

Synthesis and Evaluation of Some Novel Substituted 1, 3, 4-Oxadiazole and Pyrazole Derivatives for Antifungal Activity

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Abstract: A series of 1,3,4-oxadiazole and pyrazole derivatives have been synthesized and evaluated for antifungal activity. The structures of synthesized compounds were confirmed by IR, ¹H NMR, Mass and CHN analysis. These compounds have shown promising antifungal activity when compared with the standard drug griseofulvin by cup-plate agar diffusion method using sabouraud-dextrose agar.

INTRODUCTION

It was observed from the literature that certain five membered heterocyclic compounds possess interesting biological activities. Among them the compounds bearing 1,3,4-oxadiazole and pyrazole nucleus have wide applications in medicinal chemistry. These compounds also have been reported to have significant antifungal activity.^[1, 2] Inspired from these observations, we planned to synthesize some 1, 3, 4-oxadiazole and pyrazole derivatives.

MATERIALS AND METHODS

Methyl Salicylate, Hydrazine Hydrate, Carbon Disulfide, Phosphorus Oxychloride, Ethylacetoacetate and other compounds were procured from Merck Lab, Mumbai. Melting points were determined in open capillary method and are uncorrected. The compounds were routinely checked for their purity by TLC on silica gel G. The IR spectra were recorded on Thermo Nicolet IR 200 spectrophotometer using KBr disc method. The ¹H-NMR spectra were recorded on sophisticated multinuclear FT-NMR Spectrometer model Avance-II (Bruker) using dimethylsulfoxide-*d*₆ as solvent and tetramethylsilane as internal standard.

Synthesis of 2-hydroxy benzohydrazide (I)

A mixture of 0.1 mole (15.2 ml) methyl salicylate and 10 ml hydrazine hydrate were refluxed in 50 ml of 95% abs ethanol for 15 hrs. The resultant mixture was concentrated, cooled and poured in crushed ice. The solid mass thus separated out was filtered, dried and recrystallized from ethanol.^[3] Yield: 77%, m.p.-142-44° and Rf value: 0.49.

Synthesis of 5-(2-hydroxyphenyl)-2-mercapto-1,3,4-oxadiazole (II)

A mixture of 0.01 mole (1.52 gm) of 2-hydroxy benzohydrazide I, 0.01 mole (0.56 gm) of potassium hydroxide and 10 ml of carbon disulfide were refluxed in 50 ml of 95% abs ethanol for 12 hrs. The resultant mixture was concentrated and cooled at room temperature. Then it

was acidified with dil. HCl. The solid mass thus separated out was filtered, dried and recrystallized from ethanol.^[4] Yield: 63%, m.p.- 186-88° and Rf value: 0.56. IR (KBr disc) cm⁻¹: 3085.23(O-H Str.), 2890.30 (Ar C-H Str), 1629.08(C=N Str.) 1056.17(C-O-C Str.), 2736(C-SH); ¹H NMR (DMSO-*d*₆) δ ppm: 6.92-7.68 (m, 8H, Ar. CH), 8.03 (s, 1H, SH) 10.00(s, 1H, OH)

Synthesis of 5-(2-hydroxyphenyl)-2-(pyridinylthio)-1,3,4-oxadiazole (A₁)

A mixture of 0.005 mole (0.97 gm) of 5-(2-hydroxy phenyl)-2-mercapto-1,3,4-oxadiazole II and 0.005 mole (0.56 gm) of 2-chloro pyridine were refluxed in 25 ml of 95% abs ethanol for 2 hrs. The resultant solution was concentrated. The solid mass thus separated out was filtered, dried and recrystallized from ethanol. The compounds A₂ and A₃ were synthesized following a similar procedure.

Synthesis of 5-(2-hydroxyphenyl)-2-phenyl-1,3,4-oxadiazole (A₄)

A mixture of 0.01 mole (1.52 gm) 2-hydroxy benzohydrazide I and 0.01 mole (1.22 gm) of benzoic acid was dissolved in phosphorus oxychloride and refluxed for 18-22 hrs. The reaction mixture was slowly poured over crushed ice and kept overnight. The solid mass thus separated out was filtered, dried and recrystallized from ethanol.^[5] The compounds A₅ and A₆ were synthesized following similar procedure.

Synthesis of 1-(2-hydroxybenzoyl)-3-methyl-1H-pyrazol-5(4H)-one (III)

A mixture of 0.01 mole (1.52 gm) of 2-hydroxy benzohydrazide I and 0.1 mole (13 mL) of ethylacetoacetate were heated on water bath for 2 hrs and was stirred from time to time with glass rod. The resultant heavy reddish syrup was allowed to cool. It was washed thoroughly with ether to remove coloured impurities. The solid thus separated out was filtered, dried and recrystallized from ethanol.^[6] Yield: 75%, m.p.- 118-20° and Rf value: 0.63. IR (KBr disc) cm⁻¹: 3099.40 (O-H Str.), 3011.05 (Ar C-H Str), 3396.02 (N-H Str.), 1614.87(C=N Str.) 1698.30(C=O Str.)

Synthesis of N-((1-(2-hydroxybenzoyl)-3-methyl-5-oxo-4, 5 - dihydro - 1H - pyrazol - 4 - yl) methyl) isoncotinohydrazide (A₇)

A mixture of 0.005 mole (1.09 gm) of 1-(2-hydroxybenzoyl)-3-methyl-1H-pyrazol-5(4H)-one III, 5 ml

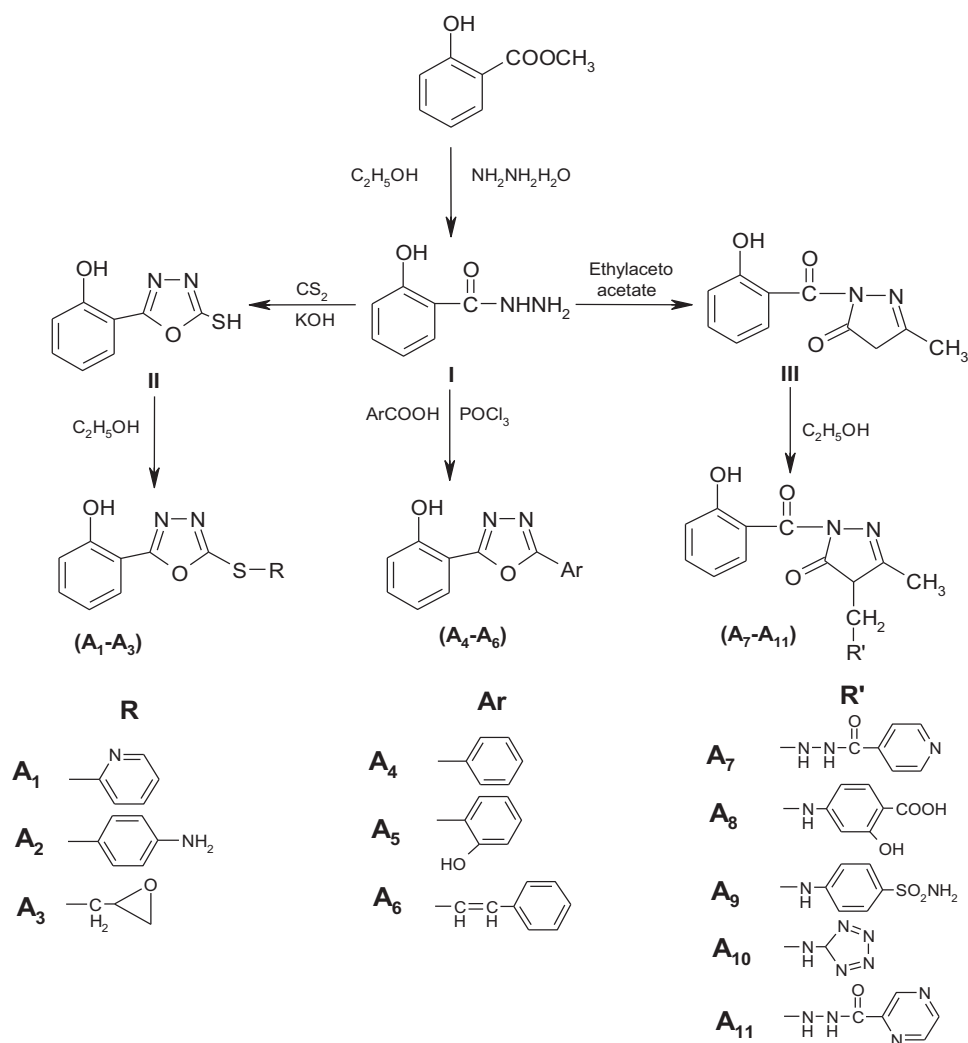
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Scheme 1: Synthesis scheme

of formaldehyde and 0.005 mole (0.68 gm) of isonicotic acid hydrazide were refluxed with 25 ml of 95% abs ethanol for 2 hrs. The resultant mixture was concentrated. The resultant solid mass was dried and recrystallized from ethanol. The compounds A₈-A₁₁ were synthesized following similar procedure.

Spectral Analysis

The compounds were synthesized as per the Scheme-I, where 2-mercapto-1,3,4-oxadiazole derivatives were synthesized by reacting salicylic acid hydrazide with carbon disulfide followed by condensation reaction. 5-(substituted aryl)-1,3,4-oxadiazole derivatives were synthesized by reacting salicylic acid hydrazide with aromatic acid. 3-methyl-pyrazol-5(4*H*)-one derivatives were synthesized by reacting salicylic acid hydrazide with ethyl acetoacetate followed by mannich reaction. The structures of the synthesized compounds were confirmed by IR, NMR, Mass and CHN analysis.

IR, NMR and Mass Spectral Data

A₁: IR (KBr disc) cm^{-1} : 3196.08(O-H Str.), 2970.70(Ar C-H Str), 1611.04(C=N Str.) 1041.47(C-O-C Str.), 688.01(C-S-C Str.)

$^1\text{H NMR}$ (DMSO- d_6) δ ppm: 6.96-7.73(m, 8H, Ar. CH), 9.25(s, 1H, OH) $m/z = 272$ [M+H]⁺

A₂: IR (KBr disc) cm^{-1} : 3157.08(O-H Str.), 3040.70 (Ar C-H Str), 3397.08(N-H Str.), 1621.39 (C=N Str.) 1057.68 (C-O-C Str.), 688.01 (C-S-C Str.)

$^1\text{H NMR}$ (DMSO- d_6) δ ppm: 7.23-7.68(m, 8H, Ar. CH), 4.6 (s, 2H, NH₂), 9.15(s, 1H, OH) $m/z = 286$ [M+H]⁺

A₃: IR (KBr disc) cm^{-1} : 3198.91 (O-H Str.), 2975.11 (Ar C-H Str), 1611.33 (C=N Str.) 1042.13 (C-O-C Str.), 687.50 (C-S-C Str.)

$^1\text{H NMR}$ (DMSO- d_6) δ ppm: 2.32-2.56(m, 4H, C-H) 7.14-7.77(m, 4H, Ar. CH), 9.81(s, 1H, OH) $m/z = 286$ [M+H]⁺

A₄: IR (KBr disc) cm^{-1} : 3201.40 (O-H Str.), 3059.90 (Ar C-H Str), 1623.70 (C=N Str.) 1069.10 (C-O-C Str.)

$^1\text{H NMR}$ (DMSO- d_6) δ ppm: 7.18-7.83(m, 9H, Ar. CH), 9.28(s, 1H, OH) 239 [M+H]⁺

A₅: IR (KBr disc) cm^{-1} : 3070.69 (O-H Str.), 2923.94 (Ar C-H Str), 1613.52 (C=N Str.) 1005.83 (C-O-C Str.)

$^1\text{H NMR}$ (DMSO- d_6) δ ppm: 6.96-7.71 (m, 8H, Ar. CH), 10.45 (s, 2H, OH) $m/z = 255$ [M+H]⁺

A₆: IR (KBr disc) cm^{-1} : 3058.90 (O-H Str.), 2927.10 (Ar C-H Str), 1632.30 (C=N Str.) 1080.80 (C-O-C Str.)

$^1\text{H NMR}$ (DMSO- d_6) δ ppm: 6.89-7.69 (m, 9H, Ar. CH), 9.87 (s, 1H, OH) $m/z = 265$ [M+H]⁺

Table 1: Analytical Data of the Synthesized Compounds (A1-A11)

Comp.	Mol. Formula	Mol. Wt.	M.P. °C	Yield %	Elemental Analyses Calcd. (Found)		
					C	H	N
A1	C ₁₃ H ₉ N ₃ O ₂ S	271	183-85	61	57.55 (57.37)	3.34 (3.22)	15.49 (15.65)
A2	C ₁₄ H ₁₁ N ₃ O ₂ S	285	89-90	68	58.93	3.89	14.73
A3	C ₁₁ H ₁₀ N ₂ O ₃ S	250	197-99	67	52.79 (52.55)	4.03 (4.21)	11.19 (11.28)
A4	C ₁₄ H ₁₀ N ₂ O ₂	238	136-38	73	70.58 (70.36)	4.23 (4.32)	11.76 (11.65)
A5	C ₁₄ H ₁₀ N ₂ O ₃	254	90-92	55	66.14	3.96	11.02
A6	C ₁₆ H ₁₂ N ₂ O ₂	264	86-88	65	72.72	4.58	10.60
A7	C ₁₈ H ₁₇ N ₅ O ₄	367	124-26	78	58.85 (58.69)	4.66 (4.45)	19.06 (19.17)
A8	C ₁₉ H ₁₇ N ₃ O ₆	383	315-17	80	59.53	4.47	10.96
A9	C ₁₈ H ₁₈ N ₄ O ₅ S	402	110-12	83	53.72 (53.57)	4.51 (4.63)	13.92 (13.97)
A10	C ₁₃ H ₁₃ N ₇ O ₃	315	80-82	61	49.52	4.16	31.10
A11	C ₁₇ H ₁₆ N ₆ O ₄	368	87-89	59	55.43 (55.29)	4.38 (4.52)	22.82 (22.67)

A7: IR (KBr disc) cm⁻¹: 3213.02 (O-H Str.), 3035.35 (Ar C-H Str), 3362.55(N-H Str.), 1600.32 (C=N Str.) 1660.70 (C=O Str.)

¹H NMR (DMSO-d₆) δ ppm: 6.78-8.78 (m, 8H, Ar. CH), 10.55 (s, 2H, OH), 5.76(s, 2H, NH), 7.96(s, 1H, CONH), 2.47(s, 3H, CH₃), 2.36-2.37(s, 2H, CH₂), 2.30(s, 1H, CH); m/z: 367 [M⁺]

A8: IR (KBr disc) cm⁻¹: 3205.40 (O-H Str.), 3062.00 (Ar C-H Str), 3425.80 (N-H Str.), 1608.60 (C=N Str.) 1697.90 (C=O Str.)

¹H NMR (DMSO-d₆) δ ppm: 6.86-8.94 (m, 7H, Ar. CH), 10.23 (s, 1H, OH), 5.54(s, 1H, NH), 7.78(s, 1H, CONH), 2.39(s, 3H, CH₃), 2.24-2.43(s, 2H, CH₂), 2.39(s, 1H, CH) m/z = 383[M⁺]

A9: IR (KBr disc) cm⁻¹: 3160.50 (O-H Str.), 3078.50 (Ar C-H Str), 3382.40 (N-H Str.), 1645.80 (C=N Str.) 1716.70 (C=O Str.)

¹H NMR (DMSO-d₆) δ ppm: 7.14-8.32 (m, 8H, Ar. CH), 10.72 (s, 1H, OH), 5.27(s, 2H, NH), 7.85(s, 1H, CONH), 2.20(s, 3H, CH₃), 2.24-2.79(s, 2H, CH₂), 2.35(s, 1H, CH) m/z = 403[M⁺]

A10: IR (KBr disc) cm⁻¹: 3208.33 (O-H Str.), 3048.19 (Ar C-H Str), 3453.21 (N-H Str.), 1611.33 (C=N Str.) 1687.91 (C=O Str.)

¹H NMR (DMSO-d₆) δ ppm: 7.26-8.49 (m, 4H, Ar. CH), 10.64 (s, 1H, OH), 5.28(s, 2H, NH), 7.85(s, 1H, CONH), 2.20(s, 3H, CH₃), 2.24-2.79(s, 2H, CH₂), 2.35(s, 2H, CH) m/z = 315[M⁺]

A11: IR (KBr disc) cm⁻¹: 3162.12 (O-H Str.), 2924.80 (Ar C-H Str), 3387.55(N-H Str.), 1600.71 (C=N Str.) 1682.98 (C=O Str.)

¹H NMR (DMSO-d₆) δ ppm: 6.97-8.86 (m, 8H, Ar. CH), 10.39 (s, 1H, OH), 5.23(s, 1H, NH), 7.76(s, 1H, CONH), 2.50(s, 3H, CH₃), 2.39-2.41(s, 2H, CH₂), 2.28(s, 1H, CH) m/z = 368[M⁺].

Antifungal Activity

The compounds were tested *in-vitro* for their antifungal activity against *Candida albicans* (ATCC 10231) and *Aspergillus niger* (ATCC 16404) by Cup-plate agar diffusion method using Sabouraud-Dextrose agar. [7, 8] Dextrose, Neopeptone and Agar procured from Hi Media Lab, Mumbai. Griseofulvin was gifted by Torrent research lab, Ahmedabad.

Sabouraud-Dextrose Agar prepared by 40 gm Dextrose, 10 gm Neopeptone and 15 gm Agar. All these components were dissolved in distilled water (1000 ml) and pH was adjusted to 5.5 – 6.0. This solution was sterilized by autoclaving at 121°C for 10 min.

One day prior to these testing, inoculations of the above fungal cultures were made in the Sabouraud-Dextrose agar and then incubated at 37°C for 18-24 hrs. A suspension of cell from this culture was made in sterile distilled water. Five colonies of >1 mm diameter were mixed with 5 ml of normal saline and vortexed for 15 sec.

Each test compound (5 mg) was dissolved in 5 ml of dimethyl formamide to give stock solution of concentration 1000 mcg/ml. Then 0.1 ml of this solution was used for testing. Standard drug griseofulvin was used with concentration of 100 mcg/ml.

Sabouraud-Dextrose agar plates were prepared by pouring 15-20 ml of the medium into each sterilized petridish and were allowed to set at room temperature. The cell suspension was standardized to a density of 530 nm using a spectrophotometer and was inoculated over the surface of medium using a sterile cotton swab. Three cups were scooped in each plate using a sterile cork borer of 6mm diameter, corresponding to control, standard and test solution. The solution of each test compound (0.10 ml/ 0.15 ml) was added in the cups by using micropipettes and these plates were subsequently incubated at 37°C for 48 hrs. The zone of inhibition was measured in mm for each organism.

RESULTS AND DISCUSSION

The synthesis of 1,3,4-oxadiazole and pyrazole derivatives were established based on the literature survey. Around 11 new derivatives were synthesized, with the standard chemicals and well established procedures.

The synthesized compounds were tested for their preliminary tests, physical constants. Spectral data and CHN analysis confirmed the structures of the final compounds. The proposed compounds were screened for their antifungal potential with the standard drugs in the well-equipped microbiology labs.

All the compounds were screened for antifungal activity. However compounds A₃, A₄, A₇, A₈ have showed maximum activity, while the remaining compounds have

Table 2: Antifungal Activity of the Synthesized Compounds (A₁-A₁₁)

S. No.	Compd.	Zone of Inhibition at 100 mcg/ml (in mm)	
		<i>A. niger</i>	<i>C. albicans</i>
1	A ₁	18	19
2	A ₂	20	21
3	A ₃	24	23
4	A ₄	23	24
5	A ₅	19	20
6	A ₆	12	15
7	A ₇	23	22
8	A ₈	24	25
9	A ₉	19	20
10.	A ₁₀	17	16
11.	A ₁₁	19	18
Standard	Griseofulvin	26	25

also shown moderate Antifungal activity, when compared with standard Griseofulvin against *Aspergillus niger* (ATCC) 16404 and *Candida albicans* (ATCC 10231).

CONCLUSION

We report here a group of 1,3,4-oxadiazole and pyrazole derivatives acting as antifungal agents. Among them, compound A₈ showed to be fungicides and were the most active against *Aspergillus niger* (ATCC) 16404 and *Candida albicans* (ATCC 10231) including clinical isolates. The proposed work has given out many active antifungal agents. Some of the compounds have showed moderate activities. These compounds with suitable modification can be explored better for their therapeutic activities in the future.

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