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Ultrasound- and Molecular Sieves-Assisted Synthesis, Molecular Docking and Antifungal Evaluation of 5-(4-(Benzyloxy)-substituted phenyl)-3-((phenylamino)methyl)-1,3,4-oxadiazole-2(3H)-thiones

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Abstract: A novel series of 5-(4-(benzyloxy)substituted phenyl)-3-((phenyl amino)methyl)-1,3,4-oxadiazole-2(3H)-thione Mannich bases **6a–o** were synthesized in good yield from the key compound 5-(4-(benzyloxy)phenyl)-1,3,4-oxadiazole-2(3H)-thione by aminomethylation with paraformaldehyde and substituted amines using molecular sieves and sonication as green chemistry tools. The antifungal activity of the new products was evaluated against seven human pathogenic fungal strains, namely, *Candida albicans* ATCC 24433, *Candida albicans* ATCC 10231, *Candida glabrata* NCYC 388, *Cryptococcus neoformans* ATCC 34664, *Cryptococcus neoformans* PRL 518, *Aspergillus fumigatus* NCIM 902 and *Aspergillus niger* ATCC 10578. The synthesized compounds **6d**, **6f**, **6g**, **6h** and **6j** exhibited promising antifungal activity against the tested fungal pathogens. In molecular docking studies, derivatives **6c**, **6f** and **6i** showed good binding at the active site of *C. albicans* cytochrome P450 enzyme lanosterol 14 α -demethylase. The *in vitro* antifungal activity results and docking studies indicated that the synthesized compounds have potential antifungal activity and can be further optimized as privileged scaffolds to design and develop potent antifungal drugs.

Keywords: 1,3,4-oxadiazoles; ultrasound; Mannich reaction; molecular sieves; antifungal activity; molecular docking

1. Introduction

Mortality due to secondary fungal infections in immunocompromised patients such as patients with AIDS, autoimmune diseases, burns, radiotherapy or chemotherapy is becoming a serious problem worldwide, with 1.5 to 2 million deaths every year [1]. Most of the mortality is caused by species belonging to four genera, namely *Candida*, *Aspergillus*, *Cryptococcus* and *Pneumocystis*. Emerging fungal pathogens and development of resistance in pathogenic fungi to currently used antifungal drugs has further aggravated the situation [2,3]. Currently, a limited repertoire of drugs from five classes, namely polyenes, azoles, echinocandins, allylamines and fluoropyrimidines are used for the treatment of fungal infections. Apart from drug resistance in pathogens, drawbacks of existing drugs such as acute

and chronic side-effects, less clinical efficiency, and effects on non-targeted cells has forced researchers around the world to search for novel and efficient antifungal drugs [2].

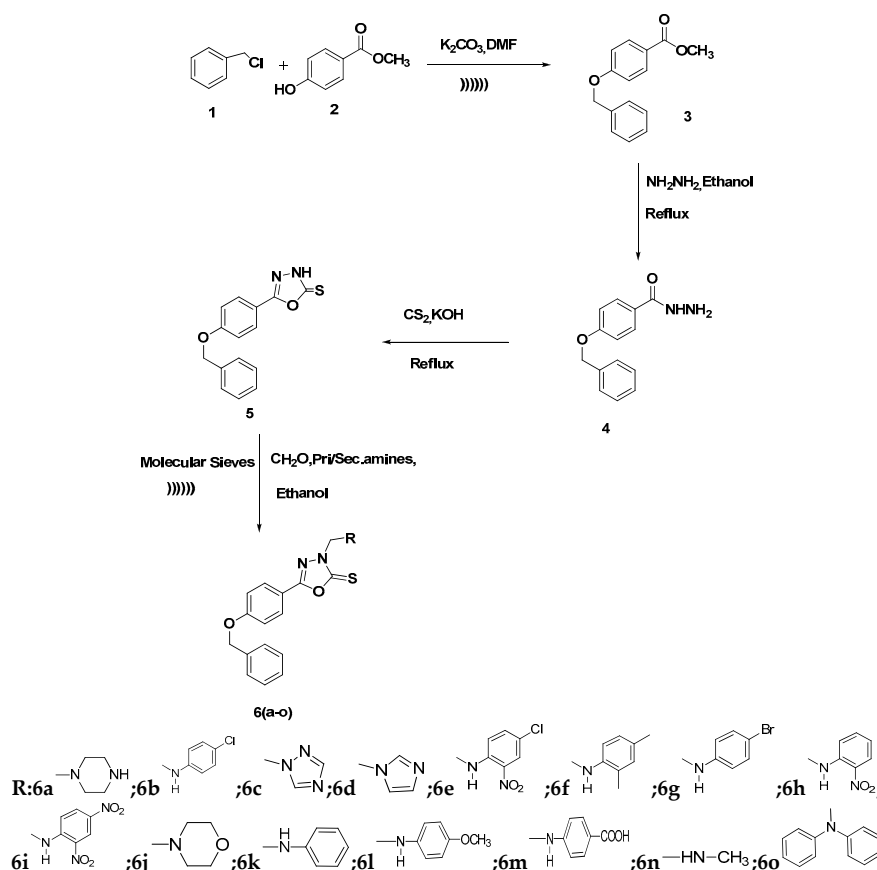
Heterocycles containing nitrogen and oxygen atoms are considered an important class of compounds in medicinal chemistry because of their versatile biological applications. The 1,3,4-oxadiazole scaffold is associated with diverse biological activities such as antifungal [4–6] antibacterial [7,8], antimycobacterial [9], anti-HIV [10], anti-hepatitis B virus [11], anticancer [12], anticonvulsant [13], anti-inflammatory [14], anti-malarial [15] and analgesic effects [16]. 5-Substituted-1,3,4-oxadiazole-2-thiones, which feature an exocyclic sulphur at C-2 of a 1,3,4-oxadiazole skeleton represent an important type of compound class in the field of coordination chemistry because of their potential multifunctional donor sites, *viz* either exocyclic sulfur or endocyclic nitrogen [17] and known to possess CNS depressant [18] and tyrosinase inhibition [19] activity. 1,3,4-Oxadiazole-2-thiones belong to the class of five-membered aromatic *N*-heterocycles substrates with the N–H reactive center involved in tautomerism with S–H [20]. The three-component condensation of a compound containing an acidic hydrogen atom with substituted primary or secondary amines and an aldehyde is known as a Mannich reaction and the product of this reaction has reported to be of significance in the preparation of natural and synthetic molecules with a remarkable biological activity. The literature survey on Mannich reaction involving oxadiazole thiones has shown that these compounds undergo either *N*-aminomethylation or *S*-aminomethylation depending on the reaction conditions [21]. The multipurpose usage of the Mannich bases in pharmaceutical chemistry [22,23] prompted us to prepare a new series of 5-(4-(benzyloxy)substitutedphenyl)-3-((phenyl amino)methyl)-1,3,4-oxadiazole-2(3*H*)-thione based aminomethyl derivatives.

Ultrasound-assisted organic synthesis is a green synthetic approach and a powerful technique for the enhancement of reaction rates and yields [24,25]. Ultrasonic irradiation is enhanced due to the formation of high energy intermediates. It offers the possibility of accomplishing potential reactions in short time cycles with cheaper reagents and less extreme physical conditions [26,27]. It can also be considered as an important tool for conservation of energy and minimization of waste as compared to the conventional techniques [28]. Microporous 3 Å molecular sieves are aluminosilicate minerals with chemical composition of $2/3K_2O \cdot 1/3Na_2O \cdot Al_2O_3 \cdot 2SiO_2 \cdot 9/2H_2O$. Since the 1990s, these molecular sieves have attracted considerable attention due to their potential use in catalysis [29]. Mannich reactions by conventional methods take 15 to 20 h for the synthesis of the same derivatives, whereas using ultrasound the reaction time is reduced to 1 to 2 h. In the present study we report the use of ultrasound- and molecular sieves-assisted Mannich reactions for the synthesis of 5-substituted 1,3,4-oxadiazole-2-thiones with primary and secondary amines. Molecular docking of the synthesized compounds and screening for antifungal potential against different yeast and filamentous fungal pathogens is also reported.

2. Results and Discussion

2.1. Chemistry

All the final compounds **6a–o** were synthesized following the procedure depicted in Scheme 1. The starting material methyl 4-(benzyloxy) benzoate (**3**) was synthesized by the reaction of methyl 4-hydroxybenzoate (**2**) and benzyl chloride(**1**) in K_2CO_3 and DMF as solvent in an ultrasonic processor for up to 4 h. Synthesis by a conventional method like stirring at room temperature required 20–28 h and by reflux nearly 8–10 h. The compound **3** obtained in good yield in step I is next refluxed with NH_2NH_2 to get 4-(benzyloxy) benzohydrazide (**4**). The reaction of the acid hydrazide **4** with carbon disulphide under basic conditions using KOH yielded 5-(4-(benzyloxy)phenyl)-1,3,4-oxadiazole-2(3*H*)-thione **5** [30]. *N*-Amino-methylation of (**5**) with various substituted primary amines and secondary amines and paraformaldehyde in presence of activated molecular sieves in ultrasonic processor under the Mannich reaction gave **6a–o** with good yields within 1–2 h (Table 1). Characterization data of the synthesized derivatives is presented in Table S1 (Supplementary Materials).



Scheme 1. Synthesis of Mannich bases 6a–o.

Table 1. Comparison of reaction kinetics of conventional and ultrasonic irradiation methods for the synthesized compounds 6a–o.

Entry	Conventional		Ultrasonic Irradiation	
	Time (h)	Yield (%)	Time (h)	Yield (%)
6a	15	68	1.5	80
6b	16	63	1.0	81
6c	15	62	1.5	78
6d	17	58	2.0	79
6e	18	45	1.0	81
6f	18	56	1.0	79
6g	19	57	2.0	81
6h	17	61	1.5	90
6i	18	53	1.0	82
6j	17	63	1.5	85
6k	15	58	1.5	80
6l	17	57	1.0	81
6m	15	61	2.0	79
6n	16	58	2.0	79
6o	15	45	2.0	78

The structure of intermediate 5-(4-(benzyloxy)phenyl)-1,3,4-oxadiazole-2(3H)-thione (5) was confirmed by spectroscopic analysis. The IR spectrum showed bands at 3235 (NH), 1621 (C=N), 1596 (C–C), 1425 (C=S), 1258 and 1093 cm^{-1} (C–O–C). The $^1\text{H-NMR}$ spectrum displayed a singlet at 3.8 ppm, assigned to the NH/SH tautomeric proton, a singlet at 5.17 ppm, integrating to 2H and assigned to the C–CH₂–O group and a 9H multiplet at 7.0–7.9 for the two aromatic rings. In the $^{13}\text{C-NMR}$ the –C=S carbon appeared at δ 189.9, along with the characteristic signals of the remaining

carbon atoms. The HRMS mass spectrum showed ($M + 1$) molecular ion peak at m/z 285, in agreement with its molecular formula, $C_{15}H_{12}O_2N_2S$.

In the IR spectrum of compound **6j**, the aromatic C–H stretching vibration was observed at 3047 cm^{-1} . The absorption band due to the CH_2 groups of the morpholine moiety was seen at $2916/2850\text{ cm}^{-1}$. The C=N and C=S moieties showed their characteristic absorption bands at 1681 and 1356 cm^{-1} , respectively. In 1H -NMR spectrum, the eight protons of the morpholine moiety resonated as two triplets at δ 2.75 and δ 3.62, while two singlet were seen at δ 5.02 and δ 5.16 for the N– CH_2 –N and – OCH_2 protons, along with a multiplet at δ 7.06–8.02 for the nine aromatic ring protons. In the ^{13}C -NMR spectrum, the signals observed at δ 67.91 and 70.12 were assigned to C2, C6 and C3, C5 of the morpholine ring, respectively. The signal due to the aryloxy methylene carbon appeared at δ 68.23. The aryloxymethine carbon atom resonated at δ 156.69, and the C=S carbon appeared at 177.14, while other aromatic carbon atoms appeared at δ 115.47, 119.51, 125.68, 128.17, 128.43, 128.61, 129.00, 131.97 and 135.64. Further, the structure of **6j** was also confirmed by recording its mass spectrum, which showed a molecular ion peak at m/z 383, in agreement with the assigned molecular formula, $C_{20}H_{21}N_3O_3S$.

2.2. In Vitro Antifungal Activity

The newly synthesized compounds **6a–o** were screened for *in vitro* antifungal activity against different yeast and filamentous fungal pathogens and were found to be fungistatic. All the compounds exhibited moderate antifungal activity against the tested yeast pathogens, *viz.* *Candida* and *Cryptococcus* strains, as compared to the standard fluconazole, the results are exhibited in Table 2. Derivatives **6i** and **6e** exhibited better antifungal activity than fluconazole against the filamentous *Aspergillus fumigatus* and *Aspergillus niger*, respectively. The antifungal activity exhibited by these compounds may be mainly due to the presence of various electron withdrawing groups such as bromine at the phenyl *para*-position for **6g** ($4\text{ }\mu\text{g/mL}$), imidazole for **6d** ($6\text{ }\mu\text{g/mL}$), morpholine for **6j** ($8\text{ }\mu\text{g/mL}$), and triazole in case of **6c** ($11.2\text{ }\mu\text{g/mL}$). Compound **6f** ($5.4\text{ }\mu\text{g/mL}$) with *ortho* and *para*-disubstituted tolyl and **6e** ($24.5\text{ }\mu\text{g/mL}$) showed good activity against *Candida glabrata* NCYC 388. The antifungal susceptibility testing results indicated that 5-(4-(benzyloxy) substituted phenyl)-3-((phenylamino)methyl)-1,3,4-oxadiazole-2(3*H*)-thione derivatives can act as a good scaffold to develop lead molecules with potent antifungal activity. The final derivatives possessing electron withdrawing groups on the phenyl ring at position 3 of the nitrogen of the 1,3,4-oxadiazole-2-thione ring acted as more potent compounds and exhibited excellent antifungal activity.

Table 2. Antifungal activity of the compounds **6a–o** (IC_{50} * in $\mu\text{g/mL}$) against different human pathogenic fungi.

Compound	<i>Candida albicans</i> ATCC 24433	<i>Candida albicans</i> ATCC 10231	<i>Candida glabrata</i> NCYC 388	<i>Cryptococcus neoformans</i> ATCC 34664	<i>Cryptococcus neoformans</i> PRL 518	<i>Aspergillus fumigatus</i> NCIM 902	<i>Aspergillus niger</i> ATCC 10578
6a	64	49.6	64	64	64	>256	27.7
6b	128	67.12	59.6	77.5	>128	24.2	24.1
6c	21.6	108	53.6	108.9	196.5	11.2	93
6d	68	159	65.6	128	>128	6	130
6e	54.4	44.6	24.5	64	14.21	25.8	47
6f	16	84.9	5.4	>256	90	256	256
6g	62	107.6	256	76.8	94.7	4	>256
6h	55.3	57.4	64	47.6	66.6	16	18.61
6i	23.3	80.6	40.3	256	93.5	24.6	>256
6j	38.4	31.5	56.6	192	39	8	128
6k	150	53.3	36.2	64.2	146.6	35.1	22.9
6l	115	95.3	42.5	49.7	145.8	81	73.2
6m	91.5	49.7	78.6	62.5	54.6	87.4	84.8
6n	30.3	43	48.8	47.5	105.6	81.5	56.9
6o	95	28.1	50.7	42.7	156.5	76.1	70.5
Fluconazole	0.12	0.11	9.4	16	4	64	46

* IC_{50} is the concentration exhibiting 50% inhibition of the growth as compared to the growth of control.

2.3. Molecular Docking Study

The azole class of antifungal drugs inhibits the cytochrome P450 lanosterol 14 α -demethylase enzyme in the ergosterol synthesis pathway. As ergosterol is the major sterol component of the fungal cell membrane, inhibition of this enzyme and subsequent ergosterol depletion by the compounds leads to loss in fungal cell integrity and function [29]. Therefore, we carried out molecular docking of our oxadiazole thiones **6a–o** and the standard drug fluconazole into the active site of cytochrome P450 lanosterol 14 α -demethylase of *Candida albicans*, using the VLife MDS 4.3 software, to understand the binding interactions. The binding energy and hydrogen bond interactions are presented in Table 3.

Table 3. Results of the docking study of the compounds.

Compound	Binding Energy	Hydrogen Bonds	Hydrophobic Bonds
6a	−41.69	LEU412-O=C	ALA343, GLY344, LEU412, MET415, PHE499, GLY508 and ALA512
6b	−49.33	LEU412-O=C	ALA343, THR347, MET415, GLY500 and HIS504
6c	−57.85	CYS506-O-CH ₂	LEU412, SER414, MET415, CYC506, ILE507 and GLY508
6d	−45.23	CYS506-O-CH ₂	ALA343, LEU412, MET415, CYC506, ILE507 and GLY508
6e	−35.50	LEU412-O=C; GLY500-N of nitro and GLY500-O-N of NO ₂	ALA343, LEU412, ILE507 and GLY508
6f	−65.25	CYS506-NH	ALA343, THR347, MET415, PHE499, HIS504, CYC506, GLY508, GLU509, ALA512 and TYR513
6g	−52.37	LEU412-O=C	THR347, LEU412, MET415, PHE499 and HIS504
6h	−47.18	VAL497 and GLY500-N of NO ₂	ALA343, GLY344, PHE499, GLY500 and GLY508
6i	−61.03	TYR154-O-N of 2-nitro; TYR168-O=C and MET415-N of 4-nitro	LEU340 and ALA343
6j	−40.29	LEU412-O-CH ₂	ALA343, GLY344, LEU412, MET415, PHE499, GLY508 and ALA512
6k	−37.85	-	ALA343, GLY344, THR347, PHE499, GLY500 and GLY508
6l	−42.91	-	LEU186, LEU240, GLY344, THR347, MET415, PHE499, GLY500, CYS506, GLY508 and PHE511
6m	−36.83	-	ALA343, LEU412 and PHE499
6n	−40.09	CYS506-O-CH ₂	ALA343, VAL440, PRO442, VAL497, GLY500 and CYC506
6o	−38.97	TYR168-O=C	LEU340, ALA343, ILE507 and GLY508
Fluconazole	−67.29	TYR168-F of phenyl	GLY500 and HIS504

The most active synthesized compounds **6c**, **6f** and **6i** showed the lowest interaction energy, *i.e.*, −57.85, −65–25 and −61.03 kcal/mol, respectively. The standard drug fluconazole also showed good interaction energy (−67.29 kcal/mol). The docking results indicated that compounds were held in the active pocket by combination of various hydrogen and hydrophobic interactions with cytochrome P450 lanosterol 14 α -demethylase. Diverse hydrophobic interactions occurred between the synthesized compounds and the active site chains of LEU186, LEU240, LEU340, ALA343, GLY344, THR347, LEU412, MET415, VAL440, PRO442, VAL497, PHE499, GLY500, HIS504, CYC506, ILE507, GLY508, GLU509, PHE511, ALA512 and TYR513. The amino acid residues such as TYR154, TYR168, LEU412, MET415, VAL497, GLY500 and CYS506 formed hydrogen bonds with the synthesized compounds. The docking interactions of the most active compounds **6c**, **6f** and **6i** are shown in Figure 1. The amino acid CYS506 formed hydrogen bonding (1.95 Å) with the −O−CH₂−oxygen of the synthesized compound **6c**. The 1,2,4-triazole ring of compound **6c** was held in the active site by forming Vander Waal's interactions with amino acid residues like PRO410, LEU412, PRO442, VAL497 and PRO498. The amino acid residue CYS506 (2.40 Å) formed a hydrogen bond with the −NH−nitrogen of compound **6f**. The amino acids

TYR154 (2.19 Å), TYR168 (2.52 Å) and MET415 (2.82 Å) all formed hydrogen bonds with the 2-NO₂ oxygen, the carbonyl oxygen and the 4-NO₂ nitrogen, respectively, of compound **6i**. On the basis of the antifungal activity and docking results, it was found that compounds **6c**, **6f** and **6i** had potential to inhibit cytochrome P450 lanosterol 14 α -demethylase of *C. albicans*.

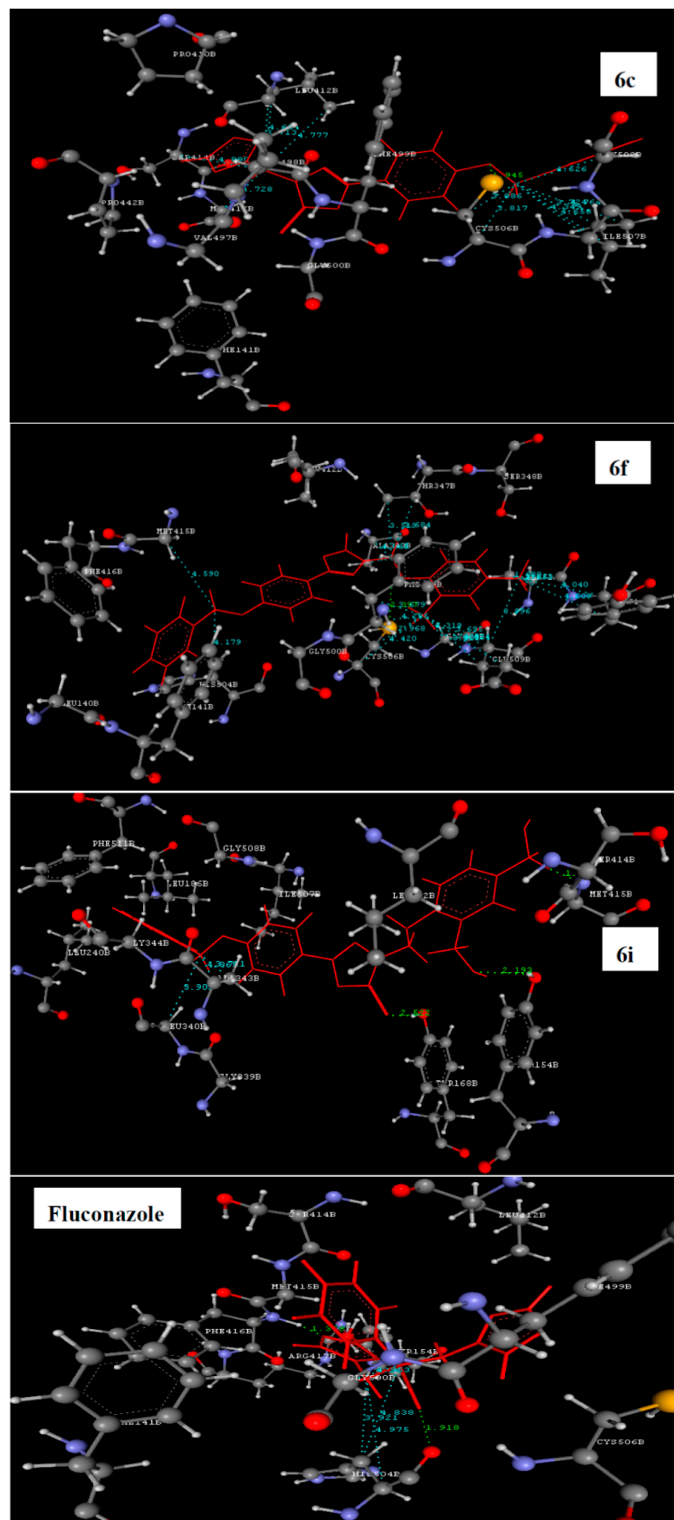


Figure 1. Docking of compounds **6c**, **6f**, **6i** and standard fluconazole. Ligands are shown in red color. Hydrogen bonds are shown in green color. Hydrophobic bonds are shown in sky blue color.

3. Materials and Methods

3.1. General Information

All the chemicals used for synthesis were procured from Merck (Mumbai, Maharashtra, India), Sigma (Mumbai), HiMedia (Mumbai) or Qualigens (Mumbai) and used without further purification. The progress of each reaction was monitored by ascending thin layer chromatography (TLC) using pre-coated silica gel F254 aluminum TLC sheets (Merck) and the spots were visualized by UV light and iodine vapors. Elemental analyses (C, H, and N) were done with a FLASH EA 112 Shimadzu' analyzer (Mumbai) and all analyses were consistent (within 0.4%) with theoretical values. Infrared (IR) spectra were recorded on a PS 4000 FTIR (JASCO, Tokyo, Japan) using KBr pellets. ¹H- and ¹³C-NMR (200 MHz) spectra were recorded on a ACF 200 spectrometer (Bruker, Billerica, MA, USA) fitted with an Aspect 3000 computer and all the chemical shifts (ppm) were referred to internal TMS for ¹H and chloroform-d for ¹³C-NMR. ¹H-NMR data are reported in the order of chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; br, broad; br s, broad singlet; m, multiplet and/or multiple resonance), number of protons. A Micro TOF-Q-II (Bruker Daltonics, Billerica, MA, USA) with electron spray ionization (ESI) was used to obtain the HRMS data. For ultrasound irradiation Vibra cell VCX-500 with solid probe was used (Sonics, Newtown, CT, USA).

3.2. Synthesis of Methyl-4-(Benzyloxy)benzoate (3)

For the synthesis of methyl-4-(benzyloxy)benzoate, the reaction between equal 0.01 mol amounts of methyl-4-hydroxybenzoate and chloromethylbenzene in *N,N*-dimethylformamide (DMF) as solvent using K₂CO₃ as mild base was carried with under an ultrasonic processor at room temperature for up to 4 h. The solution was then poured into ice-water. The solid obtained was filtered and recrystallized from ethanol. Colour: white; m.p. 105 °C (100 °C reported in chemspider)

3.3. Synthesis of 4-(Benzyloxy)benzohydrazide (4)

For the synthesis of the substituted benzohydrazide, a mixture of the corresponding ester (20 mmol), 85% hydrazine hydrate (20 mmol) in ethanol (35 mL) was refluxed for 6 h. After that, the solution was poured into ice-water. The solid was filtered and recrystallized from ethanol. Colour: white; m.p. 138 °C (140 °C reported in chemspider)

3.4. Synthesis of 5-(4-(Benzyloxy)phenyl)-1,3,4-oxadiazole-2(3H)-thione

Equimolar quantities of the substituted benzohydrazide (5 mmol) and potassium hydroxide (5 mmol) were dissolved in 95% ethanol (20 mL). The mixture was allowed to stir for several minutes at room temperature and then carbon disulfide (15 mmol) was slowly added dropwise to the reaction system and the mixture was heated to reflux. The residue obtained was dissolved in water (50 mL) and diluted hydrochloric acid was added to adjust the pH value of the solution to 5–6. The precipitate was collected washed with water for several times and dried and recrystallized from ethanol. Colour: white; m.p. 160 °C.

3.5. General Procedure for the Synthesis of 5-(4-(Benzyloxy)substituted phenyl)-3-((phenylamino)methyl)-1,3,4-oxadiazole-2(3H)-thiones 6a–o

To a methanolic solution of 5-(4-(benzyloxy) phenyl)-1,3,4-oxadiazole 2(3H) thione (5), (10 mmol), paraformaldehyde (15 mmol) and a substituted primary/secondary amine (10 mmol) in methanol 25 mL were added with constant stirring. Preactivated molecular sieves (3Å) were also added to the reaction mixture to absorb the water formed. The resulting mixture was subjected to ultrasonic irradiation for 1–2 h at room temperature. The precipitated solids were filtered, washed with ice water. The progress of the reaction was monitored by TLC using 1:4 ethyl acetate–*n*-hexane as a solvent system. The reaction was quenched with crushed ice and the solid obtained was recrystallized from methanol to yield the title compounds 6(a–o) mentioned in Table 1 (see also Table S1).

5-(4-(Benzyloxy)phenyl)-1,3,4-oxadiazole-2(3H)-thione (**5**): Recrystallized from ethanol; yield: 90%; m. p.: 160 °C. IR (KBr) ν_{\max} (cm⁻¹): 3235 (NH), 3100–3000 aromatic CH stretch, 2870–2800 aliphatic CH stretch, 1621 (C=N), 1596 (C–C), 1250 (C=S), 1258 and 1093 (C–O–C), ¹H-NMR (CDCl₃), δ ppm: 3.8 (s, 1H, NH), 5.17 (s, 2H, C–CH₂–O), 7.07.9 (m, 9H, two aromatic ring), ¹³C-NMR (CDCl₃, δ ppm): 70.8, 114.4, 116.3, 127.1, 127.6, 128.9, 130.2, 136.7, 157.0, 161.3, 189.9, *m/z*: 284.1 (100.0%), 285.1 (18.0%), 286.1 (6.5%), Molecular Formula: C₁₅H₁₂N₂O₂S. Elemental Analysis: Calculated: (C, H, N) 63.3, 4.25, 9.85 Found: 63.5, 4.22, 9.87.

5-(4-(Benzyloxy)phenyl)-3-(piperazine-1-ylmethyl)-1,3,4-oxadiazole-2(3H)-thione (**6a**): Yield: 80%; m.p.: 205 °C. IR (KBr) ν_{\max} (cm⁻¹): 3245 (NH), 3100–3000 aromatic CH stretch, 2870–2800 aliphatic CH stretch, 1623 (C=N), 1598 (C–C), 1252 (C=S), 1260 and 1095 (C–O–C), 1280 (C–N sec. amine piperazine), 1560 (N–H sec. amine piperazine), ¹H-NMR (CDCl₃), δ ppm: 1.91 (s, 1H, –NH piperazine), 2.37 (t, 4H, N–CH₂–C piperazine), 2.65 (t, 4H, C–CH₂–NH piperazine), 3.72 (s, 2H, N–CH₂–N), 5.16 (s, 2H, C–CH₂–O), 7.38–8.02 (m, 9H, aromatic ring), ¹³C-NMR (CDCl₃, δ ppm): 45.91, 54.7, 70.8, 71.0, 114.4, 116.3, 127.1, 127.5, 128.9, 130.2, 136.7, 157.0, 163.3, 177.1., *m/z* 382.15 (100.0%), 383.15 (22.8%), 384.14 (4.5%), Molecular Formula: C₂₀H₂₂N₄O₂S. Elemental Analysis: Calculated (C, H, N) 62.80, 5.80, 14.65, Found: 62.76, 5.74, 14.62.

5-(4-(Benzyloxy)phenyl)-3-(((4-chlorophenyl)amino)methyl)-1,3,4-oxadiazole-2(3H)-thione (**6b**): Yield: 81%; m.p.: 180 °C. IR (KBr) ν_{\max} (cm⁻¹): 3230 (NH), 3100–3000 aromatic CH stretch, 2870–2800, aliphatic CH stretch, 1625 (C=N), 1592 (C–C), 1235 (C=S), 1261 and 1094 (C–O–C), 3470 (N–H primary amine) 744 (C–Cl), ¹H-NMR (CDCl₃), δ ppm: 4.0 (S, 1H, –NH), 4.42 (S, 2H, N–CH₂–N–), 5.16 (S, 2H, –OCH₂), 6.54 and 8.02 (m, 13H, aromatic rings), ¹³C-NMR (CDCl₃, δ ppm): 70.8, 114.4, 114.9, 116.3, 121.1, 127.1, 127.6, 128.9, 129.6, 130.2, 136.7, 145.7, 157.0, 161.3, 177.1., *m/z* 423.08 (100.0%), 425.08 (36.9%), 424.08 (25.7%), Molecular Formula: C₂₂H₁₈ClN₃O₂S. Elemental Analysis: Calculated (C, H, N) 62.33, 4.28, 9.91, Found: 62.28, 4.22, 9.88.

3-((1H-1,2,4-Triazol-1-yl)methyl) 5-(4-(benzyloxy)phenyl)-1,3,4-oxadiazole-2(3H)-thione (**6c**): Yield: 78%; m.p.: 235 °C. IR (KBr) ν_{\max} (cm⁻¹): 3100–3000 aromatic CH stretch, 2870–2800, aliphatic CH stretch, 1621 (C=N), 1596 (C–C), 1425 (C=S), 1258 and 1093 (C–O–C), 1290 (C–N), 1622 (C=N triazole), ¹H-NMR (CDCl₃), δ ppm: 5.09 (s, 2H, N–CH₂–N), 5.16 (S, 2H, –OCH₂), 7.06–8.02 (m, 9H, aromatic rings), 8.05 and 8.68 (s, 1H, NH triazole ring), ¹³C-NMR (CDCl₃, δ ppm): 70.8, 73.6, 114.4, 116.3, 127.1, 127.6, 128.9, 130.2, 136.7, 143.8, 151.5, 157.0, 161.3, 177.1, *m/z* 365.09 (100.0%), 366.10 (19.7%), 367.09 (4.5%), Molecular Formula: C₁₈H₁₅N₅O₂S; Elemental Analysis: Calculated (C, H, N) 59.16, 4.14, 19.17, Found: 59.11, 4.10, 19.11.

3-((1H-Imidazol-1-yl)methyl) 5-(4-(benzyloxy)phenyl)-1,3,4-oxadiazole-2(3H)-thione (**6d**): Yield: 79%; m.p.: 240 °C. IR (KBr) ν_{\max} (cm⁻¹): 3100–3000 aromatic CH stretch, 2870–2800 aliphatic CH stretch, 1621 (C=N), 1596 (C–C), 1425 (C=S), 1258 and 1093 (C–O–C), 1290 (C–N), 1622 (C=N imidazole), 1625 (C=C imidazole ring), ¹H-NMR (CDCl₃), δ ppm: 5.09 (s, 2H, N–CH₂–N), 5.16 (S, 2H, –OCH₂), 6.77, 7.13 and 7.83 (s, 1H each of imidazole ring), 7.06–8.02 (m, 9H, aromatic rings), ¹³C-NMR (CDCl₃, δ ppm): 70.8, 71.7, 114.4, 116.3, 120.6, 127.1, 127.6, 128.1, 128.9, 130.2, 136.7, 137.8, 157.0, 161.3, 177.1, *m/z* 364.10 (100.0%), 365.10 (22.9%), 366.10 (5.4%), Molecular Formula: C₁₉H₁₆N₄O₂. Elemental Analysis: Calculated (C, H, N) 62.62, 4.43, 15.37 Found: 62.58, 4.40, 15.34.

5-(4-(Benzyloxy)phenyl)-3-(((4-chloro-2-nitrophenyl)amino)methyl)-1,3,4-oxadiazole-2(3H)-thione (**6e**): Yield: 81%; m.p.: 218 °C. IR (KBr) ν_{\max} (cm⁻¹): 3549(NH), 3100–3000 aromatic CH stretch, 2870–2800 aliphatic CH stretch, 1625 (C=N), 1590 (C–C), 1526 and 1346 (NO₂), 1429 (C=S), 1263 and 1089 (C–O–C), 1490 (C–NO₂), 744 (C–Cl); ¹H-NMR (DMSO-*d*₆), δ ppm: 4.0 (S, 1H, –NH), 4.42 (S, 2H, N–CH₂–N–), 5.16 (S, 2H, –OCH₂), 7.06–8.13 (m, 12H, aromatic rings). ¹³C-NMR (CDCl₃, δ ppm): 69.8, 70.8, 114.4, 115.8, 116.3, 122.0, 127.1, 127.6, 128.9, 130.2, 131.5, 135.7, 136.7, 144.8, 157.0, 161.3, 177.1, *m/z* 468.07 (100.0%), 470.06 (36.5%), 469.07 (24.9%), Molecular Formula: C₂₂H₁₇ClN₄O₄S. Elemental Analysis: Calculated (C, H, N) 56.35, 3.65, 11.95, Found 56.30, 3.61, 11.92.

5-(4-(Benzyloxy)phenyl)-3-(((2,4-dimethylphenyl)amino)methyl)-1,3,4-oxadiazole-2(3H)-thione (**6f**): Yield: 79%; mp: 230 °C. IR (KBr) ν_{\max} (cm⁻¹): 3230 (NH), 3100–3000 aromatic CH stretch, 2870–2800 aliphatic CH stretch, 1616 (C=N), 1595 (C–C), 1427 (C=S), 1252 and 1151 (C–O–C), 1460 (Aromatic C–CH₃), ¹H-NMR (CDCl₃), δ ppm: 2.10 (s, 3H, –CH₃), 2.30 (s, 3H, –CH₃), 4.0 (s, 1H, –NH), 4.42 (s, 2H, N–CH₂–N–), 5.16 (s, 2H, –OCH₂), 6.36–8.02 (m, 12H, aromatic rings), ¹³C-NMR (CDCl₃, δ ppm): 17.9, 21.6, 70.8, 71.1, 113.3, 114.4, 116.3, 126.8, 127.1, 127.6, 128.9, 130.2, 131.7, 136.2, 136.7, 143.5, 157.0, 161.3, 177.1 *m/z* 417.15 (100.0%), 418.15 (27.9%), 419.15 (5.0%) Molecular Formula: C₂₄H₂₃N₃O₂S. Elemental Analysis: Calculated (C, H, N) 69.04, 5.55, 10.06, Found: 69.00, 5.51, 10.02.

5-(4-(Benzyloxy)phenyl)-3-(((4-bromophenyl)amino)methyl)-1,3,4-oxadiazole-2(3H)-thione (**6g**): Yield: 81%; m.p.: 228 °C. IR (KBr) ν_{\max} (cm⁻¹): 3235 (NH), 3100–3000 aromatic CH stretch, 2870–2800 aliphatic CH stretch, 1621 (C=N), 1593 (C–C), 1424 (C=S), 1260 and 1123 (C–O–C), 690 (C–Br), ¹H-NMR (DMSO-*d*₆), δ ppm: 4.0 (s, 1H, –NH), 4.42 (s, 2H, N–CH₂–N–), 5.16 (s, 2H, –OCH₂), 6.59–8.02 (m, 13H, aromatic rings), ¹³C-NMR (CDCl₃, δ ppm): 70.8, 114.4, 114.5, 115.1, 116.3, 127.1, 127.6, 128.9, 130.2, 132.4, 136.7, 146.6, 157.0, 161.3, 177.1, *m/z* 469 (100%), 467 (97.4%), 470 (25.7%), 471.2 (4.3%). Molecular Formula: C₂₂H₁₈BrN₃O₂S. Elemental Analysis: Calculated (C, H, N) 56.42, 3.87, 8.97, Found: 56.38, 3.84, 8.93.

5-(4-(Benzyloxy)phenyl)-3-(((2-nitrophenyl)amino)methyl)-1,3,4-oxadiazole-2(3H)-thione (**6h**): Yield: 90%; m.p.: 220 °C. IR (KBr) ν_{\max} (cm⁻¹): 3245 (NH), 3100–3000 aromatic CH stretch, 2870–2800 aliphatic CH stretch, 1622 (C=N), 1590 (C–C), 1522 and 1345 (NO₂), 1427 (C=S), 1261 and 1088 (C–O–C), 1401 (C–NO₂), 755; ¹H-NMR (DMSO-*d*₆), δ ppm: 4.0 (s, 1H, –NH), 4.42 (s, 2H, N–CH₂–N–), 5.16 (s, 2H, –OCH₂), 7.06–8.04 (m, 13H, aromatic rings), ¹³C-NMR (CDCl₃, δ ppm): 69.8, 70.8, 114.4, 116.3, 118.0, 125.9, 127.1, 127.6, 128.9, 130.2, 131.7, 135.6, 136.7, 146.7, 157.0, 161.3, 177.1, *m/z* 431.10 (100%), 435.11 (97.4%), 436.10 (4.5%), Molecular Formula: C₂₂H₁₈N₄O₄S. Elemental Analysis: Calculated (C, H, N) 60.82, 4.18, 12.90, Found: 60.78, 4.13, 12.86.

5-(4-(Benzyloxy)phenyl)-3-(((2,4-dinitrophenyl)amino)methyl)-1,3,4-oxadiazole-2(3H)-thione (**6i**): Yield: 82%; m.p.: 145 °C. IR (KBr) ν_{\max} (cm⁻¹): 3247 (NH), 3100–3000 aromatic CH stretch, 2870–2800 aliphatic CH stretch, 1621 (C=N), 1593 (C–C), 1524 and 1344 (NO₂), 1426 (C=S), 1262 and 1083 (C–O–C), 1405–1418 (C–NO₂), ¹H-NMR (DMSO-*d*₆), δ ppm: 4.0 (s, 1H, –NH), 4.42 (s, 2H, N–CH₂–N–), 5.16 (s, 2H, –OCH₂), 7.06–8.88 (m, 12H, aromatic rings), ¹³C-NMR (CDCl₃, δ ppm): 69.8, 70.8, 114.4, 116.3, 120.8, 127.1, 127.6, 128.9, 130.2, 130.8, 135.2, 136.7, 137.2, 152.8, 157.0, 161.3, 177.1, *m/z* 479.09 (100%), 480.09 (26.7%), 481.09 (6.4%), Molecular Formula: C₂₂H₁₇N₅O₆S. Elemental Analysis: Calculated (C, H, N) 55.11, 3.57, 14.61, Found: 55.08, 3.52, 14.57.

5-(4-(Benzyloxy)phenyl)-3-(morpholinomethyl)-1,3,4-oxadiazole-2(3H)-thione (**6j**): Yield: 85%; m.p.: 200 °C. IR (KBr) ν_{\max} (cm⁻¹): 3100–3000 aromatic CH stretch, 2870–2800 aliphatic CH stretch, 1623 (C=N), 1592 (aromatic C–C), 1437 (C=S), 1222 and 1036 (C–O–C), 2916/2850 (CH₂ of morpholine), ¹H-NMR (CDCl₃), δ ppm: 2.75 (t, 4H, N–CH₂–C), 3.62 (t, 4H, O–CH₂–C), 3.72 (s, 2H, N–CH₂–N–), 5.16 (s, 2H, –OCH₂), 7.06–8.02 (m, 9H, aromatic rings), ¹³C-NMR (CDCl₃, δ ppm): 67.91, 68.23, 70.12, 115.47, 119.51, 125.68, 128.17, 128.43, 128.61, 129.00, 131.97, 135.64, 156.69, 177.14 *m/z* 383.13 (100%), 384.13 (23.7%), 385.13 (5.6%), Molecular Formula: C₂₀H₂₁N₃O₃S. Elemental Analysis: Calculated (C, H, N) 2.64, 5.52, 10.96 Found: 62.61, 5.48, 10.92.

5-(4-(Benzyloxy)phenyl)-3-((phenylamino)methyl)-1,3,4-oxadiazole-2(3H)-thione (**6k**): Yield: 80%; m.p.: 80 °C. IR (KBr) ν_{\max} (cm⁻¹): 3553 (NH), 3100–3000 aromatic CH stretch, 2870–2800 aliphatic CH stretch, 1612 (C=N), 1595 (C–C), 1428 (C=S), 1255 and 1151 (C–O–C), ¹H-NMR (CDCl₃ + DMSO-*d*₆), δ ppm: 4.0 (s, 1H, –NH), 4.42 (s, 2H, N–CH₂–N–), 5.16 (s, 2H, –OCH₂), 6.77–8.02 (m, 14H, aromatic rings), ¹³C-NMR (CDCl₃, δ ppm): 70.8, 113.5, 114.4, 116.3, 120.8, 127.1, 127.6, 128.9, 129.5, 130.2, 147.6, 157.0, 161.3, 177.1, *m/z* 383.13 (100%), 384.13 (23.7%), 385.13 (5.6%). Molecular Formula: C₂₂H₁₉N₃O₂S. Elemental Analysis: Calculated (C, H, N) 67.84, 4.92, 10.79 Found: 67.79, 4.88, 10.74.

5-(4-(Benzyloxy)phenyl)-3-(((4-methoxyphenyl)amino)methyl)-1,3,4-oxadiazole-2(3H)-thione (**6l**): Yield: 81%; m.p.: 100 °C. IR (KBr) ν_{\max} (cm⁻¹): 3550–3235 (NH), 3100–3000 aromatic CH stretch, 2870–2800 aliphatic CH stretch, 1615 (C=N), 1593 (C–C), 1425 (C=S), 1250 and 1152 (C–O–C), 1450 (C–OCH₃),

¹H-NMR (CDCl₃ + DMSO-*d*₆), δ ppm: 3.83 (s, 3H, -OCH₃), 4.0 (s, 1H, -NH), 4.42 (s, 2H, N-CH₂-N-), 5.16 (s, 2H, -OCH₂), 6.77–8.02 (m, 13H, aromatic rings), ¹³C-NMR (CDCl₃, δ ppm): 55.8, 70.8, 114.4, 115.1, 115.8, 127.1, 127.6, 128.9, 130.2, 139.9, 151.7, 157.0, 161.3, 177.1, *m/z* 419.13 (100%), 420.13 (26.9%), 421.13 (5.6%). Molecular Formula: C₂₃H₂₁N₃O₃S. Elemental Analysis: Calculated (C, H, N) 65.85, 5.05, 10.02 Found: 65.81, 5.00, 10.00.

4-(((5-(4-(Benzyloxy)phenyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl)methyl)amino)benzoic acid (**6m**): Yield: 79%; m.p.: 140 °C. IR (KBr) ν_{max} (cm⁻¹): 3100–3000 aromatic CH stretch, 2870–2800 aliphatic CH stretch, 1674 (C=O), 1613 (C=N), 1606 (C–C), 1431 (C=S), 1269 and 1185 (C–O–C), 3550–3245 (NH and COOH), ¹H-NMR (DMSO-*d*₆), δ ppm: 4.0 (s, 1H, -NH), 4.42 (s, 2H, N-CH₂-N-), 5.16 (s, 2H, -OCH₂), 6.8–8.02 (m, 13H, aromatic rings), 10.50 (s, 1H, -COOH). ¹³C-NMR (CDCl₃, δ ppm): 70.8, 112.0, 114.4, 116.3, 118.6, 127.1, 127.6, 128.9, 130.2, 131.1, 152.8, 157.0, 161.3, 169.3, 177.1, *m/z* 433.11 (100%), 434.11 (26.9%), 435.11 (5.8%). Molecular Formula: C₂₃H₁₉N₃O₄S. Elemental Analysis: Calculated (C, H, N) 63.73, 4.42, 9.69 Found: 63.67, 4.39, 9.62.

5-(4-(Benzyloxy)phenyl)-3-((methylamino)methyl)-1,3,4-oxadiazole-2(3H)-thione (**6n**): Yield: 78%; m.p.: 110 °C. IR (KBr) ν_{max} (cm⁻¹): 3552 (NH), 3100–3000 aromatic CH stretch, 2870–2800 aliphatic CH stretch, 1615 (C=N), 1593 (C–C), 1425 (C=S), 1250 and 1152 (C–O–C), 1410 (N–CH₃). ¹H-NMR (DMSO-*d*₆), δ ppm: 2.0 (s, 1H, NH), 3.26 (s, 3H, CH₃), 3.91 (s, 2H, N-CH₂-N-), 5.16 (s, 2H, -OCH₂), 7.06–8.02 (m, 9H, aromatic rings), ¹³C-NMR (CDCl₃, δ ppm): 26.0, 27.5, 70.8, 72.5, 114.5, 127.1, 127.6, 128.3, 128.4, 128.9, 129.3, 136.7, 189.8. *m/z* 327.10 (100%), 328.11 (18.7%), 329.10 (4.7%). Molecular Formula: C₁₇H₁₇N₃O₂S. Elemental Analysis: Calculated (C, H, N): 62.36, 5.23, 12.83 Found: 62.31, 5.19, 12.79.

5-(4-(Benzyloxy)phenyl)-3-((biphenylamino)methyl)-1,3,4-oxadiazole-2(3H)-thione (**6o**): Yield: 79%; m.p.: 115 °C. IR (KBr) ν_{max} (cm⁻¹): 3100–3000 aromatic CH stretch, 2870–2800 aliphatic CH stretch, 1615 (C=N), 1593 (C–C), 1425 (C=S), 1250 and 1152 (C–O–C), 1300 (C–N sec. amine), ¹H-NMR (DMSO-*d*₆), δ ppm: 4.42 (s, 2H, N-CH₂-N-), 5.16 (s, 2H, -OCH₂), 6.77–8.02 (m, 19H, aromatic rings), ¹³C-NMR (CDCl₃, δ ppm): 70.8, 80.0, 110.1, 114.4, 116.3, 119.1, 121.9, 127.6, 128.9, 129.6, 130.2, 136.7, 149.1, 157.0, 161.3, 177.1. *m/z* 465.15 (100%), 466.15 (32.2%), 467.15 (5.1%), Molecular Formula: C₂₈H₂₃N₃O₂S. Elemental Analysis: Calculated (C, H, N): 72.23, 4.98, 9.03, Found: 72.20, 4.92, 9.0.

3.6. Antifungal Screening

In vitro antifungal susceptibility testing was performed by the broth micro dilution method against seven human pathogens: *C. albicans* ATCC 24433, *C. albicans* ATCC 10231, *Candida glabrata* NCYC 388, *Cryptococcus neoformans* ATCC 34664, *Cryptococcus neoformans* PRL 518, *Aspergillus fumigatus* NCIM 902, *Aspergillus niger* ATCC 10578 following the Clinical Laboratory Standards Institute protocols M27-A3 (for yeasts) and M38-A2 (for filamentous fungi) [31–34]. Briefly, appropriate amount of compounds were dissolved in dimethyl sulfoxide to get 100× final strength. The stock was then diluted 1:50 in RPMI 1640 medium and 200 μL of this solution was added to the first row of a 96-well microtitre plate. The compounds were serially diluted two-fold in successive wells to get a range of 2–256 μg/mL. Spores of the filamentous fungi (~2 × 10⁴ spores/mL) and yeast cells freshly grown in YPG broth in logarithmic phase (~2 × 10³ cfu/mL) were suspended in the RPMI 1640 medium and 100 μL from these were inoculated in the wells of the plate. The micro titer plates were incubated for 24–48 h. Growth was checked by visual observation and measuring absorbance at 600 nm using micro titer plate reader. The IC₅₀ was defined as the concentration exhibiting 50% inhibition of the growth as compared to the growth of control.

3.7. Molecular Docking Data

The 3D model structure of cytochrome P450 lanosterol 14α-demethylase of *C. albicans* was built using homology modeling [3,33]. The amino acid sequence of the enzyme was obtained from the Universal Protein Resource (<http://www.uniprot.org/>, Accession Code: P10613) and sequence

homologs were obtained from Protein Data Bank (PDB) using Blast search. Based on the Blast search result, we used the crystal structure of human lanosterol 14 α -demethylase (CYP51) with azole as a template for homology modeling (PDB ID: 3LD6). The VLifeMDS 4.3 ProModel was used for modeling of the 3D structure of protein based on the amino acid sequences of a close homologue. Alignment of amino acid sequence of CA-CYP51 (P10613) and human CYP51 (3LD6_B) is shown in Figure S1. The Blosom-62 matrix was used with a gap penalty of 1. The model was then energy minimized using the MMFF94 force field [35]. Manual inspection was made to ensure the conserved motifs and loops were correctly aligned. The quality of generated *C. albicans* lanosterol 14 α -demethylase model was assessed by using a well validated program like PROCHECK [36]. The structural validation was performed by using Ramachandran plot and is shown in Figure 2. The further structural super imposition was performed to know the structural coordinate of target protein and RMSD value was found within standard range of 0.997607 Å. The molecular docking study of the synthesized compounds **6a–o** and the standard drugs fluconazole and miconazole were performed against homology built cytochrome P450 lanosterol 14 α -demethylase of *C. albicans* to understand the binding interactions using VLife MDS 4.3 package following standard procedures [37].

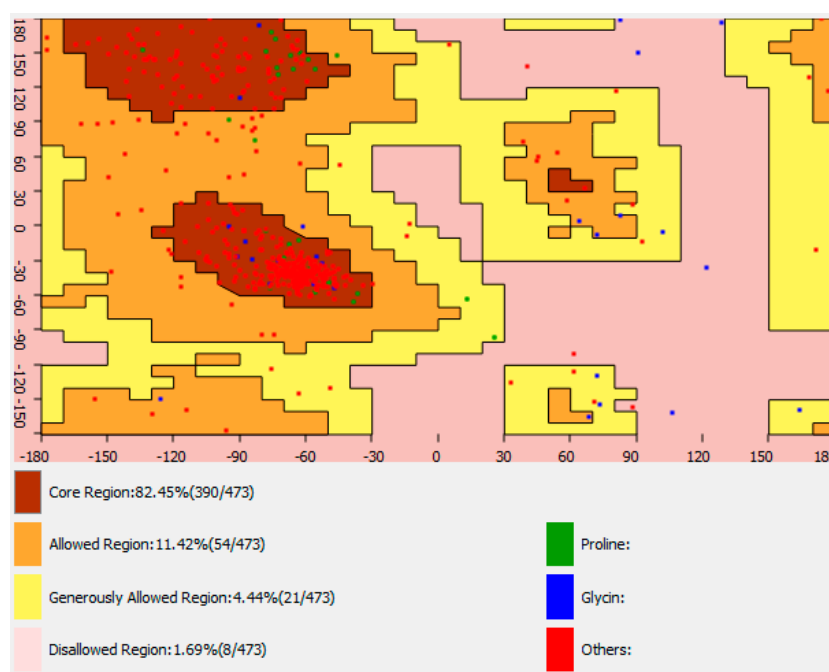


Figure 2. Ramachandran Plot for structural validation.

4. Conclusions

Fifteen novel 5-(4-(benzyloxy) phenyl)-3-((phenylamino)methyl)-1,3,4-oxadiazole-2(3*H*)-thione derivatives were successfully synthesized using molecular sieves under ultrasound irradiation giving better yields of 78%–90% after shorter reaction times of 1–2 h in contrast to conventional reactions which require 15–20 h refluxing. Compounds **6c**, **6f** and **6i** exhibited promising antifungal activity and the developed scaffold offers an attractive template for lead antifungal discovery.

Supplementary Materials: Supplementary materials can be accessed at: <http://www.mdpi.com/1420-3049/21/5/484/s1>.

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Sample Availability: Samples of the compounds are not available from the authors.



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