Bioorganic & Medicinal Chemistry Letters 25 (2015) 3730–3737

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design, synthesis and evaluation of benzotriazole derivatives as novel antifungal agents

Jay J. Shah, Vijay Khedkar, Evans C. Coutinho, Krishnapriya Mohanraj *

Bombay College of Pharmacy, Kalina, Santacruz (E), Mumbai 400098, India

ARTICLE INFO

Article history:
Received 31 December 2014
Revised 3 June 2015
Accepted 6 June 2015
Available online 14 June 2015

Keywords:
Benzotriazole derivatives
Antifungal
Docking
Ergosterol quantification

ABSTRACT

Considering the need for discovery of new antifungal drugs with greater potency and broader spectrum of activity, a new series of 5-substituted benzotriazole derivatives were designed, through structure based design, as inhibitors of fungal cytochrome P450 lanosterol 14-α demethylase. These were further optimized by a combination of iterative medicinal chemistry principles and molecular docking. Based on the best docking scores, some benzotriazole derivatives were synthesized and characterized by IR, ^{1}H NMR and MS/MS. The molecules were evaluated for their antifungal action against *Candida albicans* by cup plate method and ergosterol quantification method by UV spectroscopy. Reasonably good correlation between docking scores and antifungal activity were observed. The computational predictions were in consensus with the experimental results.

In recent years, life threatening systemic fungal infections have become increasingly common, especially in immunocompromized hosts suffering from tuberculosis, cancer or AIDS and in organ transplant cases. Development of resistance against available antifungal agents (generally azoles) is also an alarming factor.[1,2] These azole antifungal drugs mainly act by inhibiting CYP51A1 or lanosterol-14α-demethylase, an enzyme necessary in ergosterol biosynthesis, through a mechanism in which the heterocyclic nitrogen of the azole binds to the heme iron present in the enzyme. However, the increasing administration of antifungal agents and their indiscriminate usage has led to the development of fungal resistance to azoles. Genetic mutations that result in resistance to clinically used drugs, especially to fluconazole, may also result in resistance to new structurally related azoles such as voriconazole and ravuconazole.[3,4] The emergence of resistance spells the need to discover new antifungal compounds with a broad antifungal spectrum and with higher therapeutic indices than existing antifungal agents. Khabnadideh et al.,[5] Nitin et al.,[6] and Ramachandran et al.[7] have published approaches for design and synthesis of 1-substituted benzotriazole derivatives with promising antifungal activity and targeting enzyme lanosterol 14α-demethylase. We have designed 160 benzotriazole derivatives substituted at 1, 4 or 5 position as antifungal agents. Benzotriazole derivatives substituted with long alkyl chain, amino or phenylaminomethyl, phenoxymethyl, phenylmethylaminomethyl and phenylmethoxymethyl groups with unsubstituted phenyl ring or phenyl ring substituted with electron withdrawing and electron donating groups were designed and evaluated by virtual screening initially using molecular docking studies.

The chemical structures were sketched using Maestro v 9.3.5[8] in the Schrödinger Suite 2012. The ligands used in the docking studies were prepared using the LigPrep v2.5[9] module in the Schrödinger 2012 Suite. The atom types and partial charges were assigned based on the OPLS 2005 forcefield, corresponding to the physiological pH 7.4. A set of diverse conformations, and tautomeric states were generated for the ligands using the LigPrep v2.5 module.

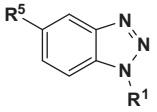
The X-ray crystal structure (PDB ID: 1E9X[10]) of lanosterol 14-α demethylase co-crystallized with ligand 4-phenylimidazole was obtained from the Protein Data Bank. Protein for the docking studies was prepared with the Protein Preparation Wizard in the Schrödinger Suite 2012. The crystallographic waters were removed, hydrogen atoms were added, atom types and partial charges were assigned based on the OPLS 2005 forcefield. Formal charges for the acidic and basic amino acids were set according to the physiological condition, that is, pH 7.4. N- and C-termini were capped with acetyl (ACE) and *N*-methyl-amino groups, respectively. This prepared system was subjected to restrained minimization to the convergence criteria of RMSD 0.005 Å for all heavy atoms.

The docking studies were performed using Glide v5.8 (Schrodinger, USA)[11–13] and GOLD suite v5.3 (CCDC, UK)[14] running on a customized workstation with CentOS Enterprise Linux 6.4. The grid for docking was generated by defining the co-crystallized ligand (4-phenylimidazole) as the grid center. The inner grid box was 10 Å × 10 Å × 10 Å while the outer grid was 20 Å × 20 Å × 20 Å in dimensions, which provided ample space for the

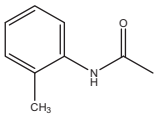
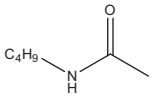
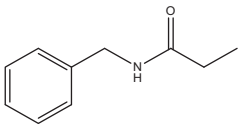
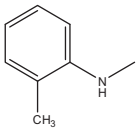
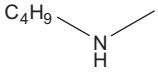
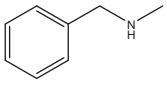
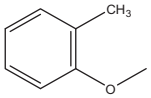
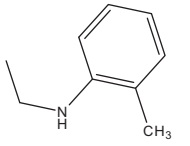
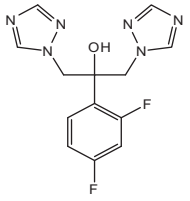
* Corresponding author. Tel.: +91 22 26670871.
E-mail address: krishnapriyamohanraj@gmail.com (K. Mohanraj).

http://dx.doi.org/10.1016/j.bmcl.2015.06.025
0960-894X/

Table 1
Docking scores of benzotriazole derivatives and standard drug fluconazole



Benzotriazole

Sr. no.	Derivative no.*	Structure		Docking score	
		R^1	R^5	Glide (XP)	GOLD
1	**4a**	H		−8.08	102.14
2	**4b**	H		−6.52	100.58
3	**4c**	H		−7.00	101.98
4	**8a**	H		−8.48	107.19
5	**8b**	H		−8.34	105.04
6	**8c**	H		−8.53	107.57
7	**9a**	H		−8.40	107.17
8	**12a**		H	−5.97	46.20
9	Fluconazole	 Fluconazole		−6.61	96.73

* Derivative number as per Schemes 1–3.

generation of ligand conformations in the binding pocket during the execution of the search and score algorithm. The van der Waals radius was scaled to 0.6 Å to soften the potential over the non-polar areas of the enzyme that lie within the grid extents and the partial atomic charges were set to 0.25. The receptor atoms beyond the extent of the grid were unscaled. To maximize the probability of forming hydrogen bonds between the residues in the enzyme active site and the ligand, the side chain hydroxyl groups of the amino acids serine, threonine and tyrosine were allowed to rotate. These settings were validated based on the protocol's ability to reproduce the X-ray conformation of the bound ligand in the enzyme active site. The receptor active site was shaped by residues in a 10 Å vicinity of ligand 4-phenylimidazole.

On completion of the docking study, it was observed that all 1-substituted benzotriazole derivatives showed very low docking scores as compared to 4 or 5-substituted benzotriazole derivatives. On comparing the docking scores of 4 and 5-substituted derivatives it was observed that 5-phenylaminomethyl, phenoxymethyl or phenylmethylaminomethyl substituted derivatives were more active than 4-substituted derivatives. It was also observed that benzotriazole derivatives with phenyl ring substituted with electron donating group (hydroxyl, amino, fluoro) substitution showed low docking scores for all three (1,4 and 5) substitution positions. Seven derivatives with good docking score (derivatives **4a**–**c**, **8a**–**c** and **9a**) were chosen for synthesis (see Table 1). It was thought appropriate to synthesize and evaluate at least one derivative with