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Short communication

Synthesis and antibacterial studies of a new series of 1,2-bis(1,3,4-oxadiazol-2-yl)ethanes and 1,2-bis(4-amino-1,2,4-triazol-3-yl)ethanes

B. Shivararna Holla[a]*, Richard Gonsalves[b], Shalini Shenoy[c]

[a]Department of Post Graduate Studies and Research in Chemistry, Mangalore University, Mangalagangothri 574 199, India
[b]St. Aloysius College, Mangalore 575 003, India
[c]Department of Microbiology, Kasturba Medical College, Mangalore 575 001, India

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Abstract – The acylhydrazones **3**, obtained by the treatment of succinic acid dihydrazide **2** with furfural, nitrofurfuraldehydediacetate and substituted arylfurfurals, on oxidative cyclization with bromine in acetic acid yielded 1,2-bis(1,3,4-oxadiazol-2-yl)ethanes **4** which are further converted into 1,2-bis(4-amino-1,2,4-triazol-3-yl)ethanes **5** with hydrazine hydrate. The newly synthesised compounds are characterised by analytical, IR, NMR and mass spectral data. Most of the newly synthesised compounds have been found to be active against both Gram positive and Gram negative bacteria at less than 6 μg/mL concentration. © 2000 Éditions scientifiques et médicales Elsevier SAS

1. Introduction

Oxadiazoles [1], triazoles [2, 3] and their derivatives are known for their biological activities [4–7]. A series of triazole derivatives containing a nitrofuryl moiety having antibacterial properties were reported from our laboratory [8]. The 1,2,4-triazole nucleus has recently been incorporated into a wide variety of therapeutically interesting drug candidates including H_1/H_2 histamine receptor blockers, cholinesterase active agents, CNS stimulants, anti-anxiety agents and sedatives [9]. Prompted by these observations, we decided to synthesise various 1,2-bis[1,3,4-oxadiazol-2-yl]ethanes **4** and 1,2-bis[4-amino-1,2,4-triazol-3-yl]ethanes **5** (*figure 1*). The results of such experiments along with their antibacterial activities are reported in this paper.

2. Chemistry

Diethyl succinate **1** in ethanol, when treated with hydrazine hydrate (80%) gave the corresponding dihydrazide **2**. This dihydrazide **2** on condensation with furfural, nitrofurfuraldhyde diacetate and substituted arylfurfurals in ethanol, in the presence of concentrated H_2SO_4 gave the corresponding bis-hydrazones (**3a–f**; *figure 1*) in good yields. Arylfurfurals were obtained by arylation of furfural as reported earlier [10]. The oxidative cyclization of **3b–g** with bromine in glacial acetic acid afforded **4b–g**. However, oxidative cyclization with ferric chloride in acetic acid failed to give title compounds **4b–g**. Compounds **4**, on refluxing with hydrazine hydrate (99%), underwent ring opening and closure reactions to yield bis[4-amino-1,2,4-triazol-3-yl]ethanes **5**. Attempts to cyclize bis-hydrazone **3a**, with an unsubstituted furan ring by oxidative cyclization were unsuccessful.

3. Results and discussion

The characterization data of the acyl hydrazones **3a–f** are given in *table I*. The formation of **3** was supported by IR bands at 3 200–3 350 (NH), 1 680 (C=O) and 1 620 (C=N). The PMR spectrum of acylhydrazone **3e** also exhibited the absence of peak due to NH_2 protons. The bromophenyl protons appeared as doublets in the region δ 7.7 (J = 8 Hz) and δ 7.8 (J = 8 Hz), while the two β protons of the furan ring resonated as doublets around δ 7.0 (J = 3 Hz) and δ 7.2 (J = 3 Hz). A singlet at δ 8.6 was attributed to the azomethine proton. The protons of the methylene groups appeared as a broad peak at δ 2.5.

*Correspondence and reprints

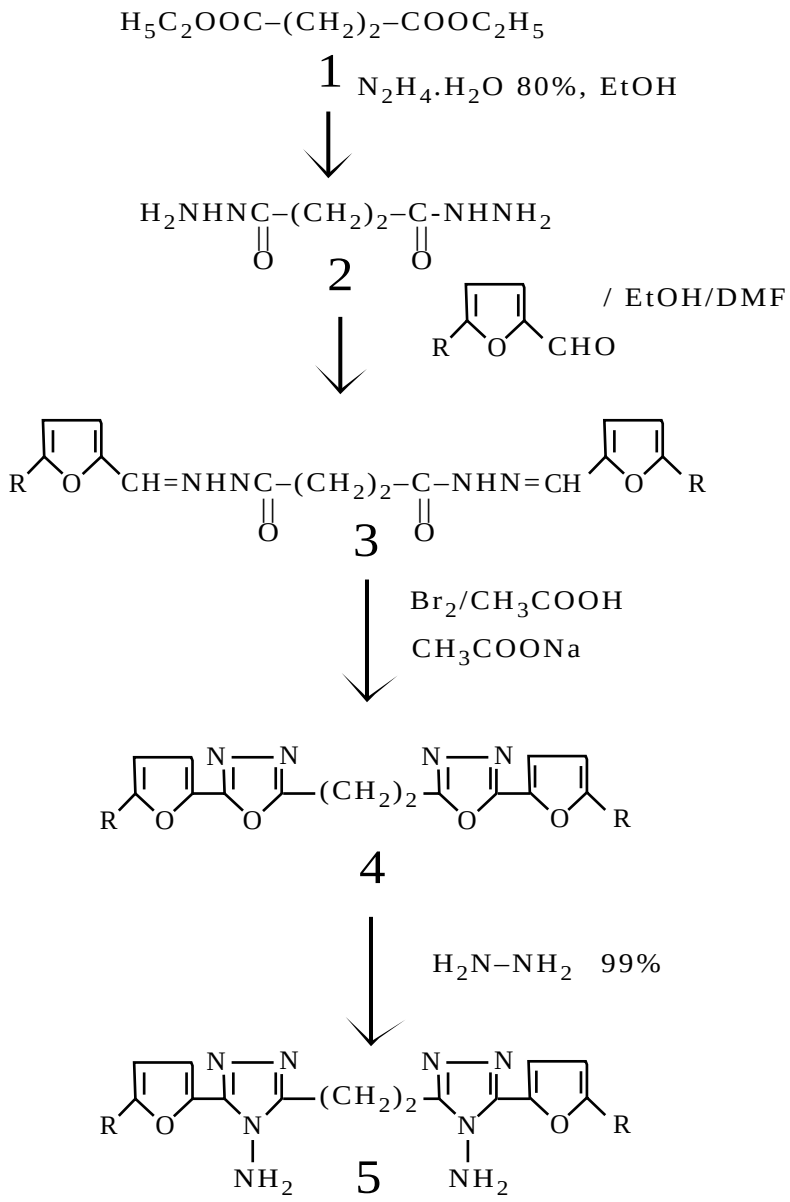

Figure 1. R = H, NO_2, p-nitrophenyl, p-chlorophenyl, p-bromophenyl,2,4-dichlorophenyl.

In the mass spectrum of **3d**, the molecular ion peak of the hydrazone was observed at m/s 522, M + 2 peak at m/z 524 and M + 4 peak at m/z 526 corresponding to its molecular formula $C_{26}H_{20}Cl_2N_4O_4$. The molecular ion of **3d** underwent fragmentation to produce a peak at m/z 177 corresponding to the formation of a chlorophenylfuran cation.

The oxidative cyclization of **3**, with bromine in glacial acetic acid in the presence of sodium acetate, yielded 1,2-bis(1,3,4-oxadiazol-2-yl)ethanes **4**. The characterization data of cyclized products **4** are given in *table I*. The PMR spectrum of 1,2-bis(1,3,4-oxadiazol-2-yl) ethane **4d** showed a doublet in the region δ 7.8 (J = 9 Hz) and δ 7.4 (J = 9 Hz) corresponding to the p-chlorophenyl moiety. The two protons of the furan ring resonated as doublets around δ 7.1 (J = 3 Hz) and δ 6.8 (J = 3 Hz). The absence of the peak due to the azomethine proton confirmed the cyclization. The signal due to protons of the methylene

Table I. Characterization data of compounds **3–5**.

Compound	R	M.p. °C	Yield (%)	Mol. formula	Analysis (%) Found [Calcd]		
					C	H	N
3a	H	100–2	80	$C_{14}H_{14}N_4O_4$	55.48	4.65	18.48
					55.62	4.63	18.54
3b	Nitro	228–30	82	$C_{14}H_{12}N_6O_8$	42.79	3.08	21.36
					42.85	3.06	21.42
3c	4-Nitrophenyl	172–74	90	$C_{26}H_{20}H_6O_8$	57.39	3.62	15.38
					57.35	3.67	15.44
3d*	4-Chlorophenyl	210–12	85	$C_{26}H_{20}Cl_2N_4O_4$	59.71	3.85	10.68
					59.77	3.83	10.72
3e**	4-Bromophenyl	168–70	86	$C_{26}H_{20}Br_2N_4O_4$	51.07	3.26	9.22
					51.14	3.27	9.18
3f	2,4-Dichlorophenyl	228–30	88	$C_{26}H_{18}Cl_4N_4O_4$	52.82	3.07	9.52
					52.88	3.05	9.49
4b	Nitro	180–82	74	$C_{14}H_8N_6O_8$	43.33	2.09	21.58
					43.29	2.06	21.64
4c^{+}	4-Nitrophenyl	288–90	76	$C_{26}H_{16}N_6O_8$	57.68	2.98	15.59
					57.77	2.96	15.55
4d^{++}	4-Chlorophenyl	180–82	75	$C_{26}H_{16}Cl_2N_4O_4$	60.18	3.11	10.86
					60.23	3.08	10.81
4e^{+++}	4-Bromophenyl	250–52	74	$C_{26}H_{16}Br_2N_4O_4$	51.45	2.61	9.29
					51.48	2.64	9.24
4f	2,4-Dichlorophenyl	179–81	78	$C_{26}H_{14}Cl_4N_4O_4$	53.19	2.42	9.59
					53.24	2.38	9.55
5b	Nitro	174–76	62	$C_{14}H_{12}N_{10}O_6$	27.83	2.94	33.62
					27.88	2.88	33.66
5c	4-Nitrophenyl	230–32	66	$C_{26}H_{20}N_{10}O_6$	54.88	3.48	24.69
					54.92	3.52	24.64
5d$^{\$}$	4-Chlorophenyl	112–14	68	$C_{26}H_{20}Cl_2N_8O_2$	57.06	3.59	20.56
					57.14	3.66	20.51
5e	4-Bromophenyl	94–96	64	$C_{26}H_{20}Br_2N_8O_2$	49.26	3.12	17.71
					49.21	3.15	17.66
5f	2,4-Dichlorophenyl	116–18	62	$C_{26}H_{18}Cl_4N_8O_2$	50.73	2.87	18.29
					50.81	2.93	18.24

*^{1}H-NMR (DMSO-d_6): δ 8.6 (s, 2H, azomethine protons), δ 7.8 (d, 4H, J = 10 Hz, aromatic protons), δ 7.5 (d, 4H, J = 10 Hz, aromatic protons), δ 7.2 (d, 2H, J = 3 Hz, 4H-furan), δ 7.0 (d, 2H, J = 3 Hz, 3H-furan), δ 2.5 (bs, 4H, methylene protons). MS. m/e (% abundance), 522/524/526 (100/66/11, M^+/M + 2/M + 4), 372 (5.9), 326 (8.1), 134 (100). **^{1}H-NMR (DMSO-d_6): δ 8.6 (s, 2H, J = 10 Hz, azomethine protons), δ 7.8 (d, 4H, J = 8 Hz, aromatic protons), δ 7.7 (d, 4H, J = 8 Hz, aromatic protons), δ 7.2 (d, 2H, J = 3 Hz, 4H-furan), δ 7.0 (d, 2H, J = 3 Hz, 3H-furan), δ 2.5 (bs, 4H, methylene protons). $^+$MS. m/e (% abundance), 524 (2.2, M–O^+), 431 (76), 403 (9.8), 252 (7.1), 216 (6.4), 214 (5.8), 148 (14.9). $^{++}$ ^{1}H-NMR (DMSO-d_6): δ 7.8 (d, 4H, J = 9 Hz, aromatic protons), δ 7.4 (d, 4H, J = 9 Hz, aromatic protons), δ 7.1 (d, 2H, J = 3 Hz, 4H-furan), δ 6.8 (d, 2H, J = 3 Hz, 3H-furan), δ 1.8 (bs, 4H, methylene protons). $^{+++}$ ^{1}H-NMR (DMSO-d_6): δ 7.8 (d, 4H, J = 8 Hz, aromatic protons), δ 7.7 (d, 4H, J = 8 Hz, aromatic protons), δ 7.2 (d, 2H, J = 3 Hz, 4H-furan), δ 7.0 (d, 2H, J = 3 Hz, 3H-furan), δ 2.5 (bs, 4H, methylene protons). $^\$$ ^{1}H-NMR (DMSO-d_6): δ 7.4 (d, 4H, J = 9 Hz, aromatic protons), δ 7.2 (d, 4H, J = 9 Hz, aromatic protons), δ 7.0 (d, 2H, J = 3 Hz, 4H-furan), δ 6.8 (d, 2H, J = 3 Hz, 3H-furan), δ 6.1 (s, 4H, methylene protons). IR (KBr) 3 400 cm^{-1} (ν, N–H), 2 900 cm^{-1} (ν, C–H), 1 633 cm^{-1} (ν, C=N), 829, 790, 731 (Ar–H def.).

groups appeared as a broad peak at δ 1.8. The mass spectrum of **4c** showed a peak at m/z 524 which corresponds to the loss of oxygen $(M–0)^{+\cdot}$. A peak at m/z 270 is attributable to the doubly charged molecular ion. A peak at m/z 216 corresponds to the formation of a p-nitrophenylfuranayl cation.

The 1,2-bis(1,3,4-oxadiazol-2-yl)ethanes **4**, on treatment with hydrazine hydrate, yielded 1,2-bis(4-amino-1,2,4-triazol-3-yl)ethanes **5**. In the IR spectrum of **5d**, a broad peak in the region 3 400–3 300 cm^{-1} was seen which is attributable to NH_2 groups present in the compound. The PMR spectrum of 1,2-bis(4-amino-1,2,4-triazol-3-yl)ethane **5d** showed a doublet in the region of δ 7.4 (J = 9 Hz) and δ 7.2 (J = 9 Hz) corresponding to a p-chlorophenyl moiety. The two protons of the furan ring resonated as doublets around δ 7.0 (J = 3Hz) and δ 6.8

Table II. Antibacterial activity data of the acyl hydrazones, bis-oxadiazolylethanes **4** and bis-triazolylethanes **5**.

	Minimum inhibitory concentration. MIC in μg/ml Antibacterial activity			
Compound	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*E. Coli*
3b	6	1.5	6	3
3c	6	6	3	3
3d	3	6	6	6
3e	6	1.5	6	6
3f	6	3	6	6
4c	6	6	3	3
4d	6	6	6	6
4e	6	6	6	12.5
5c	6	6	6	6
5e	6	6	6	6
5f	6	6	6	6
Furacin	12.5	12.5	12.5	6

B. subtilis = Bacillus subtilis; *S. aureus = Staphylococcus aureus*, *P. aeruginosa = Pseudomonas aeruginosa*, *E. coli = Escherichia coli*. Cultures of the above samples were obtained from Kasturba Medical College Hospital, Mangalore.

(J = 3Hz). A singlet at δ 6.1 is attributed to the N-NH_2 protons. The protons of the methylene group appeared as a broad peak at δ 2.3.

4. Pharmacology

Some of the acyl hydrazones **3**, oxadiazolyl ethanes **4** and triazolyl ethanes **5** were screened for their antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus subtilis* by the serial dilution method [11]. The results of such studies are given in *table II*. Furacin was used as a standard drug. Most of the compounds tested showed moderate to good antibacterial activity against all the four micro-organisms. Acyl hydrazones **3b** and **3d** showed the highest degree of antibacterial activity against *S. aureus* (*table II*). However, the cyclization of acyl hydrazones did not enhance the antibacterial activity.

5. Experimental protocols

5.1. Chemistry

Melting points were determined by a capillary method and are uncorrected. IR spectra (nujol mull) were recorded on a Perkin Elmer Infrared Spectrophotometer, PMR spectra in DMSO-d_6 were recorded on a JEOL GSX400 spectrometer, and the mass spectra on a VG-micromass spectrometer.

5.1.1. General procedure for the preparation of acylhydrazones **3**

Succinic dihydrazide (0.01 mol), prepared by refluxing diethyl succinate and hydrazine hydrate was dissolved in ethanol (30 mL) and was then treated with a solution of corresponding aldehydes (0.02 mol) in ethanol/dimethyl formamide. The mixture was heated under reflux on a water bath for 2–3 h after the addition of a few drops of concentrated sulphuric acid (*figure 1*). A solid mass was obtained on cooling the reaction mixture. It was collected by filtration and recrystallized from dimethyl formamide to yield the title compounds. Their characterization data are given in *table I*.

5.1.2. General procedure for the preparation of 1,2-bis(1,2,4-oxadiazol-2-yl)ethanes **4**

To a solution of **3** (0.01 mol) in acetic acid (20 mL), a 30% solution of bromine in acetic acid was added dropwise. A catalytic amount of sodium acetate was also added. The reaction mixture was stirred for 2 h and then poured into ice cold water (100 mL). It was allowed to stand overnight and the separated solid was filtered, washed with water, dried and recrystallized from dimethylformamide. Their characterization data are also given in *table I*.

5.1.3. General procedure for the preparation of 1,2-bis-(4-amino-1,2,4-triazol-3-yl)ethanes **5**

A solution of **4** (0.01 mol) in 99% hydrazine hydrate (10 mL) was refluxed for 1 h, cooled, poured onto crushed ice and acidified with acetic acid. The solid obtained was collected by filtration and recrystallized from ethanol to yield the title compounds.

5.2. Evaluation of antibacterial activity

The antibacterial activity of the test compounds were determined against *B. subtilis*, *S. aureus*, *P. aeruginosa* and *E. coli* by the serial dilution method. 5 mg of the test compound was dissolved in 50 mL of dimethyl formamide to prepare a stock solution of 100 μg/mL. One loopful of an 18-hour broth culture was innoculated into 5 mL of nutrient broth and this was incubated at 37 °C for 4 h. An assay was prepared by diluting 4-hour subcultures 1:1 000 in nutrient broth.

Nutrient broth (0.5 mL) was poured into tubes with labelled numbers 1–11. 0.5 mL of the solution of the test compound (100 μg/mL) was added to the first tube, the solution was mixed well and 0.5 mL of this solution was transferred into the tube no. 2. This process was repeated serially to obtain the quantities indicated in each of the test tubes. The 11th tube was taken as control. One drop of diluted broth culture of the test organism (approxi-

mately 0.05 mL) was added to all the tubes using a sterilised Pasteur pipette. The solutions were mixed gently and the incubation was carried out at 37 °C for 16–18 h. Furacin dissolved in dimethyl formamide was used as a standard. The minimum concentration at which there was no turbidity was taken as the minimum inhibitory concentration.

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