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Design, synthesis and microbiological evaluation of *N*-substituted-2-(3-oxo-3,4-dihydro-2*H*-benzo[b][1,4]thiazin-2-yl)propionamide as antifungal agent

N. P. Jain[1]* and C. D. Upasani[2]

[1] *Department of Pharmaceutical Chemistry, S N D College of Pharmacy, Babhulgaon, Yeola, Nashik, Maharashtra, India-423401,*

[2]*Department of Pharmacology, S S D J College of Pharmacy, Chandwad, Nahsik. Maharashtra, India-423101.*

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ABSTRACT:

As a part of a program to develop novel antifungal agents, a series of new *N*-Substituted-2-(3-oxo-3,4-dihydro-2*H*-benzo[b][1,4]thiazin-2-yl)propionamide was design and dock into the active pocket of enzyme lanosterol 14α-demethylase (CYP51). Genetic algorithm implemented in MDS had been successfully employed to dock inhibitors into the catalytic site of the CYP51. Compounds BTMA-50, BTMA-88 were found to have good affinity for enzyme lanosterol 14α-demethylase. The design molecules were synthesized and their *in-vitro* and *in-vivo* antifungal activity were reported. All the reaction monitored by thin layer chromatography. The structures of the synthesized compounds were established by spectral techniques (IR, ^{1}HNMR, ^{13}CNMR and Mass). *In-vitro* microbiological assay indicates that the 1,4-Benzothiazine compounds show a good antifungal activity against the tested pathogenic fungi such as *Candida albicans, Trichophyton rubrum, Epidermophyton floccosum* and *Malassazia furfur.* From the result of *in-vitro* activity of benzothiazine compounds, four compounds (BTMA-24,36,50 & 88) was selected for *In-vivo* activity were tested in a murine model of systemic *Candida albicans* infection. Compound BTMA-50 was showing potent activity in *in-vivo* antifugal testing.

Keywords: Antifungal activity, 1,4-Benzothiazine, CYP51, Molecular docking.

1. INTRODUCTION

During the past twenty years, the invasive and systemic fungal infections has increased dramatically in the population with different immunity,[1-2] fungal infection has become an important complication and a major cause of morbidity and mortality in immunocompromised patient such as patients undergoing anticancer chemotherapy or major organ transplants and patients with AIDS.[3-4] Current available therapy in treating fungal infections can suffer from drug related toxicity, hazardous drug–drug interactions, non-optimal pharmacokinetics and development of drug resistance.[5] Clinically, candidosis, aspergillosis and cryptococcosis are the three major fungal infections in immunocompromised individuals.[6] The common antifungal agents currently used in clinic are azoles (fluconazole, ketoconazole and itraconazole)[7], polyenes (amphotericin B[8] and nystatin[9]), echinocandins (caspofungin and micafungin)[10] and allylamines (naftifine and terbinafine)[11]. Among these, azoles are widely used in antifungal chemotherapy. Azole antifungals was failure in immunocompromised patient were life threating fungal infection occurs. Azole antifungals act by competitive inhibition of the lanosterol 14α-demethylase (CYP51), the enzyme that catalyzes the oxidative removal of the 14α-methyl group of

Corresponding author: Tel +912559225050; fax +912559225042;
Email-nitinpjain06@ yahoo.co.in

lanosterol to give D14, 15-desaturated intermediates in ergosterol biosynthesis.[12] In yeast and fungi, CYP51 is the key enzyme in sterol biosynthesis. Selective inhibition of CYP51 would cause depletion of ergosterol and accumulation of lanosterol and other 14-methyl sterols resulting in the growth inhibition of fungal cells.[13] From last two decade the 1,4-Benzothiazine derivatives was given versatility biological activity. 1,4-benzothiazine had antifungal activity and many evidences show that nitrogen and sulphur atom palys an important role in antifungal activity e.g. Sertaconazole, sulcanozole, tioconazole, isavuconazole, abafungin.[14]

In the present investigation, design of some new compounds *N*-Substituted-2-(3-oxo-3,4-dihydro-2*H*-benzo[b][1,4]thiazin-2-yl)propionamide, molecular modeling study of design compounds into the active site of enzyme lanosterol 14α-demethylase is reported. Design molecules were synthesize and tested for antifungal activity (*in-vitro* and *in-vivo*). In continuation to our work on drug design and antifungal research,[15-16] in the present investigation structure based design of novel 1,4-benzothiazines was described. Ligand with lower binding energy was considered to be having high affinity for the enzyme lansoterol 14α-demethylase.

2. RESULT AND DISCUSSION

2.1 Molecular modeling and docking studies:

The enzyme alpha demethylase is an important target for azole antifungal compound. Sterol 14 alpha-demethylase (CYP51), which catalyzes the conversion of lanosterol to ergosterol. As ergosterol is an essential component of the fungal cell membrane, inhibition of its synthesis results in increased cellular permeability causing leakage of cellular contents. Antifungal azoles inhibit ergosterol biosynthesis in fungi. To pre-asses the antifungal behavior of our benzothiazine derivatives on a structural basis, automated docking studies were carried out using MDS vlife 3.5 program and hydrogen bonds formed with the surrounding amino acids are used to predict their binding modes, their binding affinities and orientation of these compounds at the active site of the CYP51 were found out. Genetic algorithm implemented in MDS has been successfully employed to dock inhibitors into the catalytic site of the CYP51 and to well correlate the obtained binding score with inhibitory activities of compounds. The binding models of synthesized scaffold BTMA compounds in the enzyme active site of CYP51 were depicted in Fig. 2. Obtained results were evaluated in terms of binding score in to the catalytic site of CYP51. Smaller dock score indicates larger binding affinity of ligand for receptor. Compounds were docked into active site of enzyme demethylase and results are summarized in the Table 1. The compounds BTMA-50 and BTMA-80 showed good affinity to enzyme Sterol 14alpha-demethylase. Binding energy obtained for compounds BTMA-50, BTMA-80, BTMA-88, Ketaconazole are -13.34, -11.23, -9.83 and -11.90 respectively against demethylase enzyme.

Table no. 1 Molecular docking showing binding score of synthesized compounds in the active site of enzymes CYP51

Ligand	Dock Score
BTMA-11	-4.76
BTMA-12	-6.82
BTMA-24	-7.81
BTMA-33	-4.33
BTMA-36	-5.38
BTMA-48	-4.01
BTMA-50	-13.34
BTMA-59	-3.43
BTMA-60	-3.00
BTMA-74	-3.26
BTMA-77	-3.63
BTMA-80	-11.23
BTMA-83	-4.46
BTMA-88	-9.83
KTZ	-11.90

Ligand Binding score using GA dock
(Energy in kj/mole)

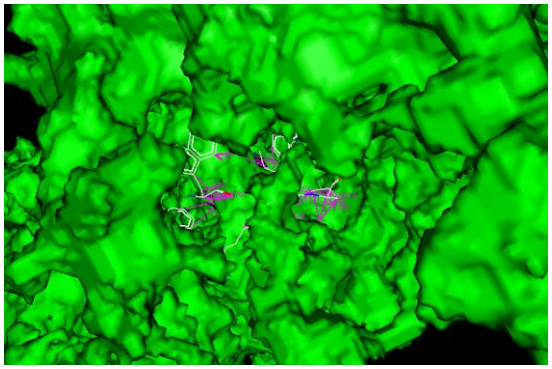

Fig no. 1a Molecule BTMA-50 into the active site of CYP51 showing a Charge interaction with surrounding amino acids. It has binding score of -13.34

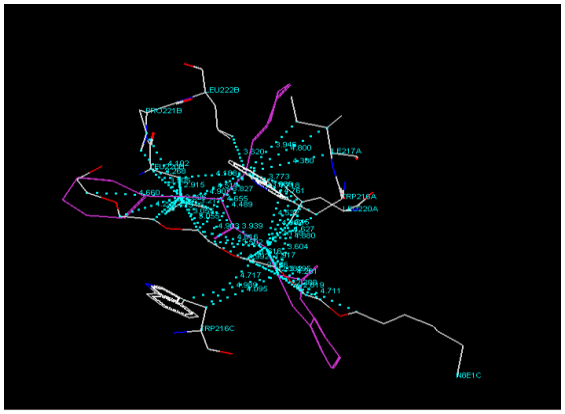

Fig no. 1b Molecule BTMA-50 into the active site of CYP51 showing a hydrophobic interaction with surrounding amino acids.

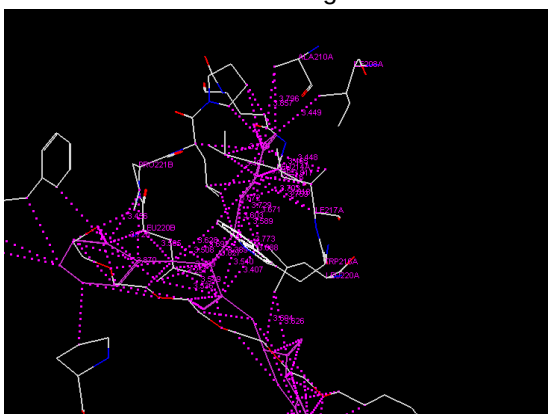

Fig no.1c Molecule BTMA-50 into the active site of CYP51 showing a Vander wall interaction with surrounding amino acids.

Fig no. 1 Best poses, orientations, hydrogen bond formed, and Van der Waals and charge interactions of synthesized compounds with enzyme CYP51.

2.2 Chemistry:

Design compounds were prepared by synthetic scheme described in fig no 2. (3-Oxo-3,4-dihydro-2*H*-1,4-benzothiazin-2-yl)propionic acid (3) was synthesized by mixing equimolar quantities of methyl maleic anhydride and *o*-aminothiophenol in THF at room temperature for 10 minute. Nucleophilic amino group addition at carbonyl carbon of unhydrided and opening of ring take place. Further michal type addition at carbon carbon double bond by thio group take place form 1,4-Beznothiazine ring. Obtained 3, reflux with methanol in the presence of sulphuric acid for 6 h to prepare methyl ester (4) of carboxylic acid (3). The resultant methyl ester (4) were condense with different amine into *N*-substituted-2-(3-oxo-3,4-dihydro-*2H*-benzo[b][1,4]thiazin-2-yl)propionamide (BTMA) (5) by simple heating in methanol for 6 h. All compounds were obtaining in good yield. Reactions were monitored by thin-layer chromatography. In structure 3, 4 & 5, there are two chiral centers each, which means there is a possibility for 2n = 2*2 = 4 isomers i.e two diastereomers and two pairs of enantiomers, we not separating and isolating the stereo isomer of 3, 4 & 5. The spectral data (IR and ^{1}HNMR, ^{13}CNMR and Mass spectra) are consistent with assign structures of the compounds.

The formation of 1,4-Benzothiazine ring is confirm by IR spectra of 3 with COOH (-OH) peak at 3000-2550 broad & strong and C=O peak at 1710. The NMR spectra of acid (3) were show aromatic proton in the range of δ 6.98-7.450, lactam proton on nitrogen at δ 8.700 *s*, acid proton at δ 10.450 *s*, The ester of 3 with methanol also confirm with IR by the absence of OH peak at 3000-2550 and in NMR show the absence of carboxylic proton at δ 10.450 ppm and presence of singlet at 3.860 for the methoxy group. The formation of amide linkage in 1,4-Benzothiazine derivatives is confirm by NH-CO peak at 1700-1650 and N-H peak at 3200-3400 and in NMR two singlet peak around at δ 8.5-9.5 and 10.0- 11.0 ppm for the NH of amide linkage and lactam.

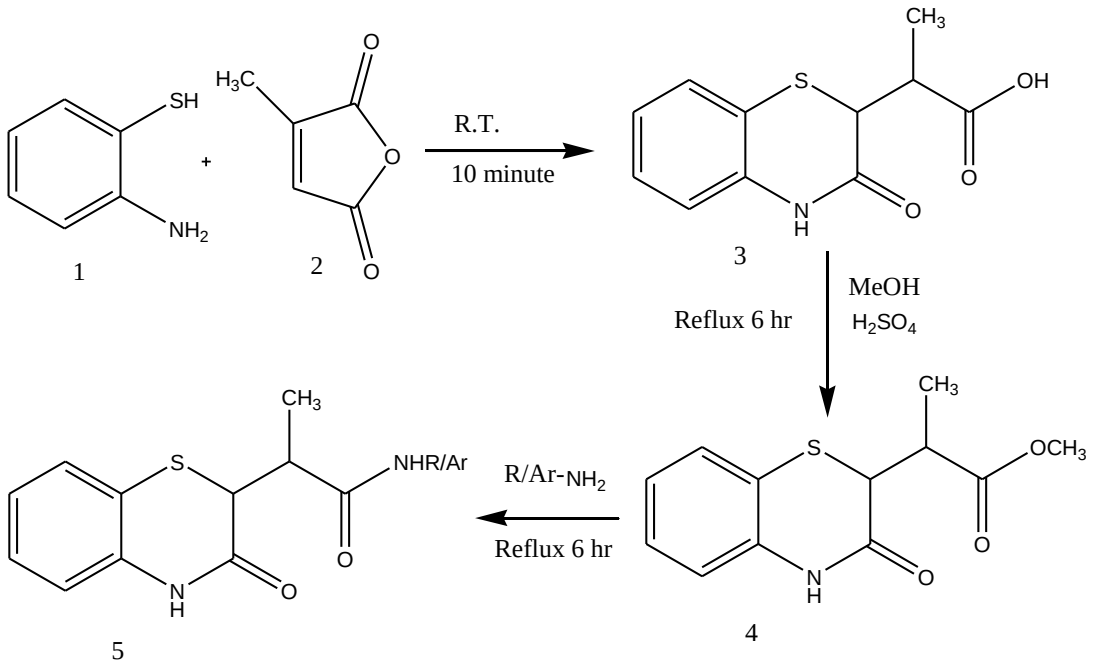

Fig no.2: Scheme of synthesis of 1,4-benzothiazine compounds

***2.3 In-vitro* antifungal activity:**

Antifungal activity of synthesized BTMA series compounds were evaluated against four microbial species, viz. *C. albicans* (ATCC 14053) *E. floccosum* (ATCC 18397), *T. rubrum* (ATCC 28188), and *M. furfur* (ATCC 14521), using tube-dilution method and MIC (minimum inhibitory concentration) of compounds was determined. It was found that BTMA-24 (MIC-

Table no.2 Microbiological Activity data of BTMA series derivatives

		MIC[a]			
Sr.No.	Compound Code	*C. albicans*	*E. Floccusom*	*T. rubrum*	*M. rubrum*
1	BTMA-11	0.250	0.250	0.250	0.250
2	BTMA-12	0.125	0.125	0.250	0.125
3	BTMA-24	0.125	0.0625	0.0312	0.0312
4	BTMA-33	0.250	0.250	0.250	0.250
5	BTMA-36	0.0625	0.0625	0.250	0.125
6	BTMA-48	0.250	0.250	0.250	0.250
7	BTMA-50	0.0625	0.0625	0.125	0.125
8	BTMA-59	0.250	0.250	0.250	0.250
9	BTMA-60	0.250	0.250	0.250	0.250
10	BTMA-74	0.250	0.250	0.250	0.250
11	BTMA-77	0.125	0.250	0.250	0.250
12	BTMA-80	0.250	0.250	0.250	0.125
13	BTMA-83	0.250	0.250	0.250	0.250
14	BTMA-88	0.250	0.250	0.0625	0.0625
15	Ketaconazole	0.0625	0.0625	0.0625	0.0625
16	DMSO	Nil	Nil	Nil	Nil

[a]All MIC value in µmol/ml

Each result represents the average of triplicate reading.

E. Floccusom= Epidermophyton. floccusom; M. ruburum = Microsporum. Rubrum; *M. furfur = Malassazia furfur*

0.0312 µmole/ml), BTMA-88, (MIC-0.0625 µmole/ml) against *T. rubrum,* BTMA-24, BTMA-36, BTMA-50 (MIC-0.0625 µmole/ml) against *E. floccosum*, BTMA-24 (MIC-0.0625 µmole/ml), BTMA-88 (MIC-0.0312 µmole/ml) against *M. furfur* and BTMA-36, BTMA-50 (MIC-0.0625 µmole/ml) against *C. albicans* showed significant activity. From above result it was found that cyclohexamine and dicyclohexamine substituted derivatives are high active as ketoconazole so hydrophobic group is important for the activity. Antifungal activity results of all synthesized compound reported in table no 2.

***In-vivo* activity:**

From the results of *in-vitro* activity of benzothiazine compounds, four compounds (BTMA-24,36,50 & 88) was selected for *In-vivo* activity were tested in a murine model of systemic *Candida albicans* infection. To this end, mice were treated intraperitoneally with compounds 2 h before systemic challenge with *C. albicans* and once daily for eight consecutive days. The results were reported in Table 3. There was a good *in-vivo* activity of BTMA-50 (MST = 25, D/T = 4/6) comparable to that of KTZ (MST = 20, D/T = 4/6).

Table no. 3 *In-vivo* antifungal activity of four compounds against *Candida albicans.*

Treatment[a]	MST[b]	D/T[c]
Diluent[d]	30.5	6/6
BTMA-24	15	3/6
BTMA-36	10.5	2/6
BTMA-50	25	4/6
BTMA-88	5	1/6
Ketaconazole (KTZ)	20	4/6

[a] Diluent, KTZ, and indicated compounds were tested for in vitro activity (MIC values) or were given intraperitoneally at the dose of 10 mg/kg 2 h before intravenous challenge with C. albicans and once for eight consecutive days after challenge.

[b] Median survival time.

[c] Dead mice at 60 days over total animals infected.

[d] EtOH/H2O 1:4.

3. CONCLUSIONS

The synthesized *N*-substituted-2-(3-oxo-3,4-dihydro-*2H*-benzo[b][1,4]thiazin-2-yl)propionamide derivatives by given scheme in fig no 2 with good yield and shown promising *in-vitro* antifungal activity against various fungal species viz., *Candida albicans, Trichophyton rubrum, Epidermophyton floccosum* and *Malassazia furfur* and *in-vivo* antifungal activity against *Candida albicans*. Compound BTMA-36 and BTMA-50 were found to be potent antifungals with MICs 0.625 µmol/ml against *Candida albicans* and *Epidermophyton floccusom* and also compound BTMA-88 was found to be potent antifungal against *Trichophyton rubrum* and *Malassazia rubrum* with MIC 0.625 µmol/ml. Compound BTMA-50 has appreciable antifungal activity *in-vivo* in murine model comparative to ketaconazole. Compounds BTMA-50, BTMA-80, BTMA-88 had more affinity for receptor as well as antifungal activity compared with standard ketaconazole and also found a good correlation in molecular modeling studies with the *in-vitro* and *in-vivo* antifungal activity.

4. EXPERIMENTAL

4.1 Material and methodology

All chemicals were purchased from Research laboratory are used without purification. All solvents were used for chromatography are AR grade and purchased from Rankem. Melting points of all the synthesized compounds determined on a Thomas-Hoover melting point apparatus and are uncorrected. The progress of reaction was monitored by TLC using silica gel adsorbent on coated aluminum plates from Merck and UV light as a visualizing agent. Analytical sample was prepared by column chromatograph. The IR spectra were recorded on Shimadzu FTIR spectrometer in the range of 4000-400 cm^{-1}. ^{1}HNMR spectra were scanned at 300 MHz on Varian-NMR-Mercury 300 FT NMR spectrometer using DMSO d_6 as solvent and tetramethylsilane (TMS) as an internal standard. ^{13}CNMR spectra on Bruker Advance-II spectrometer were scanned using DMSO-d_6 as solvent and TMS as internal standard and mass spectra recorded on Applied Biosystems 4800 plus MALDI TOF analyzer.

3.2 In-silico molecular docking

Sterol 14 alpha-demethylases (CYP51) are essential enzymes in sterol biosynthesis in eukaryotes and drug targets in antifungal therapy. Molecular docking of BTMA compounds into the three-dimensional X-ray structure of CYP51 was carried out using the molecular design Suite (MDS) software package (3.5). The protein–ligand complex was constructed based on the X-ray structure CYP51 (PDB code-3G1Q) (16). All compounds were built using Chem Draw Ultra v.8.0. All the structures are clean and 3D optimized. Energy minimization and geometric optimization conducted using the Merck Molecular Force Field. Keeping program parameters to their default values, the docking was performed using Molecular Design Suite (MDS) into the 3D model of the catalytic site of CYP51. Genetic algorithm implemented in MDS has been successfully employed to dock inhibitors into the catalytic site of the enzyme. Obtained results were evaluated in terms of binding score in to the catalytic site enzyme lanosterol 14α-demethylase.

3.3 Chemistry

3.3.1 General Procedure:(3-Oxo-3,4-dihydro-2*H*-1,4-benzothiazin-2-yl)propionic acid (3)

To a solution of methyl maleic anhydride (1) (5.7g, 0.05 mole) in THF (30mL) a solution of *o*-aminothiophenol (2) (6.25g, 0.05mole) in THF (15mL) was added with stirring and the solution was stirring continue for 10 minutes at room temperature, the crystalline product that precipitated out, was filtered off and washed with THF.

Yield; 9.5g (86%), Melting point: 150-152 ^{O}C, IR (cm^{-1}): 3000-2550 (COOH) Broad, 3274 (NH) *s*, 3020 (CH) *s*, 1710 (C=O) *s*, ^{1}HNMR (DMSO-d_6, 300 *MHz*): δ 0.95 (d, 3H, CH_3 J=3.2 Hz), 2.85 (m, 1H, CH), 3.800 (d, H, CH, J=5.5 Hz), 6.98-7.450 (m, 4H. ArH), 8.70 (s, 1H, NH), 10.450(s, 1H, COOH).

3.3.2 Methyl 2-(3-oxo-3,4-dihydro-2*H*-1,4-benzothiazin-2-yl)propionate (4)

(3-Oxo-3,4-dihydro-2*H*-1,4-benzothiazin-2-yl)propionic acid **(3)** (0.01 mol), dry methanol (25 mL) and few drops of Conc. H_2SO_4 (98%,) were taken in a 100 mL round bottomed flask and was heated at reflux for 4 hr on a water bath. Progress of reaction was monitored by TLC. The reaction mixture was then concentrated, cooled, the crystalline product was separated by filtration, dried.

Yield: 80 %, Melting point: 122-125 ^{O}C, IR (cm^{-1}): 3350 (NH) m, 2950 (CH) m, 1750 (C=O) *s*, ^{1}HNMR (DMSO-d_6, 300 *MHz*): δ 1.015 (d, 3H, CH_3, J=3.5 Hz) 2.660 (m, 1H, CH), 3.250 (d, 1H, CH, J= 5.7 Hz), 3.860 (s, 1H, CH_3), 7.160-7.541 (*m*, 4H. ArH), 10.140 (*s*, 1H, NH).

3.3.3 General procedure for synthesis of *N*-[Alkyl or un/substituted phenyl]-2-(3-oxo-3, 4-dihydro-*2H*-benzo[b][1,4]thiazin-2-yl)propionamide (BTMA) (5)

In a round bottomed flask (100 mL) fitted with a reflux condenser, a mixture of Methyl 2-(3-oxo-3,4-dihydro-2*H*-1,4-benzothiazin-2-yl)propionate (0.01mole) (**4**) and alkyl/ arylamine (0.01mole) in dry methanol (50 mL) was heated on a water bath for 6 hr. After completion of the reaction (monitored by TLC), the reaction mixture was poured into cold water and extract with dichloromethane and wash with dil HCl. Extract was dry with magnesium sulphate and concentrated, cooled and recrystallized from alcohol to get the product. Analytical sample was prepared by column chromatograph eluting with methanol: chloroform (3:7).

***3.3.4 N*-(2-Flourophenyl)-2-(3-oxo-3,4-dihydro-*2H*-benzo[b][1,4]thiazin-2-yl)propionamide (BTMA-11):** Yield: 50%, Melting Point: 160-165 ^{O}C, IR (cm^{-1}): 3445 (N-H) s, 1650(–CONH-) *s*, 1710 (-CO-) *m,* ^{1}HNMR (DMSO-d_6, 300 MHz,): δ 0.95 (d, 3H, CH_3, J=3.4 Hz) 2.900 (m, 1H, CH), 3.856(d, 1H, CH, J=5.2 Hz), 6.980-7.440 (m, 8H, ArH), 9.150 (s, 1H, NH amide), 10.650 (s, 1H, NH lactam).

***3.3.5 N*-(4-Flourophenyl)-2-(3-oxo-3,4-dihydro-*2H*-benzo[b][1,4]thiazin-2-yl)propionamide (BTMA-12):** Yield: 55%, Melting Point: 238-240 ^{O}C, IR (cm^{-1}): 3390 (N-H) m, 2990 (CH) s, 1630 (–CONH-) *m*, 1720 (-CO-) *s*, ^{1}HNMR (DMSO-d_6, 300 MHz,): δ 1.110 (d, 3H, CH_3 J=3.5 Hz), 2.885(d, 1H, CH), 3.787(d, 1H, CH, J=6.2 Hz), 6.970-7.450(m, 8H, ArH), 8.800 (s, 1H, NH amide), 10.650 (s, 1H, NH lactam). ^{13}CNMR (DMSO-d_6, 500 MHz,): δ 18.654 (CH3), 33.333 (CH), 37.208 (CH ring), 116.733, 123.040, 125.373.125.690, 126.916, 130.670, 131.182, 134.829, 136.423, 155.341 (10 peak of aromatic), 158.355 (C=O, lactam), 168.627 (C=O, amide), Mass (m/e) : M^+ = 330.08, M^+ +1 = 331.09, 235.05, 236.06, 164.02, 165.02, 110.

***3.3.6 N*-Cyclohexyl-2-(3-oxo-3,4-dihydro-*2H*-benzo[b][1,4]thiazin-2-yl)propionamide (BTMA-24)**

Yield: 45%, Melting Point: 138-140 ^{O}C, IR (cm^{-1}): 1770(-CO-) *m*, 1625 (–CONH-) *m*, 2990(-CH_3) *s*, 3265 (NH) *s* , ^{1}HNMR (DMSO-d_6, 300 MHz,): δ 0.9221 (d, 3H, CH_3, J=3.5 Hz), 1.750-2.360 (m, 11H, Cyclohexane), 2.719 (m, 1H, CH), 3.721 (d, 1H, CH, J=5.3 Hz), 7.010-.590 (m, 4H, ArH), 8.523 s, 1H, NH amide). 10.450 (s, 1H, NH lactam); ^{13}CNMR (DMSO-d_6, 500 MHz,): δ 23.025, 25.219, 27.980, 51.640 (4 peak of Cyclohexane), 24.920 (CH_3), 33.370 (CH), 37.185 (CH ring), 112.2, 123, 126.2,127.4,135.5, 136.7 (6 peak of aromatic) 155.5 (C=O, lactam), 165.4 (C=O, amide); Mass (m/e): M^+ = 318.43, 235.04, 236.04, 237.97, 164.20, 165.21, 166.01.

***3.3.7 N*-(3-Chlorophenethyl)-2-(3-oxo-3,4-dihydro-*2H*-benzo[b][1,4]thiazin-2-yl)propionamide (BTMA-33)**

Yield: 62%, Melting Point: 120-122 ^{O}C, IR (cm^{-1}): 1710(-CO-) *s*, 1620(–CONH-) *s*, 2950 (CH) *s*, 3410 (NH) *m*; ^{1}HNMR (DMSO-d_6, 300 MHz,): δ 1.10 (d, 3H, CH_3, J=4.2 Hz), 1.900-2.650 (m, 4H, CH_2-CH_2) 2.800 (d, 1H, CH, J=5.2), 3.670 (d, 1H, CH, J=5.4), 7.120-7.400 (m, 8H, ArH), 9.100 (s, 1H, NH amide), 10.580 (s, 1H, NH lactam).

***3.3.8 N*-(2-Chloro-4-Hydroxyphenyl)-2-(3-oxo-3,4-dihydro-*2H*-benzo[b][1,4]thiazin-2-yl)propionamide (BTMA-36)**

Yield: 71%, Melting Point: 130-135 ^{O}C, IR (cm^{-1}): 1750 (C=O) *s,* 1620(–CONH-) m, 2950 (CH) *s*, 3600-3300 (-OH) Broad *s*, 3180 (NH) w, ^{1}HNMR (DMSO-d_6, 300 MHz,): δ 0.989 (d, 3H, CH_3, J=3.5 Hz), 2.750 (m, 1H, CH), 3.831 (d, 1H, CH, J=5.5 Hz), 4.130 (s, 1H, OH), 6.940-7.380 (m, 7H, ArH), 9.150 (s, 1H, NH amide), 10.350 (s, 1H, NH lactam); ^{13}CNMR (DMSO-d_6, 500 MHz): δ 24.145 (CH3), 33.285 (CH), 37.066 (CH ring), 104.143, 112.050, 117.066, 117.510, 123.955, 126.285, 127.031, 127.639, 130.184, 131.635, 135.441, 150.177 (12 peak of aromatic) 165.205 (C=O, lactam), 171.031 (C=O, amide); Mass (m/e): M^+ = 362.57, M^+ +2 = 364.57, 235.27, 164.36, 165.22, 127.00, 129.00

***3.3.9 N*-(3-Chloro-4-flourophenyl)-2-(3-oxo-3,4-dihydro-*2H*-benzo[b][1,4]thiazin-2-yl)propionamide (BTMA-48)** Yield: 55%, Melting Point: 222-228 ^{O}C, IR (cm^{-1}): 1645(–CONH-) *s*, 1730(-CO-) s, 3265 (NH) m, 2925(CH) m ; ^{1}HNMR (DMSO-d_6, 300 MHz,): δ 1.050 (d, 3H, CH_3, J=3.5 Hz), 2.700 (m, 1H, CH), 3.801 (d, 1H, CH, J=5.3 Hz), 6.850-7.450 (m, 7H, ArH), 8.900 (s, 1H, NH amide). 10.200 (s, 1H, NH lactam); ^{13}CNMR (DMSO-d_6, 500 MHz,): δ 26.185 (CH3), 33.440 (CH), 37.177 (CH ring), 104.285, 112.056, 117.143, 117.955, 123.031, 126.285, 127.184, 127.639, 130.510, 136.635, 145.441, 150.066 (12 peak of aromatic) 165.285 (C=O, lactam), 170.086 (C=O, amide); Mass (m/e): M^+ = 364.87, M^+ +2 = 366.57, 235.25, 200.54, 164.35, 165.27, 131.00, 129.00.

***3.3.10 N*-(Dicyclohexyl)-2-(3-oxo-3, 4-dihydro-*2H*-benzo[b][1, 4]thiazin-2-yl)propionamide (BTMA-50)**

Yield: 58%, Melting Point: 265-257 ^{O}C, IR (cm^{-1}): 1640(–CONH-) *m*, 1660(-CO-) *s,* 3215 (NH) m, 2935(CH) m; ^{1}HNMR (DMSO-d_6, 300 MHz,): δ 1.050 (d, 3H, CH_3, J=4.2 Hz), 1.250 (m, 10H, Cyclohexane), 2.720 (m, 1H, CH), 3.720 (d, 1H, CH, J=5.2 Hz), 6.910-7.590 (m, 4H, ArH), 8.700 (s, 1H, NH amide), 10.600 (s, 1H, NH lactam).

***3.3.11 N*-(2-Chlorophenyl)-2-(3-oxo-3,4-dihydro-*2H*-benzo[b][1,4]thiazin-2-yl)propionamide (BTMA-59)**

Yield: 70%, Melting Point: 208-210 ^{O}C, IR (cm^{-1}): 1725 (C=O) s, 1620 (–CONH-) *s*, 3000 (CH) *m*, 3430(NH) *m*; ^{1}HNMR (DMSO-d_6, 300 MHz,): δ 0.95 (d, 3H, CH_3, J=3.4Hz) 2.900 (m, 1H, CH), 3.856 (d, 1H, CH, J=5.3Hz), 7.000 -7.540 (m, 8H, ArH), 8.400 (s, 1H, NH amide), 10.435 (s, 1H, NH lactam).

***3.3.12 N*-(3-Chlorophenyl)-2-(3-oxo-3,4-dihydro-*2H*-benzo[b][1,4]thiazin-2-yl)propionamide (BTMA-60)**

Yield: 42%, Melting Point: 193-198 ^{O}C, IR (cm^{-1}): 1730(-CO-) *s*, 1625(-CONH-) *s*, 2950 (CH), 3200 (NH) *m*; ^{1}HNMR (DMSO-d_6, 300 MHz,): δ 1.100 (d, 3H, CH_3, J=5.3Hz), 2.885 (m, 1H, CH), 3.787 (d, 1H, CH, J=5.3Hz), 6.870-7.450 (m, 8H, ArH), 8.650 (s, 1H, NH amide), 10.225(s, 1H, NH lactam).

***3.3.13 N*-(3,4-Dichlorophenyl)-2-(3-oxo-3,4-dihydro-*2H*-benzo[b][1,4]thiazin-2-yl)propionamide (BTMA-74)**

Yield: 67%, Melting Point: 228-230 ^{O}C, IR (cm^{-1}): 1685 (-CO-) *m*, 1630(-CONH-) *s*, 3010 (=CH) s, 3195 (NH) *w*, 2900 (CH) s; ^{1}HNMR (DMSO-d_6, 300 MHz,): δ 0.989 (d, 3H, CH_3, J=3.4Hz), 2.750 (m, 1H, CH), 3.831 (d, 1H, CH, J=5.3Hz), 6.840-7.380 (m, 7H, ArH), 9.100 (s, 1H, NH amide). 10.150 (s, 1H, NH lactam).

***3.3.14 N*-(2,5-Dichlorophenyl)-2-(3-oxo-3,4-dihydro-*2H*-benzo[b][1,4]thiazin-2-yl)propionamide (BTMA-77)**

Yield: 32%, Melting Point: 238-240 ^{O}C, IR (cm^{-1}): 1690 (-CO-) *s*, 1630 (-CONH-) *s*, 2925 (CH) s, 3280 (NH) *m;* ^{1}H NMR (DMSO-d_6, 300 MHz,): δ 0.900 (d, 3H, CH_3, J=3.4Hz), 2.650 (m, 1H, CH), 3.820 (d, 1H, CH, J=5.3Hz), 6.950-7.320 (m, 7H, ArH), 8.900 (s, 1H, NH amide), 10.100 (s, 1H, NH lactam).

***3.3.15 N*-(2,3-Dichlorophenyl)-2-(3-oxo-3,4-dihydro-*2H*-benzo[b][1,4]thiazin-2-yl)propionamide (BTMA-80)**

Yield: 38%, Melting Point: 234-235 ^{O}C, IR (cm^{-1}): 1735(-CO-) *s* , 1615 (-CONH-) *m*, 2910 (CH) *w*, 3250 (NH) s; ^{1}HNMR (DMSO-d_6, 300 MHz,): δ 1.250 (d, 3H, CH_3, J=3.4Hz), 2.800 (m, 1H, CH), 3.720 (d, 1H, CH, J=5.3Hz), 7.180-7.410 (m, 7H, ArH), 9.100 (s, 1H, NH amide). 10.150 (s, 1H, NH lactam).

***3.3.16 N*-(2,4-Diflourophenyl)-2-(3-oxo-3,4-dihydro-*2H*-benzo[b][1,4]thiazin-2-yl)propionamide (BTMA-83)**

Yield: 50%, Melting Point: 206-210 ^{O}C, IR (cm^{-1}): 1740(-CO-) *s*, 1655 (-CONH-) *m*, 2910, (CH) *s*, 3260 (NH) *m*; ^{1}HNMR (DMSO-d_6, 300 MHz,): δ 0.980 (d, 3H, CH_3, J=3.4Hz), 2.800 (m, 1H, CH), 3.830 (d, 1H, CH, J=5.3Hz), 7.110-7.480 (m, 7H, ArH), 9.200 (s, 1H, NH amide), 10.200 (s, 1H, NH lactam).

***3.3.17 N*-(4-Methylbenzyl)-2-(3-oxo-3,4-dihydro-*2H*-benzo[b][1,4]thiazin-2-yl)propionamide (BTMA-88)**

Yield: 60%, Melting Point: 110-112 ^{O}C, IR (cm^{-1}): 1710 (-CO-) *s*, 1610 (-CONH-) *m*, 2980, (CH) *w*, 3310 (NH) *m*; ^{1}HNMR (DMSO-d_6, 300 MHz,): δ 1.10 (d, 3H, CH_3, J=3.4Hz), 2.1 (s, 3H, CH_3) 2.480 (s, 2H, CH_2), 2.800 (m, 1H, CH), 3.670 (d, 1H, CH, J=5.3Hz), 7.120-7.400 (m, 8H, ArH), 9.100 (s, 1H, NH amide), 10.520 (s, 1H, NH lactam).

3.4 Antimicrobial activity:

3.4.1 In-vitro activity:

A group of synthesized compounds was screened for antifungal activity against *Candida albicans*, (ATCC 14053) *Epidermophyton floccosum,* (ATCC 18397) *Trychophyton rubrum,* (ATCC 28188) *and Malassazia furfur* (ATCC 14521). The evaluation of antifungal activity was carried out by the tube dilution method (turbidometric method). The turbidometric method depends upon the inhibition of growth of a microbial culture in the uniform solution of drug in a fluid medium (broth) that is favorable for its growth. The nutrient medium used for fungi was *Sabouraud's broth*. Composition of Double Strength Sabouraud's broth is

i. Peptone – 20gm
ii. Dextrose – 80gm
iii. Distilled water –Qs to 1000 mL

Peptone and dextrose were dissolved in distilled water with heating. Then it was cooled and the P_H was adjusted to 5.4 with lactic acid and filtered.

The prepared medium and the suspension tubes were sterilized at 120^{o}C for 15 minutes in autoclave. The stock solution 1µmole/ml compound (equimolar) was prepared in DMSO.

All the compounds were screened individually by making serial dilutions containing 0.5, 0.25, 0.125, 0.0625, 0.0312 and 0.0156 µmole/ml of the compound. To each tube containing 2mL of Sabouraud's liquid medium 2mL of the drug solution (1µmole/mL) was added. The tubes were inoculated using microbial suspension in saline solution. The standard used was Ketoconazole (1 µmole/mL). The positive control (Organism + broth + DMSO) and the blank (broth + DMSO) were also prepared. The dermatophytes were incubated at 28 ^{0}C. Growth, MIC was determined at 24 h for *C. albicans*, at 72 h for *T. rubrum, E. floccosum* and *M. furfur*. The growth in the tubes was observed visually for the turbidity.

***3.4.2 In-vivo* activity:**

Mice

Female mice (8–10 weeks old, weighing 25–30 g) were obtained from Serum Laboratories (pune, India). The yeasts (*C.albicans*) were grown at 28 ^{0}C in Sabouraud's dextrose agar. Under these conditions the organisms grew as a pure yeast-phase population. Before use, yeast cells were harvested from a 24 h culture, suspended in pyrogen-free saline, washed twice, quantified by hemocytometry and adjusted to the desired concentration. Animal ethical committee approval no. SND/IAEC/16/2013-14.

Systemic candidiasis model

Mice were infected intravenously (iv) with $7X10^5$ *C. albicans* via the lateral tail vein. Diluent ($EtOH/H_2O$ 1:4), compounds and KTZ were administered intraperitoneally (ip) at a dose of 10 mg/kg of body weight 2 h before infection and then once daily for eight consecutive days. For survival studies, mice were observed for 60 days.

Statistical analysis

Differences in median survival time were determined by the Mann–Whitney U-test. The student t test was used to evaluate the significance of all other data. Each experiment was repeated three times.

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REFERENCES

1. Imwidthaya P, Poungvarin. Cryptococcosis in AIDS. Postgrad Med J 2000;76:85-88.
2. Steenbergen JN, Casadevall A, Development and Evaluation of a Molecular Viability Assay for *Pneumocystis carinii.* Journal of Clinical Microbiology 2000;38:1974-1976.
3. Fridkin SK, Jarvis WR. Epidemiology of nosocomial fungal infections. Clin. Microbiol. Rev. 1996;9:499-511.
4. Watkins WJ, Renau TE, Chemotherapeutic agents in: Abraham DJ (Ed), Burger's Medicinal Chemistry and Drug Discovery. Sixth edition, John Wiley & Sons Inc., New York, 2003;5:882-884.
5. Maertens JA, Boogaerts MA. Fungal cell wall inhibitors: emphasis on clinical aspects. Curr Pharm Des 2000;6:225-239.
6. Latge JP. *Aspergillus fumigatus* and Aspergillosis. Clin Microbiol Rev 1999;12:310-350.
7. Sheehan DJ, Hitchcock CA, Sibley CM, Current and emerging azole antifungal agents. Clin. Microbiol. Rev., 1999;12:40.
8. Gallis HA, Drew RH, Pickard WW. Amphotericin B: 30 years of clinical experience. Rev. Infect. Dis. 1990;12:308-329.
9. Arikan S, Rex JH. Nystatin LF (Aronex/Abbott). Curr Opin Invest Drugs. 2001;2:488-495.
10. Denning DW. Echinocandins: a new class of antifungal. J Antimicrobial Chemotherapy. 2002;49:889-891.
11. Birnbaum JE. Pharmacology of the allylamines. J. Am. Acad. Dermatol. 1990;23:782-785.
12. Aoyama Y, Yoshida Y, Sato R. Yeast cytochrome P-450 catalyzing lanosterol 14 alpha-demethylation II Lanosterol metabolism by purified P-450(14)DM and by intact microsomes. J Biol Chem 1984;259:1661-1666.
13. Lamb DC, Kelly DE, Venkateswarlu K, Manning NJ, Bligh HF, Schunck WH, Kelly, SL. Generation of a complete, soluble, and catalytically active sterol 14α-demethylase-reductase complex. Biochemistry. 1999;38:8733-8738.
14. Rathore BS, Kumar M. Synthesis of 7-chloro-5-trifluoromethyl/7-fluoro/7-trifluoromethyl-4H-1,4-benzothiazines as antimicrobial agents. Bioorg Med Chem. 2006;14:5678-5682
15. Jain NP, Upasani CD, Jain UN. Synthesis and antifungal Activity of 1,4-Benzothiazine Derivatives. Am J PhamTech Res 2013;3(5):548-558.
16. Jain NP, Upasani CD, Jain UN. 3DQSAR Studies of *N*-[Alkyl or Un/substituted phenyl]-2-(3-oxo-3,4-dihydro-2*H*-benzo[b][1,4]thiazin-2-yl)acetamide. International J. Pharmaceutical & Phytopharmacological Research., 2013;3(2):124-133.