SUMMARY OF PROJECT COMPLETION REPORT (FINAL)

UGC MAJOR RESEARCH PROJECT

(July 2012 - December 2015)

Submitted to

UNIVERSITY GRANTS COMMISSION

BAHADUR SHAH ZAFAR MARG

NEW DELHI – 110 002

Project Title: "Synthesis & pharmacodynamic studies in the efficacy of new triazole and diketo acid based antifungal agents"

File No.: **41-277/2012(SR) dated 16 July 2012**

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1. Name of the Faculty/ Department: **Department of Biosciences, F/o Natural Sciences**

2. Project Title: " Synthesis & pharmcodynamic studies in the efficacy of new triazole and diketo acid based antifungal agents**"**

3. PI (name, affiliation and photograph):

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4. Co-PI (if any) (name, affiliation and photograph): **NA**

5. Funding Agency: **University Grant Commission, New Delhi**

6. Amount funded: **Rs. 9,85,567/-**

7. Duration of the project: **Three Years**

8. Starting date of the Project (and date of completion of projects for projects under category (II): **16/07/2012**

9. Project objectives (max 100 words):

The main objectives of the proposed research programme are:

1. Synthesis of some diketo esters and their corresponding acids
2. Synthesis of *N*-2-aryl-substituted- 1 ,2,3-triazole derivatives
3. Synthesis of amino acid-triazole hybrids
4. Synthesis of triazole derivatives of natural precursors
5. Assessment of in vitro antifungal activity
6. Evaluation of toxicity effect of lead molecules by haemolytic assay as well as by MTT assay

Patents Granted/Published

1. Triazole-amino acid based hybrid as potential inhibitor for candida infection. Application Number 201611008628, **Publication date 15/09/2017** (**GRANTED**)
2. Triazole-amino acid based hybrids as potential antifungal drug for candida infection. Application Number 201611006284, **Publication date 25/08/2017** (Under examination).

PI Publications:

1. Irfan, M., Khan, M., Manzoor, N., & **Abid, M***. (2014). Synthesis of N-2-aryl-substituted-1, 2, 3-triazole Derivatives as Novel Inhibitors of *Entamoeba histolytica*. ***Open Journal of Organic Chemistry***, 2, 21-28, DOI:10.12966/ojoc.04.02.2014.

2. Irfan, M., Aneja, B., Yadava, U., Khan, S. I., Manzoor, N., Daniliuc, C. G., & Abid, M*. (2015). Synthesis, QSAR and anticandidal evaluation of 1,2,3-triazoles derived from naturally bioactive scaffolds. ***European Journal of Medicinal Chemistry***, 93, 246–254. DOI:10.1016/j.ejmech.2015.02.007.

3. Aneja, B., Kumar, B., Jairajpuri, M. A., & **Abid, M***. (2016). A structure guided drug-discovery approach towards identification of Plasmodium inhibitors. ***RSC advances***, 6(22), 18364-18406. DOI : 10.1039/C5RA19673F.

4. Alam, S., Hasan, P., Aneja, B., Ahmad, M. B., & **Abid, M*** (2016). POCl3 Mediated Staudinger Reaction of Imines with Ketenes: Synthesis of Monocyclic β-Lactam and 1, 3-Oxazinone Derivatives. ***Rasayan J. Chem***. (RJC), 9(2), 101-111. DOI :

5. Masood, M. M., Aneja, B., Azam, A., & **Abid, M*** (2016). Efficient multistep synthesis and spectral characterization of dihydropyrrolo [3, 2-c] pyridine-4-one derivatives. ***Rasayan J. Chem***, 9(2), 234-242.

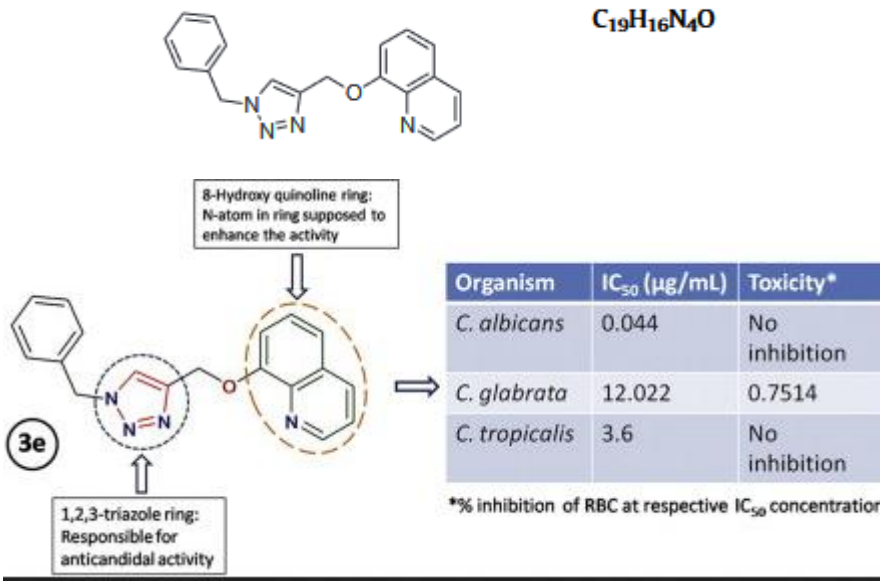
6. Aneja, B., Irfan, M., Kapil, C., Jairajpuri, M. A., Maguire, R., Kavanagh, K., & **Abid, M***. (2016). Effect of novel triazole–amino acid hybrids on growth and virulence of Candida species: in vitro and in vivo studies. ***Organic & Biomolecular Chemistry***, 14(45), 10599-10619. DOI :10.1039/C6OB01718E.

7. Alam, S., Hasan, P., & Aneja, B., Yadava, U, Abid, M* (2016) Synthesis and Crystal Structure Analysis of Monocyclic β-Lactam Derivatives. ***Struct Chem Crystallogr Commun***, 2, 1. DOI : 10.21767/2470-9905.100019

8. Aneja, B., Kumari, M., Azam, A., Kumar, A., **Abid, M*.**, & Patel, R. (2018). Effect of triazole-tryptophan hybrid on the conformation stability of bovine serum albumin. Luminescence, 33(3), 464-474. DOI : 10.1002/bio.3435.

Summary and Findings:

Novel 1,2,3-triazole derivatives (3a-h) synthesized of eight natural precursors (1a-h) showed promising antimicrobial activities. The reaction was carried out in following sequences, the precursor compounds (1a-h) were converted to their respective alkyne (2a-h) followed by addition of freshly prepared benzyl azide. Structural elucidation of all the triazole derivatives was done using FT-IR, (1)H, (13)C NMR, mass and elemental analysis techniques. The single crystal X-ray diffraction for **3d** was also recorded. The result of in vitro anticandidal activity performed against three different strains of Candida showed that compound **3e** was found superior/comparable to fluconazole (FLC) with IC_{50} values of 0.044 µg/mL against *Candida albicans* (ATCC 90028), 12.022 µg/mL against *Candida glabrata* (ATCC 90030), and 3.60 µg/mL against *Candida tropicalis* (ATCC 750).

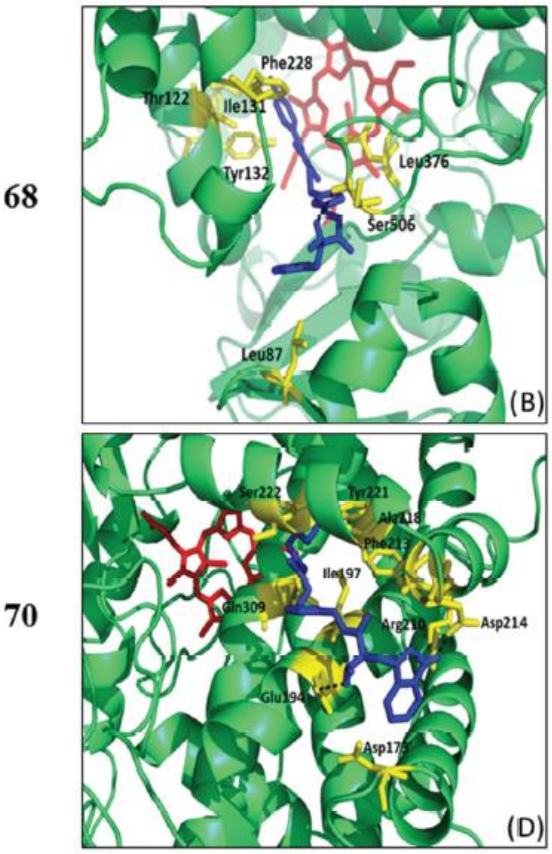


Structure of compound **3e**

Moreover, at their IC_{50} values, compounds 3e and 3h showed <5% hemolysis which indicates the non-toxic behavior of these inhibitors. Cytotoxicity assay was also performed on VERO cell line and all the derivatives were found non-toxic up to the concentration of 10.0 µg/mL. The *in silico* technique of 3D-QSAR was applied to establish structure activity relationship of the synthesized compounds. The results reveal the molecular fragments that play an essential role in improving the anticandidal activity.

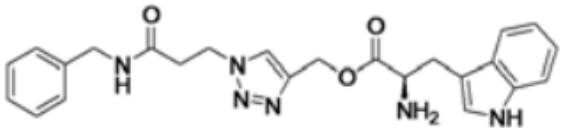
Study further focused on synthesis of novel triazole–amino acid hybrids with potent *in vitro* and *in vivo* inhibitory activity against Candida species. These novel compounds might help in overcoming resistant to existing chemotherapies. The increasing incidence of human candidiasis and the

tendency of Candida species to become resistant to existing chemotherapies are well-recognized health problems. Particularly, compounds named 68 and 70 showed potent in vitro activity against fluconazole (FLC) resistant as well as sensitive clinical isolates of *Candida albicans*. Further analysis includes time kill curve analysis of lead inhibitors 68 and 70 showed their fungistatic nature. Secretion of hydrolytic enzymes, mainly proteinases and phospholipases, decreased considerably in the presence of 68 and 70 indicating their interference in fungal virulence. TEM analysis of Candida cells exposed to compounds 68 and 70 clearly showed morphological changes and intracellular damage as their possible mode of action. A mechanistic study was carried out on the two most effective inhibitors (68 and 70), the results revealed the inhibition of ergosterol biosynthesis thereby causing the cells to lose their integrity and viability. Cytotoxicity assay was also performed and the results showed no cytotoxicity up to a concentration of 200 μg/mL in the HEK293 cell line. Binding of the lead compounds were showed by in silico analysis of 68 and 70. Their binding to a modeled C. albicans CYP51 showed critical H-bonding as well as hydrophobic interactions with the important active site residues indicating the basis of their anti-Candida role. Studies on the larvae of *Galleria mellonella* showed that the selected inhibitors (68 and 70) were non-toxic, did not provoke an immune response and significantly reduced *Candida* proliferation in vivo.



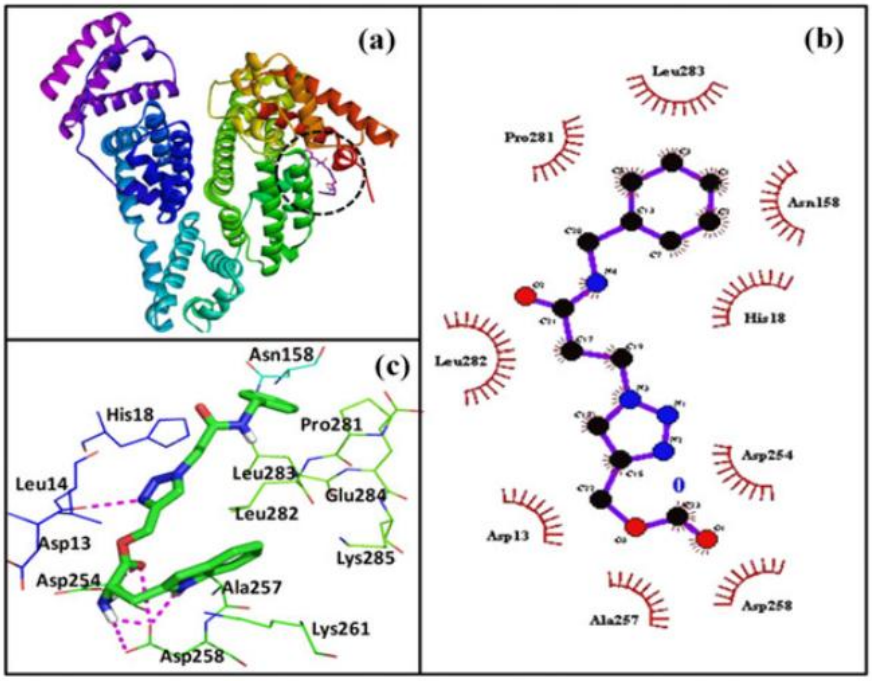
1. The plausible mode of binding of 68 in the active site of modeledCYP51
2. The possible mode of binding of 70 in the active site of modeled CYP51;

The effect of binding of the above synthesized potent antimicrobial compound bearing 1,2,3-triazole core and a tryptophan tail, triazole-tryptophan hybrid (TTH) with bovine serum albumin (BSA) have been explored using various spectroscopic and molecular docking methods.



Structure of triazole - tryptophan hybrid (TTH)

The experimental data in the study supports the fact that TTH strongly quenches the intrinsic fluorophore of BSA by a static quenching mechanism. Time-resolved fluorescence spectra further confirmed the involvement of static quenching for TTH–BSA system. The calculated thermodynamic parameters; ΔH, ΔS, and ΔG showed that the binding process was spontaneous, exothermic and entropy driven. Synchronous fluorescence, three-dimensional (3D) fluorescence and circular dichroism data revealed that TTH induces the structural alteration in BSA and enhances its stability. In silico study of TTH–BSA system showed that it binds with BSA at the site I of subdomain IIA. Both the experimental and in silico study showed that the hydrophobic and electrostatic interactions play a major role in TTH–BSA binding.



Schematic representation of BSA–TTH docked structure. (a) Three-dimensional structure of BSA with docked TTH. The image was visualized using PyMol. (b) Docked TTH and surrounding amino acid residues of binding pocket of BSA interacting through hydrophobic interactions with TTH. (c) Docked TTH showing hydrogen bond with Asp254 and Asp258 residue of BSA