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Rational Modification of a Lead Molecule: Improving the Antifungal Activity of Indole – Triazole – Amino Acid Conjugates

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Abstract. The modification of a molecule that was identified as highly efficacious in the previous studies could considerably improve the biological activity of the resulting compounds. While targeting lanosterol 14-α demethylase, the molecular modelling studies convinced that the extension of the phenyl ring of compound **1** deep into the hydrophobic pocket of the enzyme may increase the enzyme – ligand interactions and hence improve the anti-fungal profile of the molecules. As a result, the newly designed compounds **2** were synthesized and screened for their anti-microbial properties and these compounds were found to exhibit considerably better activity than the previous molecule **1**. Some of the compounds in this series exhibited MIC_{90} 16 μg mL^{-1} and 32 μg mL^{-1} against *Candida albicans* and *Aspergillus niger*, respectively as against 312 μg mL^{-1} for compound **1**.

Introduction

The use of antineoplastic and immunosupressive agents along with the broad spectrum of antibiotics, cancer treatment and AIDS [1-5] is responsible for the increasing incidences of suprafacial and invasive fungal infections [6-7]. The pathogenic microbes including *Candida* sp, *Cryptococcus* sp and *Aspergillus* sp are the most common causative agents of invasive fungal diseases [8-10]. Besides the lack of complete understanding of the bio-mechanistic pathways of the fungal cells, the similarity in the fungal cell structure and the human host has made the invention of new antifungal agents quite problematic [11-12]. Thus a limited number of chemotherapeutic agents for fungal infections are available and among the existing antifungal agents, most of them act as fungistatic rather than fungicidal [13]. Furthermore, the over use of the present antifungals, prevalence of mutations and development of biofilms by the fungus are certain factors contributing for developing resistance to the antifungal drugs in the pathogenic fungi [14-17].

Out of the available antifungals; the polyenes (amphotericin-B) and 5-fluorocytosine do not impact on the synthesis of ergosterol. Allylamines (terbinafine) act upstream of the ergosterol biosynthesis pathway whereas azoles, along with the other classes such as morpholines and ployenes, act downstream while binding to ergosterol [18-23]. For the azole class of antifungals (the imidazoles and triazoles); the imidazoels were the first one to be used but with their poor bioavailability and high toxicity they had only limited formulations and could not be used systemically [24]. Improved over the imidazoles, recently, 1,3-thiazolidin-4-one derivatives have been reported with high anti-fungal potency and low toxicity [25]. Owing to the favourable pharmacokinetics and safety profile, the triazoles have been used frequently in treating invasive infections [26-28]. Triazoles act by deactivating the cytochrome P450 dependent enzyme lanosterol 14-α demethylase, consequently minimizing the synthesis of ergosterol. The depletion of ergosterol and accumulation of toxic methyl

sterols alters the cell membrane fluidity, thereby disrupting the activity of membrane bound enzymes and leading to the inhibition in the growth and replication of fungal cells [29-31].

Out of the recently reported series of indole – azole conjugates, compound **1** (chart 1) was the most potent anti-fungal agent with MIC_{80} 312.5 μg mL^{-1} [32]. Mechanistically, compound **1** was found to target lanosterol 14-α demethylase. Motivated by these findings, and in continuation of our efforts endowed with the discovery of amino acid appended indole conjugates with potential chemotherapeutic properties; it was planned to modify compound **1** so that its anti-fungal activity gets improved.

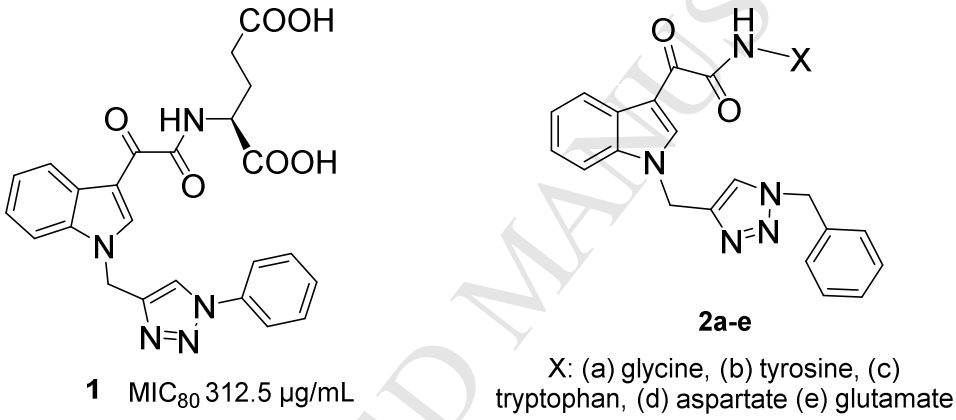


Chart 1

Results and Discussion

Since the computational techniques have made it possible to investigate the crystal coordinates of the enzyme as well as the enzyme substrate complexes and thence helping in the design of new chemical entities, the molecular docking studies of compound **1** in the active site pocket of lanosterol 14-α demethylase were performed. The enzyme – compound interactions were compared with the enzyme – fluconazole interactions. Significantly more contacts of compound **1** in the active site of the enzyme were observed (Figure 1 A, 1B). In addition to parallel with fluconazole for interacting with R96 and F83; compound **1** also exhibited interactions with F255, F78, Y76, R326 and H392. Since a large part of the enzyme pocket is hydrophobic (Figure 1C) and these interactions play critical role in the drug-enzyme

binding, it was planned to extend the phenyl moiety, present on the azole ring, deep into the hydrophobic pocket of the protein constituted by Y76 and F78. Consequently, the phenyl ring present on the azole moiety of compound **1** was replaced with the benzyl group and compound **2** (Chart 1) were designed. The docking of compound **2e** in the active site pocket of lanosterol 14-α demethylase clearly showed that the phenyl unit of benzyl group occupies the hydrophobic pocket of the enzyme and characteristically, the indole part of the molecule exhibits cation-π interactions with R96 (Figure 1D). Hence compounds **2** were synthesized and investigated for their antimicrobial activity by screening against *Candida albicans* (MTCC 3958) and *Aspergillus niger* (MTCC 9933).

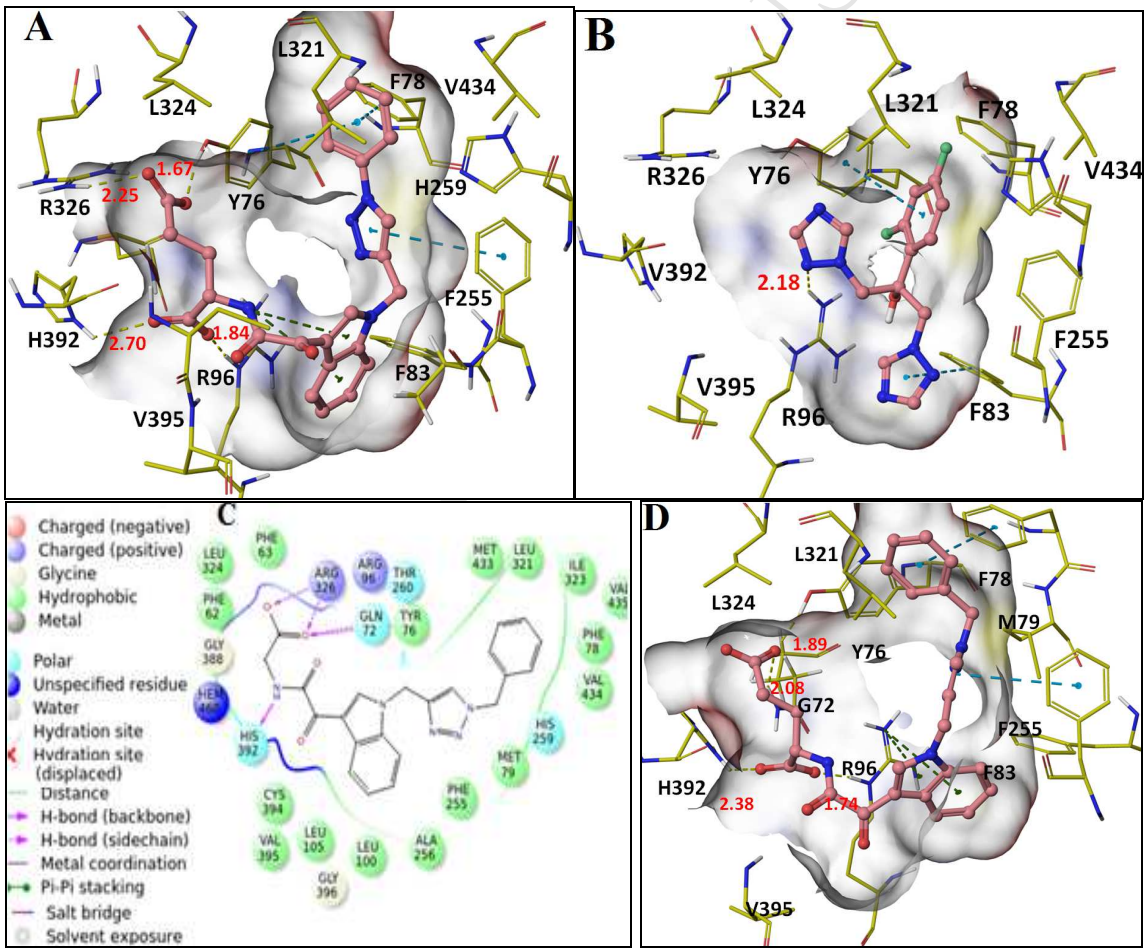
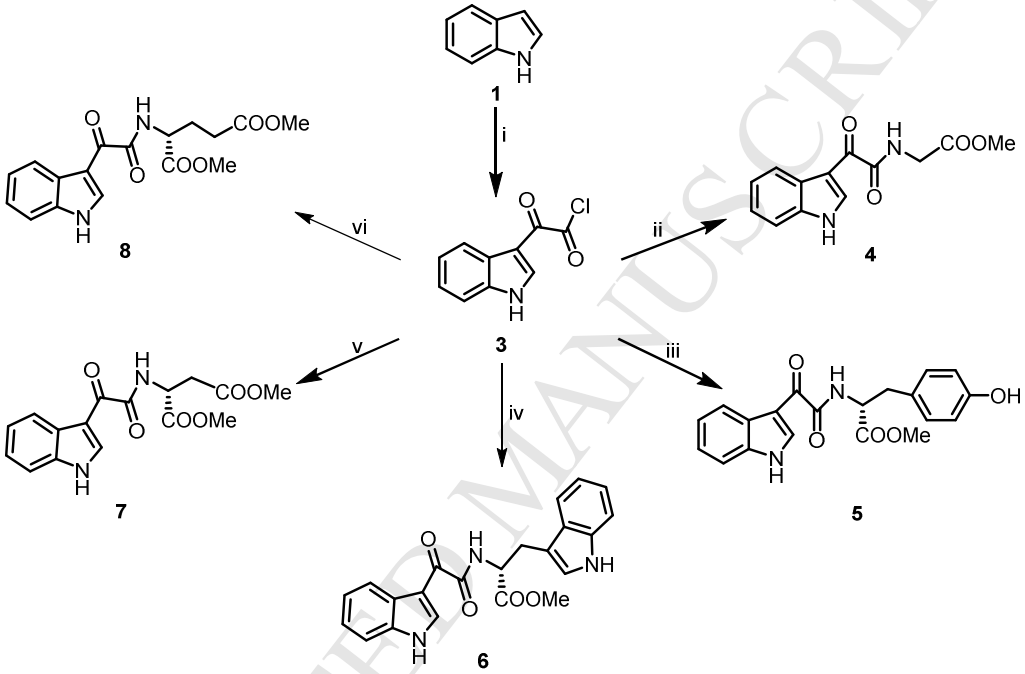


Figure 1. (A) Compound **1** docked in the active site pocket of lanosterol 14-α demethylase. (B) Fluconazole docked in the active site of lanosterol 14-α demethylase. (C) 2D view of the docking pose of compound **1** in the active site pocket of the enzyme showing hydrophobic residues. (D) Compound **2e** docked in the active site pocket of lanosterol 14-α demethylase.

Chemistry

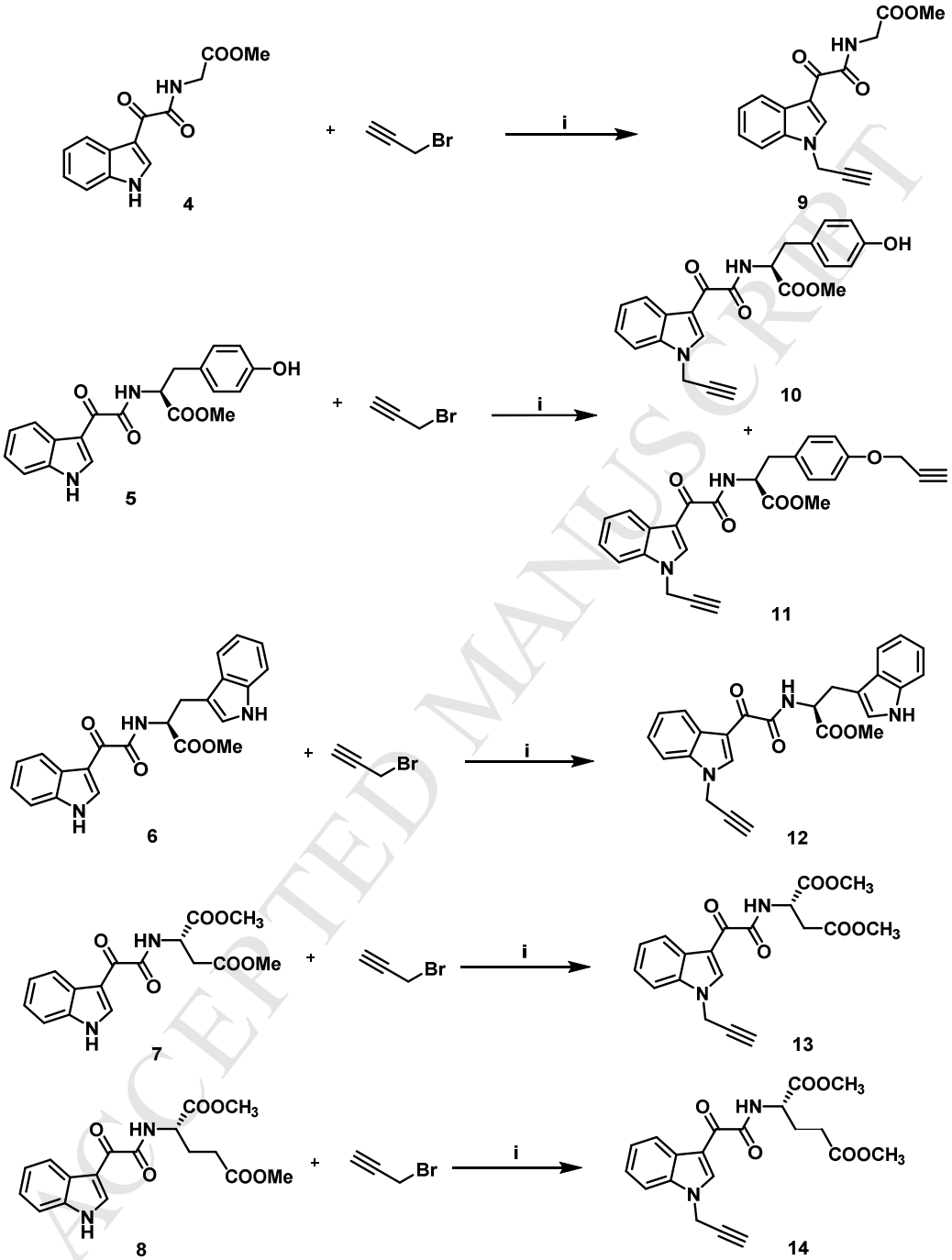
Reaction of indole (**1**) with oxalyl chloride at 0-5 ºC resulted in the formation of a yellow colored compound **3**. Compound **3** was then made to react with K_2CO_3 pretreated glycine methyl ester/L-tyrosine methyl ester/L-tryptophan methyl ester/L-aspartate methyl diester/L-glutamic methyl diester in dry acetonitrile in the presence of K_2CO_3 and triethylamine and compounds **4** – **8** were procured (Scheme 1).



Scheme 1. Synthesis of compounds **4** – **8**.

Further reaction of compound **4** with propargyl bromide in the presence of sodium hydride in dry acetonitrile at 0 – 10 ºC provided compound **9** (Scheme 2). Using the same procedure, compounds **10 – 14** were obtained by the reaction of **5 – 8** with propargyl bromide. During the propargylation of compound **5**, compounds **10** and **11** with propargyl group at indole-N only and at both indole N and tyrosine-O, respectively were isolated. For compound **6**, propargylation occurred at one indole N only and it did not go to Trp residue as it was

confirmed with HMBC, HSQC, COSY and TOCSY NMR experiments with compound **18** (Figure S42-S48).



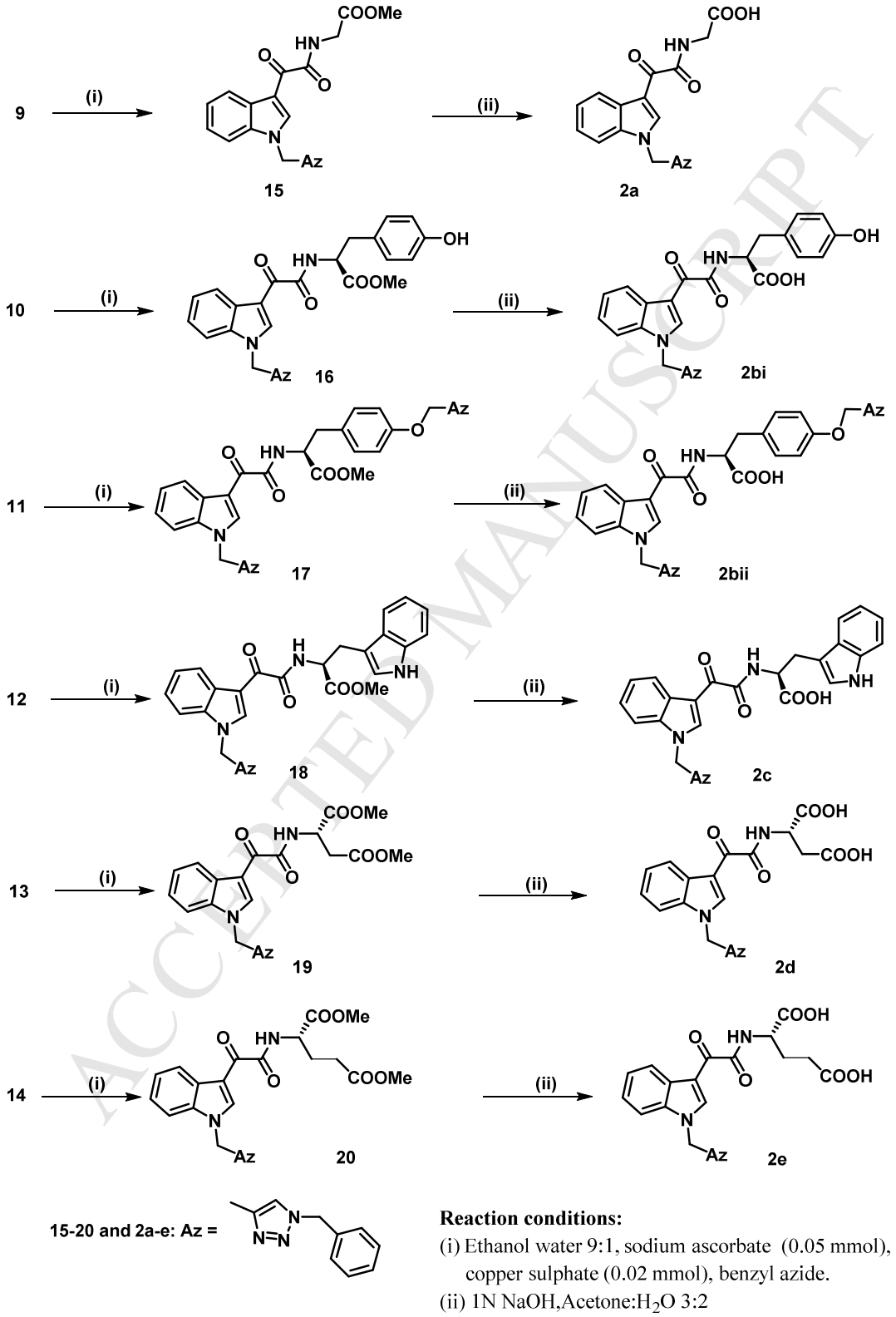
Reaction conditions:

(i) Dry ACN, NaH (1.5 mmol), propargyl bromide (1.5 mmol), 0-5 ^{o}C

Scheme 2. Synthesis of compounds **9 – 14**.

Reaction of compound **9** with benzyl azide in the presence of sodium ascorbate and copper sulphate in ethanol:water (9:1) gave compound **15** (Scheme 3). Similarly, reaction of

10 – **14** with benzyl azide led to the formation of respective compounds **16** – **20**. The ester hydrolysis of compounds **15** – **20** provided the target compounds **2a**-**e** (Scheme 3).



Scheme 3. Synthesis of compounds **2.**

Biological studies

All the synthesized compounds were screened for *in-vitro* antifungal activity against two fungal strains; *Candida albicans* (MTCC 3958) and *Aspergillus niger* (MTCC 9933). Antifungal activity of the test compounds was expressed in terms of ZOI (Zone of Inhibition) and MIC (Minimum Inhibitory Concentration). Fluconazole was used as reference drug for the study and 5% DMSO was used as a negative control.

Table 1. Zone of inhibition and MIC_{90} of compounds against *Candida albicans* and *Aspergillus niger*.

Compound	*Candida albicans*		*Aspergillus niger*	
	ZOI (mm)	MIC_{90} (µg/mL)	ZOI (mm)	MIC_{90} (µg/mL)
4	10	128	-	-
5	-	-	9	>128
6	14	32	-	-
7	15	32	13	64
8	-	-	9	>128
9	11	64	-	-
10	11	64	10	128
11	13	64	13	64
12	10	128	9	>128
13	13	64	-	-
14	9	128	-	-
15*	12	64	11	64
16	8	128	10	128
17	-	-	13	64
18*	9	128	12	64
19	11	128	-	-
20	10	64	11	64
2a	13	32	10	128
2bi	15	16	13	>32
2bii	16	16	15	32
2c	14	32	14	>32
2d	15	32	13	64
2e	14	32	12	>32
Fluconazole	19	16	18	8

*Compounds showed precipitation at the test concentration 128 µg/50 µL while determination of ZOI value, therefore, 10% DMSO was used for both these compounds instead of 5%. The comparative anti-fungal activities of 5 and 10 % DMSO in the negative control groups showed no significant difference.

A significant improvement in the anti-fungal activity of compounds **2** over compound **1** was observed. The different fragments of the molecule viz. amino acid, propargyl group, azole part were found to influence the activity of the molecule. In the category of compounds **4**–**8**; compounds **6** and **7** bearing respectively Trp and Asp residues showed better anti-fungal activity. Replacement of Asp with Glu in compound **8** did not improve the activity of the compound in comparison to compound **7**, even not at the propargylated stage (compare MIC of compound **13** and **14**). Presence of propargyl group at N-1 position in compounds **6** and **7** increased the MIC of the resulting compound **12** and **13**. However, the propargylation of compound **5** resulted into the improvement in MIC of the compounds **10** and **11**. Amongst the azole group carrying compounds **15**–**20**; compound **20** exhibited MIC 64 μg mL^{-1} and the MIC was further improved when ester group of compound **20** was hydrolyzed to the corresponding acid in compound **2e**. In fact, compounds **2a-e** showing ZOI 13-16 mm exhibited better anti-fungal activity in comparison to their corresponding precursor compounds. Overall, the ZOI for compounds **2b-e** was comparable to the standard drug fluconazole. In general, the compounds were active against both *Candida albicans* and *Aspergillus niger*.

Cytotoxicity of compound 2bii

MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] cellular toxicity assay was performed for checking the bio-safety of compound **2bii** against sheep blood cells. Reduction of MTT dye to formazan can directly be co-related with the metabolically active cells, so that the absorbance can be interpreted with the living state of the cells. At a concentration of 1 mg mL^{-1} (conc much higher than the MIC_{90} of this compound), compound **2bii** was found to be non-cytotoxic. The cytotoxicity was comparable to the untreated (control) sample and the cell viability of 85 % was observed.

3D Quantitative Structure Activity Relationship (QSAR) Studies Model

In order to correlate the structural features viz, steric, electrostatic, hydrophobic, HBD and HBA of the molecules with biological activity, Gaussian based QSAR model was generated by using PLS with three factors. R^2 CV value of 0.54 was derived from the LOO cross-validation method and the non-cross validation analysis provided R^2 0.97 with standard error of estimate 0.09 and an F ratio 50.3. The steric factor contribution is 0.41 whereas the electrostatic, hydrophobic, HBA and HBD contribute 0.16, 0.17, 0.14 and 0.10, respectively towards the antimicrobial activity of the molecules. The green colored contours in Fig. 2a favor the steric effect of bulky substituent whereas the yellow color represents negative effect of the steric substituent in this region. The positive effect of the bulky substituent in green region was clearly visible in case of compounds **2c**, **2d**, **2e**, **2bi**, **2bii**. The hydrophobic contours (Figure 2b) showed the light yellow (positive saturation) and white (negative saturation) colored regions. This can be effectively seen by the presence of indole scaffold and phenyl rings in the light yellow colored region – thus increase in the activity. Also in case of compound **2bii** the presence of additional phenyl rings in the yellow colored region lead to increase in its IC_{50} value. The electrostatic contour (Figure 2c) showed red and blue regions with positive and negative saturation respectively. Red region favored the presence of electronegative groups and blue region favored electropositive groups. The presence of acid forms of the amino acids in this region produced favorable effect in comparison to the ester forms of the compounds. In case of hydrogen bond acceptor (HBA) contour map (Figure 2d), the presence of maroon colored region (positive saturation) in the amide bond area complemented their positive effect (increase in the activity) whereas the magenta colored area proved to be detrimental for the HBA group present there. The effect of hydrogen bond donor parameter was represented with purple and cyan contours (Figure 2e). The purple colored areas were suitable for donors and cyan colored negative regions found not suitable

for hydrogen bond donors. The contribution of this region was limited (0.10) (Table 3). The QSAR model also showed the 3 fitness graph amongst the observed and predicted activity for (a) training set (b) test set (c) and both (training and test set combined) of molecules (supporting information). Therefore, the results obtained from QSAR studies are clearly supporting the design of the molecules. The steric factor along with the hydrophobic parameters is the major contributors for the biological activity of the molecules under present investigation.

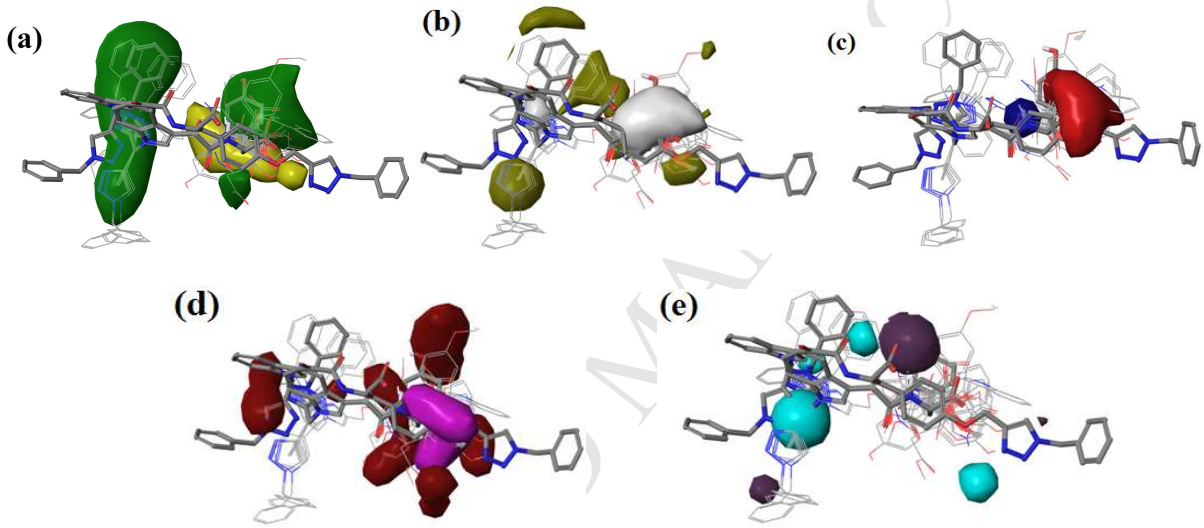


Figure 2. Visualization of QSAR effects of different parts of the molecule. (a) Steric contour map (yellow, negative saturation; Green, positive saturation); (b) Hydrophobic contour map (white, negative saturation; Light yellow, positive saturation); (c) Electrostatic contour map (red, negative saturation; blue, positive saturation); (d) H-bond acceptor contour map (magenta, negative saturation; maroon, positive saturation); (e) H-bond donor contour map (cyan, negative saturation; purple, positive saturation). The molecules with better anti-fungal activity are represented as tube sketches.

Table 2. PLS Statistical Parameters of the Selected 3D-QSAR Model[a]

PLS-Factors	SD	R^2	R^2 CV	R^2 scramble	Stability	F	P	RMSE	Q^2	Pearson-r
1	0.1835	0.6853	0.0035	0.6009	0.168	32.7	4.09e-05	0.16	0.6501	0.8971
2	0.1409	0.8268	0.1160	0.8259	0.419	33.4	4.68e-06	0.15	0.6716	0.9026
3	0.0990	0.9206	0.0122	0.9050	0.218	50.3	2.07e-07	0.18	0.5755	0.8660

[a]SD, standard deviation of regression; R, squared value for R^2 for regression; F, variance ratio (a large value of F indicates more statistical regression); P, significance level of variance ratio (smaller values indicate a greater degree of confidence); RMSE, root mean squared error; Q^2, value for the predicted activities; Pearson-r, value for the predicted activities of the test set.

Table 3. Contribution of various factors towards the biological activity of the molecule.

PLS-Factors	Steric	electrostatic	hydrophobic	HBA	HBD
1	0.421	0.154	0.186	0.151	0.086
2	0.439	0.157	0.169	0.143	0.090
3	0.412	0.161	0.170	0.146	0.1086

Conclusions

Guided by the results of the molecular modeling studies, the rational modification of a lead molecule led to the development of highly efficacious anti-fungal agents. Amongst the different series of compounds obtained during the synthesis of compounds **2**; it was observed that compounds **2** were the most potent. Compounds **2bi** and **2bii** with MIC_{90} 16 μg mL^{-1} were identified as the most suitable candidates for further investigation. Under the experimental procedures, the MIC_{90} of these two compounds was comparable to that of standard drug fluconazole. The 3D contour model obtained by QSAR studies indicating the role of steric, hydrophobic and electrostatic parameters will be helpful in further improving the structure of the compound.

Experimental section

All materials were procured from commercial suppliers and used as such without any further notification unless otherwise required. Diethyl ether was dried by distilling over

anhydrous calcium chloride and further dried by passing sodium wire. Acetonitrile was dried by refluxing over phosphorous pentaoxide (P_2O_5) and stored over activated 4 Å molecular sieves. Melting points were determined in open capillaries and are uncorrected. All reactions were carried out on oven dried glass wares. Reactions were monitored by thin layer chromatography (TLC) performed on glass plates coated with silica gel (GF254) and further visualized under UV light and iodine atmosphere. Column chromatography was performed with silica gel 60 – 120 mesh size and hexane : ethyl acetate as eluents. ^{1}H, ^{13}C, DEPT-NMR spectra were recorded on Bruker 500 MHz, JEOL 400 MHz and 125 MHz, 100 MHz NMR spectrometer, respectively using $CDCl_3$ and DMSO-d_6 as solvents and TMS as internal standard. Chemical shifts are reported in ppm relative to trimethyl silane, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet, dd = double doublet, br s = broad singlet) and coupling constant (*J* in Hz). Data for ^{13}C and DEPT-135 NMR spectra are given in chemical shift; +ve signals correspond to CH_3 and CH carbons, -ve signals correspond to CH_2 while absent means a quaternary carbon. Mass spectra were recorded on Bruker Micro TOF QII mass spectrometer. Machine was calibrated with sodium formate using KdScientific automated pump, with flow rate 180 µL/h, 50 µM solution in ACN-water formic acid (7:2.9:0.1) was injected to electrospray ionization source. Desolvation was performed with dry N_2 gas heated at 180 ºC. Various parameters of the mass spectrometer were optimized for maximum ion abundance. Typically the capillary voltage was 4500 V and vacuum was maintained at $3\text{-}4\times10^{-7}$ mbar. Sodium formate was used as internal calibrant.

General procedure for synthesis of compounds 4-8 (Procedure A)

Indole (**1**) (1 g, 8.5 mmol) was dissolved in dry ether (50 mL) and oxalyl chloride (732 µL, 8.5 mmol) was added drop-wise keeping the temperature between 0-5 ^{o}C. The reaction mixture was stirred for 2-3 min. The solid product was filtered under vacuum to get compound **3**. Compound **3** was then dissolved in dry ACN (50 ml), to which oven dried

K_2CO_3 (1 equiv) was added. Further, the ice cooled solution of neutralized L-amino acid methyl ester (neutralized with K_2CO_3) was added to the ACN solution of compound **3**. The reaction was then stirred for 3 h maintaining the temperature 0-5 ^oC. The reaction was quenched by adding 20 mL ice cold water and extracted with ethyl acetate (4×25 ml). The combined organic layers were then washed with water and brine solution, dried over anhydrous sodium sulphate and solvent was removed by distillation. Crude compound thus obtained was purified through column chromatography using hexane-ethyl acetate as the eluent (60:40).

General procedure for synthesis of compounds 9 -14 (Procedure B)

NaH (28 mg, 1.1 mmol) after washing with dry hexane was suspended in dry ACN followed by the addition of compound **4** (260 mg, 1.0 mmol). After the change in colour of the reaction mixture from colourless to yellow, propargyl bromide (178 mg, 1.5 mmol) was added drop-wise. Reaction mixture was stirred for 2 h, maintaining the temperature 0-5 °C. After the completion of the reaction (TLC), 10 mL ice cooled water was added to the reaction mixture and extracted with ethyl acetate (4×25 mL). The combined organic layers were then washed with water and brine solution and dried over anhydrous sodium sulphate. The organic layer was concentrated under vacuum and the crude product was purified through column chromatography using hexane – ethyl acetate as eluent to procure product **9**. Compounds **10** – **14** were prepared from compounds **5** – **8** by using the same procedure.

General procedure for the synthesis of compounds 15-20 (Procedure C)

Compound **9** (298 mg, 1.0 mmol) was dissolved in ethanol - water (50 mL, 9:1 v/v). Sodium ascorbate (9 mg, 0.05 mmol) was added to the above solution and the reaction mixture was allowed to stir for 5 – 10 min at room temperature. Then copper sulphate (2 mg, 0.01 mmol) was added to the reaction mixture followed by the addition of benzyl azide (133 mg 1.0 mmol) and the reaction mixture was stirred for 12 h. After the completion of reaction (TLC),

the reaction mixture was washed with water and brine followed by extraction with ethyl acetate (4×25 ml). The combined organic layers were then dried over anhydrous sodium sulphate and concentrated under vacuum. Crude material obtained was then purified through column chromatography using hexane:ethyl acetate as eluents to obtain pure product **15**. Compounds **16** – **20** were obtained from compounds **10** – **14** by using same experimental protocol.

General procedure for the synthesis of compounds 2 (Procedure D)

Compound **15** (431 mg, 1.0 mmol) was taken in acetone - water (20 mL, 2:1 v/v) containing 1N NaOH. The reaction mixture was stirred for 30 min (TLC). Acetone was removed under vacuum and 10 mL 1N HCl was added drop-wise until the solution becomes acidic. The precipitates formed were filtered and left for drying to obtain pure product **2a**. Similarly, the ester group of compounds **16** – **20** was hydrolyzed.

Methyl (2-(1*H*-indol-3-yl)-2-oxoacetyl)glycinate (4). Compound **4** was synthesized following general procedure A. White solid, yield 70%, mp 163 ºC. IR (KBr): 3369, 3324, 3149, 2952, 1736, 1490, 1423 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 3.68 (s, 3H, OCH_3), 4.01 (d, J = 5.85 Hz, 2H, CH_2), 7.27-7.30 (m, 2H, ArH), 7.54-7.56 (m, 1H, ArH), 8.24-8.26 (m, 1H, ArH), 8.75-8.76 (m, 1H, ArH), 9.09 (t, J = 5.90 Hz, 1H, NH) 12.26 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 41.0 (-ve, CH_2), 52.3 (+ve , OCH_3), 112.6 (ArC), 121.7 (+ve, ArCH), 121.7 (+ve, ArCH), 123.1 (+ve, ArCH), 124.0 (+ve, ArCH), 126.5 (ArC), 136.7 (ArC), 139.0 (+ve, ArCH), 164.4 (C=O), 170.2 (C=O), 181.7 (C=O). HRMS (micro TOF-QII, MS, ESI) Calcd for $C_{13}H_{12}N_2O_4$ ($[M+H]^+$) 261.0870, found 261.0762.

Methyl (2-(1*H*-indol-3-yl)-2-oxoacetyl)tyrosinate (5). Compound **5** was synthesized following general procedure A. White solid, yield 70%, mp 180-182 ^{o}C. IR (KBr): 3369, 3324, 3149, 3140, 2952, 1736, 1490, 1423 cm^{-1}. ^{1}H NMR (500 MHz, DMSO- d_6) δ: 3.00 (dd, J =14.19, 5.13 Hz, 1H, CH_2), 3.08 (dd, J = 14.02, 5.30 Hz, 1H, CH_2), 3.67 (s, 3H, OCH_3),

4.57 (m, 1H, CH), 6.66 (d, *J* = 8.3 Hz, 2H, ArH), 7.04 (d, *J* = 8.24 Hz, 2H, ArH), 7.26-7.27 (m, 2H, ArH), 7.53 (d, *J*=7.19 Hz, 1H, ArH), 8.19-8.21 (m ,1H, ArH), 8.56 (s, 1H, ArH), 8.92 (d, *J* = 8.9 Hz, 1H, NH), 9.24 (s, 1H, NH), 12.25 (s, 1H, OH). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 35.8 (-ve, CH_2), 52.5 (+ve, CH), 54.1 (+ve, OCH_3), 112.5 (ArC), 113.0 (+ve, ArCH), 115.5 (+ve, ArCH), 121.6 (+ve, ArCH), 123.1 (+ve, ArCH), 123.9 (+ve, ArCH), 126.4 (ArC), 127.6 (ArC), 130.4 (+ve, ArCH), 136.7 (ArC), 138.8 (+ve, ArCH), 156.7 (C=O), 164.0 (C=O), 171.9 (C=O), 181.9 (C=O). HRMS (micro TOF-QII, MS, ESI) Calcd for $C_{20}H_{18}N_2O_5$ ($[M+H]^+$) 367.1288, found 367.1290.

Methyl (2-(1*H*-indol-3-yl)-2-oxoacetyl)tryptophanate (6). Compound **6** was synthesized following general procedure A. White solid, yield 75%, mp 170-172 ºC, IR (KBr): 3436, 3310, 3272, 2952, 1733, 1498, 1468 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 3.30-3.34 (m, 2H, CH_2), 3.8 (s, 3H, OCH_3), 4.68-4.73 (m, 1H, CH), 6.99 (t, *J* = 7.84 Hz, 1H, ArH), 7.08 (t, *J* = 7.25 Hz, 1H, ArH), 7.21 (d, *J* = 2.20 Hz, 1H, ArH), 7.26-7.28 (m, 2H, ArH), 7.36 (d, *J* = 8.15, 1H, ArH), 7.53-7.55 (m, 2H, ArH), 8.20-8.22 (m, 1H, ArH), 8.59 (d, *J* = 3.02 Hz, 1H, ArH), 8.89 (d, *J* = 7.87 Hz, 1H, NH), 10.88 (s, 1H, NH), 12.24 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 26.9 (-ve, CH_2), 52.5 (+ve, OCH_3), 53.4 (+ve, CH), 109.8 (ArC), 111.9 (+ve, ArCH), 112.5 (ArC), 113.0 (+ve, ArCH), 118.4 (+ve, ArCH), 118.9 (+ve, ArCH), 121.5 (+ve, ArCH), 121.7 (+ve, ArCH), 123.1 (+ve, ArCH), 123.9 (+ve, ArCH), 124.3 (+ve, ArCH), 126.5 (ArC), 127.5 (ArC), 136.6 (ArC), 136.7 (ArC), 138.9 (+ve, ArCH), 163.9 (C=O), 172.1 (C=O), 181.8 (C=O). HRMS (micro TOF-QII, MS, ESI) Calcd for $C_{22}H_{19}N_3O_4$ $[M+Na]^+$ 412.1267, found 412.1384.

Dimethyl (2-(1*H*-indol-3-yl)-2-oxoacetyl)aspartate (7). Compound **7** was synthesized following general procedure A. White solid, yield 75%, mp 168 -170 ºC, IR (KBr) 3336, 3142, 3000, 1736, 3145, 2952, 1736 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.95 (dd, *J* = 17.27, 4.88 Hz, 1H, CH_2), 3.13 (dd, *J* = 17.11, 5.10 Hz 1H, CH_2), 3.74 (s, 3H, OCH_3), 3.81 (s, 3H,

OCH_3), 4.96-5.00 (m, 1H, CH), 7.31-7.37 (m, 2H, ArH), 7.45-7.47 (m, 1H, ArH), 8.32 (d, *J* = 8.54 Hz, 1H, ArH), 8.42 (d, *J* = 7.72 Hz, 1H, ArH), 9.0 (s, 1H, NH), 9.13 (s, 1H, NH). ^{13}C NMR (125 MHz $CDCl_3$) δ**:** 36.1 (-ve, CH_2), 48.6 (+ve, CH), 52.2 (+ve, OCH_3), 52.9 (+ve, OCH_3), 111.65 (+ve, ArCH), 113.2 (ArC), 122.4 (+ve, ArCH), 123.4 (+ve, ArCH), 124.2 (+ve, ArCH), 126.5 (ArC), 135.7 (ArC), 138.1 (+ve, ArCH), 162.1 (C=O), 170.6 (C=O), 170.9 (C=O), 179.50 (C=O). HRMS (micro TOF-QII, MS, ESI) Calcd for $C_{16}H_{16}N_2O_6$ ($[M+H]^+$) 333.1081, found 333.3973.

Dimethyl (2-(1*H*-indol-3-yl)-2-oxoacetyl)glutamate (8). Compound **8** was synthesized following general procedure A. White solid, yield 75%, mp 143-145 ºC. ^{1}H NMR (500 MHz, DMSO-*d*6) δ: 2.01-2.07 (m, 1H, CH_2), 2.12-2.18 (m, 1H, CH), 2.41-2.44 (m, 2H, CH_2), 3.59 (s, 3H, OCH_3), 3.68 (s, 3H, OCH_3), 4.43-4.48 (m, 1H, CH), 7.27-7.30 (m, 2H, ArH), 7.54-7.56 (m, 1H, ArH), 8.23-8.25 (m, 1H, ArH), 8.66 (d, *J* = 2.48 Hz, 1H, ArH), 9.10 (d, 7.87 Hz, 1H, NH), 12.28 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 25.9 (-ve, CH_2), 30.2 (-ve, CH_2), 51.6 (+ve, OCH_3), 51.8 (+ve, OCH_3), 52.6 (+ve, CH), 112.6 (ArC), 113.1 (+ve, ArCH), 121.6 (+ve, ArCH), 123.1 (+ve, ArCH), 124.0 (+ve, ArCH), 126.5 (ArC), 136.7 (ArC), 138.8 (+ve, ArCH), 164.6 (C=O), 172.0 (C=O), 173.1 (C=O), 182.1 (C=O). HRMS (micro TOF-QII, MS, ESI): Calcd for $C_{17}H_{18}N_2O_6$ ($[M+H]^+$) 347.1238, found 347.1381.

Methyl ((2-oxo-2-(1-(prop-2-yn-1-yl)-1*H*-indol-3-yl) acetyl) glycinate (9). Compound **9** was synthesized following general procedure B**.** Yellow solid, yield 70%, mp 117-119 ºC, IR (KBr) 3369, 3265, 3216, 2952, 2120, 1736 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.54 (t, *J* = 2.45, 1H, CH), 3.8 (s, 3H, OCH_3), 4.18 (d, *J* = 5.74 Hz, 2H, CH_2), 4.93 (d, *J* = 2.5 Hz, 2H, CH_2), 7.37-7.39 (m, 2H, ArH), 7.46–7.48 (m, 1H, ArH), 8.01 (t, *J* = 5.90 Hz, 1H, ArH), 8.43-8.44 (m, 1H, ArH), 9.07 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 41.1 (-ve, CH_2), 42.4 (-ve, CH_2), 52.56 (+ve, OCH_3), 60.4 (+ve, CH), 110.3 (+ve, CH), 112.3 (ArC), 122.7 (+ve, CH), 123.7 (+ve, ArCH), 124.2 (+ve, ArCH), 127.6 (ArC), 136.2 (ArC), 140.9

(+ve, ArCH), 142.6 (ArC), 162.8 (C=O), 169.5 (C=O), 179 (C=O). HRMS (micro TOF-QII, MS, ESI) Calcd for $C_{16}H_{14}N_2O_4$ ($[M+Na]^+$) 321.0845, found 321.0831.

Methyl (2-oxo-2-(1-prop-2-yn-1-yl)-1*H*-indol-3-yl)acetyl)tyrosinate (10). Compound **10** was synthesized following general procedure B. Yellow solid, yield 40%, mp 117 ºC. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.50 (t, *J* = 2.54, 1H, CH), 3.08 (dd, *J* = 14.17, 6.72 Hz, 1H, CH_2), 3.16 (dd, *J* = 14.17, 5.45 Hz, 1H, CH_2), 3.78 (s, 3H, OCH_3), 4.84-4.87 (m, 1H, CH), 4.89 (d , *J* = 2.42 Hz, 2H, CH_2), 5.64 (br, 1H, OH), 6.74 (d, *J* = 8.4 Hz, 2H, ArH), 7.03 (d, *J* = 8.39 Hz , 2H, ArH), 7.37-7.39 (m, 2H, ArH), 7.46-7.48 (m, 1H, ArH), 7.90 (d, *J* = 8.50 Hz, 1H, ArH), 8.41-8.43 (m, 1H, ArH), 8.99 (s, 1H, NH). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 36.8 (-ve, CH_2), 37.3 (-ve, CH_2), 52.4 (+ve, CH), 53.5 (+ve, OCH_3), 75.5 (+ve, CH), 75.7 (C), 110.1 (+ve, ArCH), 112.3 (ArC), 115.7 (+ve, ArCH), 122.8 (+ve, ArCH), 123.8 (+ve, ArCH), 124.2 (+ve, ArCH), 127.4 (ArC), 127.7 (ArC), 130.4 (+ve, ArCH), 135.9 (ArC), 140.5 (+ve, ArCH), 155 (ArC), 161.9 (C=O), 171.2 (C=O), 179.3 (C=O). HRMS (micro TOF-QII, MS, ESI) Calcd for $C_{23}H_{20}N_2O_5$ $[M+H]^+$ 405.1445, found 405.0878.

Methyl-2-(2-oxo-2(1-(prop-2-yn-1-yl)-1*H*-indol-3-yl)acetamido)-3-(4-(-2yn-1-yloxy) phenyl)propanoate (11). Compound **11** was synthesized following general procedure B. Yellow thick oil, yield 50%. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.48 (t, *J* = 2.41 Hz, 1H, CH), 2.51 (t, *J* = 2.56, 1H, CH), 3.12 (dd, *J* = 14.43, 6.61 1H, CH_2), 3.18 (dd, *J* = 14.26, 5.56 Hz, 1H, CH_2), 3.73 (s, 3H, OCH_3), 4.64 (d, *J* =2.4, 2H, CH_2), 4.87 (m, 1H, CH), 4.91 (d, *J* = 2.49 Hz, 2H, CH_2), 6.89-6.91 (m, 2H, ArH), 7.10-7.11 (m, 2H, ArH), 7.35-7.37 (m, 2H, ArH), 7.45-7.46 (m, 1H, ArH), 7.86 (d, *J* = 8.33 Hz, 1H, ArH), 8.40-8.41 (m, 1H, ArH), 9.01 (s, 1H, NH). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 36.8 (-ve, CH_2), 37.41 (-ve, CH_2), 52.4 (+ve, CH), 53.4 (+ve, CH), 55.7 (-ve, CH_2), 55.8 (+ve, OCH_3), 75.4 (+ve, CH), 75.5 (+ve, CH), 75.7 (C), 78.5 (C), 110.08 (+ve, ArCH), 112.3 (ArC), 115.2 (+ve, ArCH), 122.8 (+ve, ArCH), 123.7 (+ve, ArCH), 124.1 (+ve, ArCH), 127.7 (ArC), 128.6 (ArC), 130.2 (+ve, ArCH), 135.9 (ArC),

140.4 (+ve, ArCH), 156.8 (ArC), 161.8 (C=O), 171.1 (C=O), 179.9 (C=O). HRMS (micro TOF-QII, MS, ESI) Calcd for $C_{40}H_{36}N_8O_5$ $[M+Na]^+$ 465.2512, found 465.2592.

Methyl (2-oxo-2-(1-(prop-2-yn-1-yl)-1*H*-indol-3-yl)acetyl-L-tryptophanate (12). Compound **12** was synthesized following general procedure B**.** Yellow solid, yield 60%, mp 119-121 ºC. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.52 (t, *J* = 2.26, 1H, CH), 3.42-3.43 (m, 2H, CH_2), 3.71 (s, 3H, OCH_3), 4.92-4.93 (d, *J* = 1.70 Hz, 2H, CH_2), 4.96-5.00 (m, 1H, CH), 7.11-7.15 (m, 2H, ArH), 7.20 (t, *J* =7.43 Hz, 1H, ArH), 7.36-7.39 (m, 3H, ArH), 7.48 (m, 1H, ArH), 7.60 (d, *J* =8.05 Hz, 1H, ArH), 7.98 (d, *J* = 8.12 Hz, 1H, ArH), 8.16 (br, 1H, NH), 8.41-8.43 (m, 1H, ArH), 9.05 (s, 1H, NH). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 27.8 (-ve, CH_2), 36.8 (-ve, CH_2), 52.5 (+ve, OCH_3), 53.4 (+ve, CH), 75.4 (+ve, CH), 75.7 (C), 109.8 (C), 110.0 (+ve, ArCH), 112.5 (C) 118.6 (+ve, ArCH), 119.7 (+ve, ArCH), 122.3 (+ve, ArCH), 122.8 (+ve, ArCH), 123.7 (+ve, ArCH), 124.1 (+ve, ArCH), 127.3 (ArC), 127.7 (ArC), 135.9 (ArC), 136.9 (ArC), 140.4 (+ve, ArCH), 161.9 (C=O), 171.5 (C=O), 179.8 (C=O). HRMS (micro TOF-QII, MS, ESI) Calcd for $C_{25}H_{21}N_3O_4$ $[M+H]^+$ 428.4274, found 428.1600.

Dimethyl (2-oxo-2-(1-(prop-2-yn-1-yl)-1*H*-indol-3-yl)acetyl)aspartate (13). Compound **13** was synthesized following general procedure B. Yellow solid, yield 60%, mp 115-117 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.53 (t, *J* = 2.7 Hz , 1H), 2.92 (dd, *J* = 17.2, 5.0 Hz, 1H, CH_2), 3.12 (dd, *J* = 17.4, 4.8 Hz, 1H, CH_2), 3.72 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 4.92-4.97 (m, 2H+1H), 7.37-7.39 (m, 2H, ArH), 7.46–7.48 (m, 1H, ArH), 8.30 (d, *J* = 8.9 Hz, 1H, ArH), 8.43-8.45 (m, 1H, ArH), 9.06 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 36.1 (-ve, CH_2), 36.9 (-ve, CH_2), 48.5 (+ve, OCH_3) 52.3 (+ve, OCH_3), 53.0 (+ve, CH), 75.6 (+ve, CH), 75.8 (C), 110.1 +ve, ArCH), 112.4 (ArC), 122.9 (+ve, ArCH), 123.9 (+ve, ArCH), 124.2 (+ve, ArCH), 127.8 (ArC), 135.9 (ArC), 140.5 (+ve, ArCH), 162.0 (C=O), 170.5 (C=O), 170.9 (C=O), 179.14 (C=O). HRMS (micro TOF-QII, MS, ESI) Calcd for $C_{19}H_{18}N_2O_6$ $[M+H]^+$ 371.1237, found 371.1193.

Dimethyl (2-oxo-2-(1-(prop-2-yn-1-yl)-1*H*-indol-3-yl)acetyl)glutamate (14). Compound **14** was synthesized following general procedure B. Yellow solid, yield 60%, mp 121-123 ºC, 60%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.10-2.19 (m, 1H, CH_2), 2.28-2.39 (m, 1H, CH_2), 2.41-2.48 (dd, *J* = 16.0 Hz, 4.0 Hz, 2H, CH_2), 2.52 (t, *J* = 2.8 Hz, 1H, CH), 3.68 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 4.65-4.73 (m, 1H, CH), 4.93 (d, *J* = 2.7 Hz, 2H, CH_2), 7.36-7.38 (m, 2H, ArH), 7.46-7.48 (m, 1H, ArH), 8.03 (d, *J* = 8.7 Hz, 1H, ArH), 8.42-8.44 (m, 1H, ArH), 9.06 (br, 1H, NH). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 27.3 (-ve, CH_2), 30.1 (-ve, CH_2), 36.9 (-ve, CH_2), 51.7 (+ve, CH), 52.0 (+ve, OCH_3), 52.7 (+ve, OCH_3), 75.8 (C), 110.1 (+ve, ArCH), 112.4 (ArC), 122.8 (+ve, ArCH), 123.9 (+ve, ArCH), 124.2 (+ve, ArCH), 127.8 (ArC), 135.9 (ArC), 140.5 (+ve, ArCH), 162.2 (C=O), 171.4 (C=O), 172.9 (C=O), 179.2 (C=O). HRMS (micro TOF-QII, MS, ESI) Calcd for $C_{20}H_{20}N_2O_6$ $[M+Na]^+$ 407.1213, found 407.1025.

Methyl (2-(1-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)methyl)-1*H*-indol-3yl)-2-oxoacetyl)glycinate (15). Compound **15** was synthesized following general procedure C. Yellow solid, yield 75%, mp 143-145 ºC. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.80 (s, 3H, OCH_3), 4.16 (d, *J* = 5.8 Hz, 2H, CH_2), 5.45 (s, 2H, CH_2), 5.47 (s, 2H, CH_2), 7.20-7.21 (m, 2H, ArH), 7.29-7.34 (m, 6H, ArH), 7.48 (d, *J* = 7.6 Hz, 1H, ArH), 7.98 (t, *J* = 5.5 Hz, 1H, ArH), 8.41 (d, *J* = 7.0 Hz, 1H, ArH), 8.99 (s, 1H, NH). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 41.0 (-ve, CH_2), 43.1 (-ve, CH_2), 52.4 (+ve, OCH_3) 54.3 (-ve, CH_2), 110.6 (+ve, ArCH), 112.3 (ArC), 122.2 (+ve, ArCH), 122.6 (+ve, ArCH), 123.7 (+ve, ArCH), 124.1 (+ve, ArCH), 127.6 (ArC), 128.04 (+ve, ArCH), 129.1 (+ve, ArCH), 134.1 (ArC), 136.1 (ArC), 140.8 (+ve, ArCH), 162.6 (C=O), 169.4 (C=O), 179.1 (C=O). HRMS (micro TOF-QII, MS, ESI) Calcd for $C_{23}H_{21}N_5O_4$ $[M+Na]^+$ 454.1485, found 454.1636.

Methyl (2-1((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-indol-3-yl)-2-oxoacetyl)-L-tyrosinate (16). Compound **16** was synthesized following general procedure C. Yellow solid, yield 40 %, mp 141-143 ºC. ^{1}H NMR (500 MHz, $CDCl_3$) δ: ^{1}H NMR (500 MHz, $CDCl_3$) δ:

2.91-2.95 (m, 1H, CH_2), 3.18 (dd, *J* = 14.0, 4.9 Hz, 1H, CH_2), 3.77 (s, 3H, OCH_3), 4.84 (m, 1H, CH), 5.43 (s, 2H, CH_2), 5.45 (d, *J* = 10.7 Hz, 2H, CH_2), 6.75 (d, *J* = 8.2 Hz, 2H, ArH), 6.99 (d, *J* = 8.5 Hz, 2H, ArH), 7.19-7.22 (m, 3H, ArH), 7.28-7.32 (m, 3H, ArH), 7.33-7.36 (m, 3H, ArH), 7.42 (d, *J* = 7.9 Hz, 1H, ArH), 7.73 (d, *J* = 8.5 Hz, 1H, ArH), 8.34 (d, *J* = 7.3 Hz, 1H, NH), 8.60 (s, 1H, OH). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 38.1 (-ve, CH_2), 42.7 (-ve, CH_2), 52.5 (+ve, OCH_3), 53.6 (+ve, CH), 54.4 (-ve, CH_2), 110.4 (+ve, ArCH), 112.6 (ArC), 115.9 (+ve, ArCH), 122.4 (+ve, ArCH), 122.7 (+ve, ArCH), 123.6 (+ve, ArCH), 124.1 (+ve, ArCH), 127.3 (ArC) 127.6 (ArC) 128.1 (+ve, ArCH), 128.9 (+ve, ArCH), 129.2 (+ve, ArCH), 130.5 (+ve, ArCH), 133.9 (+ve, ArCH), 136.0 (ArC), 140.9 (+ve, ArCH), 142.8 (ArC), 155.5 (ArC), 162.1 (C=O), 162.7 (ArC), 171.2 (C=O), 179.8 (C=O). HRMS (micro TOF-QII, MS, ESI) Calcd for $C_{30}H_{27}N_5O_5$ $[M+Na]^+$ 560.1904, found 560.5723.

Methyl (*S*)-3-(4-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-2-(2-(1-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-indol-3-yl)-2-oxoacetamido)propanoate (17). Compound **17** was synthesized following general procedure C. Yellow thick oil, yield 50%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.12 (dd, *J* = 13.2, 6.6 Hz, 2H, CH_2), 3.72 (s, 3H, OCH_3), 4.78-4.82 (m, 1H, CH), 5.13 (s, 2H, CH_2), 5.43 (s, 2H, CH_2), 5.45 (s, 2H, CH_2), 5.51 (s, 2H, CH_2), 6.87 (d, *J* = 8.5 Hz, 2H, ArH), 7.07 (d, *J* = 8.5 Hz, 2H, ArH), 7.18-7.20 (m, 2H, ArH), 7.29-7.32 (m, 5H, ArH), 7.34-7.38 (m, 5H, ArH), 7.46 (d, *J* = 7.6 Hz, 1H, ArH), 7.50 (s, 1H, ArH), 7.85 (d, *J* = 8.5 Hz, 1H, ArH), 8.37 (d, *J* = 7.6 Hz, 1H, ArH), 8.93 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 37.2 (-ve, CH_2), 43.1 (-ve, CH_2), 52.4 (+ve, OCH_3), 53.4 (+ve, CH), 54.2 (-ve, CH_2), 54.3 (-ve, CH_2), 62.1 (-ve, CH_2), 110.6 (+ve, ArCH), 112.3 (ArC), 115.1 (+ve, ArCH), 122.0 (+ve, ArCH), 122.5 (+ve, ArCH), 122.6 (+ve, ArCH), 123.6 (+ve, ArCH), 124.1 (+ve, ArCH), 127.6 (ArC), 128.0 (+ve, ArCH), 128.1 (+ve, ArCH), 128.2 (ArC), 128.7 (+ve, ArCH), 128.8 (+ve, ArCH), 129.1 (+ve, ArCH), 129.1 (+ve, ArCH), 130.2 (+ve, ArCH), 134.1 (ArC), 134.4 (ArC), 136.1 (+ve, ArCH), 140.8 (+ve, ArCH), 143.1

(ArC), 144.5 (ArC), 157.4 (ArC), 161.9 (C=O), 162.52 (C=O), 171.1 (C=O), 179.2 (C=O). HRMS (micro TOF-QII, MS, ESI) Calcd for $C_{40}H_{36}N_8O_5$ $[M+Na]^+$ 709.2881, found 709.1780.

Methyl (2-(1-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-indol-3-yl)-2-oxoacetyl)-L-tryptophanate 18. Compound **18** was synthesized following general procedure C. Yellow solid, yield 60%, mp 144-146 ºC. ^{1}H NMR (500 MHz) δ: 3.38 (d, *J* = 5.87 Hz, 2H CH_2), 3.69 (s, 3H, OCH_3), 4.91-4.95 (m, 1H, CH), 5.44 (s, 4H, 2×CH_2), 7.08-7.12 (m, 2H, ArH), 7.16 - 7.20 (m, 3H, ArH), 7.27-7.35 (m, 7H, ArH), 7.45 (d, *J* = 7.59 Hz, 1H, ArH), 7.58 (d, *J* = 7.89 Hz, 1H, ArH), 7.92 (d, *J* = 8.05 Hz, 1H, CON(H), 8.22 (br, 1H, NH Tryptophan), 8.36 (d, *J* = 7.44 Hz, 1H, ArH), 8.91 (s, 1H, ArH). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 27.9 (-ve, CH_2), 43.1 (-ve, CH_2), 52.5 (+ve, OCH_3), 52.6 (+ve, CH), 54.4 (-ve, CH_2), 109.8 (ArC), 110.6 (+ve, ArCH), 111.2 (+ve, ArCH), 112.3 (ArC), 118.6 (+ve, ArCH) 119.7 (+ve, ArCH), 122.3 (+ve, ArCH), 122.6 (+ve, ArCH), 122.9 (+ve, ArCH), 123.6 (+ve, ArCH), 124.1 (+ve, ArCH), 127.3 (+ve, ArCH), 127.6 (ArC), 128.0 (ArC), 128.9 (+ve, ArCH), 129.2 (+ve, ArCH), 134.0 (+ve, ArCH), 136.1 (ArC), 136.2 (ArC), 140.8 (ArC), 162.0 (C=O), 171.5 (C=O), 179.5 (C=O). HRMS (micro TOF-QII, MS,ESI): Calcd for $C_{32}H_{28}N_6O_4$ ($[M+Na]^+$) 583.2064, found 583.2153.

Dimethyl (2-(1-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-1H-indol-3-yl)-2-oxoacteyl)–L-aspartate (19). Compound **19** was synthesized following general procedure C. Yellow solid, yield 60%, mp 145-147 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.89 (dd, *J* = 15.9, 5.0 Hz, 1H, CH_2), 3.09 (dd, *J* = 16.2, 4.8 Hz, 1H, CH_2), 3.70 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 4.89-4.91 (m, 1H, CH), 5.43 (s, 2H, CH_2), 5.46 (s, 2H, CH_2), 7.17-7.21 (m, 2H, ArH), 7.27-7.34 (m, 6H, ArH), 7.45-7.46 (m, 1H, ArH), 8.26 (d, *J* = 8.8 Hz, 1H, NH), 8.38-8.40 (m, 1H, ArH), 8.97 (s, 1H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 36.1 (-ve, CH_2), 43.2 (-ve, CH_2), 48.5 (+ve, CH), 52.3 (+ve, OCH_3), 53.0 (+ve, OCH_3), 54.4 (-ve, CH_2), 110.6 (+ve, ArCH),

112.4 (ArC), 122.7 (+ve, ArCH), 123.8 (+ve, ArCH), 124.3 (+ve, ArCH), 127.8 (ArC), 128.1 (+ve, ArCH), 129.0 (+ve, ArCH), 129.2 (+ve, ArCH), 134.1 (ArC), 136.1 (ArC), 140.8 (+ve, ArCH), 143.1 (ArC), 162.1 (C=O), 170.5 (C=O), 170.9 (C=O), 179.0 (C=O). HRMS (micro TOF-QII, MS, ESI) Calcd for $C_{16}H_{16}N_2O_6$ $[M+H]^+$ 504.1877, found 504.1550.

Dimethyl (2-(1-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-indol-3-yl)-2-oxoacetyl) glutamate (20). Compound **20** was synthesized following general procedure C. Yellow solid, yield 60%, mp 146-148 ºC. 1H-NMR (400 MHz, $CDCl_3$) δ: 2.10-2.17 (m, 1H, CH_2), 2.30-2.39 (m, 1H, CH_2), 2.42-2.49 (dd, *J* = 16 Hz, 4 Hz, 2H, CH_2), 3.68 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 4.64-4.70 (m, 1H, CH), 5.45-5.47 (s, 4H, 2xCH_2), 7.30-7.36 (m, 6H, ArH), 7.47 (d, *J* = 8.0 Hz, 1H, ArH), 8.01 (d, *J* = 8.2 Hz, 1H, ArH). 8.40 (d, *J* = 8.0 Hz, 1H, ArH), 8.98 (br, 1H, NH). ^{13}C NMR (100 MHz normal/DEPT-135, $CDCl_3$) δ: 27.2 (-ve, CH_2), 30.0 (-ve, CH_2), 43.2 (-ve, CH_2), 51.6 (+ve, OCH_3), 51.9 (+ve, OCH_3), 52.7 (+ve, CH), 54.4 (-ve, CH_2), 110.6 (+ve, ArCH), 112.3 (ArC), 122.7 (+ve, ArCH), 123.7 (+ve, ArCH), 124.2 (+ve, ArCH), 127.7 (ArC), 128.0 (+ve, ArCH), 128.9 (+ve, ArCH), 129.2 (+ve, ArCH), 134.0 (ArC), 136.1 (ArC), 140.8 (ArC), 162.2 (C=O), 171.3 (C=O), 172.9 (C=O), 179.0 (C=O). HRMS (micro TOF-QII, MS, ESI) Calcd for $C_{27}H_{27}N_5O_6$ $[M+H]^+$ 518.2034, found 540.1350.

(2-(1-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-indol-3-yl)-2-oxoacetyl)glycine (2a). Compound **2a** was synthesized following general procedure D. Pink solid, yield 80%, mp 157 ºC, IR (KBr) 3127, 3034, 2929, 1736 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 3.90 (d, *J*=6.24 Hz, 2H, CH_2), 5.56 (s, 2H, CH_2), 5.63 (s, 2H, CH_2), 7.26- 7.27 (m, 2H, ArH), 7.29- 7.33 (m, 4H, ArH), 7.35-7.37 (m, 1H, ArH), 7.72- 7.74 (m, 1H, ArH), 8.23- 8.26 (m, 2H, NH, ArH), 8.97- 8.99 (m, 2H, ArH), 12.67- 12.76 (br, 1H, COOH). ^{13}C (125 MHz, DMSO- d_6) d: 41.1 (-ve, CH_2), 42.0 (-ve, CH_2), 53.2 (-ve, CH_2), 111.8 (ArC), 112.0 (+ve, ArCH), 122.0 (+ve, ArCH), 123.5 (+ve, ArCH), 124.0 (+ve, ArCH), 124.4 (+ve, ArCH), 127.3 (ArC), 128.3 (+ve, ArCH), 128.6 (+ve, ArCH), 129.2 (+ve, ArCH), 136.3 (ArC), 136.5 (ArC), 141.5 (+ve,

ArCH), 142.9 (ArC), 163.7 (C=O), 171.0 (C=O), 181.2 (C=O).HRMS (micro TOF-QII, MS, ESI)**:** Calcd for $C_{22}H_{19}N_5O_4$ $[M+Na]^+$ 440.1329, found 440.1845.

(2-(1-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-indol-3-yl)-2-oxoacetyl)tyrosine (2bi). Compound **2bi** was synthesized following general procedure D. Brown solid, yield 80%, mp 168 ºC. $[\alpha]_D^{25}$ (1, DMSO) = - 60º. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 3.02-3.15 (m, 2H, CH_2), 4.51-4.54 (m, 1H, CH), 5.07 (s, 2H, CH_2), 5.56-5.59 (m, 2H, CH_2), 6.65 (d, *J* = 8.06 Hz, 1H, ArH), 6.92 (d, *J* = 8.06 Hz, 1H, ArH), 7.04 (d, *J* = 7.87 Hz, 1H,ArH), 7.18 (d, *J* = 8.06 Hz, 1H, ArH), 7.26-7.27 (m, 2H, ArH), 7.29-7.33 (m, 4H, ArH),7.35-7.36 (m, 2H, ArH), 7.52 (d, *J* = 6.87 Hz, 1H ArH), 8.19-8.25 (m, 1H NH, 1H ArH), 8.57 (br, 1H, OH), 8.77-8.79 (br, 1H, ArH), 12.25 (br, 1H, COOH). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 35.9 (-ve, CH_2), 53.6 (-ve, CH_2), 54.3 (+ve, CH), 61.8 (-ve, CH_2), 112.8 (+ve, ArCH), 113.3 (+ve, ArCH), 115.2 (+ve, ArCH), 115.8 (+ve, ArCH), 122.0 (+ve, ArCH), 123.4 (+ve, ArCH), 124.3 (+ve, ArCH), 125.3 (+ve, ArCH), 126.8 (ab, ArC), 128.6 (+ve, ArCH), 128.7 (+ve, ArCH), 128.9 (+ve, ArCH), 129.5 (+ve, ArCH), 130.6 (ab, ArC),130.8 (+ve, ArCH), 130.9 (+ve, ArCH), 136.6 (ab, ArC), 136.7 (ab, ArC), 137.0 (ab, ArC), 139.2 (+ve, ArCH), 143.8 (ab, ArC), 157.5 (ab, ArC), 164.2 (C=O), 173.1 (C=O), 182.3 (C=O). HRMS (micro TOF-QII, MS, ESI)**:** Calcd for $C_{29}H_{25}N_5O_5$ $[M+H]^+$ 524.1928, found 524.1761.

3-(4-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-2-(2-(1-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-indol-3-yl)-2-oxoacetamido)propanoic acid (2bii). Compound **2bii** was synthesized following general procedure D. Brown solid, yield 80%, mp 166 ºC. $[\alpha]_D^{25}$ (1, DMSO) = - 20º. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 3.03-3.12 (m, 2H, CH_2), 4.50-4.57 (m, 1H, CH), 5.07 (s, 2H, CH_2), 5.56 (s, 2H, CH_2), 5.56-5.61 (m, 4H, CH_2), 6.66 (d, *J* = 8.62 Hz, 2H, ArH), 6.93 (d, *J* = 8.31, 2H, ArH), 7.05 (d, *J* = 8.16 Hz, 2H, ArH), 7.19 (d, *J* = 8.16 Hz, 2H, ArH), 7.26-7.35 (m, 5H, ArH), 7.72 (d, *J* = 7.39 Hz, 2H, ArH), 8.22-8.24 (m, 2H, ArH), 8.70 (d, *J* = 8.31 Hz, 1H, ArH), 8.76-8.81 (m, 3H, ArH), 9.25 (br, 1H, NH), 12.92

(br, 1H, COOH). ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 35.6 (-ve, CH_2), 35.7 (-ve, CH_2), 41.9 (-ve, CH_2), 53.1 (-ve, CH_2), 54.1 (+ve, CH), 61.4 (-ve, CH_2), 111.8 (ArC), 112.0 (+ve, ArCH), 114.9 (+ve, ArCH), 115.5 (+ve, ArCH), 121.9 (+ve, ArCH), 123.6 (+ve, ArCH), 124.0 (+ve, ArCH), 124.5 (+ve, ArCH), 125.07(+ve, ArCH), 127.2 (ArC), 128.0 (ArC), 128.3 (+ve, ArCH), 128.4 (+ve, ArCH), 128.6 (+ve, ArCH), 129.2 (+ve, ArCH), 130.5 (+ve, ArCH), 130.6 (+ve, ArCH), 136.3 (ArC), 136.4 (ArC), 136.5 (ArC), 141.4 (+ve, ArCH), 142.9 (ArC), 143.5 (ArC), 156.4 (ArC), 157.2 (+ve, ArCH), 163.3 (C=O), 172.9 (C=O), 181.4 (C=O). HRMS (micro TOF-QII, MS, ESI)**:** Calcd for $C_{39}H_{34}N_8O_5$ $[M+H]^+$ 695.2724, found 695.2498.

(2-(1-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-indol-3-yl)-2-oxoacetyl) tryptophan (2c). Compound **2c** was synthesized following general procedure D. Brown solid, yield 80%, mp 160 ºC. $[\alpha]_D^{25}$ (1, DMSO) = - 30^o. IR (KBr) 3127, 3034, 2929, 1736 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 3.16-3.33 (m, 2H, CH_2), 4.61-4.65 (m, 1H, CH), 5.48 (s, 4H, 2×CH_2), 6.93 (t, *J* = 7.55 Hz, 1H, ArH), 7.03 (t, *J* = 7.55 Hz, 1H, ArH), 7.14 (s, 1H, ArH), 7.20-7.22 (m, 2H, ArH), 7.26-7.29 (m, 5H, ArH), 7.32-7.33 (m, 2H, ArH), 7.51 (d, *J* = 8.19 Hz, 1H, ArH), 7.62-7.63 (m, 1H, ArH), 8.12-8.15 (m, 2H, ArH), 8.54 (d, *J* = 7.93 Hz, 1H, NH), 8.65 (s, 1H, NH), 10.88 (br s, 1H, COOH). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 26.9 (-ve, CH_2), 41.8 (-ve, CH_2), 53.3 (+ve, CH), 53.4 (-ve, CH_2), 110.3 (ArC), 111.6 (ArC), 111.8 (+ve, ArCH) 112.0 (+ve, ArCH), 118.5 (+ve, ArCH), 119.2 (+ve, ArCH), 121.7 (+ve, ArCH), 121.9 (+ve, ArCH), 123.9 (+ve, ArCH), 124.2 (+ve, ArCH), 124.5 (+ve, ArCH), 126.9 (ArC), 127.4 (ArC), 128.2 (+ve, ArCH), 128.8 (+ve, ArCH), 129.3 (+ve, ArCH), 135.8 (ArC), 136.3 (ArC), 136.5 (ArC), 141.3 (+ve, ArCH), 142.7 (ArC), 163.2 (C=O), 173.2 (C=O), 181.1 (C=O). HRMS (micro TOF-QII, MS, ESI): Calcd for $C_{31}H_{26}N_6O_4$ ($[M+Na]^+$) 569.1907, found 569.1858.

(2-(1-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-indol-3-yl)-2-oxoacetyl)aspartic acid. Compound **2d** was synthesized following general procedure D. White solid, yield 80% , mp 165 ºC. $[\alpha]_D^{25}$ (1, DMSO) = - 10º. IR (KBr) 3142, 3309, 2926, 2363, 1722 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ: 2.77-2.88 (m, 2H, CH_2), 4.69-4.72 (m, 1H, CH), 5.57 (s, 2H, CH_2), 5.64 (s, 2H, CH_2), 7.26-7.27 (m, 2H, ArH), 7.30-7.32 (m, 2H, ArH), 7.34-7.37 (m, 3H, ArH), 7.72-7.74 (m, 1H, ArH), 8.23-8.25 (m, 2H, ArH), 8.91 (d, *J* = 8.50 Hz, 1H, ArH), 8.99 (br, 1H, NH), 12.66 (br, 2H, 2×COOH). ^{13}C NMR (125 MHz DMSO-d_6) δ: 36.1 (-ve, CH_2), 42.3 (-ve, CH_2), 49.1 (+ve, CH), 52.8 (-ve, CH_2), 111.8 (ArC), 112.1 (ArCH), 121.9 (+ve, ArCH), 123.6 (+ve, ArCH), 124.1 (+ve, ArCH), 124.5 (+ve, ArCH), 127.3 (ArC), 128.3 (+ve, ArCH), 128.6 (+ve, ArCH), 129.2 (+ve, ArCH), 136.3 (ArC), 136.5 (ArC), 141.6 (+ve, ArCH), 142.9 (ArC), 162.9 (C=O), 172.3 (C=O), 172.4 (C=O), 181.0 (C=O). HRMS (micro TOF-QII, MS, ESI): Calcd for $C_{24}H_{21}N_5O_6$ ($[M+Na]^+$) 498.1384, found 498.1389.

(2-(1-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-indol-3-yl)-2-oxoacetyl) glutamic acid (2e). Compound **2e** was synthesized following general procedure D. Pink solid, yield 80%, mp 161 ºC. $[\alpha]_D^{25}$ (1, DMSO) = - 20º. ^1H NMR (400 MHz, DMSO-d_6) δ: ^1H NMR (400 MHz, DMSO-*d*6) δ: 1.90-1.98 (m, 1H, CH_2), 2.05-2.12 (m, 1H, CH_2), 2.26-2.30 (t, J = 8.0 Hz, 2H, CH_2), 4.29-4.35 (m, 1H, CH), 5.52 (s, 2H, CH_2), 5.59 (s, 2H, CH_2), 7.21-7.33 (m, 7H, ArH), 7.67-7.70 (m, 1H, ArH), 8.18-8.21 (m, 2H, ArH), 8.87 (s, 1H, ArH), 8.89 (s, 1H, NH), 12.25-12.75 (2H, COOH), ^{13}C NMR (125 MHz normal/DEPT-135, DMSO-d_6) δ: 25.6 (-ve, CH_2), 30.1 (-ve, CH_2), 41.5 (-ve, CH_2), 51.3 (+ve, CH), 52.8 (-ve, CH_2), 111.4 (ArCH), 111.5 (+ve, ArC), 121.3 (+ve, ArCH), 123.1 (+ve, ArCH), 124.0 (+ve, ArCH), 127.9 (+ve, ArCH), 128.1 (ArCH), 128.7 (+ve, ArCH), 135.8 (+ve, ArC), 136.0 (ArC), 140.9 (ArC), 142.5 (ArC), 163.4 (C=O), 172.6 (C=O), 173.8 (C=O), 181.0 (C=O). HRMS (micro TOF-QII, MS, ESI)**:** Calcd for $C_{25}H_{23}N_5O_6$ $[M+H]^+$ 490.1721, found 490.1791.

Anti-fungal activity

Compounds were screened *in-vitro* for antifungal activity against strains; *Candida albicans* (MTCC 3958) and *Aspergillus niger* (MTCC 9933), their antifungal activities were expressed in terms of ZOI (Zone of Inhibition) and MIC (Minimum Inhibitory Concentration). In this study, Fluconazole was used as reference drug while 5% DMSO in triple distilled water was used as a negative control. Tests were performed in triplicate. The procedure followed for determining ZOI is described briefly: autoclaved respective fungal growth mediums; Malt Yeast Agar (HiMedia) for *Candida albicans* and Czapek Yeast Extract Agar-CYA (HiMedia) for *Aspergillus niger* were poured into sterile petri plates. Next, plates were swabbed with 100 µL inocula of each fungal (1×10^5 CFU/mL) culture under aseptic condition. After the adsorption, wells of 6 mm diameter were made by the sterile metallic borer and the solution of working compound (128 µg/50 µL) in 5% DMSO was poured into the wells. Two compounds **12** and **15** exhibited less solubility and not produced clear solution in 5% DMSO at the mentioned concentration (128 µg/50µL). Hence, the solutions of both these compounds were prepared in 10% DMSO. The plates were incubated at 28 ^{o}C for 48 h and ZOI was determined as mean of triplicate values. The comparative studies of the two negative control sets (5 and 10 % DMSO) not exhibited any significant difference in their anti-fungal activity.

MIC assay was performed to determine the lowest concentrations of the test compound required to inhibit the visible growth of selected fungal strain and for this purpose, the compounds at 128, 64, 32, 16 and 8 µg/mL concentration were used. Yeast Malt Broth (Himedia) for *Candida albicans* and Czapek Dox Broth (Himedia) for *Aspergillus niger* were prepared in triple distilled autoclaved water and transferred in to set of test tubes with net 9.5 ml in each tube. Further, compounds were added by dissolving in DMSO attaining a concentration of 128, 64, 32, 16 and 8 µg/mL in such a way that the net percentage of DMSO remains 5%. To obtain this, the set of five test tubes containing respective media were added with DMSO of volume 400 µL, 450 µL, 475 µL, 487.5 µL and 493.75 µL. Further, to this set

of test tubes, the test compounds 100 µL, 50 µL, 25 µL, 12.5 µL and 6.25 µL, respectively were added from the stock solution (12.8 mg/mL in DMSO). The content of the test tubes was mixed thoroughly.

In the similar way, the working concentration of positive control Fluconazole was prepared. 5% DMSO was taken as negative control. The tubes were inoculated with 100 µL of inocula (1×10^5 CFU/mL) of respective fungal cultures aseptically to obtain final size 1×10^3 CFU/mL. Finally, the culture of *C. albicans* and *A. Niger* were incubated on a rotary shaker (180 rpm) at 28 ºC for 48 and 72 h, respectively under dark conditions. The concentrations which inhibited visible fungal growth were determined and data were compared with that of reference drug fluconazole [33-35].

Cytotoxicity assay

The bio-safety of compound **2bii** was evaluated using MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] cellular toxicity assay [36]. The sheep blood cells were taken in 10:3 ratios with the anticoagulant solution (Alsevier solution) i.e. 10 mL of sheep blood in 3 mL of Alsevier solution. The plasma was then separated from blood cells by centrifugation at 16000 rpm at 25 °C for 20 min. The supernatant was discarded and 6 mL of 1X PBS was added to the cellular pellet. Washing with 1X PBS was repeated thrice and the blood cells were re-suspended in 6 mL 1X PBS. The final suspension was serially diluted in PBS and blood cells observed under a light microscope and counted with the help of a Haemocytometer so as to obtain cell density of 1×10^5 cells/mL. 100 µL of the above suspension was taken in ELISA microplate well and incubated at 37 °C for 24 h. The supernatant was removed and the wells were treated with 200 µL of compound **2bii** and further incubated at 37 °C for 24 h. After incubation, supernatant was discarded again and 20 µL of MTT solution (5mg/ml in 1X PBS) was added. It was kept for 4 h of incubation at 37 °C and for 4 h in an orbital shaker at 60 rpm. After incubation the supernatant was removed

carefully and 50 µL of DMSO was added to each well so as to dissolve the formazan crystals formed. The absorbance was measured at 595 nm using a microplate reader (Biorad 680-XR, Japan). The wells with diluents (10% DMSO) treated cells served as control.

Conflict of interests

Authors declare no conflict of interest for the research work reported here.

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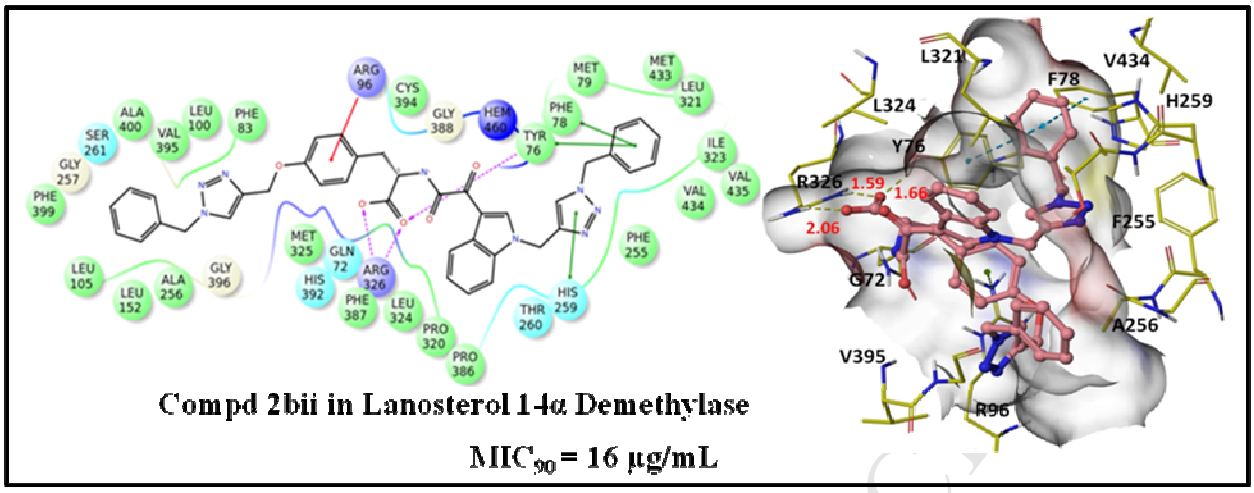
Graphical abstract



Highlights

- Molecular modelling based modification of the lead molecule
- Significant improvement in the anti-fungal activity of the molecules
- QSAR studies further supported the design of molecules