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Antimicrobial activity of novel 3-substituted- 5-(pyridine-4-yl)-3H-1,3,4-oxadiazole-2-thione derivatives

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5-(Pyridine-4-yl)-3H-1,3,4-oxadiazole-2-thione was prepared from isonicotinic acid hydrazide (isoniazid). The Mannich reaction of 5-(pyridine-4-yl)-3H-1,3,4-oxadiazole-2-thione was carried out with formaldehyde and various 1-substituted piperazine derivatives. All the synthesized compounds were characterized by IRS and ^{1}H NMR spectral feature. Their thiol-thione tautomeric equilibrium is described. All the compounds were also screened for their antimicrobial activity.

Key words: Isonicotinic acid hydrazide, 1,3,4-oxadiazole-2-thione, Mannich base reaction, spectral studies, antimicrobial activity.

INTRODUCTION

Many drugs like antibiotics, antimycotics, circulatory system and antiparasitic ones contain piperazine ring [1–7]. Thus piperazine derivatives play the pivotal role in therapeutical application. 1,3,4,-oxadiazole-2-thione has been reported to exhibit antifungal, antibacterial, antileishmanial and insecticidal activities [8–13]. One of the anti-TB drugs i.e. isoniazide affords heterocyclic compounds, which have good biological properties [14]. Its Mannich bases were also reported recently for important biological properties [15]. In view of the important biological properties it was planned to synthesize Mannich bases with 5-(pyridin-4-yl)-3H-1,3,4-oxadiazole-2-thione with piperazine moiety. The route is given in Scheme 1.

EXPERIMENTAL

Melting points were determined by the open capillary method and were uncorrected. The IR spectra were recorded in KBr pellets on a Nicolet 760D spectrometer and ^{1}H NMR spectra were recorded in DMSO with TMS as internal standard on a Brucker spectrometer at 400 MHz.

Preparation of 5-(pyridine-4-yl)-3H-1,3,4-oxadiazole-2-thione (**2**). Compound (**2**) was prepared according to a reported method [16]. A mixture of 1 (0.01 mol), potassium hydroxide (0.015 mol), Carbon disulphide (0.015 mol) and ethanol (50 ml, 95%), was refluxed for 6 h. Excess of solvent was distilled off and the remaining residue was poured in ice cold water and acidified with acetic acid. The yellow coloured compound, thus obtained, was filtered and dried.

Preparation of 3-substituted-5-(pyridine-4-yl)-3H-1,3,4-oxadiazole-2-thione (**3a**–**i**). Compounds (**3a**–**i**) were prepared by a known method [15]. Amount of 5.6 mmol of substituted piperazine derivatives were added to mixture of 5.6 mmol of 2 in 30 ml of absolute ethanol. Portion of 5.6 mmol 37% formaldehyde were added dropwise to the above stirred suspension and the reaction mixture was heated under reflux for 24 h. After concentration under reduced pressure, the residue was recrystallized from absolute ethanol.

Preparation of S-methyl-5-(pyridine-4-yl)-3H-1,3,4-oxadiazole-2-thione (**4**). Compound (**4**) was prepared according to known method [17]. A mixture of **2** (0.005 mol), sodium hydroxide (0.005 mol) and methyl iodide (0.006 mol) was stirred in water for 14 h. The resulting solution was removed by vacuum evaporation, the products were collected by filtration and washed with water.

BIOLOGICAL SCREENING

Antibacterial activities

Antibacterial activities of all the compounds were studied against Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative (*E. coli*, *Salmonelly typhi*,) at a concentaration of 50 µg/ML by agar cup plate method. A DMSO system was used as a control in this method. Under similar conditions, using tetracycline as a standard for comparison, we carried out a control experiment. The area of inhibition of zone was measured in mm. Compounds **3d** and **3e** were found to be more active

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against the above microbes. Other compounds were found to be less or moderately active than tetracycline. (Table 2).

Antifungal activities

The antifungal activities of all the compounds were studied at 1000 ppm concentration *in vitro*. Plant pathogenic organisms used were *Nigrospora Sp*., *Aspergillus niger*, *Rhizopus nigricum*, *Botrydepladia thiorbomine* and *Penicillium expansum*. The antifungal activity of all compounds was measured on each of these plant pathogenic strains on a potato dextrose agar (PDA) medium. Such a PDA medium contained potato 200 g, dextrose 20 g, and agar 20 g and 1 L of water. Five day old cultures were employed. The compounds to be tested were suspended (1000 ppm) in a PDA medium and autoclaved at 120ºC for 15 min at 15 atm pressure. These media were poured into sterile Petri plates and the organisms were inoculated after cooling down the Petri plates. The percentage inhibition for fungi was calculated after five days using the formula given below:

$$\text{Percentage of inhibition} = 100(X–Y)/X$$

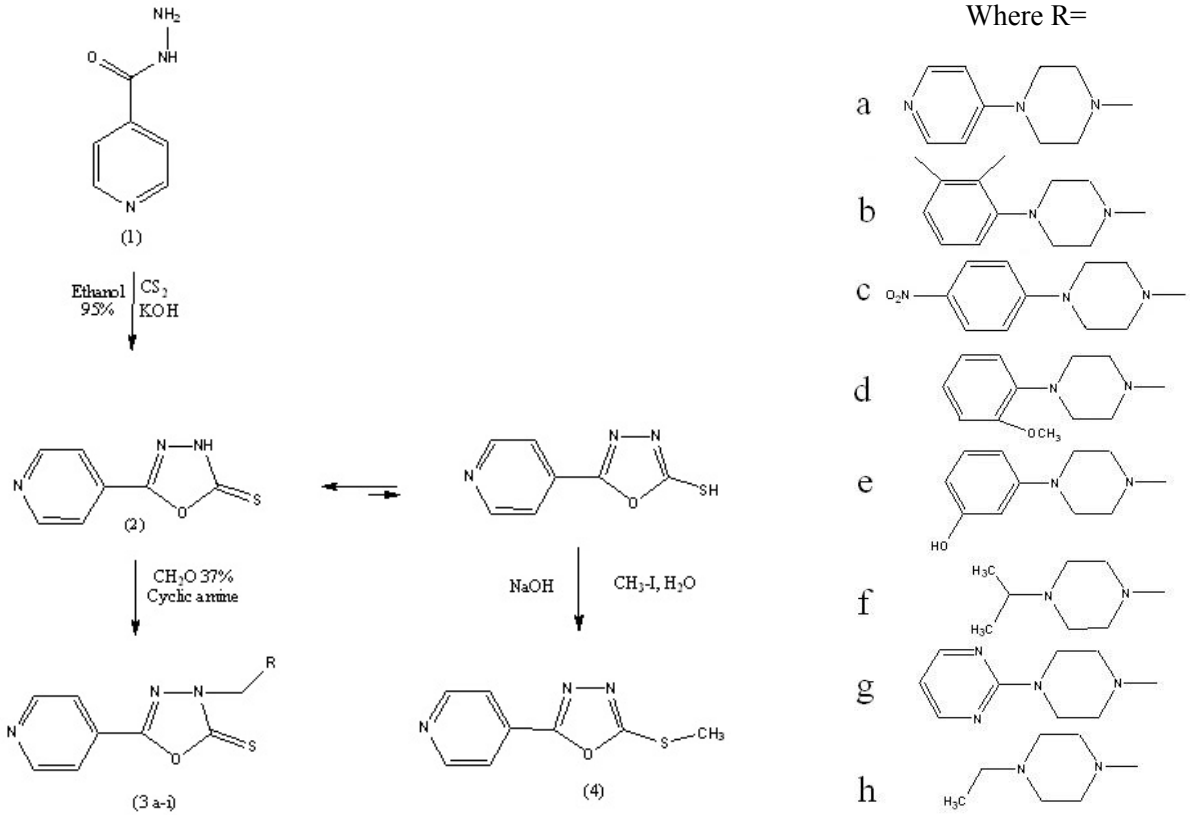
where X = area of colony in control plate; and Y = area of colony in test plate.

The fungicidal activity displayed by various compounds is shown in Table 3.

RESULTS AND DISCUSSION

The Mannich base reaction of 5-(pyridine-4-yl)-3H-1,3,4-oxadiazole-2-thione has been performed successfully with 1-substituted piperazine derivatives. Both moieties have important applications in medicine. The structure of 3-substiuted- 5-(pyridine-4-yl)-3H-1,3,4-oxadiazol-2-thione was confirmed by elemental analysis and IR spectra showing absorption bands at 1625–1645 cm^{-1} (C=N), 1240–1260 cm^{-1} (C=S), 1040–1080 cm^{-1} (C–O), 3035–3085 cm^{-1} (C–H of Ar), 1480–1525 cm^{-1} (C=C of Ar), 1040–1080 cm^{-1} (N–N of 1,3,4-oxadiazole-2-thione). Additional bands appear due to substitution in the piperazine moiety, as follows: 1520 cm^{-1} ($–NO_2$), 2830 cm^{-1} (–CH of $–OCH_3$), 3550 cm^{-1} (–OH).

The examination of the data reveals that the elemental contents (Table 1) are consistent with the predicted structure shown in Scheme 1. The IRS and NMR data (Table 1) also allow a direct assignment of the predicted structure.



Scheme 1

Table 1. Analytical spectral data of compounds **2**, (**3a**–**i**) and **4**.

Comp.	Molecular formula (mol. mass)	Yield %	M.P., ºC	Elemental analysis, % Found (Calcd)				1H NMR δ, ppm
				C	H	N	S	
2	$C_7H_5N_3OS$ (179.20)	69	262	46.90 (46.92)	2.77 (2.81)	23.40 (23.45)	17.82 (17.89)	8.02 (d, 2H, Pyr.), 8.77 (d, 2H, pyr.), 14.14 (s, 1H, –NH), 1.9 (s, 1H, SH)
3a	$C_{17}H_{18}N_6OS$ (354.44)	58	102–103	54.00 (54.02)	4.28 (4.34)	23.60 (23.62)	12.00 (12.02)	8.02, 8.77 (four d, 8H, Ar–H.), 5.03 (s, 2H CH_2 exocyclic), 2.50, 3.19 (two t, 8H, pip.).
3b	$C_{20}H_{23}N_5OS$ (381.49)	60	103–105	62.90 (62.97)	6.00 (6.08)	18.30 (18.36)	8.35 (8.41)	8.00, 8.65 (two d, 4H, Ar–H.), 7.03–7.50 (m, 3H, Ar–H.), 4.95 (s, 2H CH_2 exocyclic), 2.48, 3.15 (two t, 8H, pip.).
3c	$C_{18}H_{18}N_6O_3S$ (398.44)	60	174–176	54.21 (54.26)	4.50 (4.55)	20.89 (21.09)	7.90 (8.05)	8.12, 8.87 (two d, 4H, Ar–H.), 7.03–7.50 (m, 4H, Ar–H.), 5.02 (s, 2H CH_2 exocyclic), 2.52, 3.17 (two t, 8H, pip.).
3d	$C_{19}H_{21}N_5O_2S$ (383.47)	63	116–118	59.50 (59.51)	5.4 (5.52)9	18.21 (18.26)	8.30 (8.36)	8.02, 8.77 (two d, 4H, Ar–H.), 7.02–8.00 (m, 4H, Ar–H), 5.11 (s, 2H CH_2 exocyclic), 2.52, 3.17 (two t, 8H, pip.), 3.10 (s, 3H –OCH3).
3e	$C_{18}H_{19}N_5O_2S$ (369.44)	55	111–115	58.50 (58.52)	5.10 (5.18)	18.90 (18.96)	8.60 (8.68)	8.05, 8.75 (two d, 4H, Ar–H.), 7.02–8.77 (m, 4H, Ar–H), 4.99 (s, 2H CH_2 exocyclic), 2,44, 3.11 (two t, 8H, pip.), 6.90 (s, H –OH)
3f	$C_{15}H_{21}N_5OS$ (319.43)	70	182–184	56.38 (56.40)	6.59 (6.63)	21.89 (21.92)	9.92 (10.04)	8.02, 8.77 (two d, 4H, Ar–H.), 5.11 (s, 2H CH_2 exocyclic), 2.52, 3.17 (two t, 8H, pip.), 2.69 (m, 1H –CH), 1.3 (m, 6H, $–CH_3$)
3g	$C_{16}H_{17}N_7OS$ (355.42)	65	139–141	53.99 (54.07)	4.79 (4.82)	27.59 (27.50)	9.00 (9.02)	8.02, 8.77 (two d, 4H, Ar–H.), 7.00–7.80 (m, 3H, Ar–H.), 5.10 (s, 2H CH_2 exocyclic), 2.59, 3.24 (two t, 8H, pip.),
3h	$C_{14}H_{19}N_5OS$ (305.40)	65	134–136	55.00 (55.06)	4.95 (4.88)	22.93 (22.90)	10.50 (10.48)	8.01, 8.71 (two d, 4H, Ar–H.), 5.05 (s, 2H CH_2 exocyclic), 2.52, 3.17 (two t, 8H, pip.), 2.38 (m, 2H $–CH_2$), 1.1 (t, 3H, $–CH_3$)
3i	$C_{15}H_{21}N_5OS$ (319.43)	63	93–98	56.35 (56.40)	6.59 (6.63)	21.90 (21.92)	10.04 (10.00)	8.02, 8.77 (two d, 4H, Ar–H.), 5.05 (s, 2H CH_2 exocyclic), 2.52, 3.17 (two t, 8H, pip.), 1.2 (t, 3H, CH_3), 1.5 (m, 2H, CH_2), 3.3 (t, 2H, CH_2)
4	$C_8H_7N_3OS$ (193.23)	50	130–132	49.70 (49.73)	3.59 (3.65)	21.70 (21.75)	16.50 (16.59)	8.00, 8.70 (two d, 4H, Ar–H.), 2.8 (s, 3H, CH_3)

Table 2. Antibacterial activity of compounds (**3a**–**i**) and **4**.

Comp.	Gram-positive		Gram-negative	
	Staphylococcus aureus	*Bacillus subtilis*	*E. coli*	*Salmonelly typhi*
3a	25	21	20	23
3b	22	23	22	19
3c	31	22	28	27
3d	45	46	44	41
3e	41	40	40	43
3f	29	27	26	28
3g	19	17	22	23
3h	21	22	25	30
3i	20	19	23	26
4	22	23	19	21
Tetracycline	40	41	42	39

Table 3. Antifungal activity of compounds (**3a**–**i**) and **4**.

Comp.	Zone of Inhibition at 1000 ppm, %				
	Nigrospora Sp.	*Aspergillus niger*	*Botrydepladia thiobromine*	*Rhizopus nigricum*	*Penicillium expansum*
3a	15	19	20	21	22
3b	21	23	15	14	20
3c	34	33	29	30	30
3d	46	46	41	40	40
3e	40	42	42	40	40
3f	22	26	22	29	30
3g	19	18	21	20	18
3h	18	17	20	23	25
3i	28	23	25	21	24
4	26	21	22	28	24

There are some studies on electronic structures and thiol-thione tautomeric equilibria of heterocyclic thione derivatives [18–20]. We observed that extensive thiol-thione tautomerism exists in compound **2**. In the 1H NMR signals of the SH protons were recorded although they were very weak and also the ready synthesis of the Mannich bases **3a**–**i** and compound **4** confirmed the tautomerism. It has been reported that the crystal structure of compounds like **2** corresponds to the thiol form too [21–23]. Finally, the crystal structure of **2** corresponded to the thione form, but there is thiol-thione tautomerism in solution.

The antibacterial activity of compounds was tested against some strains of bacteria. The results show that the prepared compounds are toxic or moderately toxic against the bacteria. The comparison of the antibacterial activity of some com-

pounds with tetracycline shows that these compounds have almost similar activity.

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АНТИМИКРОБНА АКТИВНОСТ НА НОВИ 3-ЗАМЕСТЕНИ 5-(ПИРИДИН-4-ИЛ)-3Н-1,3,4-ОКСАДИАЗОЛ-2-ТИОНОВИ ПРОИЗВОДНИ

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(Резюме)

Синтезиран е 5-(пиридин-4-ил)-3Н-1,3,4-оксадиазол-2-тион от хидразид на изоникотинова киселина (изониазид). Проведена е реакция на Маних на синтезираното съединение с формалдехид и различни 1-заместени пиперазинови производни. Всички синтезирани съединения са охарактеризирани с ИЧС и ^{1}Н ЯМР. Описано е тяхното тавтомерно равновесие тиол-тион. Всички съединения са изследвани за антимикробна активност.