“Molecular Sieves And Ultrasound-Assisted Synthesis Of Novel 1,3,4-Oxadiazole-2-Thiones Derivatives As Potential Antifungal Agents.”

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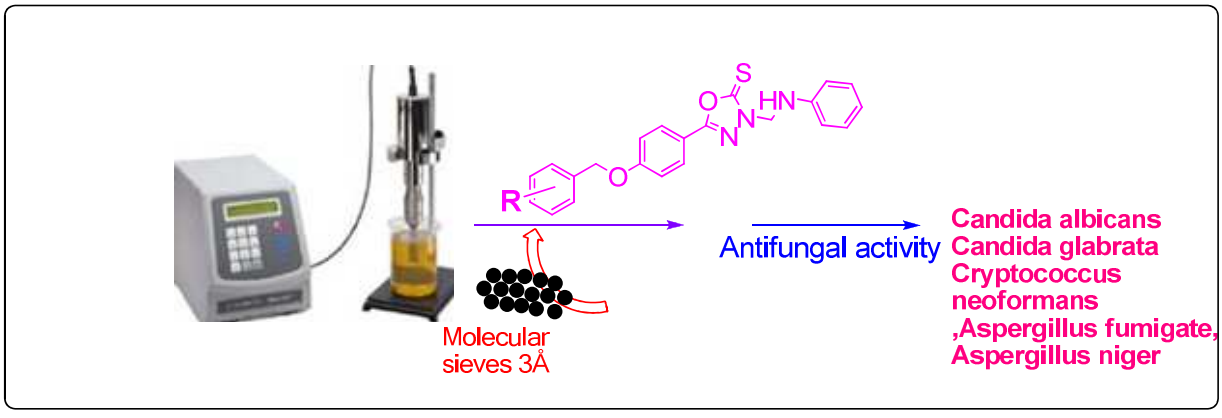


5-(4-(Benzyloxy)substituted phenyl)-3-((phenylamino)methyl)-1,3,4-oxadiazole-2(3H)-thione as antifungal agent.

Abstract:

In the category of microorganism, fungi are considered as the special class of microbes responsible for opportunistic pathogenic infections in humans species. Due to the side effects of commercially available antifungal drugs and the emergence of new drug resistant fungal species in the past few years, has forced the researchers to search for novel and efficient antifungal drug molecules. The 1,3,4-oxadiazoles scaffold is associated with diverse biological activities. The multipurpose use of the Mannich bases in pharmaceutical chemistry promote us to prepare a new series of 5-(4-(benzyloxy)substituted phenyl)-3-((phenyl amino)methyl)-1,3,4-oxadiazole- 2(3H)-thione as antifungal agents. Herein, we report molecular sieves and ultrasound assisted synthesis of novel series of Mannich bases of the 5-substituted 1,3,4-oxadiazole-2-thiones by amino methylation with paraformaldehyde and substituted primary / secondary amines and their evaluation for antifungal activity .The structures of the newly synthesized compounds were determined by IR, NMR and Mass spectral study. The synthesized compounds exhibited interesting moderate to excellent antifungal activity against *Candida albicans* (NCIM 3557),*Candida albicans*(NCIM3471), *Candida glabrata*(NCIM3237), *Cryptococcus neoformans* (NCIM 3542),*Cryptococcus neoformans*(NCIM 3378),*Aspergillus fumigates*(NCIM 902), *Aspergillus niger*(NCIM 628) using Flucanazole as a standard reference drug. The synthesised compounds 6d, 6f ,6g, 6h and 6j exhibited promising antifungal activity as antifungal agents.

Key words: 1,3,4-oxadiazoles, Mannich reaction, Ultrasound –assisted, Molecular sieves, Antifungal activity.

Introduction:

In the category of microorganism, fungi are considered as the special class of microbes responsible for opportunistic pathogenic infections in plant, animals and humans species. Commercially available broad-spectrum antifungal drugs includes fluconazole, itraconazole, miconazole and voriconazole in which the mechanism of action is on target CYP51 which get inhibited and in turn switch off the biosynthesis of ergo sterols[1] .But the frequent use of these antifungal drugs in immune compromised patients who are undergoing the long term treatment of broad-spectrum antibiotics may be in cases of HIV infection, grafting surgery or anticancer therapy [2], has led to the development of resistance fungal strains. The emergence of new drug resistant fungal species in the past few years has forced the researchers around the world to search for novel and efficient antifungal drug molecules. [3,4,5]. The 1,3,4-oxadiazoles scaffold is associated with diverse biological activities such as antifungal[6a-b] antibacterial [7], anti myco bacterial [8], anti HIV [9], anti-hepatitis B virus[10] anticancer [11] anticonvulsant [12], anti-inflammatory [13], anti malarial [14] analgesic [15] etc. 5-Substituted-1,3,4-oxadiazole-2-thiones represent an important type of compound in the field of coordination chemistry because of their potential multifunctional donor sites, viz either exocyclic sulfur or endocyclic nitrogen[16,17] and possess CNS depressant [18]and tyrosinase inhibition [19] property. The multipurpose use of the Mannich bases in pharmaceutical chemistry [20,21] promote us to prepare a new series of 1,3,4-oxadiazole based amino methyl derivatives .

The use of ultrasound to endorse chemical reactions is called “Sonochemistry”. Ultrasonic-assisted organic synthesis is a green synthetic approach and it is a powerful technique towards the increase in reaction rate and yield [22, 23]. It offers the potential reaction in small time cycles, cheaper reagents and milder physical conditions [24, 25]. It can also be considered as important tool for conservation of energy and minimization of waste as compared to the conventional techniques [26].Herein, we introduced ultrasound- promoted Mannich reaction of the 5-substituted 1,3,4-oxadiazole-2-thiones with primary and secondary amines and its antifungal screening because of the structural resemblance with established antifungal molecule triazoles. As the majority of fungal infections are caused by Candida, Yeast, Cryptococcus Aspergillus species, [27, 28]. So we have screened the synthesized compound against the selected strains of these species.

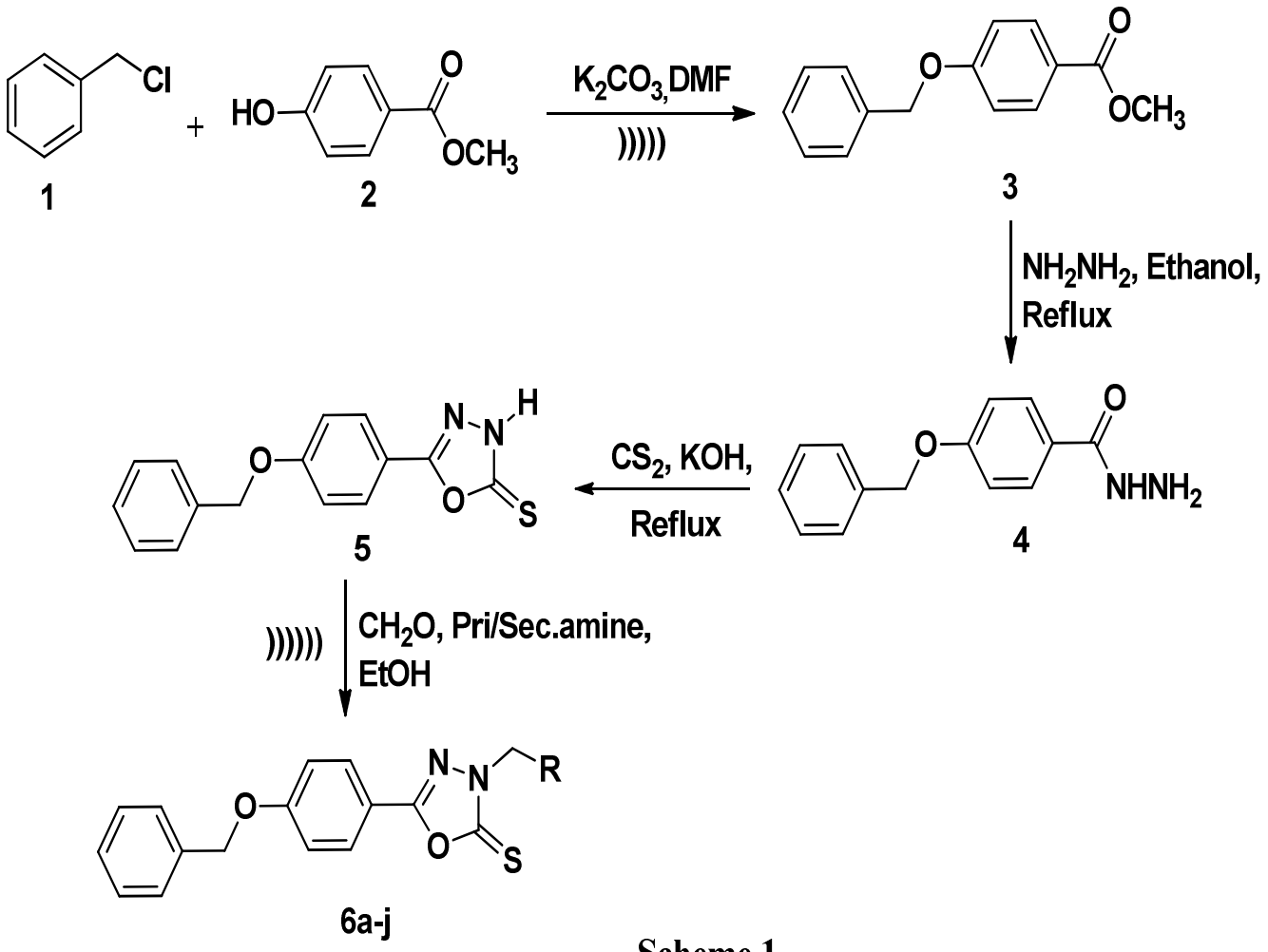
Result and Discussion:

We herein report the synthesis and antifungal evaluation of some novel structural scaffolds (6a-o) as illustrated in **Scheme 1**.

The starting material methyl 4-(benzyloxy) benzoate (3) was synthesized by reaction of methyl4-hydroxybenzoate and chloromethyl benzene in K_2CO_3 and DMF as solvent in ultrasonic processor up to 4hr. The compound Methyl 4-(benzyloxy) benzoate obtained with good yield in step I, is refluxed with NH_2NH_2 to get 4-(benzyloxy) benzohydrazide (4).

The reaction of the above acid hydrazides (4) with carbon disulphide under basic conditions using KOH yielded 1, 3, 4-oxadiazol-2-thiones (5), which underwent N-amino methylation by the Mannich reaction in ultrasonic processor and various substituted primary and some secondary amines in presence of paraformaldehyde.

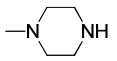
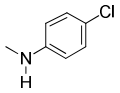
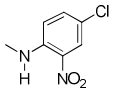
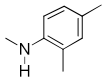
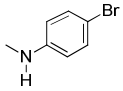
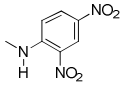
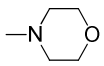
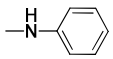
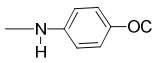
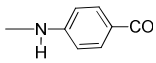
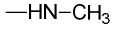
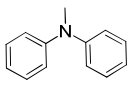
The structure of intermediate 1, 3, 4 oxadiazole-2-thiones (5) was confirmed by its spectroscopic analysis. The IR spectrum showed a weak SH stretching absorption at 2592 cm^{-1}. The absorption bands due to -C=N was observed at 1681 cm^{-1}. A broad downfield signal observed at d 11.8 in the ^{1}H NMR spectrum was assigned to NH/SH tautomeric proton. In ^{13}C NMR spectrum, -C=S carbon appeared at δ 177.13 in addition to other characteristic signals of remaining carbon atoms. The HRMS mass spectrum showed (M+1) molecular ion peak at m/z 285 in agreement with its molecular formula, $C_{15}H_{13}O_2N_2S$.In the IR spectrum of compound **6j**, the aromatic C-H stretching vibration was observed at 3047 cm^{-1}. The absorption band due to CH_2 group of morpholine moiety was seen at 2916/2850 cm^{-1}. The C=N and C=S moieties showed their characteristic absorption bands at 1681 and 1356 cm^{-1}, respectively. The ^{1}H NMR spectrum showed a sharp singlet at δ 5.02 for N-CH_2-N protons. Eight protons of morpholine moiety resonated as two triplets at δ 2.75 and δ 3.62 with J ¼ 6.80 Hz. In ^{13}C NMR spectrum, the signals observed at δ 67.91 and 70.12 were assigned to C2, C6 and C3, C5 of morpholine ring respectively. The signal due to aryloxy methylene carbon appeared at δ 68.23. The aryloxy methine carbon atom resonated at δ, 156.69, while other aromatic carbon atoms appeared at δ 115.47, 119.51, 125.68, 128.17, 128.43, 128.61, 129.00, 131.97 and135.64. Further, the structure of **6j** was also confirmed by recording its mass spectrum, which showed a molecular ion peak at m/z 383. The observed molecular mass is in agreement with the assigned molecular formula, $C_{20}H_{21}N_3O_3S$. Similarly, compound **6h** shows molecular ion peak at 457, M+1 peak at 458 and M+2 peak at 459.



Scheme 1

Table 1.Characterization data of Mannich bases (6a-o).

Derivatives	R	(Mol. wt)	Yield (%)	m.p. (^{o}C)	Analysis (%) Found (calculated)

					C H N
a		382.48	80	205	62.80 5.80 14.65 (62.76)(5.74)(14.62)
b		423.92	81	180	62.33 4.28 9.91 (62.28)(4.22)(9.88)
c		365.41	78	235	59.16 4.14 19.17 (59.11)(4.10)(19.11)
d		364.42	79	240	62.62 4.43 15.37 (62.58)(4.40)(15.34)
e		468.91	81	218	56.35 3.65 11.95 (56.30)(3.61)(11.92)
f		417.52	79	230	69.04 5.55 10.06 (69.00)(5.51)(10.02)
g		468.37	81	228	56.42 3.87 8.97 (56.38)(3.84)(8.93)
h		434.47	90	220	60.82 4.18 12.90 (60.78)(4.13)(12.86)
i		479.47	82	145	55.11 3.57 14.61 (55.08)(3.52)(14.57)
j		383.46	85	200	62.64 5.52 10.96 (62.61)(5.48)(10.92)
k		389.47	80	80	67.84 4.92 10.79 (67.79)(4.88)(10.74)
l		419.13	81	100	65.85 5.05 10.02 (65.81)(5.00)(10.00)
m		433.48	79	140	63.73 4.42 9.69 (63.67)(4.39)(9.62)
n		327.40	78	110	62.36 5.23 2.83 (62.31)(5.19)(2.79)
o		465.57	79	115	72.23 4.98 9.03 (72.20)(4.92)(9.00)

Biological evaluation

In vitro antifungal susceptibility testing was performed by broth micro dilution method according to the Clinical and Laboratory Standards Institute (CLSI) to find out IC_{50} concentration and

minimum inhibitory concentration (MIC_{90}) of the compounds. The activities of compounds were tested against human pathogens *Candida albicans* NCIM 3557, *Candida albicans* NCIM3471, *Candida glabrata* NCIM 3237, *Cryptococcus neoformans* NCIM 3542,*Cryptococcus neoformans* NCIM 3378,*Aspergillus fumigates* NCIM 902, *Aspergillus niger* NCIM 628.

- Among the synthesised compounds **6g**(4µg/ml),6d(6µg/ml),**6j**(8µg/ml),**6c**(11.2µg/ml),**6h**(16µg/ml),**6b**(24.2µg/ml),**6i**(24.6µg/ml),**6e**(25.8µg/ml) & **6k**(35.1µg/ml) give **excellent** activity against the strain *Aspergillus fumigatus* (NCIM 902) ,standard drug fluconazole (64 µg/ml).

- Similarly compounds **6h**(18.61µg/ml),**6k**(22.9µg/ml),**6b**(24.1µg/ml) & **6a**(27.7µg/ml) gives **excellent** activity against the *Aspergillus niger*.(NCIM 628),whereas 6e(47µg/ml) give good activity against the same strain, standard drug fluconazole (46 µg/ml).

- Compound **6f** (5.4 µg/ml) gives **excellent** activity against *Candida glabrata*(NCIM 3237) and **6e**(24.5µg/ml)shows **good** activity.Fluconazole is used as a standard drug (9.4 µg/ml).

- Other synthesized compounds have shown good to moderate activity for the remaining strain of fungi.

Table 2. Antifungal activity (IC_{50}) of compounds 6(a-o)

Entry	*Candida*	*Candida*	*Candida*	*Cryptococc*	*Cryptococcu*	*Aspergillusfu*	*Aspergillusn*

	albicans (NCIM 3557)	*albicans*(NCIM 3471)	*glabrata*(NCIM 3237)	*us neoformans* (NCIM 3542)	*s neoformans* (NCIM 3378)	*migatus*(NCIM 902)	*iger*. (NCIM 628)
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
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

Table 3. **Antifungal activity (MICs) of compounds 6(a-o)**

Entry	*Candida albicans*(NCIM 3557)	*Candida albicans*(NCIM 3471)	*Candida glabrata*(NCIM 3237)	*Cryptococcus neoformans* (NCIM 3542)	*Cryptococcus neoformans* (NCIM 3378)	*Aspergillusfumigatus*(NCIM 902)	*Aspergillusniger*. (NCIM 628)
6a	>256	>64	64	>256	>256	>256	>**64**
6b	>256	128	128	>256	>256	**128**	>256
6c	128	128	128	>256	>256	**128**	>256
6d	>256	>256	>128	>256	>256	256	>256
6e	64	64	64	256	>256	**128**	256
6f	64	256	**8**	>256	>256	256	256
6g	128	256	256	256	>256	256	256
6h	128	>256	256	>256	>256	256	>256
6i	**32**	128	64	256	256	256	**128**
6j	128	256	256	256	>256	>256	256
6k	>256	>256	>256	>256	>256	>256	>256
6l	>256	>256	>256	>256	>256	>256	>256
6m	>256	>256	>256	>256	>256	>256	>256
6n	>256	>256	>256	>256	>256	>256	>256
6o	128	**32**	128	**64**	>256	**128**	**128**
Fluconazole	**0.25**	**0.25**	**16**	**32**	**8**	**>256**	**>256**

Experimental section:

a)General procedure for the synthesis of 4-(benzyloxy)benzoate(3):

For the synthesis of methyl-4-(benzyloxy) benzoate, methyl-4-hydroxybenzoate and chloromethyl benzene were taken in equal ratio (0.01mol). in N,N- dimethyl formamide (DMF) as solvent and reaction is carried out in K_2CO_3 ultrasonic processor up to 4hr. After that, the solution was poured into ice-water. The precipitate was filtered and recrystallized from ethanol. Colour: White M.P.105^0C

b) General procedure for the synthesis of 4-(benzyloxy) benzohydrazide (4):

For the synthesis of substituted benzo hydrazines, a mixture of corresponding esters (20 mmol), 85% hydrazine hydrate (20 mmol) in ethanol (35 ml) was heated to reflux for 6 h. After that, the solution was poured into ice-water. The precipitate was filtered and recrystallized from ethanol. Colour: White M.P.80^0C

c) General procedure for the synthesis of 5-phenyl-1,3,4-oxadiazole-2-thione:

Equimolar quantities of the substituted benzo hydrazine (5 mmol) and potassium hydroxide (5 mmol) were dissolved in 20 mL of 95% ethanol. The mixture was allowed to stir for several minutes at room temperature and then carbon disulfide (15 mmol) was slowly added drop wise 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 values of the solution to 5-6. Then the precipitate was collected washed with water for several times and dried and recrystallized from ethanol. Colour: White M.P.160^0C.

d)General procedure for the synthesis of Mannich Base Derivatives of 1,3,4-Oxadiazole-2-thiones (6a-o):

1,3,4-Oxadiazole-2-thiones (10 mmol) was dissolved in methanol, para formaldehyde (15mmol) and primary/secondary amine (10 mmol) in methanol were added with constant stirring. The resulting mixture was kept in ultrasonic processor for 2-4hrs. The precipitated solids were filtered, washed with water and recrystallized from methanol to yield the title compounds 6a-o (Table 1).

The physical characterization and spectral data of synthesized derivatives is as follows:

5-(4-(benzyloxy) phenyl)-3-(((4-chlorophenyl)amino)methyl)-1,3,4-oxadiazole-2(3H)-thione:

Recrystallization from methanol; yield: 81%; m p: 180^0C; IR (KBr), mmax/cm^{-1}: 3235 (NH), 1621 (C=N), 1596 (C-C), 1425 (C=S), 1258 and 1093 (C–O–C), 820, 744; ^{1}H NMR (CDCl3), δ

ppm: 4.0 (S,1H, -NH), 4.42(S,2H, N-CH_2-N-) 5.16 (S,2H, -OCH_2), 6.54–8.02 (m, 13H, aromatic rings).

5-(4-(benzyloxy)phenyl)-3-(((4-chloro-2-nitrophenyl)amino)methyl)-1,3,4-oxadiazole-2(3H)-thione:

Recrystallization from methanol; yield: 81%; mp: 218^0C; IR (KBr), mmax/cm^{-1}: 3545–3245 (OH and NH), 1620 (C=N), 1592 (C-C), 1524 and 1343 (NO2), 1427 (C=S), 1261 and 1088 (C–O–C),864, 755; ^{1}H NMR (DMSO-d6), δppm: 4.0 (S,1H, -NH), 4.42(S,2H, N-CH_2-N-) 5.16 (S,2H, -OCH_2), 7.06–8.13 (m, 12H, aromatic rings).

5-(4-(benzyloxy)phenyl)-3-(((2,4-dimethylphenyl)amino)methyl)-1,3,4-oxadiazole-2(3H)-thione:

Recrystallization from methanol; yield: 79%; mp: 230^0C;) IR (KBr), mmax/cm^{-1}3230 (NH), 1616 (C=N), 1595 (C-C), 1427 (C=S),1252 and 1151 (C–O–C), 805, 753; ^{1}H NMR (CDCl3),δppm: 4.0 (S,1H, -NH), 4.42(S,2H, N-CH_2-N-) 5.16 (S,2H, -OCH_2), 6.36–8.02 (m, 12H, aromatic rings), 2.30 (s, 3H,-CH_3), 2.10 (s, 3H,-CH_3)

5-(4-(benzyloxy)phenyl)-3-(((4-bromophenyl)amino)methyl)-1,3,4-oxadiazole-2(3H)-thione:

Recrystallization from methanol; yield: 81%; mp: 228^0C; IR (KBr), mmax/cm^{-1}: 3235 (NH), 1621 (C=N), 1593 (C-C), 1424 (C=S),1260 and 1123 (C–O–C), 815, 740; ^{1}H NMR (DMSOd6),δppm: 4.0 (S,1H, -NH), 4.42(S,2H, N-CH_2-N-) 5.16 (S,2H, -OCH_2), 6.59–8.02 (m, 13H, aromatic rings)

5-(4-(benzyloxy)phenyl)-3-(((2-nitrophenyl)amino)methyl)-1,3,4-oxadiazole-2(3H)-thione:

Recrystallization from methanol; yield: 90%; mp: 220^0C; IR (KBr), mmax/cm^{-1}: 3245 (NH), 1622 (C=N), 1590 (C-C), 1522 and 1345 (NO2), 1427 (C=S), 1261 and 1088 (C–O–C),864, 755; ^{1}H NMR (DMSO-d6), δ ppm: 4.0 (S,1H, -NH), 4.42(S,2H, N-CH_2-N-) 5.16 (S,2H, -OCH_2), 6.06-8.13 (m, 13H, aromatic rings).

5-(4-(benzyloxy)phenyl)-3-(((2,4-dinitrophenyl)amino)methyl)-1,3,4-oxadiazole-2(3H)-thione:

Recrystallization from methanol; yield: 82%; mp: 145^0C; IR (KBr), mmax/cm^{-1}: 3247(NH), 1621 (C=N), 1593 (C-C), 1524 and 1344 (NO2), 1426 (C=S), 1262 and 1083 (C–O–C),

864, 755; ^{1}H NMR (DMSO-d6), δ ppm: 4.0 (S,1H, -NH), 4.42(S,2H, N-CH_2-N-) 5.16 (S,2H, -OCH_2), 6.06–8.88(m, 12H, aromatic rings).

5-(4-(benzyloxy)phenyl)-3-(morpholinomethyl)-1,3,4-oxadiazole-2(3H)-thione:

Recrystallization from methanol; yield: 85%; mp: 200^{0}C;IR(KBr), mmax/cm^{-1}: 1623 (C-N), 1592 (aromatic C-C), 1437 (C=S), 1222 and 1036 (C–O–C), 741; ^{1}H NMR (CDCl3), δ ppm: 2.50 (t, 4H,N-CH_2-C), 3.65 (t, 4H, O-CH_2-C), 3.72(S,2H, N-CH_2-N-) 4.0 (S,1H, -NH), 5.16 (S,2H,-OCH_2),7.06-8.02(m,9H,aromaticrings).

5-(4-(benzyloxy)phenyl)-3-((phenylamino)methyl)-1,3,4-oxadiazole-2(3H)-thione

Recrystallization, from methanol; yield: 80%; mp: 80^{0}C. IR (KBr), mmax/cm^{-1}: 3550–3235 (NH), 1615 (C=N), 1593 (C-C), 1425 (C=S), 1250 and 1152 (C–O–C), 761, 739, 705; ^{1}H NMR (CDCl3 + DMSO-d6), δ ppm: 4.0 (S,1H, -NH), 4.42(S,2H, N-CH_2-N-) 5.16 (S,2H, -OCH_2), 6.77–8.02 (m, 14H, aromatic rings)

4-(((5-(4-(benzyloxy)phenyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl)methyl)amino)benzoic acid:

Recrystallization from methanol; yield: 79%; mp: 140^{0}C; . IR (KBr), mmax/cm$^{-1:}$

3550–3245 (NH and COOH), 1674 (C=O), 1613 (C=N), 1606 (C-C), 1431 (C=S), 1269 and 1185 (C–O–C), 856, 739; ^{1}H NMR (DMSO-d6), δ ppm:4.0 (S,1H, -NH), 4.42(S,2H, N-CH_2-N-) 5.16 (S,2H, -OCH_2), 6.8–8.02 (m, 13H, aromatic rings), 10.50 (s, 1H, -COOH).

Antifungal activity:

Stock solution of each derivatives were prepared in DMSO at concentration of 12800 µg/ml. Compound stocks were serially diluted two fold in micro titer plate and 4µl of this is used for assay to get a final concentration in the range of 256-2 µg/ml. Spores of the filamentous fungi (~2x10^4 spores/ml) and yeast cells freshly grown in YPG broth in logarithmic phase (~2x10^{3}cfu/ml) were suspended in the RPMI 1640 medium and 196 µl from these were inoculated in the wells of the plate. The micro titer plate were incubated for 24-48hr. Growth was checked by visual observation and measuring absorbance at 600 nm using micro titer plate reader. The IC_{50} was defined as the concentration exhibiting 50% inhibition of the growth as compared to the growth of control. Whereas Minimum Inhibitory Concentration MIC_{90} was the concentration causing>90% inhibition of the growth as compared to the growth of control.

Conclusion:

Fifteen novel derivatives of **5-(4-(Benzyloxy)substituted phenyl)-3-((phenylamino)methyl)-1,3,4-oxadiazole-2(3H)-thione** were successfully synthesized under ultrasound irradiation giving better yield of 75-95% and in shorter duration of 2-4 hrs in contrast to conventional reactions which requires refluxing of 10-12 hrs. The structures of all the compounds were confirmed by recording their ^{1}H NMR, ^{13}C NMR, Mass and IR spectra. All the newly synthesized compounds were screened for their in vitro antifungal properties and were found to be fungi-static. Among the screened samples, nearly nine derivatives exhibited excellent antifungal activity against ***Aspergillus fumigatus*** (NCIM 902), fluconazole used as standard drug, (64 µg/ml).The excellent antifungal activity exhibited by the following compounds may be due to the presence of various electron withdrawing groups. All the mentioned derivatives give excellent activity, such as **6g**(4µg/ml) having bromine at para position of phenyl act as strong withdrawing groups is found to be the most potent, **6d**(6µg/ml) having imidazole, **6j**(8µg/ml) morpholine, **6c**(11.2µg/ml) having triazole, **6h**(16µg/ml) haqving nitro at para position of phenyl and **6b(**24.2µg/ml) having para substituted chlorophenyl, **6i**(24.6µg/ml) show two nitro phenyl group at ortho and para position, **6e**(25.8µg/ml) ortho nitro and para chloro phenyl and **6k**(35.1µg/ml) having un substituted phenyl. Other compounds which exhibited excellent antifungal activity **6h**(18.61µg/ml),6k(22.9µg/ml),**6b**(24.1µg/ml) & **6a(**27.7µg/ml) having piperazine as a substituent, **6e**(47µg/ml) against ***Aspergillus niger***.(NCIM 628) and fluconazole was used as a standard drug (46 µg/ml). Some compounds exhibited excellent to good activity against ***Candida globrata***(NCIM3237) when compared with the same standard drug fluconazole (9.4 µg/ml) such as **6f** (5.4 µg/ml) ortho and para di substituted toluyl; **6e**(24.5µg/ml)shows good activity .

With these excellent result, here we can conclude that **5-(4-(Benzyloxy)substituted phenyl)-3-((phenylamino)methyl)-1,3,4-oxadiazole- 2(3H)-thione** derivatives can act as a potent fungi static scaffold to develop newer drugs possessing antifungal activity. The final derivatives possessing electron withdrawing groups on phenyl ring at position 3 of nitrogen of 1,3,4-Oxadiazole-2-thiones ring are acting as more potent compounds and exhibit excellent antifungal activity .

Acknowledgements:

The authors are thankful to the Mrs. Fatima Rafiq Zakaria Chairman Maulana Azad Educational Trust, Dr. Maqdoom Farooqui, Principal Maulana Azad Postgraduate & Research Cenre, Aurangabad and Dr. Zahid Zaheer, Principal, Y.B. Chavan College of Pharmacy, Dr. Rafiq Zakaria Campus, Aurangabad 431 001 (M.S.), India for providing the laboratory facility. The authors are thankful to the Dr. M. Deshpande National Chemical Laboratory, Pune for cooperation in antifungal screening.

References:

[1] A.G. Nikalje , M. S. Ghodke, F. A. K. Khan, J. N. Sangshetti Chinese Chemical Letters 26 (2015) 108–112

[2] D.P. Kontoyiannis, Lancet 359 (2002) 1135-1144.

[3] J.H. Block, J.M. Beale (Eds.), Wilson and Griswold's Textbook of Organic Medicinal and Pharmaceutical Chemistry, 11th ed., Lippincott's Williams & Wilkins Publication, 2004, p. 240

[4] D.L. Hawksworth, Mycol. Res. 105 (2001) 1422-1432.

[5] J. Morschhauser, Fungal Genet. Biol. 47 (2010) 94-106.

[6] **a**Liu, F.; Luo, X. Q.; Song, B. A.; Bhadury, P. S.; Yang, S.; Jin, L. H.; Xue, W.; Hu, D. Y. Bioorg. Med. Chem. Lett. 2008, 16, 3632.6

bChen, C. J.; Song, B. A.; Yang, S.; Xu, G. F.; Bhadury, P. S.; Jin, L. H.; Hu, D. Y.; Li, Q. Z.; Liu, F.; Xue, W.; Lu, P.; Chen, Z. Bioorg. Med. Chem. Lett. 2007, 15, 3981.

[7] Jin, L.; Chen, J.; Song, B.; Chen, Z.; Yang, S.; Li, Q.; Hu, D.; Xu, R. Bioorg. Med. Chem. Lett. 2006, 16, 5036.

[8] F. Macaev, G. Rusu, S. Pogrebnoi, A. Gudima, E. Stingaci, L. Vlad, N. Shvets, F. Kandemirli, A.Dimoglo, R. Reynolds, Bioorg. Med. Chem. 13(2005) 4842-4850.

[9] S.G. Kucukguzel, E.E. Oruc, S. Rollas, F. Sahin,A. Ozbek, Eur. J. Med. Chem. 37 (3) (2002) 197-206.

[10] T.M.C. Tan , Y. Chen, K. H. Kong, Jing Bai, Y. Li,S.G. Lim, T. H.Ang, Y. Lam Antiviral Research 71 (2006) 7–14

[11] J.J. Bhat, B.R. Shah, H.P. Shah, P.B. Trivedi,N.K. Undavia, N.C. Desai, Indian J. Chem. 33B(1994) 189-192.

[12] A. Zarghi, A. Sayyed, M. Tabatabai, A. Faizi, P.Ahadian, V. Navabi, A. Zanganeh, Shafiee, Bioorg. Med. Chem. Lett. 15 (2005) 1863-1865.

[13] E. Palaska, G. Sahin, P. Kelicen, N.T. Durlu, G.Altinok, Il Farmaco 57 (2002) 101-107.

[14] P.R. Kagthara, N.S. Shah, R.K. Doshi, H.H.Parekh, Indian J. Chem. 38B (1999) 572-576.

[15] M. Amir, K. Shikha, Eur. J. Med. Chem. 39(2004) 535-545.

[16]. Singh M, Pandey A K, Butcher R J and Singh N K, Polyhedron, 2009, 28(3), 461-466;

[17] Pandey O.P, Sengupta S K and Tripathy S C, Bull Chem Soc Jpn., 1985, 58, 2395-2398.

[18] N. Soni, J.P. Barthwal, A.K. Saxena, K.P.Bhargava, S.S. Parmar, J. Heterocycl. Chem. 19 (2009) 29-32.

[19] S. Yamamoto, Jpn. Patent (1993), 05124948.Chem. Abstr. 119 (1993) 146370.

[20] A. Sasse, X. Ligneau, B. Sadek, S. Elz, H. Pertz,C.R. Ganelin, J.M. Arrang, J.C. Schwartz, W.Schunack, Arch. Pharm. Med. Chem., 334(2001) 45.

[21] A. Mitsch, P. Wibner, I. Sattler, M. Schlitzer, Arch. Pharm. Med. Chem. 334 (2001) 40.

[22] Lindley J, Lorimer JP, Mason TJ..Ultrasonics, 1986; 24: 292–293.

[23] Bang K, Lee K, Park YK, Lee PH. Bull Korean Chem Soc, 2002; 23: 1272–1276 Pharm. Med. Chem. 334 (2001) 40.

[24] Park SY, Han HJ, Yoo B, Choi K, Yoon HJ, Yoo MC, Woo B. Bull Korean Chem Soc, 2005; 26: 878–879.

[25] Suslick KS. Sonochemistry, Science 1990; 247: 1439-1445.

[26] Saleh TS, Abd EL-Rahman NM. Ultrason Sonochem, 2009; 16: 237–242

[27] D.P. Kontoyiannis, Lancet 359 (2002) 1135-1144.

[28] F.C. Odds, A. Brown, N. Gow, Trends Microbiol. 11 (2003) 272-279.