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Synthesis and vasodilating properties of *N*-alkylamide derivatives of 4-amino-3-furoxancarboxylic acid and related azo derivatives

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Abstract

A series of *N*-alkylamide derivatives of 4-amino-3-furoxancarboxylic acids **5a**–**11a** and their oxidation products, the azo derivatives **5b**–**11b**, were synthesised and studied for their vasodilating properties. All the products were able to release rat aorta strips precontracted with (−)noradrenaline. Azo derivatives proved to be 20–200 times more potent than the parent amines. The large variation of lipophilicity within the two series does not seem to influence significantly the activity. Experiments carried out in the presence of oxyhaemoglobin (HbO_2) suggest the involvement of nitric oxide ($NO^{\bullet}$) in the vasodilation.

Keywords: Aminofuroxans; Azofuroxans; Furoxancarboxylic acids; NO; Vasodilation

1. Introduction

Nitric oxide is an important biological messenger which elicits a surprisingly wide range of physiological and pathophysiological effects. Among other properties, it helps to maintain micro- and macrovascular homeostasis by a number of mechanisms involving activation of soluble guanylate cyclase, such as dilation of blood vessels, inhibition of platelet adherence and aggregation as well as attenuation of leukocyte adherence [1]. Today, there is an increasing interest in molecules able to release NO[1] in physiological conditions [2]. They are traditionally used in the treatment of cardiovascular diseases; typical examples are organic nitrates and nitrites, which were introduced in therapy many years before it was appreciated that their action involved NO-release. There is consolidated evidence that furoxan derivatives (1,2,5-oxadiazole 2-oxide (**1**), Fig. 1) can release NO in physiological conditions, under the action of thiol cofactors [2,3]. The NO-release mechanism appears to be complex, and it is not yet fully understood; it could involve more than one NO-redox form. In addition, an enzymatic pathway of NO-generation in the tissues cannot be excluded either [3]. This finding brought about a renewed interest in this minor heterocyclic system principally known for an argument on its structure and for its intriguing chemistry [4,5]. Amino and related azofuroxans constitute important but little explored families in the wide class of furoxan derivatives. Recently, the Russian team reported the preparation of 4-amino-3-furoxancarbonyl azide (**2**) [6]. This compound is able to transfer the 4-amino-3-furoxancarbonyl moiety to nucleophiles, under appropriate conditions. This makes it possible to obtain 4-aminofuroxans 3-substituted with a variety of COR groups. In this paper, we describe synthesis and properties of a new series of *N*-alkylamides of 4-amino-3-furoxancarboxylic acid (der.s **5a**–**11a**) obtained by reacting **2** with a number of amines with different lipophilicity. $KMnO_4$ oxidation of these products easily affords the corresponding azofuroxans (der.s **5b**–**11b**). All these com-

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[1] The term nitrogen monoxide (NO) is frequently used as family name embrassing the three redox forms nitric oxide ($NO^{\bullet}$), nitroxyl anion (NO^{-}) and nitrosonium ion (NO^{+}) [J.S. Stamler, D.J. Singel, J. Loscalzo, Biochemistry of nitric oxide and its redox-activated forms, Science, 258 (1992), 1898–1902]

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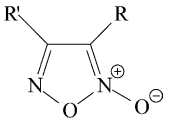

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Fig. 1. General structure of furoxan derivatives.

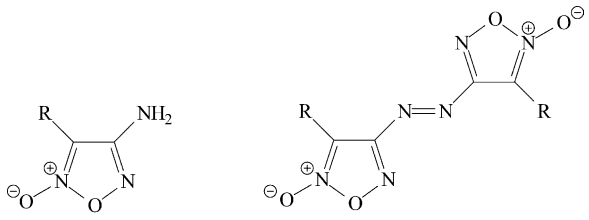
pounds, along with amines **3a**, **4a** and the related azo derivatives **3b**, **4b** (Fig. 2), were assayed for their vasodilating activity on rat aorta strips.

2. Chemistry

All the new products were synthesised according to the pathway reported in Scheme 1. Azido derivative **2** was treated in a mixture of THF/water with the appropriate amine at room temperature to give the expected amides in good yields. Room temperature $KMnO_4$ oxidation of these substances in a mixture of CH_2Cl_2/water containing hydrochloric acid gave the corresponding azo derivatives in moderate-good yields. We assume the prevalent trans configuration for these products, analogously to the one previously demonstrated in the case of 4,4′-dinitro-3,3′-diazenofuroxan [7]. Analytical and spectral data of the final compounds are collected in Table 1.

3. Pharmacology

Vasodilating activities of all the products were evaluated on rat thoracic aorta strips precontracted with (−)noradrenaline. Concentration-response curves were determined for each compound and the potency, expressed as EC_{50}, was calculated (Table 2). The experiments were repeated in the presence of 10 μM oxyhaemoglobin (HbO_2), a $NO^{\bullet}$ scavenger, and in some selected cases (**7a**, **7b**) also in the presence of 1 μM ODQ (1*H*-[1,2,4]-oxadiazolo[4,3-*a*]quinoxalin-1-one), an inhibitor of soluble guanylate cyclase (sGC). Potencies determined in these experiments, expressed as EC_{50} values, are entered in Table 2.



3a R = CH_3 **3b**

4a R = C_6H_5 **4b**

Fig. 2. Structure of reference compounds **3a**, **3b**, **4a**, **4b**.

4. Results and discussion

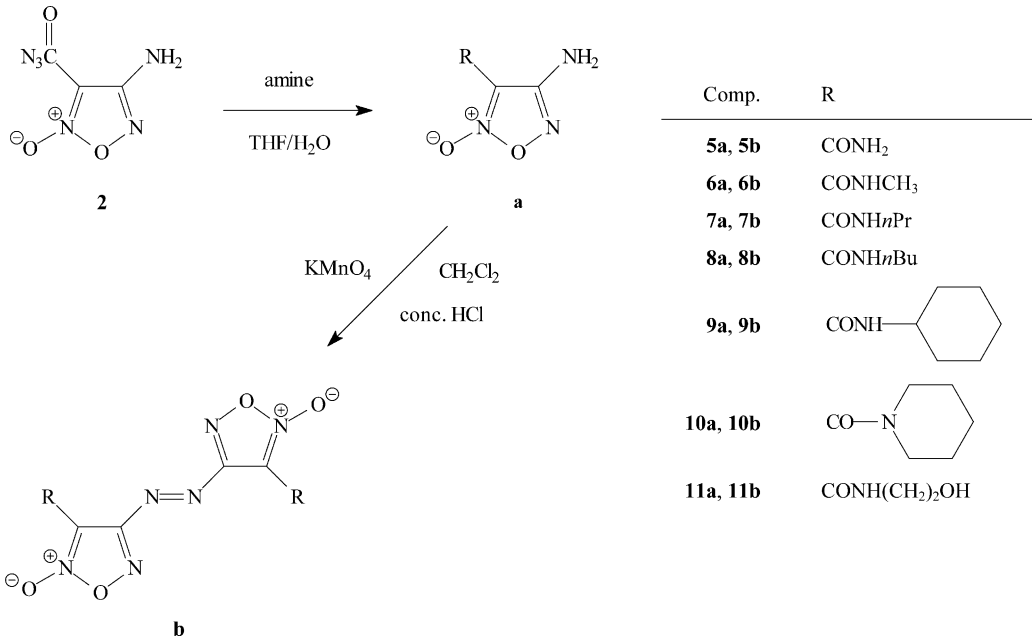
Analysis of Table 2 shows that all amino derivatives, with the exception of references **3a**, **4a**, which do not contain the electron-withdrawing amido group at the ring, display vasodilating activity. They were able to relax rat aorta strips precontracted with (−)noradrena-)noradrenaline in a concentration-dependent fashion. When the experiments were repeated in the presence of HbO_2, a significant rightward shift of the curves was observed. This decrease in the potency suggests that $NO^{\bullet}$ (or one of its bioequivalent forms) is involved in the dilation of the tissue. The EC_{50} values of the products, with the exception of **10a**, lie within a limited range (3–15 μM); this indicates that the large variation of lipophilicity in the series (see Table 2) does not significantly influence the activity. The most active compounds were the amides **8a** and **9a** bearing *n*-butyl and cyclohexyl group, respectively. Interestingly, the least potent amino derivative was **10a**, suggesting that the presence of hydrogen on amide nitrogen could play a role in the vasodilating action. Different behaviours were observed moving to azo derivatives as far as vasodilating activity is concerned. These compounds were definitely more potent (about 20–200-fold) than the parent amines and a larger decrease in the potency was observed in the presence of HbO_2. In this class also, the reference derivatives **3b**, **4b**—unlike the parent amino derivatives **3a**, **4a**—display good vasodilating activity, indicating an important role of the azo function in the dilation. The most active products were **7b** and **9b**, bearing on the amide nitrogen a *n*-propyl and a cyclohexyl group, respectively. These two latter compounds display a potency similar to the one of glyceryl trinitrate (GTN). Again, the least potent compound was the piperidinocarbonyl derivative **10b**. For the couple of *n*-propyl derivatives **7a**, **7b** the vasodilating experiments were repeated in the presence of ODQ. The decrease in the potency that occurs in these conditions again suggests the involvement of $NO^{\bullet}$ in the dilation of the tissue in both series.

In conclusion, the *N*-alkylamide derivatives of 4-amino-3-furoxancarboxylic acid described in the present work display rather good vasodilating properties, which are strongly improved in the corresponding azo analogues.

5. Experimental procedures

5.1. Chemistry

Melting points were determined on a Büchi B-540 apparatus and are uncorrected. All the compounds were routinely checked by IR (UR-20 spectrometer, KBr pellets) and ^1H NMR (Bruker WM-250). NMR spectra



Scheme 1. Synthetic pathway to amino and azo derivatives.

were detected in DMSO-d_6 and chemical shifts were referred to internal TMS. The following abbreviations were used to indicate the peak multiplicity: s, singlet; d, doublet; t, triplet; qt, quartet, m, multiplet. Mass spectra were recorded on a Finnigan MAT INCOS-50 instrument and are in keeping with the proposed structures. UV spectra (λ, nm) were recorded in MeOH on a Specord UV–Vis spectrophotometer. Analytical samples were purified either by recrystallisation or flash chromatography on silica gel. All the compounds were analysed for C, H, N and the results were within $\pm 0.4\%$ of the theoretical values. Products used for pharmacological tests were checked by HPLC. Chromatographic analysis was achieved using a Merck LiChrospher RP-18 column (250 × 4 mm; 5 μm particles size) termostatised at 40 °C, eluting (1 ml/min) with acetonitrile/water 60/40 (v/v).

Products **2** [6], **3a** [6,8], **3b** [9], **4a** [10], **4b** [9], **5a** [11], **6a** [12] were synthesised according to procedures reported in literature.

5.1.1. General method for the preparation of N-alkylamides of 4-amino-3-furoxancarboxylic acid 7a–11a

To a stirred solution of 4-amino-3-azidocarbonylfuroxan (**2**) (1.0 g, 5.9 mmol) in a mixture of THF (1 ml) and water (5 ml), 12 mmol of the appropriate amine were added dropwise. The reaction mixture was stirred for 1 h at room temperature (r.t.). After this time the formed precipitate was collected by filtration, washed twice with cold water and dried on air. The products **7a**, **10a** were purified by flash chromatography using as eluents 40–60 petroleum ether 3: dichloromethane 7 and dichloromethane, respectively. Compound **7a** was further recrystallised from hexane. The remaining products were obtained as pure compounds.

5.1.2. General method for the preparation of azofuroxans 5b–11b

A solution of $KMnO_4$ (1.5–2.0 mmol) in 16–20 ml of water was added drop-wise to a stirred water-cooled mixture (15–20 °C) of the appropriate aminofuroxan (2.0 mmol), CH_2Cl_2 (8 ml) and conc. HCl (10 ml). The stirring was continued for 3 h at r.t., then oxalic acid was added until MnO_2 disappeared. The precipitate was collected by filtration, washed twice with cold water and then with a small amount of CH_2Cl_2. The obtained product was dried on air. Derivative **11b** was obtained as a pure compound. The remaining derivatives were purified using the following procedure: **5b**, recrystallised from a mixture of methanol–ethanol; **6b**, flash-chromatographed using as eluent ethyl acetate 0.5:dichloromethane 9.5, then recrystallised from methanol; **7b**, **8b**, flash-chromatographed using as eluent 40–60 petroleum ether 3:dichloromethane 7; **9b**, **10b**, recrystallised from acetone.

5.2. Vasodilating activity assay

Thoracic aortas were isolated from male Wistar rats weighing 180–200 g. The endothelium was removed and the vessels were helically cut: three strips were obtained from each aorta. The tissues were mounted under 0.7 g tension in organ baths containing 30 ml of Krebs-bicarbonate buffer with the following composition (mM): NaCl 111.2, KCl 5.0, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.0, $NaHCO_3$ 12.0, glucose 11.1, maintained at

Table 1
Analytical and spectral data of amino- (**7a**–**11a**) and azofuroxans (**5b**–**11b**)

Comp.	Yield (%)	M.p. (°C)	IR	^{1}H NMR	Elem. anal. (%)	Found	Calc.
7a	88	118–119	3416, 3360, 3312 (NH), 2968 (CH), 1668 (C=O), 1624 (NH), 1580, 1500, 1480, 1416 (f-cycl.), 1256 ($C_{c.}$–NH_2)	0.92 t (3H, CH_3) 1.57 m (2H, CH_2) 3.28 m (2H, N–CH_2) 6.47 br.s (2H, NH_2) 8.21 br.s (1H, NH)	C H N	41.8 5.5 24.9	42.1 5.3 24.6
8a	75	97–98	3460, 3360, 3330, 3235 (NH), 2970, 2945, 2880 (CH), 1670 (C=O), 1625 (NH), 1585, 1515, 1476, 1382 (f-cycl.), 1233 ($C_{c.}$–NH_2)	0.89 t (3H, CH_3) 1.32 m (2H, CH_2) 1.50 m (2H, CH_2) 3.30 m (2H, N–CH_2) 6.50 br.s (2H, NH_2) 8.24 br.s (1H, NH)	C H N	45.15 6.2 22.4	45.4 6.0 22.7
9a	79	121–122	3440, 3328, 3224 (NH), 2944, 2856 (CH), 1668 (C=O), 1620 (NH), 1584, 1544, 1492, 1452 (f-cycl.), 1212 ($C_{c.}$–NH_2)	1.2–1.9 m (10H, $5CH_2$) 3.81 m (1H, N–CH) 6.47 s, br (2H, NH_2) 7.82 d (1H, NH)	C H N	45.2 6.3 22.9	45.4 6.0 22.7
10a	86	115–116	3440, 3327, 3235 (NH), 2960, 2875 (CH), 1645 (C=O), 1600, 1511, 1452, 1378 (f-cycl.), 1232 ($C_{c.}$–NH_2)	1.57 m (6H, $3CH_2$) 3.27–3.54 s (4H, 2N–CH_2) 6.46 s (2H, NH_2)	C H N	47.5 5.9 25.0	47.8 6.2 24.8
11a	94	157–158 (dec.)	3432, 3376, 3312(NH), 2992, 2936, 2888 (CH), 1644(C=O), 1623(NH), 1584, 1536, 1488, 1440, 1416(f-cycl.), 1368(OH), 1204($C_{c.}$–NH_2), 1048(C–O)	4.87 t (1H, OH) 3.39 m (2H, N–CH_2) 3.53 m (2H, O–CH_2) 6.50 s (2H, NH_2) 8.18 t (1H, NH)	C H N	45.5 5.4 26.2	45.3 5.7 26.4
5b	64	187–188 (dec.)	3408, 3344, 3192(NH), 1684(C=O), 1632 (NH), 1596, 1520, 1432, 1396(f-cycl.), 1472(N=N)	8.07, 8.33 2s (2H, NH_2)	C H N	25.1 1.3 39.7	25.4 1.4 39.4
6b	74	133–134 (soft.) 159–160 (dec.)	3304(NH), 2944(CH), 1709, 1676(C=O), 1628(NH), 1552, 1518, 1412, 1332(f-cycl.), 1480(N=N)	2.86 d (3H, CH_3) 8.62 qt (1H, NH)	C H N	30.6 2.8 36.2	30.8 2.6 35.9
7b	57	168–169 (dec.)	3288, 3096(NH), 2976, 2960, 2936, 2872(CH), 1668(C=O), 1612(NH), 1572, 1440, 1328(f-cycl.), 1480(N=N)	0.92 t (3H, CH_3) 1.54 qt (2H, CH_2) 3.21 m (2H, N–CH_2) 8.82 t, br (1H, NH)	C H N	42.3 4.4 25.1	42.6 4.2 24.8
8b	42	168–169 (dec.)	3352(NH), 2960, 2936, 2864(CH), 1664(C=O), 1616(NH), 1592, 1552, 1440, 1328(f-cycl.), 1472(N=N)	0.96 t (3H, CH_3) 1.37 m (2H, CH_2) 1.52 m (2H, CH_2) 3.37 qt (2H, N–CH_2) 8.62 t (1H, NH)	C H N	45.6 5.2 23.1	45.9 5.0 22.9
9b	45	205–206 (dec.)	3264, 3088(NH), 2936, 2856(CH), 1660(C=O), 1616(NH), 1564, 1440, 1336(f-cycl.), 1488(N=N)	1.17–1.93 m (10H, $5CH_2$) 3.70 br.s (1H, N–CH) 8.72 d (1H, NH)	C H N	45.7 5.3 23.2	45.9 5.0 22.9
10b	40	201–202 (dec.)	2960, 2936, 2920, 2864(CH), 1660(C=O), 1608, 1448, 1420, 1304 (f-cycl.), 1488(N=N)	1.52 m (2H, CH_2) 1.67 m (4H, $2CH_2$) 3.35 m (2H, N–CH_2) 3.60 m (2H, N–CH_2)	C H N	47.9 5.5 25.3	48.2 5.4 25.0
11b	34	137–138 (dec.)	3280(NH), 2944, 2888(CH), 1700, 1664(C = O), 1604(NH), 1536, 1432, 1392, 1328(f-cycl.), 1468(N = N), 1048(C–O)	3.32 m (2H, N–CH_2) 3.53 m (2H, O–CH_2) 4.60 br.s (1H, OH) 8.87 s (1H, NH)	C H N	45.4 5.0 26.4	45.7 4.8 26.7

37 °C and gassed with 95% O_2–5% CO_2 (pH 7.4). The aortic strips were allowed to equilibrate for 2.5 h and then contracted with 1 μM (−)noradrenaline. When the response to the agonist reached a plateau, cumulative concentrations of the vasodilating agent were added. Results are expressed as $EC_{50} \pm SEM$ (μM). The effects of both 10 μM HbO_2 and 1 μM ODQ on relaxation were evaluated in separate series of experiments in which

Table 2
In vitro evaluation of vasodilating activity

Comp.	EC_{50} (μM)	EC_{50} (μM) + HbO_2 (+ODQ)	CLOGP
GTN	0.022 ± 0.004		
3a	inactive	–	−0.32
3b	0.15 ± 0.02	7.0 ± 0.3	1.08
4a	inactive	–	1.42
4b	0.50 ± 0.10	1.2 ± 0.2	4.73
5a	15 ± 1	32 ± 3	−0.32
5b	0.26 ± 0.04	3.3 ± 0.6	−1.3
6a	11 ± 1	20 ± 1	−0.01
6b	0.15 ± 0.02	2.6 ± 0.1	−0.83
7a	5.9 ± 0.6	8.2 ± 0.2 (58 ± 8)	1.05
7b	0.042 ± 0.008	1.1 ± 0.2 (2.0 ± 0.1)	1.29
8a	5.5 ± 0.5	9.7 ± 0.6	1.58
8b	0.10 ± 0.02	1.1 ± 0.1	2.35
9a	3.1 ± 0.3	5.5 ± 0.9	2.02
9b	0.057 ± 0.012	0.66 ± 0.08	3.23
10a	79 ± 8	182 ± 35	0.26
10b	0.40 ± 0.06	3.3 ± 0.2	0.16
11a	7.7 ± 1.2	13 ± 1	−0.22
11b	0.26 ± 0.01	5.8 ± 0.4	−1.95

CLOGP [13] values are also reported.

HbO_2 was added immediately, while ODQ 5 min before the contraction.

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