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Synthesis and evaluation of antimitotic activity of alkylated 2-amino-1,3,4-oxadiazole derivatives

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Abstract

Series of *N*-alkylated-2-amino-1,3,4-oxadiazoles (**2**) were synthesized via alkylation of 2-amino-1,3,4-oxadiazoles (**1**) under phase transfer condition. This paper also describes the study of antimitotic activity of these molecules by onion root tip method.

Keywords: Antimitotic activity; *N*-alkylated-2-amino-1,3,4-oxadiazoles; Onion root tip method

1. Introduction

Substituted 1,3,4-oxadiazoles [1] have wide variety of uses, in particular as biologically active compounds in medicine and as dye stuffs, fluorescent whiteners, herbicides, fungicides, hypnotics and as sedatives. These compounds also showed antibacterial, analgesic, antimalarial, antiinflammatory, anticonvulsant and diuretic activity. In particular 2-amino-1,3,4-oxadiazoles have been reported to possess promising antitumor activity [2].

Normally 2-amino-1,3,4-oxadiazoles alkylate at the amino group under alkaline condition. For instance the anion derived from *N*-acetylamino-1,3,4-oxadiazole react with ethyl bromoacetate and phenacyl bromide to give *N*-alkyl derivatives [1].Montgomery et al. [3] reported that *N*-mustards undergo ring closure to form strained ring systems, which subsequently act to alkylate a critical cell constituent on an NH, SH or OH functions and thereby blocks the function of such a cell constituent. Biochemically, the alkylation could destroy the activity of an essential cell constituent. Prompted by these findings and in continuation of our studies on 1,3,4-oxadiazoles [4,5], a number of *N*-bromoalkylated-5-aryl-2-amino-1,3,4-oxadiazoles were prepared and were screened for their antimitotic activities. Results of such studies are discussed in this paper.

2. Chemistry

For the present work, 5-aryl-2-amino-1,3,4-oxadiazoles were prepared according to the reported procedure [4]. In combination with the antimitotic activity of 2-amino-1,3,4-oxadiazoles and *N*-mustards, it was considered worthwhile to alkylate 5-aryl-2-amino-1,3,4-oxadiazoles (**1**), hoping to get the alkylated derivative with promising antimitotic activity. In a typical procedure, a mixture of 2-amino-5-aryl-1,3,4-oxadiazole (**1**, 1 mmol), 1,3-dibromopropane (2 mmol) and tetrabutylammonium bromide (0.1 mmol) in tetrahydrofuran were treated with powdered KOH (1 mmol) at room temperature and stirred for 15 h. After 15 h of stirring, the TLC of the reaction mixture showed three spots corresponding to the starting material, a minor spot may be due to allyl substituted product and the major spot due to the expected product. The expected product was then isolated by usual workup and later was purified by column chromatography to get the desired *N*-mustard derivatives (**2**) in 70–80% yield. Structure proof for alkyl derivatives were provided by NMR studies. NMR showed the absence of NH protons in the region 3.30–3.35 ppm. ^{1}H NMR of all the bromoalkylated aminooxadiazoles showed peaks due to

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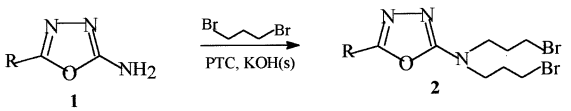
aromatic protons and other substituents in the expected region. The substituted oxadiazoles gave significantly stable two molecular ion peaks with relative intensity ranging from 20–35% and 21–36%, respectively. The two molecular ion peaks are due to the presence of bromine atom in the molecule. All underwent similar fragmentation pattern and gave aryl nitrile radical ion as the base peak.



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R: a) Ph; b) p-(MeO)C_6H_4; c) $(MeO)_3C_6H_2$; d) m-$(NO_2)C_6H_4$; e) Thienyl

3. Experimental

3.1. Chemistry

Melting points were determined in open capillaries on a Buchi oil melting point apparatus and are uncorrected. NMR spectra were taken on a Varian 60 MHz instrument (chemical shifts in δ-scale downfield from TMS standard) and the mass spectra in Hitachi RMU-61 spectrophotometer and important fragments are given with the relative intensities (in the brackets). For TLC, plates coated with silica gel G were used and the spots were developed with iodine.

3.1.1. General procedure for the preparation of 2-N,N-di(3′-bromopropyl)amino-1,3,4-oxadiazoles (2)

A typical procedure, freshly powdered KOH (0.28g, 5 mmol) was added to a mixture of 2-amino-5-phenyl-1,3,4-oxadiazole (**1a**, 0.81 g, 5 mmol), 1,3-dibromopropane (2.4 g, 9.90 mmol) and tetrabutylammonium bromide (0.32 g, 1 mmol) in tetrahydrofuran (15 ml) followed by stirring for 15 h. The solvent was evaporated under reduced pressure, followed by extraction with ether. The organic layer was washed with brine solution, dried over anhydrous sodium sulphate and evaporated. The residue was then dissolved in minimum chloroform and was diluted with petroleum ether. The alkylated derivative (**2a**) crystallizes out as white crystalline solid in 75% (1.50 g) yield, m.p. 216–17°C, ^{1}H NMR ($CDCl_3$): δ 1.80–1.92 (m, 4H, CH_2), 3.50–3.60 (t, 4H, NCH_2), 4.80–4.95 (t, 4H, CH_2Br), 7.50–7.80 (m, 5H, ArH); MS: m/z 405 (M^+, 20), 401 (M^+, 20), 302 (08), 298 (09), 103 (100), 77 (2); *Anal*. Calc. for $C_{14}H_{17}N_3OBr_2$: C, 41.71; H, 4.25; N, 10.42; Br, 39.64. Found: C, 41.68; H, 4.22; N, 10.45; Br, 39.67%.

Complex **2b** was obtained as white crystalline solid in 70% (1.52 g) yield, m.p. 225–29°C from **1b** (0.96 g, 5 mmol), ^{1}H NMR ($CDCl_3$):1.80–1.90 (m, 4H, CH_2), 3.50–3.60 (t, 4H, NCH_2), 3.95 (s, 3H, OCH_3), 4.75–4.95 (t, 4H, CH_2Br), 7.10–7.12 (d, 2H, ArH), 7.70–7.72 (d, 2H, ArH); MS: m/z 435 (M^+, 25), 431 (M^+, 26), 302 (11), 298 (11), 133 (100), 107 (5); *Anal*. Calc. for $C_{15}H_{19}N_3O_2Br_2$: C, 41.59; H, 4.42; N, 9.70; Br, 36.89. Found: C, 41.55; H, 4.40; N, 9.73; Br, 36.92%.

Complex **2c** was obtained as white crystalline solid in 76% (1.87 g) yield, m.p. 192–93°C from **1c** (1.23 g, 5 mmol), ^{1}H NMR ($CDCl_3$): 1.85–1.95 (m, 4H, CH_2), 3.40–3.56 (t, 4H, NCH_2), 3.94 (s, 3H, OCH_3), 3.98 (s, 6H, OCH_3), 4.90–5.10 (t, 4H, CH_2Br), 7.05 (s, 2H, ArH); MS: m/z 495 (M^+, 34), 491 (M^+, 36), 302 (18), 298 (19), 193 (100), 167 (12); *Anal*. Calc. for $C_{17}H_{23}N_3O_4Br_2$: C, 41.40; H, 4.70; N, 8.52; Br, 32.40. Found: C, 41.35; H, 4.66; N, 8.55; Br, 32.45%.

Complex **2d** was obtained as white crystalline solid in 70% (1.70 g) yield, m.p. 144–46°C from **1d** (1.00 g, 5 mmol), ^{1}H NMR ($CDCl_3$): 1.80–1.90 (m, 4H, CH_2), 3.60–3.65 (t, 4H, NCH_2), 4.72–4.95 (t, 4H, CH_2Br), 7.45–7.50 (d, 2H, ArH), 7.70–7.80 (d, 2H, ArH); MS: m/z 450 (M^+, 21), 446 (M^+, 20), 302 (06), 298 (06), 148 (100), 122 (4); *Anal*. Calc. for $C_{14}H_{16}N_4O_3Br_2$: C, 37.52; H, 3.60; N, 12.50; Br, 35.66. Found: C, 37.48; H, 3.54; N, 12.54; Br, 35.70%.

Complex **2e** was obtained as white crystalline solid in 80% (1.64 g) yield, m.p. 134–35°C from **1e** (0.82 g, 5 mmol), ^{1}H NMR ($CDCl_3$): 1.80–1.90 (m, 4H, CH_2), 3.50–3.60 (t, 4H, NCH_2), 4.70–4.95 (t, 4H, CH_2Br), 7.40–7.70 (m, 3H, ArH); MS: m/z 411 (M^+, 32), 407 (M^+, 33), 302 (10), 298 (11), 109 (100); *Anal*. Calc. for $C_{12}H_{15}N_3OSBr_2$: C, 35.39; H, 3.71; N, 10.32; Br, 38.79; S, 7.86. Found: C, 35.36; H, 3.66; N, 10.37; Br, 38.83; S, 7.89%.

3.2. Antimitotic activity

The antimitotic activity of the synthesized products were studied by Onion Root Tip method [6]. The ID_{50} (concentration for 50% inhibition of mitosis) was determined as follows. After germinating the onion root tips for 2 days by immersing the onion to an extent of about 0.5 cm in a sample tube containing a test solution (prepared by dissolving known weight of synthetic derivative in 3 ml of ethanol and diluted with distilled water to 250 ml in a standard flask), germinated root tips were then removed and placed on the tube containing fixing solvent (ethanol–acetic acid 3:1 v/v) for 24 h. It was then kept in the preservating solvent (70% ethanol). Root tips were placed on the microslide, a drop of stain solution (Orcein in acetic acid: 0.2 N HCl 7:1 v/v) was added and the root tips were squashed by a blade. The slide was then mounted for observation under a microscope. The total number of cells and the number of dividing cells were counted. The percent of the number of dividing cells compared to the control and the percent inhibition of mitosis by the test antimitotic agent at a given concentration against a control were calculated. A graph of concentration versus per-

Table 1
Antimitotic activity of 5-aryl-2-*N*,*N*-dialkylamino-1,3,4-oxadiazoles

Compound	Concentration in 10^{-6} mol	No. of dividing cells	Total no. of cells	% of dividing cells	% of dividing cells when compared to the control	% of inhibition when compared to control	ID_{50} (mol l^{-1}) ($\times 10^{-6}$)
Control	235	1320	17.80	100	0		
1a	5.254, 13.824, 24.217	145, 104, 90	929, 802, 784	15.61, 12.95, 11.48	87.69, 72.81, 64.49	12.31, 27.19, 35.51	37.8
1c	6.038, 15.520, 25.538	126, 126, 91	915, 1028, 857	13.77, 12.26, 10.61	77.35, 67.88, 60.00	22.65, 31.12, 40.00	34.5
1e	8.522, 17.258, 22.028	140, 88, 65	956, 728, 656	14.64, 12.08, 9.91	82.24, 67.86, 55.67	17.76, 32.14, 44.33	24.5
2a	4.963, 9.926, 24.814	93, 88, 86	695, 822, 993	13.38, 10.70, 8.66	75.17, 60.13, 48.65	24.83, 39.87, 51.35	24.0
2b	4.619, 9.238, 23.095	113, 113, 85	931, 960, 1021	12.14, 11.77, 8.33	68.20, 66.12, 46.80	31.80, 33.88, 53.20	20.0
2c	4.057, 8.114, 20.284	107, 95, 73	962, 1036, 943	11.12, 9.17, 7.74	62.47, 51.52, 43.48	37.53, 48.48, 56.52	12.5
2d	2.232, 4.464, 11.161	154, 144, 154	912, 895, 1068	16.88, 16.08, 14.42	94.89, 90.34, 81.01	5.11, 9.66, 18.99	24.0
2e	2.445, 4.890, 12.225	279, 89, 115	2200, 1056, 2491	12.68, 8.43, 4.62	71.24, 47.36, 25.96	28.76, 52.67, 74.04	7.1

cent inhibition for each test compound was drawn. The concentration needed for 50% inhibition (ID_{50}) was got from extrapolating the line in the graph to 50% inhibition. ID_{50} values for the synthetic derivatives for antimitotic activity are tabulated in Table 1.

4. Results and discussion

All of the prepared compounds, as 2-amino-1,3,4-oxadiazole derivatives, showed some degrees of antimitotic activity. It is worth of note that some of the new products have showed increased antimitotic activity, notably the oxadiazole (**2e**) with thienyl moiety (ID_{50} value 7.1×10^{-6} M) when compared to the parent compound (**1e**) with a ID_{50} value 24.5×10^{-6} M. It was observed that, when aryl group possess electron donating group, the molecule showed some activity when compared to phenyl or *m*-nitrophenyl group. One of the possible mode of action could be that *N*,*N*-di(bromopropyl)aminooxadiazoles undergo ring closure to form strained azetidinium ion, which subsequently act to alkylate on critical cell constituents such as NH, SH or OH functions, thereby blocks the function of cell constituent. Chemically, the alkylation process would be favoured by the opening of azetidinium ion. The increased activity may be due to the presence of thienyl moiety in the molecule, which increase the nucleophilicity of amine nitrogen and facilitate the formation of azetidinium ion.

Acknowledgements

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