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Original Article

ANTIMICROBIAL AND *IN SILICO* ADMET SCREENING OF NOVEL (*E*)-*N*-(2-(1*H*-INDOL-3-YL-AMINO) VINYL)-3-(1-METHYL-1*H*-INDOL-3-YL)-3-PHENYLPROPANAMIDE DERIVATIVES

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ABSTRACT

Objective: Synthesis, *in silico* absorption, distribution, metabolism, excretion, toxicity (ADMET) and *in vitro* antimicrobial screening of (*E*)-*N*-(2-(1*H*-indol-3-ylamino) vinyl)-3-(1-methyl-1*H*-indol-3-yl)-3-phenylpropanamide derivatives.

Methods: (*E*)-*N*-(2-(1*H*-indol-3-ylamino) vinyl)-3-(1-methyl-1*H*-indol-3-yl)-3 phenylpropane-amide derivatives were synthesized by combining indole ethanolamine and substituted Meldrum's adduct. The synthesized compounds were subjected to *in vitro* antimicrobial study by cup plate method and *in silico* ADMET properties using ACD/I-Lab 2.0.

Results: The *in vitro* antimicrobial screening against precarious pathogenic microorganisms *viz*, *Pseudomonas aureginosa*, *Staphylococcus aureus, Escherichia coli, Vibrio cholerae*, and the antifungal activity against *Candida albicans, Aspergillus niger*, *Penicillin chrysogenum* and *Cladosporium oxysporum* strains. The results revealed that compounds 5b, 5c, 5d and 5e showed good antimicrobial property and obeyed the *in silico* pharmacokinetic parameters.

Conclusion: The encouraging results exhibited by the compounds (*E*)-*N*-(2-(1*H*-indol-3-ylamino) vinyl)-3-(1-methyl-1*H*-indol-3-yl)-3-phenyl propanamide derivatives, 5(a-e) can be explored as possible hits in antimicrobial therapy. The molecules obey the Lipinski rule of five when tested *in silico* and can be used in understanding the quantitative structure-activity relationship (QSAR) parameters.

Keywords: Antimicrobial, ADMET, Phenylpropanamide, Meldrum's adduct, Indole and Nitro methane

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INTRODUCTION

Indole derivatives have been found to possess a wide range of biological activities. Many indole derivatives were reported as antimicrobial agents in several studies, among them, ethyl-3-indolylacrylate, and 5-bromo-3-(2-cyanovinyl) indole and 3(2-nitrovinyl)-indole were found to be active against a wide variety of pathogenic microorganisms [1]. Literature study revealed that halo indole was also found highly active [2], some other indole derivatives such as 3-acyl-4, 7-dihydroxy indoles possess antibacterial properties against *Escherichia coli* and *Streptococcus pyogenes* [3]. 1-morpholino-3-carbethoxy-5-hydroxy-2-methylindole was reported to be highly active against *Escherichia coli* and *Bacillus cirroflagellosu* [4] and 1-(4-phenyl), and (1-naphthyl-4*H*-1, 2, 4-triazole-5-thion-3-yl) indole exhibited strong antibacterial and antifungal activities [5].

Based on the above reports, we explored the synthesis of new indole propanamide derivatives 5(a–e) to be potent antibacterial and antifungal agents when tested against precarious pathogenic microorganisms such as *Pseudomonas aureginosa*, *Escherichia coli, Vibrio cholerae*, *Staphylococcus aureus* and antifungal activity against *Candida albicans, Aspergillus niger*, *Penicillin chrysogenum* and *Cladosporium oxysporum*. The rationality in investigating the biopotency of indole-3-propanamide derivatives is due to its use as precursors to obtain several biologically active molecules, especially for the treatment of brain disorder [6], as tyrosine kinase inhibitors, inhibitors of epidermal growth factor (EGF) receptor [7], immunosuppressive activity [8] anti-allergic [9] and inflammation inhibitors [10]. Thus, indole-3-propanamides have become an important precursor for various pharmaceutically important compounds [11-13], and there is flexibility in exploring indole-3-propanamide derivatives randomly for various biological activities to get suitable hits.

The synthesized compound 5(a-e) was subjected for *in silico* ADMET properties to predict the pharmacological actions such as aqueous solubility (PlogS), blood-brain barrier penetration (QPlogBB), intestinal absorption (logHIA), hepatotoxicity, Caco-2 cell permeability (QPPCaco) which helps to understand drug metabolism studies of the molecules.

MATERIALS AND METHODS

The chemicals and reagents were obtained from Sd-Fine, Hi-Media India, Sigma-Aldrich chemical company and were used as received. Melting points were determined in open capillary and are uncorrected. The purity of the compounds was checked by TLC using precoated silica gel plates procured from Merck. Further the synthesized compounds were purified by column chromatography using silica gel (60-120 mesh). FTIR was recorded (400–4000 cm^{-1}) using a KBr pellet method on the Shimadzu-8400S spectrometer; ^{1}H and ^{13}C NMR spectra were recorded on a Bruker supercon FT NMR (400 *MHz*) spectrometer in $CDCl_3$ using TMS as an internal standard. The chemical shifts are expressed in δ units. Mass spectral data were obtained on a JEOL SX 102/DA-6000 (10 KV) FAB mass spectrometer.

Synthesis of (*E*)-2-(1*H*-indol-3-yl) ethenamine (3)

An equimolar mixture of indole-3-carbaldehyde (1) (0.8 mol) and nitromethane (2) (1 mol) in acetic acid with ammonium chloride base was refluxed for 6-7 h at 70-80 °C. After completion of the reaction, the mixture was neutralized with sodium bicarbonate; the product obtained was isolated from ethyl acetate solvent. The product obtained after evaporation was purified by column chromatography using silica gel (60-120 mesh, mobile phase petroleum ether: ethyl acetate, 9:1 v/v) as an adsorbent to get the pure (*E*)-2-(1*H*-indol-3-yl) ethenamine (3).

Synthesis of (*E*)-*N*-(2-(1*H*-indol-3-ylamino) vinyl)-3-(1-methyl-1*H*-indol-3-yl)-3-phenyl propanamide derivatives (5a)

Substituted Meldrum's adduct [14] (4) (1 mol) and (*E*)-2-(1*H*-indol-3-yl) methenamine 3 (1 mol) were accurately mixed together in 5 ml

pyridine and was refluxed 4-6 h at 80 °C. The progress of the reaction was monitored by using pre-coated silica gel plates (Merck). After completion, the product obtained was treated with copper sulphate solution and extracted using ethyl acetate. The crude product isolated was purified by column chromatography using silica gel (60-120 mesh, mobile phase petroleum ether: ethyl acetate 9:1 v/v) to get the pure (*E*)-*N*-(2-(1*H*-indol-3-yl-amino) vinyl)-3-(1-methyl-1*H*-indol-3-yl)-3-phenylpropanamide (5a). Similarly, other derivatives 5(b-e) were prepared by adopting the same procedure mentioned above.

Spectral data

(*E*)-*N*-(2-(1*H*-indol-3-yl) vinyl)-3-(1-methyl-1*H*-indol-3-yl)-3-phenylpropanamide (5a)

^{1}H-NMR(400 MHz, DMSO-d6): δ = 2.97 (d d, *J* = 6.88, 13.44 *Hz*, 1H), 3.06 (d d, *J* = 7.56, 13.44 *Hz*, 1H), 3.67 (s, 3H), 3.76 (q, *J* = 6.96 *Hz*, 1H), 4.81 (d, *J* = 11.12 *Hz*, 1H), 6.98-6.99 (m, 2H), 7.11 (t, *J* = 8.24 *Hz*, 5H), 7.19 (t, *J* = 7.04 *Hz*, 2H), 7.26 (t, *J* = 8.08 *Hz*, 1H), 7.34 (t, *J* = 7.24 *Hz*, 2H), 7.62 (s, 1H), 7.69 (d, *J* = 38.00 *Hz*, 1H), 8.56 (s, 1H), 9.65 (s, 1H), 10.90 (s, 1H) ppm; ^{13}C NMR (100 *MHz*, DMSO-*d6*): δ = 39.38, 39.59, 39.80, 40.01, 40.63, 41.76, 42.70, 58.00, 110.09, 112.03, 116.72, 117.88, 118.74, 119.45, 121.43, 123.13, 126.89, 127.17, 128.45, 129.48, 130.74, 133.39, 137.20, 138.00, 140.79, 141.04, 171.00 ppm; IR (KBr: ν_{max}/cm): 3,356(N-H), 3,109(C-H), 2,995(C-C), 1,564(C=C), 1,678(C=O), 1,295(C-N); MS: *m/z* = 420.30.

(*E*)-*N*-(2-(1*H*-indol-3-yl) vinyl)-3-(4-chlorophenyl)-3-(1-methyl-1*H*-indol-3yl) phenylpropanamide (5b)

^{1}H-NMR(400 MHz, DMSO-d6): δ = 2.97 (d d, *J* = 6.76, 13.40 *Hz*, 1H), 3.06 (d d, *J* = 7.56, 13.44 *Hz*, 1H), 3.67 (s, 3H), 3.76 (q, *J* = 6.92 *Hz*, 1H), 4.88 (d, *J* = 7.56 *Hz*, 1H), 6.98-6.99 (m, 2H), 7.11 (t, *J* = 8.12 *Hz*, 5H), 7.19 (t, *J* = 7.04 *Hz*, 2H), 7.26 (t, *J* = 8.08 *Hz*, 1H), 7.33 (d, *J* = 7.24 *Hz*, 2H), 7.49 (d, *J* = 104.48 *Hz,* 1H), 7.69 (d, *J* = 38.04 *Hz*, 1H), 8.57 (s, 1H), 9.67 (s, 1H), 10.81 (s, 1H) ppm; ^{13}C NMR (100 *MHz*, DMSO-*d6*): δ = 39.36, 39.57, 39.78, 40.63, 41.79, 42.72, 58.07, 110.00, 112.04, 116.73, 117.89, 118.75, 119.46, 121.42, 123.14, 126.89, 127.18, 128.46, 129.48, 130.74, 137.23, 138.00, 140.79, 141.07, 171.03 ppm; IR (KBr: ν_{max}/cm): 3,350(N-H), 2,975(C-C), 1,536(C=C), 1,650(C=O), 1,247(C-N), 853(C-Cl); MS: m/z = 456.87(m+2).

(*E*)-*N*-(2-(1*H*-indol-3-yl) vinyl)-3-(4-bromophenyl)-3-(1-methyl-1*H*-indol-3yl) phenylpropanamide (5c)

^{1}H-NMR(400 MHz, DMSO-d6): δ = 2.97 (d d, *J* = 6.76, 13.36 *Hz*, 1H), 3.07 (d d, *J* = 7.72, 13.40 *Hz*, 1H), 3.67 (s, 3H), 3.76 (q, *J* = 7.08 *Hz*, 1H), 4.83 (d, *J* = 4.28 *Hz*, 1H), 6.98-6.99 (m, 2H), 7.11 (t, *J* = 7.88 *Hz*, 5H), 7.19 (t, *J* = 7.40 *Hz*, 2H), 7.26 (t, *J* = 7.80 *Hz*, 1H), 7.33 (d, *J* = 7.28 *Hz*, 2H), 7.49 (d, *J* = 104.64 *Hz*, 1H), 7.69 (d, *J* = 37.92 *Hz*, 1H), 8.37 (s, 1H), 9.56 (s, 1H), 10.00 (s, 1H) ppm; ^{13}C NMR (100 *MHz*, DMSO-*d6*): δ = 41.79, 42.75, 58.04, 110.05, 112.07, 116.73, 117.89, 118.72, 119.46, 121.42, 123.18, 126.89, 127.14, 128.39, 129.57, 130.79, 133.36, 137.23, 138.00, 140.70, 141.07, 171.08 ppm; IR (KBr: ν_{max}/cm): 3,364(N-H), 3,109(C-H), 2,992(C-C), 1,584(C=C), 1,674(C=O), 764(C-Br); MS: *m/z* = 501.26(m+2).

(*E*)-*N*-(2-(1*H*-indol-3-yl) vinyl)-3-(1-methyl-1*H*-indol-3yl)-3-(4-nitrophenyl) phenylpropanamide (5d)

^{1}H-NMR(400 MHz, DMSO-d6): δ = 2.97 (d d, *J* = 6.88, 13.32 *Hz*, 1H), 3.07 (d d, *J* = 7.56, 13.60 *Hz*, 1H), 3.67 (s, 3H), 3.76 (q, *J* = 6.44 *Hz*, 1H), 4.88 (d d, *J* = 7.52, *Hz*, 1H), 6.98-6.99 (m, 5H), 7.11 (t, *J* = 8.28 *Hz*, 2H), 7.19 (t, *J* = 7.28 *Hz*, 1H), 7.26 (t, *J* = 8.08 *Hz*, 2H), 7.33 (d, *J* = 7.28 *Hz*, 1H), 7.49 (d, *J* = 104.60 *Hz*, 1H), 7.69 (d, *J* = 38.04 *Hz*, 2H), 8.52 (d, *J* =-236.08 *Hz*, 1H), 8.52 (s, 1H), 9.67 (s, 1H), 10.05 (s, 1H) ppm; ^{13}C NMR (100 *MHz*, DMSO-*d6*): δ = 32.26, 39.89, 41.04, 42.00, 110.59, 116.80, 117.01, 118.22, 119.63, 121.76, 123.13, 126.75, 127.38, 128.59, 129.80, 130.01, 133.22, 137.43, 140.63, 141.76, 171.70 ppm; IR (KBr: ν_{max}/cm): 3,358(N-H), 2,931(C-C), 3,088(C-H), 1,536(C=C), 1,649(C=O), 1,297(C-N), 1,536(C-No_2); MS: *m/z* = 465.52.

(*E*)-*N*-(2-(1*H*-indol-3-yl) vinyl)-3-(4-aminophenyl)-3-(1-methyl-1*H*-indol-3yl) phenylpropanamide (5e)

^{1}H-NMR(400 MHz, DMSO-d6): δ = 2.97 (d d, *J* = 6.88, 13.44 *Hz*, 1H), 3.06 (d d, *J* = 7.56, 13.44 *Hz*, 1H), 3.67 (s, 3H), 3.76 (q, *J* = 6.72 *Hz*, 1H), 3.08 (d, *J* = 7.64 *Hz*, 1H), 6.98-6.99 (m, 2H), 7.11 (t, *J* = 8.24 *Hz*, 5H), 7.19 (t, *J* = 7.04 *Hz*, 2H), 7.26 (t, *J* = 8.08 *Hz*, 1H), 7.33 (d, *J* = 7.24 *Hz*, 2H), 7.49 (d, *J* = 104.64 *Hz*, 1H), 7.69 (d, *J* = 38.00 *Hz*, 1H), 3.67 (s, 1H), 3.67 (s, 1H), 10.05 (s, 1H) ppm; ^{13}C NMR (100 *MHz*, DMSO-*d6*): δ = 32.75, 39.09, 41.76, 42.70, 110.09, 116.72, 117.88, 118.74, 119.45, 121.43, 123.13, 126.89, 127.17, 128.45, 129.48, 130.74, 133.39, 137.20, 140.79, 141.04, 171.00 ppm; IR (KBr: ν_{max}/cm): 3,351(N-H), 2,989(C-H), 2,931(C-C), 1,647(C=O), 1,556(C=C), 1,297(C-N); MS: *m/z* = 435.23.

Antimicrobial screening

Antibacterial study

The antibacterial activity of the title compounds were evaluated by cup-plate method using four different bacterial strains *viz*. *Staphylococcus aureus*, *Vibrio cholerae*, *Pseudomonas auriginosa*, and *Escherichia coli* (Bacterial species were obtained from Microbiology Department, Kuvempu University, Jnana Sahyadri, Shankarghatta, Shimoga) using nutrient agar (NA) medium A. The test solutions were prepared in DMSO and diluted using double distilled water to get concentrations of 1000, 500 and 250µg/ml. The test microorganisms maintained in slant tubes as solid culture. At first, the solid slants were inoculated from the original stock culture and finally the liquid media was inoculated from the solid slants. The microbes were allowed to incubate for 24 h at 37 °C. 20 ml of liquid agar media was taken in McCartney bottle and sterilized in autoclave at 121 °C for 15 min at 15 psi.

The sterilized media was then poured into sterilised petri plates aseptically in a horizontal laminar air flow chamber. The layers of media were uniformly distributed and were allowed to solidify in the aseptic chamber, followed by inoculating the bacterial strains separately in the petri plates. The spore suspension was adjusted with sterile saline to a concentration of approximately 1.0×10^7 CFU/ml. In order to determine the antibacterial activity, the inoculated petri plates were divided into three quarters and to each quarter a well was made in the media with the help of a sterilized cork borer (9 mm). The known concentrations of the standard drug amoxicillin and the test compounds were added to the respective labelled wells. Thus, applied plates were then kept in refrigerator for 10 min. followed by incubation for 24 h at 37 °C [15-17].

Antifungal study

The antifungal activity of the synthesized compounds evaluated by cup-plate method using potato dextrose agar medium B (glucose 4%, peptone 1%, agar 2% and distilled water 1000 ml) with a pH 5.4. Four different fungal strains used in this process were *Candida albicans, Aspergillus niger*, *Penicillin chrysogenum* and *Cladosporium oxysporum* (Fungal species were obtained from Microbiology Department, Kuvempu University, Jnana Sahyadri, Shankarghatta, Shimoga). Three different concentrations of test sample solutions were prepared using DMSO and dilute with distilled water at the strength of 1000, 500 and 250µg/ml. The test microorganisms were maintained in slant tubes as solid culture and in test tube (containing 2.5 ml of media). First the solid slants were inoculated from the original stock culture and finally the liquid media were inoculated from the solid slants. The microbes were allowed to incubate for 24 h at a temperature of 28 °C. 20 ml of liquid agar media was taken in McCartney bottle and sterilized in autoclave at 121 °C for 15 min at 15 psi.

The sterilized media was poured into petri plates in aseptic condition. The layers of media were made uniform; the media was allowed to solidify followed by inoculating the fungal strains separately in the petri plates. The spore suspension was adjusted with sterile saline to a concentration of approximately 1.0×10^7 in a final volume of 20 µl per well. Antifungal activity was determined by cup-plate method**.** For this purpose the inoculated petri plates were divided into three quarters and to each quarter a well was made in the media with the help of a sterilised cork borer (9 mm). The known concentrations of the standard drug and the test compounds were added to the wells so that the volume fills up the well uniformly. Thus applied plates were then kept in refrigerator for 10 min, followed by incubation for 72 h at 28 °C [15-17].

***In silico* ADME-toxicity prediction**

The synthesized molecules were subjected for *in silico* ADMET parameters to predict the physicochemical properties and optimize using QSAR parameters. The QSAR properties of the compounds significantly help to understand pharmacokinetics behavior and to predict possible biological activity such as absorption, distribution, metabolism, excretion and toxicity (ADMET). The ADMET QSAR [18] help to evaluate biologically active molecules and eliminate the biologically poor molecules containing undesirable functional groups based on Lipinski rule and further statistical calculation of the molecules help to understand biological behavior of the synthesized molecules. The ADMET study was carried by ACD/I-Lab-2.

RESULTS AND DISCUSSION

In this work series of (*E*)-*N*-(2-(1*H*-indol-3-yl-amino) vinyl)-3-(1-methyl-1*H*-indol-3-yl)-3-phenylpropanamide derivatives 5(a-e) were synthesized, allowed for *in vitro* antimicrobial screening against precarious pathogenic microorganisms *viz*, *Pseudomonas aureginosa*, *Escherichia coli, Vibrio cholerae, Staphylococcus aureus* and antifungal activity against *Candida albicans, Aspergillus niger*, *Penicillin chrysogenum* and *Cladosporium oxysporum* strains. There are several therapeutic options now exist to cure infectious diseases, but due to their poor response rate, adverse effects and the development of resistant mutants highlights the need for new alternative drugs, which are most specificity in their mechanism of action while used as therapeutic agents.

In this regard, indole derivatives have exhibited antimicrobial activity [1]; Hence serendipity way of identification of new leads that can play an important role to discover new drug molecules. Among the series of synthesized compounds 5c, 5d and 5e were potent against both bacterial as well as fungal strains. These results were correlated with ADMET parameters, gives reliable efficacy in all physiological properties; it is due to the presence of electron withdrawing (chlorine, bromine, nitro) and donating groups (amine) on aryl ring.

The incorporation of halogen substituent into lead analogues to improve the lipophilic nature, the lipophilicity of the derivatives it is due to the presence of chlorine, bromine, nitro and amine functionalities. The chlorine, bromine and nitro groups are electron withdrawing; the amine functionalities are involved intra molecular hydrogen bonding, this increases the non-polar property of the lead analogues; it helps to easy penetration of the drug into the lipid membranes. The chemical reactivity of halogen atoms depends on their position of attachment to the lead and nature of the halogen. Compound 5a was synthesized without substitution and is necessary to compare the substitution effect on the aryl ring. The replacement of a hydrogen atom in an active molecule by a substitute (halogen, nitro and amino) can modify the potency, duration of action and the nature of the pharmacological effect [24].

The substitutes can affect various parameters of a drug molecule, such as partition coefficient, electronic density, steric environment, bioavailability, pharmacokinetics and ability to establish direct interactions between the substituent and the receptor or the enzyme; the compounds rich in electron density are exerted mesomeric effect, which are able to soluble in lipids, the lipid solubility is directly proportional to efficacy and their by pharmacological effect, it might be involved in the inhibition of cell wall synthesis.

Antimicrobial activity

The compounds 5(a-e) were tested for *in vitro* antimicrobial activity against *Pseudomonas aureginosa*, *Escherichia coli, Vibrio cholerae*, *Staphylococcus aureus using amoxicillin* as a standard and for their antifungal activity against *Candida albicans, Aspergillus niger*, *Penicillin chrysogenum* and *Cladosporium oxysporum* using flucanazole as standard.

The compounds were dissolved in DMSO at three different concentrations 1000, 500 and 250 µg/ml. The inhibition values of the compounds were determined by the twofold serial dilution technique using nutrient agar medium A and Potato dextrose agar for bacteria and fungi, respectively. Table 1 and table 2 represent the results of the *in vitro* activity determined by the cup plate method [15]. The antimicrobial result reveals that the compound 5a, 5b showed moderate activity at 1000 µg/ml against *Pseudomonas auriginosa Staphylococcus aureus* and Escherichia coli, 5c, 5d and 5e are potent at (250 and 1000 µg/ml) against *Pseudomonas auriginosa, Staphylococcus aureus and Escherichia coli* respectively, when compares to standard amoxicillin. The compounds 5a, 5b, 5d and 5e showed moderate activity against *Cladosporium oxysporum*, *Penicillin chrysogenum* and *Candida albicans*, 5c reveals good activity at 250µg/ml as compared to rest of the test samples with flucanazole. In general, the compound 5c exhibit potent antimicrobial activity.

N-substituted indole with halogen functions of the aryl ring at 3-position enhances the antimicrobial activity. Some simple indole derivatives with mixed antifungal and antibacterial activities were converted by Whitehead and Whitesitt (1974) to antibacterial compounds by introducing an appropriate diarylmethyl substituent. These investigators also increased the antifungal activity of 5-bromoindole 2 to 25 times by adding the diphenylmethyl group to the 3-position. The compound 5-bromoindole inhibited six of the fungal species and was considered as an effective antifungal agent of the simple indoles. Inspired by these studies, insertion of a halogen-substituted aromatic ring 3-position of indole especially with bromine exhibited good activity.

Table 1: The antibacterial potential of (*E*)-*N*-(2-(1*H*-indol-3-ylamino) vinyl)-3-(1-methyl-1*H*-indol-3-yl)-3-phenylpropanamide and its derivatives 5(a-e)

Compound	Conc. of test sample (µg/ml)	Zone of inhibition in mm (mean±SD) n=3			
		PA	SA	VC	EC
5a	1000	18±0.20	15±0.10	00	16±0.20
	500	16±0.20	14±0.10	00	15±0.10
	250	15±0.10	14±0.10	00	15±0.20
5b	1000	18±0.20	17±0.10	17±0.20	22±0.10
	500	14±0.10	16±0.10	13±0.10	18±0.10
	250	12±0.10	16±0.20	12±0.10	18±0.10
5c	1000	24±0.10	22±0.20	22±0.20	22±0.20
	500	21±0.20	20±0.10	18±0.20	18±0.20
	250	20±0.10	20±0.20	14±0.20	16±0.10
5d	1000	22±0.30	22±0.10	21±0.20	20±0.20
	500	19±0.20	17±0.20	19±0.10	16±0.20
	250	15±0.20	13±0.20	13±0.10	12±0.10
5e	1000	22±0.20	20±0.10	21±0.10	20±0.30
	500	19±0.30	17±0.30	19±0.30	16±0.20
	250	15±0.10	10±0.20	13±0.20	12±0.20
Standard (Amoxicillin)	1 mg/ml	32±0.20	26±0.20	28±0.10	34±0.20

PA: Pseudomonas auriginosa, SA: Staphylococcus aureus, VC: Vibrio cholera, EC: Escherichia coli, Standard: Amoxicillin, Bore Size: 9 mm, SD: Standard deviation.

Table 2: The antifungal potential of (*E*)-*N*-(2-(1*H*-indol-3-ylamino) vinyl)-3-(1-methyl-1*H*-indol-3-yl)-3-phenylpropanamide and its derivatives 5(a-e)

Compound	Conc. of test sample (µg/ml)	Zone of inhibition in mm (mean±SD) n=3			
		CO	PC	CA	AN
	1000	16±0.20	10±0.30	18±0.30	00
5a	500	08±0.30	00	10±0.20	00
	250	00	00	00	00
	1000	18±0.40	12±0.30	18±0.30	00
5b	500	10±0.30	08±0.20	12±0.30	00
	250	04±0.20	08±0.20	08±0.20	00
	1000	22±0.20	26±0.20	20±0.20	12±0.30
5c	500	18±0.30	20±0.30	12±0.20	08±0.50
	250	16±0.30	17±0.40	08±0.40	00
	1000	14±0.20	12±0.30	14±0.40	00
5d	500	10±0.30	08±0.40	10±0.50	00
	250	10±0.20	00	00	00
	1000	18±0.20	12±0.20	18±0.40	00
5e	500	14±0.20	10±0.20	12±0.30	00
	250	10±0.20	08±0.40	08±0.30	00
Standard (Flucanazole)	1 mg/ml	28±0.30	32±0.20	24±0.20	20±0.40

CO: Cladosporium oxysporum, PC: Penicillin chrysogenum, CA: Candida albicans, AN: Aspergillus niger, Standard: Flucanazole, Bore Size: 9 mm, SD: Standard deviation.

***In silico* ADME-toxicity prediction**

The QSAR properties of the compounds significantly help to understand pharmacokinetics behavior to predict possible biological activity such as absorption, distribution, metabolism, excretion and toxicity (ADMET). The ADMET QSAR [18] help to evaluate biological active molecules and eliminate the biologically poor molecules containing undesirable functional groups based on Lipinski rule.

The *in silico* results revealed that molecules have moderate effect on reliability index and LD50 dose [19] on different routes of drug administration is found to be safe enough. The compounds were found to be good with P log BB, log HIA, P Caco, GI, log PGI poor P log S and solubility and greater membrane permeability aqueous solubility (P log S), blood-brain barrier penetration (Q P log BB), intestinal absorption (log HIA) [20, 21] Hepato toxicity, Caco-2 cell permeability (QPP Caco) also help to understand drug metabolism studies of the molecules [22]. To predict the toxicity of lead molecules with intra peritoneal, oral, intravenous and subcutaneous toxic effects of blood, cardiovascular system, gastrointestinal, kidney, liver and lungs to calculate sensitivity, specificity and area under the curves (AUC) that predicts the linearity of compounds [23].

The absorption, distribution, metabolism and excretion, parameters influence the drug levels and kinetics of drug exposure to the tissues and perform the pharmacological activity of the compounds as a drug. According to *in silico* ADME analysis, the resulting compounds showed good blood/brain barrier partition coefficient power with PlogBB value and good cell permeability value of nearly 1 with PCaco, good intestinal absorption properties with log HIA value 1 of the compound 5e, good glycoprotein substrates and glycoprotein inhibitors (0.8-1.0), the compounds showed moderate aqueous soluble property with better LD50 and drug reliability index. The pharmacokinetic parameters help to further explore the molecules on to other parameters of drug discovery and minimise the time in the drug development process.

Table 3: ADME and pharmacological parameters prediction for the ligands (*E*)-*N*-(2-(1*H*-indol-3-yl-amino) vinyl)-3-(1-methyl-1*H*-indol-3-yl)-3-phenylpropanamide derivatives 5(a-e) using ADMET SAR toolbox

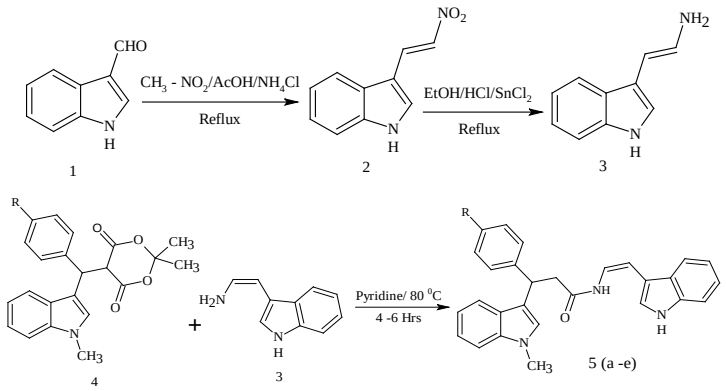
Ligand	PlogBB[a]	$\log_{HIA}$[c]	P Caco[b]	logpGI (substrate)[d]	logPGI (non-inhibitor)[e]	PlogS[f]	logpapp[g]
5a	0.9785	0.9970	0.5334	0.5051	0.5911	-3.5569	0.9480
5b	0.9782	0.9975	0.5000	0.5050	0.6641	-4.2428	0.8702
5c	0.9541	0.9953	0.5465	0.5105	0.7480	-3.7250	0.8485
5d	0.9836	0.9945	0.5292	0.5402	0.6834	-3.6698	0.8505
5e	0.9773	0.9963	0.5204	0.5062	0.5507	-4.1127	0.8307
Indomethacin	0.9381	0.9509	0.5857	0.6360	0.9313	-4.6825	0.6287
Camptothecin	0.6345	0.8410	0.5555	0.6039	0.7852	-3.0369	1.1839
Tetracycline	0.9841	0.8006	0.7439	0.7910	0.8025	-3.0575	0.7655
Tretinoin	0.9311	0.9925	0.7603	0.6144	0.8912	-3.0895	1.7734
Levostatin	0.9287	0.9452	0.5484	0.7861	0.7046	-5.9475	0.8127
Metronidazole	0.9297	0.9805	0.5365	0.5141	0.8954	-1.3229	0.8033

[a]Predicted blood/brain barrier partition coefficient (1-high penetration, 2-medium penetration and 3-Low penetration), [b]Predicted Caco-2 cell permeability in nm/s (acceptable range: -1 is poor, 1is great), [c]Predicted Human intestinal absorption in nm/s (acceptable range: 0 poor,>1 great), [d]Predicted P-glycoprotein substrate in nm/s (acceptable range of-5 is poor, 1 is great), [e]Predicted P-glycoprotein inhibitor in nm/s (accepted range: 0 to 1), [f]Predicted aqueous solubility, (Concern value is 0-2 highly soluble), [g]Predicted probability of Caco-2 cell permeability in cm/s (Concern value is-1 to 1).

Table 4: LD_{50} and probability of health effects of (*E*)-*N*-(2-(1*H*-indol-3-yl-amino) vinyl)-3-(1-methyl-1*H*-indol-3-yl)-3-phenylpropanamide derivatives 5(a-e) using ACD/I-Lab 2.0

ADME-TOX Paramet-ers	Intraperitoneal a	Oral a	Intra -venous a	Subcutaneous a	Blood Effect b	Cardiovascr system effect b	Gastro intestnal effect b	Kidney effect b	Liver effect b	Lung effect b
5a	180(0.32)	780(0.35)	20(0.29)	230(0.43)	0.95	0.95	0.65	0.75	0.03	0.97
5b	210(0.32)	920(0.43)	20(0.31)	210(0.42)	0.96	0.99	0.66	0.82	0.94	0.97
5c	290(0.33)	850(0.42)	19(0.35)	250(0.4)	0.96	0.99	0.98	0.92	0.93	0.98
5d	150(0.3)	920(0.37)	16(0.36)	200(0.36)	0.93	0.95	0.91	0.81	0.95	0.99
5e	190(0.32)	840(0.36)	21(0.32)	190(0.16)	0.94	0.96	0.94	0.85	0.94	0.97

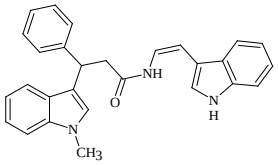
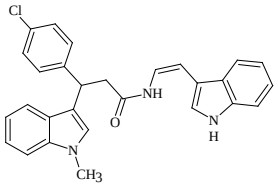
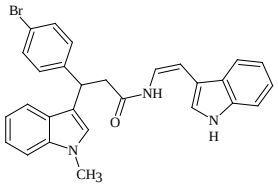
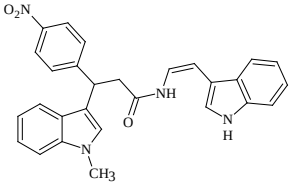
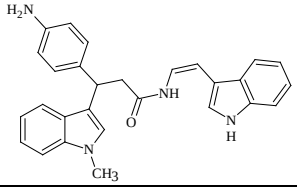
[a]Estimated LD_{50}-mouse value in mg/kg after intra peritoneal, oral, intravenous and subcutaneous administration, [b]Estimated probability of blood, gastrointestinal system, kidney, liver and lung effect at therapeutic dose range of compounds, The drugs with moderate effect on reliability index (>0.5), The drugs with border line effect on reliability index (>0.3,<0.5).



R = H, Cl, NO_2, NH_2, Br

Scheme: Synthesis of (*E*)-*N*-(2-(1*H*-indol-3-yl-amino) vinyl)-3-(1-methyl-1*H*-indol-3-yl)-3-phenylpropanamide derivatives 5(a-e)

Table 5: Physical properties of synthesis of (*E*)-*N*-(2-(1*H*-indol-3-yl-amino) vinyl)-3-(1-methyl-1*H*-indol-3-yl)-3-phenylpropanamide derivatives 5(a-e)

Entry	Products	M. P (°C)	Yield(%)[a]
5a		103-105	84
5b		117-119	89
5c		220-223	73
5d		126-128	78
5e		119-121	69

CONCLUSION

The results revealed that compounds 5b, 5c, 5d and 5e showed good antimicrobial potential and were obeying the *in silico* pharmacokinetic parameters. In case of compound 5e less activity was observed against fungus than 5c. The encouraging results exhibited by the title compounds 5(a-e), can be explored as lead molecules in antimicrobial therapy.

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CONFLICT OF INTTERESTS

No conflicts of interest

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