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SYNTHESIS OF NOVEL AZOLE HETEROCYCLES WITH THEIR ANTITUBERCULAR AND ANTIFUNGAL EVALUATION

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ABSTRACT

Azoles exhibits wide range of pharmacological properties. A limited number of drugs are currently available to treat microbial infections that too have serious toxic effects the existing drugs has been suffering from the development of resistance and thus newer drugs are required with better activity profile. In the present investigation, some 2, 5-disubstituted-1,3,4-oxadiazole derivatives and a triazole derivative derived from 3-hydroxy-2-naphthoic acid were synthesized and subjected to antitubercular and antifungal activity. The structures of targeted compounds were confirmed by spectroscopic techniques like ^{1}H NMR and mass spectroscopy. Compound **5b** exhibited best antifungal activity while compound **5d** was found active against mycobacterium tuberculosis $H_{37}Rv$.

Key words: 3-Hydroxy-2-naphthoic acid, 1, 3, 4-Oxadiazole, Antitubercular activity.

INTRODUCTION

Several five membered aromatic systems having three hetero atoms at symmetrical positions have been studied because of their interesting biological properties. Oxadiazole derivatives, which belong to an important group of heterocyclic compounds, have been the subject of extensive study in the recent past. In particular, 2,5-disubstituted-1,3,4-oxadiazoles have received the most attention during last three decades as potential biomolecules[1]. Numerous reports have highlighted their chemistry and use[2,3]. A review on 1,3,4-oxadiazoles reveals that they possess wide range of biological activities like anticancer, fungicidal, herbicidal, pesticidal, analgesic, anticonvulsant, anti-HIV, antibacterial and plant growth regulator activities[4]. Further 1, 2, 4-triazoles also have a wide range of therapeutic properties. This nucleus has been incorporated into many therapeutically important agents, mainly displaying antimicrobial activities[5]. Thus 1, 3, 4-oxadiazole and 1, 2, 4-triazole

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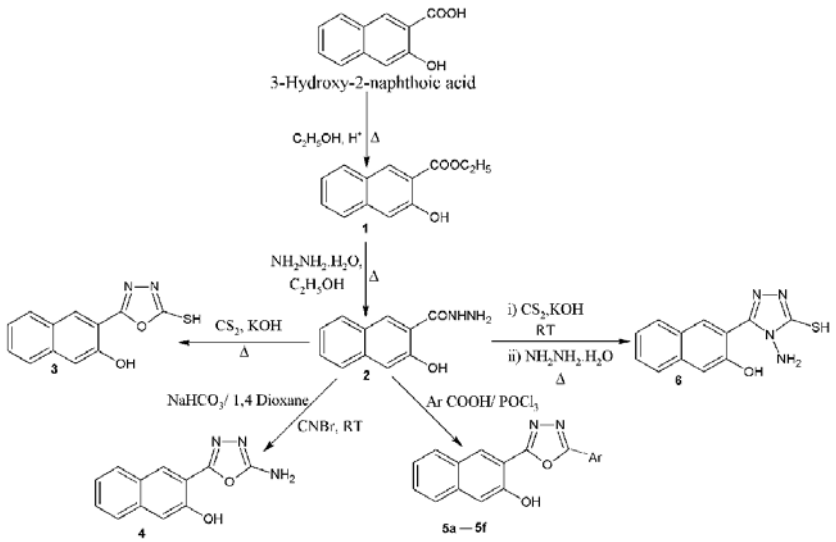
derivatives are expected to show chemotherapeutic intervention against bacteria, virus and other pathogens[6]. Over the past several years the emergence of bacterial resistance to nearly all the classes of antimicrobial agents has become a serious public health concern. Hence there is always a need for search of novel backbone, which can overcome this problem. Our continued efforts to exploit potential of azole backbone prompted us to undertake the present study[7-9]. We herein report synthesis and antifungal and antitubercular evaluation of few of such azoles.

EXPERIMENTAL

Melting points were determined by open capillary methods on a 'Veego' VMP-D apparatus and are uncorrected. TLC was performed using silica gel G plates of size 3x8 cm (Sigma-Aldrich) and visualized by UV or in an iodine chamber. Column chromatography, wherever necessary was performed on a neutral silica column (2.5x45 cm) using appropriate eluent. The IR spectra (KBr) were determined on FTIR 8400S, Shimadzu spectrometer and the values are expressed in cm^{-1}. Mass spectra were recorded on Thermo Fisher Scientific mass spectrometry instruments and ^1H NMR spectra were recorded at 400 MHz in either $CDCl_3$ or DMSO-d_6 solvents using TMS as an internal reference standard at sophisticated analytical instrument facility (SAIF), IIT Mumbai. Elemental analyses were carried out on thermoquest EA-1112 elemental analyzer at IIT, Mumbai.



Where Ar = Phenyl **(5a)**; 4-Aminophenyl **(5b)**; 2-Hydroxyphenyl **(5c)**; 4-Pyridyl **(5d)**; 4-Nitrophenyl **(5e)**; 4-Methylphenyl **(5f)**.

Scheme: Synthesis of 1,3,4-oxadiazole derivatives

Synthesis of 3-hydroxy ethyl-2-naphthalate (1)

In a mixture of 3-hydroxy-2-naphthoic acid (28.28 g, 0.15 mole) in absolute ethanol (36 mL), conc. sulphuric acid (38 mL) was added slowly under stirring. It was then refluxed on steam bath for 7-8 h, cooled and poured onto crushed ice under stirring. The pH was adjusted to 7 using 10% $NaHCO_3$ and the mixture was then extracted with 5 x 25 mL of diethyl ether. Combined ethereal extracts were dried over anhydrous $MgSO_4$ and solvent was removed under reduced pressure to yield yellow color solid[10].

Yield: 77.18%, m.p.: 145-148^{0}C, IR (KBr, cm^{-1}): 3400 (-OH str); 3056, 2856 (Ar-CH, str); 1745 (C=O str, ester); 752, 702 (Ar-CH, bend).

Synthesis of 3-hydroxy-2-naphthyl carbohydrazide (2)

In a solution of **1** (21.6 g, 0.1 mole) in absolute ethanol (40 mL) 98% hydrazine hydrate (7.5 g, 0.15 mole) was added. This mixture was refluxed for about 7-8 h, cooled, concentrated in vacuum and diluted with sufficient ice cold water. The white precipitate thus obtained was filtered, washed with ice cold water, dried and recrystallized from absolute ethanol[11].

Yield: 74.25%, m.p.: 126-128^{0}C, IR (KBr, cm^{-1}): 3321 (-OH str); 3051 (-NH str); 2929, 2852 (Ar-CH, str); 1631(-C=O str, amide); 744, 622 (Ar-CH, bend).

Synthesis of 5-(3'-hydroxynaphthalen-2-yl)-2-mercapto-1,3,4-oxadiazole (3)

To an equimolar ethanolic solution of **2** (2.02 g, 0.01 mole) a solution of potassium hydroxide (0.5 g, 0.01 mole) in water (5 mL) was added under stirring. To this, carbon disulphide (0.76 g, 0.01 mole) was added and the reaction mixture was refluxed till the evolution of H_2S ceased. After removal of excess of solvent, the residue was poured into ice-cold water and acidified with dilute (10%) HCl to obtain the white solid. The product was filtered, washed with ice-cold water and recrystallized from absolute ethanol to give white crystals.

Yield: 65.57%, m.p.: 168-170^{0}C, IR (KBr, cm^{-1}): 3326 (-OH str); 2927 (Ar-CH, Str); 2792, 2663 (-SH str); 1627, 1573 (C=N str); 1311 (-SH bend); 1184, 1087, 1049 (C-O-C, oxadiazole str); 892, 840, 730 (Ar CH, bend); MS: m/e (%): 245 (M + 1, 100%), 227 (M-17, 10%).

Synthesis of 5-(3'-hydroxynaphthalen-2-yl)-2-amino-1,3,4- oxadiazole (4)

To a mixture of **2 (**2.02 g, 0.01 mole) in 1, 4 dioxane (47 mL), a solution of sodium bicarbonate (0.78 g, 0.01 mole) in water (47 mL) was added. Cyanogen bromide (1.08 g, 0.01 mole) was then added to the resulting mixture and stirred for 4 hr at room temperature. It was then concentrated, diluted with water and filtered to obtain reddish brown colored solid which was recrystallized from absolute ethanol.

Yield: 62.76%, m.p.: 138-140^{0}C, IR (KBr, cm^{-1}): 3352 (-OH str); 3209 (-NH str); 3056, 2923 (Ar-CH, str); 1604 (C=N str); 1475 (-NH bend); 1176, 1095, 927 (C-O-C, oxadiazole str); 837, 788, 740 (Ar-CH, bend); ^{1}H NMR ($CDCl_3$): δ 10.06 (s, 1H, OH), 7.53-7.26 (dd, 4H, naphthyl ring B), 7.11 (s, 2H, naphthyl ring A), 6.62 (s, 2H, -NH_2).

Synthesis of 2, 5-disubstituted-1, 3, 4-oxadiazole (5a-5f)

A mixture of **2** (1.01 g, 0.005 mole) and appropriate aromatic acid (0.005 mole) was dissolved in phosphorus oxychloride (3.83 mL, 0.025 mole) and refluxed for 6-8 h on a steam bath. Then reaction mixture was cooled to room temperature and neutralized with ice cold solution of 10% sodium bicarbonate. The solid thus precipitated was filtered, washed with water, dried and recrystallized from absolute ethanol.

2-(3'-Hydroxynaphthyl)-5-phenyl-1,3,4-oxadiazole (5a)

Yield: 83.33%, m.p.: 163-165^{0}C, IR (KBr, cm^{-1}): 3388 (-OH str); 3116 (Ar-CH, str); 1600, 1512 (C=N str); 1168, 1060, 1006 (C-O-C, oxadiazole str); 835, 721, 680 (Ar-CH, bend); ^{1}H NMR ($CDCl_3$): δ 10.06 (s, 1H, OH), 8.22 (s, 2H, naphthyl ring A), 7.89-7.61 (dd, 2H, naphthyl ring B), 7.50 (m, 5H, phenyl), 7.39 (d, 2H, naphthyl ring B).

2-(3'-Hydroxynaphthyl)-5-(4-aminophenyl)-1,3,4-oxadiazole (5b)

Yield: 80%, m.p.: 142-145^{0}C, IR (KBr, cm^{-1}): 3334 (-OH str); 3220 (-NH str); 3056, 2929 (Ar-CH, str); 1633, 1602 (C=N str); 1172, 1068, 1010 (C-O-C, oxadiazole str); 844, 750 (Ar-CH, bend).

2-(3'- Hydroxynaphthyl)-5-(2-hydroxyphenyl)-1,3,4-oxadiazole (5c)

Yield: 83%, m.p.: 170-172^{0}C, IR (KBr, cm^{-1}): 3400 (-OH str); 3112, 2921 (Ar-CH, str); 1596, 1515 (C=N str); 1218, 1155, 1076 (C-O-C, oxadiazole str); 887, 837, 663 (Ar-CH, bend). Anal. Calc.: C, 71.05; H, 3.97; N, 9.21 %. Found: C, 71.00; H, 3.85; N, 9.11%.

2-(3'-Hydroxynaphthyl)-5-(pyridine-4-yl)-1,3,4-oxadiazole (5d)

Yield: 79%, m.p.: 178-180^{0}C, IR (KBr, cm^{-1}): 3334 (-OH str); 3056, 2929, 2854 (Ar-CH str); 1633, 1602 (C=N str); 1172, 1068, 1010 (C-O-C, oxadiazole str); 894, 844, 750 (Ar-CH, bend); ^{1}H NMR ($CDCl_3$): δ 8.98 (s, 1H, OH), 7.97 (d, 2H, pyridyl), 7.79 (d, 2H, pyridyl), 7.58-7.53 (m, 6H, naphthyl ring A and B). MS: m/e (%): 290 (M+1, 53%), 211 (M-78, 16%), 171 (M-118, 100%), 170 (M-119, 10%).

2-(3'-Hydroxynaphthyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole (5e)

Yield: 83.33%, m.p.: 228-230^{0}C, IR (KBr, cm^{-1}): 3326 (-OH str); 3055, 2929 (Ar-CH, str); 1627, 1575 (C=N str); 1174, 1089, 1022 (C-O-C, oxadiazole str); 894, 746 (Ar-CH, bend); ^{1}H NMR ($CDCl_3$): δ 9.88 (s, 1H, OH), 8.76 (s, 1H, naphthyl ring A), 8.43-8.39 (s, 2H of phenyl and 1H of naphthyl ring A), 7.98-7.78 (s, 2H, phenyl), 7.67-7.53 (m, 4H, naphthyl ring B). MS: m/e (%): 334 (M+1, 25%), 291 (M-42, 100%), 171 (M-162, 21%).

2-(3'-Hydroxynaphthyl)-5-(4-methylphenyl)-1,3,4-oxadiazole (5f)

Yield: 79.77%, m.p.: 128-130^{0}C, IR (KBr, cm^{-1}): 3321 (-OH str); 3051, 2929 (Ar-CH, str); 1631, 1589 (C=N str); 1236, 1146, 1070 (C-O-C, oxadiazole str); 831, 744 (Ar-CH, bend); ^{1}H NMR ($CDCl_3$): δ 8.82 (s, 1H, OH), 8.24 (d, 1H, naphthyl ring B), 8.21 (d, 1H, naphthyl ring B), 7.82 (s, 1H, naphthyl ring A), 7.69 (s, 1H, naphthyl ring A), 7.60 (t, 1H, naphthyl ring B), 7.60 (t, 1H, naphthyl ring B), 7.37 (d, 2H, phenyl), 7.34 (d, 2H, phenyl), 2.49 (s, 3H, CH_3).

Synthesis of 3-mercapto-5-(3'-hydroxynaphthalen-2-yl)-4-amino-1,2,4-triazole (6)

Synthesis of potassium salt of 3-hydroxy-2-naphthyl carbohydrazide

In a 500 mL conical flask, carbon disulphide (1.14 g, 0.015 mole) was added to a solution of potassium hydroxide (0.85 g, 0.15 mole), absolute ethanol (40 mL) and hydrazide (**2**, 2.02 g, 0.15 mole). This mixture was diluted with absolute ethanol (100 mL) and agitated for 18-20 h. It was then diluted with dry ether (250 mL) and the product thus obtained was filtered and vacuum dried at 65-70 ^{0}C. The salt prepared as described above was obtained in quantitative yields and were used without further purification.

Synthesis of 3-Mercapto-5-(-3'-hydroxynaphthalen-2-yl)-4-amino-1,2,4-triazole (6)

A suspension of above mentioned potassium salt (2 g, 0.01 mole), 98% hydrazine hydrate (2.25 mL, 0.02 mole) and water (45 mL) was refluxed with stirring until the evolution of H_2S gas ceased. It was then diluted with cold water (20-30 mL) and carefully

acidified with conc. hydrochloric acid. The white solid thus separated was filtered, washed with cold water, dried and recrystallized from absolute ethanol.

Yield: 77.51%, m.p.: 215-220^{0}C, IR (KBr, cm^{-1}): 3294 (-OH str); 3174 (-NH str); 2931 (Ar-CH, str); 2765 (-SH str); 1641 (-NH bend); 1531, 1502 (C=N str); 1303 (-SH bend); 873, 744, 676 (Ar-CH, bend); ^{1}H NMR (DMSO-d_6): δ 13.93 (s, 1H, SH), 10.62 (s, 1H, OH), 8.04 (s, 2H, naphthyl ring A), 7.89-7.86 (d, 2H, naphthyl ring B), 7.32 (d, 2H, naphthyl ring B), 5.60 (s, 2H, NH_2).

Biological evaluation

Antifungal activity

The antifungal activity was performed *in vitro* by agar well diffusion method against *C. albicans* and *A. niger* using Fluconazole as standard[12]. The cultures of 48 h old grown on potato dextrose agar (PDA) were used for inoculation of fungal strain on PDA plates. An aliquot (0.02 mL) of inoculum was introduced to molten PDA and poured into a petri dish. After solidification, the appropriate wells were made on agar plate by using cork borer (size 6.0 mm). Plates were incubated for 24–48 h at 28^{0}C. The antifungal activity was evaluated by measuring zones of inhibition of fungal growth. The complete antifungal analysis was carried out under strict aseptic conditions.

Table 1: Antifungal activity of synthesized compounds

Compound	Minimum inhibitory concentration (MIC) (µg/mL)	
	C. albicans	***A. niger***
3	8	62.5
4	31.25	31.25
5a	62.5	1
5b	1	1
5c	16	1
5d	16	1
5e	16	1
5f	31.25	250
6	16	1
Fluconazole	16	8

Antitubercular activity

Antitubercular activity was carried out by tube dilution method against *Mycobacterium tuberculosis*, using Middlebrook 7H-9 broth against $H_{37}Rv$, a standard strain of *Mycobacterium tuberculosis*[13]. The basal medium was prepared according to manufacturer's instructions (Hi-Media) and sterilized by autoclaving. 4.5 mL of broth was poured into each one of the sterile bottles; 0.5 mL of ADC supplement was added. This supplement contains catalase, dextrose and bovine serum albumin fraction v. Then a stock solution of the compound was prepared (10 mg/mL). From this appropriate amount of solution was transferred to media bottles to achieve final concentrations of 25, 50, 75 µg/mL, finally 10 µL suspension of Mtb strain (100000 organisms/mL, adjusted by McFarland's turbidity standard) was transferred to each of the tubes and incubated at 37°C. Along with this one growth control without compound and drug controls were also set up. The bottles were inspected for growth twice a week for a period of three weeks. The appearance of turbidity was considered as growth and indicated resistance to the compound. The growth was confirmed by making a smear from each bottle and performing a ZN stain. Streptomycin and Pyrazinamide were used as reference standards.

Table 2: Antitubercular activity of synthesized compounds

Compound	Antitubercular activity (µg/mL)		
	5	**10**	**25**
3	R	S	S
4	R	R	R
5a	R	R	R
5b	R	R	R
5c	R	S	S
5d	S	S	S
5e	R	R	R
5f	R	S	R
6	R	R	R
Streptomycin	R	S	S
Pyrazinamide	R	S	S

RESULTS AND DISCUSSION

As outlined in the reaction scheme, ester **(1)** and hydrazide **(2)** were obtained in quantitative yields and confirmed by spectral data. Hydrazide **(2)** was then converted to various 2, 5-disubstituted 1, 3, 4-oxadiazole derivatives **(3**, **4** and **5a-5f)** and a 1, 2, 4-triazole derivative **(6)**. The appearance of C-O-C str in between 1236-927 cm^{-1} and C=N str in between 1650-1500 cm^{-1} in IR showed the formation of 1, 3, 4-oxadiazole ring and appearance of C=N str at 1531, 1502 cm^{-1} exhibited the formation of 1, 2, 4-triazole ring. Few of these compounds were also confirmed by their mass spectra. The mass spectrum of compound **5d** exhibited M + 1 peak at m/z 290 (M + 1, 53%) and other peaks at 211 (M-78, 16%), 171 (M-118, 100%), 170 (M-119, 10%). The mass spectrum of compounds **3** and **5e** also exhibited their M + 1 peak, which confirmed their formation.

Antifungal activity

The results are reported in Table 1. The antifungal activity showed that MIC of some of the compounds like **5a**, **5b**, **5c**, **5d**, **5e** and **6** is less than that of the standard, underlining their potential against these fungi.

Antitubercular activity

The antitubercular study was carried out against *Mycobacterium tuberculosis* H_{37} *Rv.* The results are shown in Table 2. Compound **5d** was found active at concentration of 5 µg/mL, compounds **3**, **5c**, **5f** were found to be active at concentration of 10 µg/mL and compounds **4**, **5a**, **5b**, **5e** and **6** were inactive as the bacteria exhibited resistance to these compounds at all tested concentrations.

Thus, compounds **3**, **5c** and **5d** exhibited good antifungal and antitubercular activities in comparison with respective standards.

CONCLUSION

Some novel 1, 3, 4-oxadiazole **(3**, **4**, **5a-5f)** and 1, 2, 4-triazole **(6)** derivatives of 3-hydroxy-2-naphthoic acid were synthesized. Spectroscopic and elemental analysis confirmed the proposed structures of these compounds. Thus, from antimicrobial study, it is evident that incorporation of azole heterocyclic rings into bioactive molecules like 3-hydroxy-2-naphthoic acid could enhance the biological potential upto highest extent.

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