were confirmed by their spectral data (Supplementary information).

Antimicrobial activity:

The results of antimicrobial activities are depicted in Table-1, which shows that all the compounds had varied degree of activities against test organisms. Among the synthesized derivatives, compounds 4 and 9 showed antibacterial activity equivalent to that of standard (Ciprofloxacin) against tested organisms.

However, compound-7 showed better activity against *E. coli* than *B. cirroflagellosus*. In case of antifungal activities, compound-7 was found to be most active with zone of inhibition equivalent to that of standard Griseofulvin, against both *A. Niger* and *C. albicans*. Whereas compounds 4, 5, 8 and 9 showed greater antifungal activity against *A. Niger* alone.

***In silico*Predictions**

Physico-chemical Properties:

The Lipinski's rule-of-five [18]. On drug-likeness has been utilized most often to determine if a chemical entity would make it into orally active drug in humans or not. Calculations of molecular parameters such as miLogP, TPSA and others were obtained using Mo inspiration web server [19].The drug-likeness scores of each compound is given in Table-3. The miLogP values of compounds 1, 3, 4 and 8 were found to be lower than 5, thus were more likely to be bio available than other derivatives. Only compound-1 had TPSA below 60 $Å^2$, thus predicted to have both good intestinal absorption and Blood-brain barrier (BBB) penetration [20]. All the synthesized compounds 1-9 had less than 10 H-bonds acceptors (HBA) and less than 5 H-bond donors (HBD). Except for compounds 2, 5 and 6, molecular conformation flexibility (nrotb) for all other compounds were ≤10, which presumes good oral bioavailability. Lastly, all the compounds except 5, 6, 7 and 9 in this series had molecular weight (MW) below 500. Overall based on this study, only compounds 1, 3, 4 and 8 were compliant with Lipinski's rule-of-five, hence were predicted to have good oral bioavailability. Among these, only compound-1 had the least polar surface area therefore assumed to have best intestinal absorption and BBB penetration capability.

Furthermore, bioactivity scores (Table-4) were calculated for their drug-likeness asGPCR ligands (GPCRL), ion channel modulators (ICM), kinase inhibitors (KI), nuclear receptorligands (NRL), protease inhibitors (PI) and enzyme inhibitors (EI). The scores were categorized as >0 indicate high activity, between 0 to -0.5 indicate moderate activity and scores <-0.5 indicate inactivity [21]. The results indicate that Ciprofloxacin, compounds 4, 8 and 1 showed high activity scores for EI respectively. Based on our results, only compound-2 showed positive as NRL and compound-8 for GPCR. These receptors being important therapeutic targets in many metabolic and inflammatory diseases such as diabetes, cirrhosis and fibrosis [22-23]. These can be potential ligands for cell surface or nuclear receptors with pharmacological implications.

Pharmacokinetic Properties:

Absorption properties of compounds (1-9) were predicted by admetSAR (Supplementary data, Table-S1). The results suggested that all the synthesized compounds might be able to cross blood-brain barrier (BBB+) and deliver to CNS system. The study also predicted that all the compounds were capable of being absorbed by human intestine, while only compound-1 and 2 had Caco-2 permeability. Among all, compound-4 was predicted as the only non-substrate for P-glycoprotein, which effluxes drugs for clearance from the system. All the tested compounds were predicted to have high CYPinhibitory promiscuity, as it inhibited most of the isoforms of cytochrome P450 [24]. Hence, in general the xenobiotic metabolism of these compounds and there *in vivo* dosage requirements needs to be studied further for considering their therapeutic efficacy.

Toxicological Properties:

In silico toxicological analysis of the synthesized compounds 1-9 were predicted by admetSAR. The analysis projected all the compounds as weak HERG (Human Ether-a-go-go-Related Gene) inhibitors, non-AMES toxic (except Ciprofloxacin) and non-carcinogenic (except Griseofulvin) but highly toxic for fish, *Tetrahymena pyriformis* and not readily biodegradable. Depending on the risk of acute oral toxicity, all the synthesized compounds were predicted as category III (as per WHO-IARC Monographs) with LD_{50} values greater than 500 mg/kg but less than 5000 mg/kg. According to predictions on rat carcinogenicity derived from carcinogenic potency database (CPDB), all the synthesized compounds were foreseen to be non-carcinogenic chemicals (Supplementary data Table-1).

Molecular docking analysis:

The present study of *in silico* analysis was undertaken to identify whether the molecular docking of the lead molecule, 1-phenylethyl-2-methyl-3-ethoxycarbonyl-5-hydroxyindole (Compound-1) with G6P target provides any correlating information with respect to their *in vitro* antimicrobial activity. Computationally the molecular docking investigations revealed lower binding energy values of the lead when compared to ciprofloxacin (Table-2), indicating lower binding affinity with G6P. The molecular docking analysis revealed that among all the ligands (data not shown), the lead with the least binding energy of -4.218 kcal/mol exhibited most favourable hydrogen-bond interactions with the

active site residues of G6P (Table-2). The lead was able to interact with most of the active site residues Cys 1,Trp 74 and Gly 99 of G6P identical to ciprofloxacin(Figure-1), suggesting their strong inhibitory potential. Along with hydrogen bonds, the lead was able to make a π-π stacking with Arg 26 that makes the lead different from ciprofloxacin. Figure-2, shows the interaction pattern of lead with G6P. Though ciprofloxacin makes a similar kind of interactions with G6P with the same key residues, the G-score seems to be significantly higher than that of the lead. Thus may be a reason for lowered antimicrobial activity compared to ciprofloxacin.

Table 1. Antimicrobial activity of synthesized compounds 1-9.

Compound No.	Concentration:1mg/ml Zone of inhibition in mm after 48hr		Compound No.	Concentration:1mg/ml Zone of inhibition in mm after 48hr	
	E. coli	*B. cirroflagellosus*		*Candida albicans*	*Aspergillus niger*
1	++	++	1	++	++
2	+	++	2	+	++
3	+	+	3	+	+
4	+++	+++	4	++	+++
5	++	++	5	++	+++
6	+	+	6	+	+
7	+++	++	7	+++	+++
8	++	++	8	++	+++
9	+++	+++	9	++	+++
Ciprofloxacin	+++	+++	Griseofulvin	+++	+++

Symbols: Zone diameter of growth inhibition (-): Inactive; (+): Weak active (12-16mm); (++): Moderately active (16-21mm); (+++): Highly active (>21mm).

Table 2. Molecular docking of compounds with glucosamine-6-phosphate synthase (G6P).

Compound	GScore	H-Bonds
Compound-1 (Lead molecule)	-4.218	CYS 1, TRP 74, GLY 99[#]
Ciprofloxacin	-3.813	CYS 1, TRP 74, GLY 99

 # additionally the lead could make a π-π stacking with ARG 26 that makes even better interaction than ciprofloxacin.

TABLE 3. Physicochemical parameters for the synthesized compounds and standards.

Compound ID	miLogP	TPSA	MW	HBA	HBD	nrotb
Ciprofloxacin	-0.70	74.57	331.35	6	2	3
Griseofulvin	1.57	71.08	352.77	6	0	3
Compound-1	3.89	51.47	323.39	4	1	6
Compound-2	5.49	66.78	437.54	6	0	11
Compound-3	2.89	95.59	423.51	7	3	9
Compound-4	4.12	82.30	465.57	7	1	9
Compound-5	5.35	93.62	558.70	8	3	13
Compound-6	6.68	77.23	522.65	7	1	11
Compound-7	5.71	74.09	540.69	7	1	10
Compound-8	4.33	79.40	433.51	7	0	9
Compound-9	5.36	74.50	501.63	7	1	10

TPSA- topological polar surface area; HBA- H-bond acceptors; HBD- H-bond donors; nrotb- conformational flexibility (number of rotatable bonds)

TABLE 4. Estimation of drug ability for the synthesized compounds and standards.

Compound ID	GPCR Ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Ciprofloxacin	0.12	-0.04	-0.07	-0.19	-0.20	0.28
Griseofulvin	-0.34	-0.30	-0.72	-0.04	-0.26	-0.03
Compound-1	-0.02	-0.25	-0.21	-0.14	-0.34	0.01
Compound-2	-0.18	-0.26	-0.28	0.04	-0.28	-0.02
Compound-3	-0.19	-0.60	-0.33	-0.41	-0.30	-0.03
Compound-4	-0.20	-0.52	-0.47	-0.37	-0.46	0.12
Compound-5	-0.36	-0.76	-0.55	-0.60	-0.42	-0.31
Compound-6	-0.14	-0.44	-0.34	-0.34	-0.31	-0.12
Compound-7	-0.21	-0.64	-0.54	-0.43	-0.43	-0.28
Compound-8	0.04	-0.27	-0.23	-0.06	-0.18	0.11
Compound-9	-0.14	-0.55	-0.30	-0.33	-0.38	-0.16

Figure (a). The 2D interaction map of G6P-Lead (Compound-1) complex.

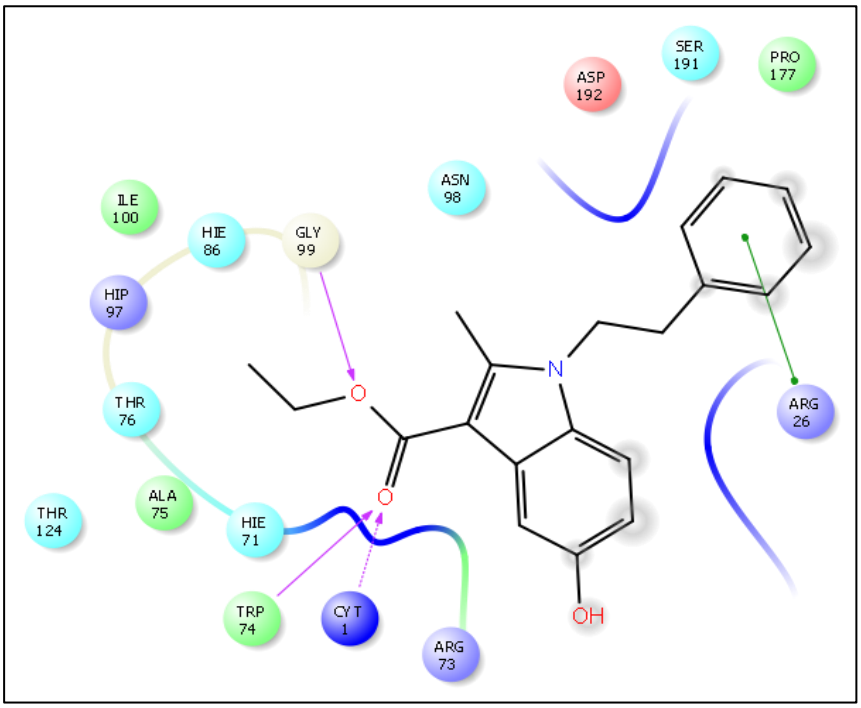


Figure (b). (A-C). The interaction pattern of G6P-Lead (Compound-1) contacts.

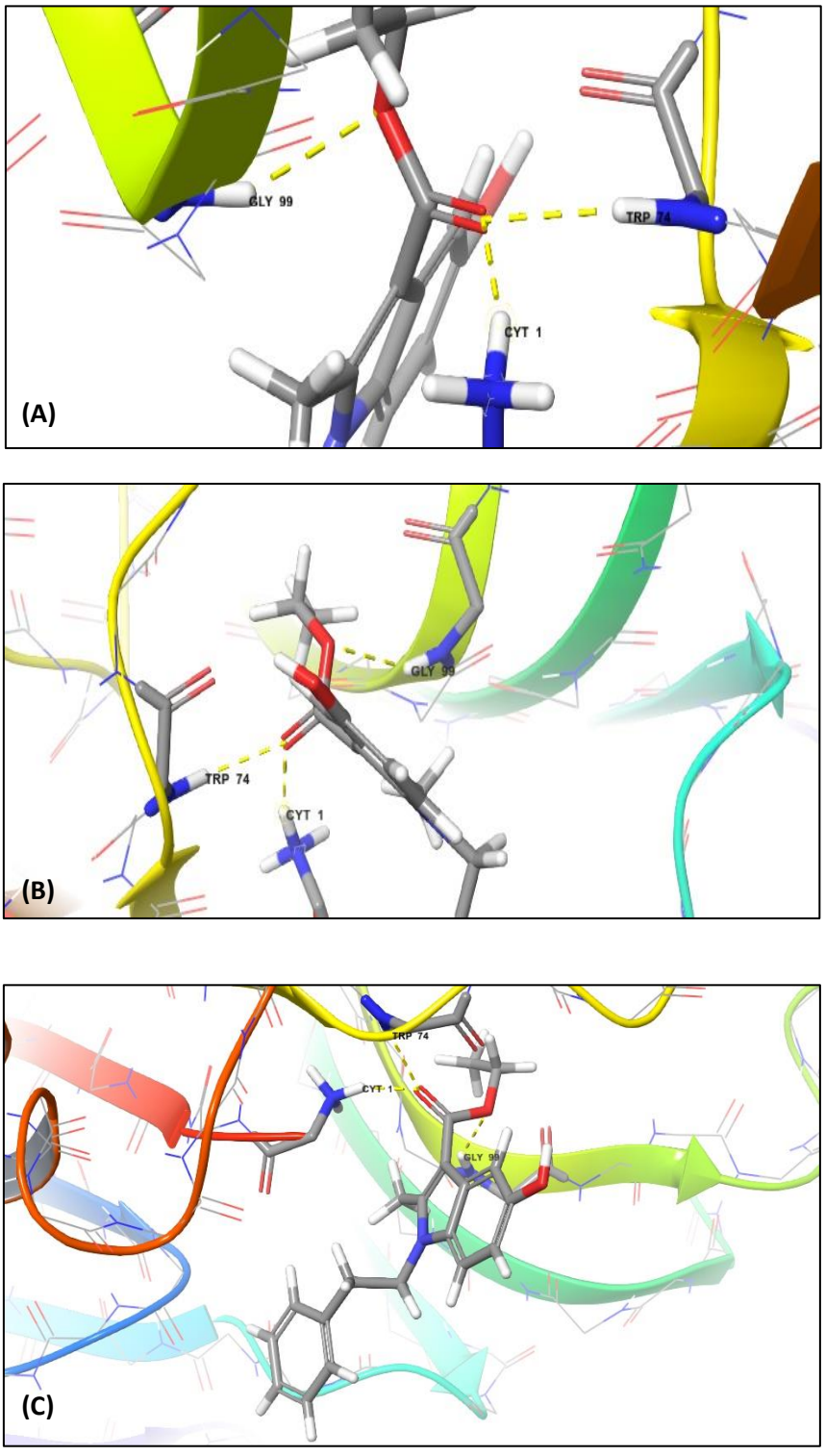


Figure (c). The 2D interaction map of G6P- Ciprofloxacin complex.

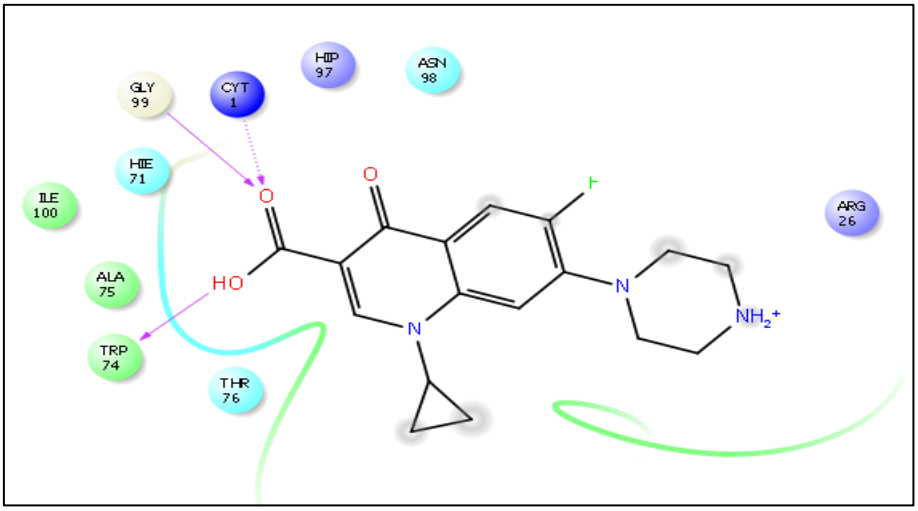
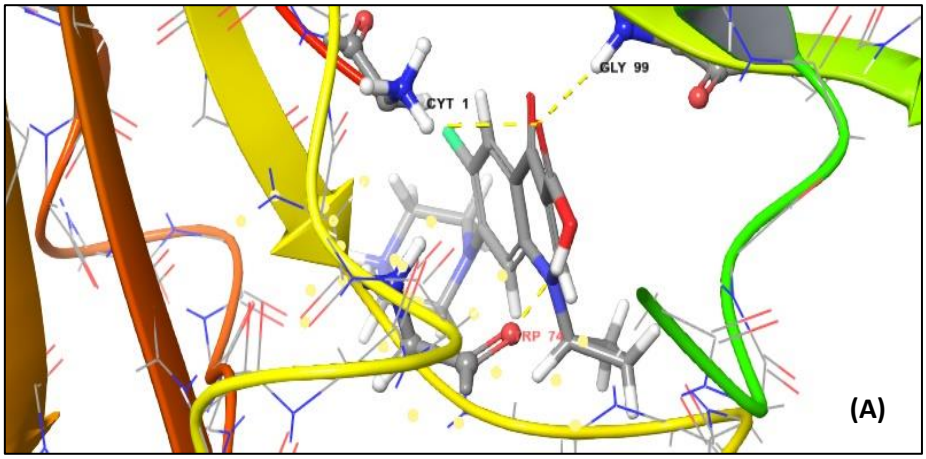
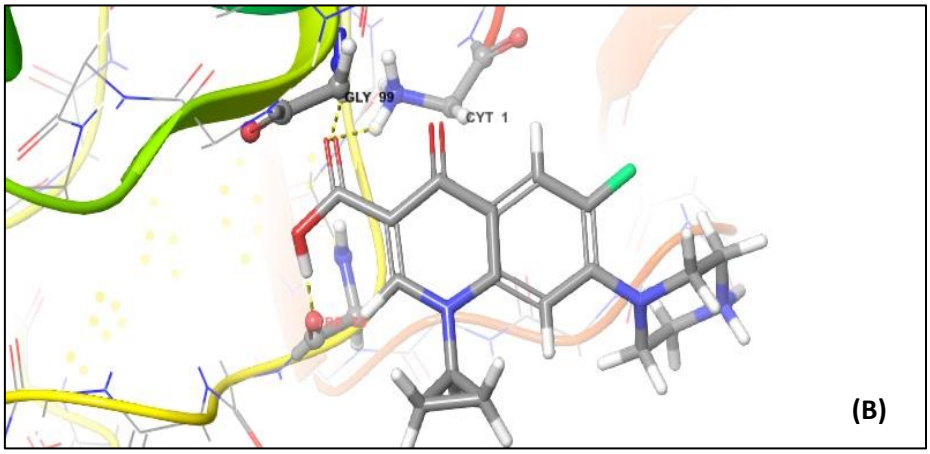


Figure (d). (A-B). The interaction pattern of G6P-Ciprofloxacin contacts.





CONCLUSION:

New series of 1-phenylethyl-2-methyl-3-ethoxycarbonyl-5-hydroxyindole derivatives (compound 1-9) were prepared. The antifungal activity of the newly synthesized compounds was tested against *C. albicans*and *A. niger*, antibacterial activity was tested against *E.coli* and *B. cirroflagellosus*. Amongst listed compounds- 4, 7, and 9 showed promising antimicrobial activity. The synthesized compounds were confirmed by ^{1}HNMR, FT-IR, Mass, ^{13}CNMR spectral analyses. Computationally, molecular docking investigations revealed lower binding energy values of lead molecule (compound-1), when compared to ciprofloxacin indicating lower binding energy with Glucosamine-6-phosphate synthase (G6P). The molecular docking analysis revealed that among all the ligands, compound-1showed least binding energy of -4.218 kcal/mol and exhibited most favourable hydrogen bond interactions with active site residues of G6P. *In silico* ADMET analysis also predicted that the lead compound may be less likely toxic and has favourable human intestinal absorption capability. In summary, the lead molecule and its subsequent derivatives may have the potential to be promising therapeutic agents.

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