Synthesis of 1-aminobenzo[*d*]imidazol-2-ylmethanol derivatives and their reactivity towards DT-diaphorase

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New nitro substituted 1-arylmethylideneaminobenzo[*d*]imidazol-2-ylmethanols (**2 a–i**) were synthesized by the condensation of 1-aminobenzo[*d*]imidazol-2-ylmethanol (**1**) with various nitroaromatic aldehydes. The obtained derivatives **2 b–i** were investigated for their reactivity towards the enzyme DT-diaphorase by kinetic methods. Some changes in the hydroxy group of 1-aminobenzo[*d*]imidazol-2-ylmethanol were made.

Key words: benzimidazole, 1-aminobenzo[*d*]imidazol-2-ylmethanol, nitroaromatic compounds, enzymatic reduction, Shiff bases, DT-diaphorase

INTRODUCTION

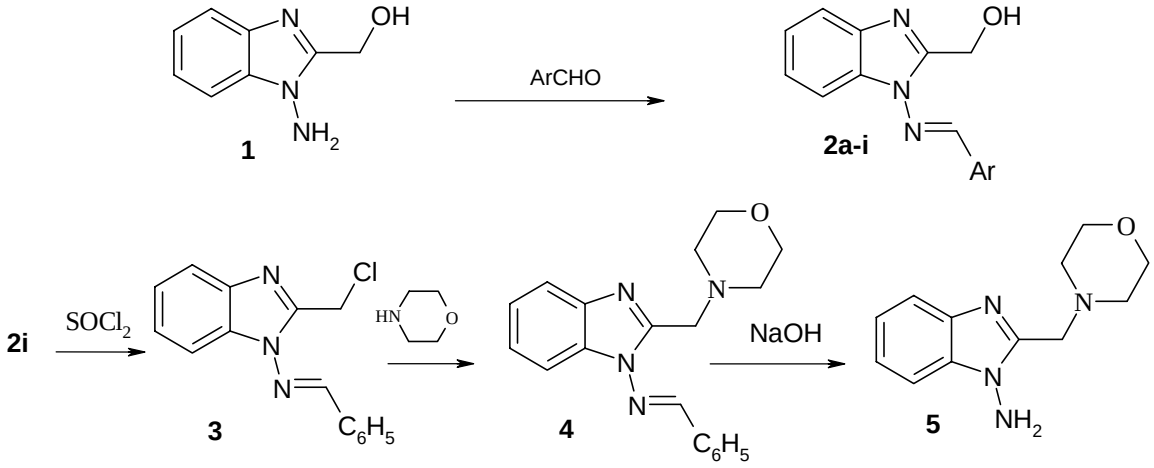
Bifunctional benzo[*d*]imidazoles are important intermediates for the synthesis of heterocyclic compounds containing fused heterosystems, which sometimes exhibit anti-cancer activity [1, 2]. Continuing our interest in the synthesis and investigation of benzo[*d*]imidazoles and in the search for biologically active compounds, we have synthesized a series of nitro derivatives of 1-[phenylmethylideneamino]-1*H*-benzo[*d*]imidazol-2-ylmethanol. The hydroxy group of protected 1-aminobenzo[*d*]imidazol-2-ylmethanol (**2a**) was changed to chloro and then to morpholino groups. The reduction of nitroaromatic compounds by DT-diaphorase to cytotoxic hydroxylamines seems to be important for toxicity towards certain types of cancer cells in which the content of DT-diaphorase is increased 10–100 times [3]. For this reason, the reactivity of nitroaromatic compounds towards this enzyme was investigated.

RESULTS AND DISCUSSION

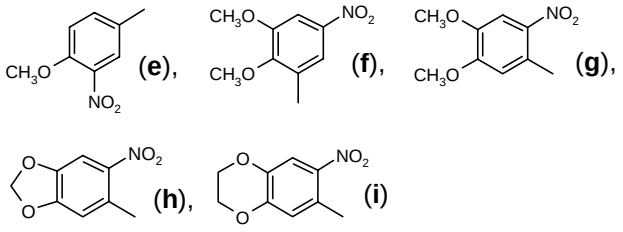
1-Aminobenzo[d]imidazol-2-ylmethanol (**1**) was synthesized by the methods as described in [4]. The amino group of compound **1** was protected by condensation with benzaldehyde. The hydroxy group of the obtained derivative **2a** was converted to a chloro group by the treatment of thionyl chloride. The obtained chloro derivative **3** reacts with morpholine to give compound **4**. The amino group in the 1st position of compound **4** was easily deprotected by the treatment with aqueous sodium hydroxide solution. Shiff bases **2 b–i** were synthesized by the condensation of amine **1** with various nitro substituted benzaldehydes (Scheme).

Table 1 presents the kinetic parameter values obtained for the two-electron reduction of nitroaromatic compounds **2 b–i** by rat liver DT-diaphorase (NADP(H):quinone oxidoreductase EC 1.6.99.2). The catalytic constants (k_{cat}) obtained for compounds **2 b–h** were close to previously determined k_{cat} values for 3,5-dinitrobenzamide or nitrobenzaldehyde [5]. Surprisingly, the $k_{cat}/K_{m(\text{nitroaromatic compound})}$ parameters obtained for compounds **2 b–h** were significantly higher than those for 3,5-dinitrobenzamide or nitrobenzaldehydes. The data of our work and previous observations [5] showed that the rates of DT-diaphorase reactions with nitrocompounds do not depend on the NADPH concentration, thus pointing to the low values of K_m for NADPH. Since nitroaromatic compounds are competitive inhibitors with respect to NADPH in the 2-methyl-1,4-naphthoquinone reaction of DT-diaphorase [5], the inhibition

Scheme



2a-i: Ar= C_6H_5 (**a**) 2-$NO_2C_6H_4$ (**b**), 3-$NO_2C_6H_4$ (**c**), 4-$NO_2C_6H_4$ (**d**),



properties of these compounds were tested as well. The competitive inhibition constants $K_{inhib.}$ determined for nitroaromatic compounds **2f** and **2h** were 140 μM and 230 μM, respectively.

A minimal kinetic scheme for the two-electron reduction of aromatic nitrocompounds by DT-diaphorase can be presented as follows:

$$E_0 + NADPH \xrightarrow{k_1} E_r + NADP^+,$$

$$E_r + ArNO_2 \underset{k_{-2}}{\overset{k_2}{\leftrightarrow}} E_rArNO_2 \xrightarrow{k_3} E_o + ArNO.$$

For this model, the hyperbolic form of the steady-state rate equation is:

$$v/[E] = k_3/\{1 + k_3/k_1[NADPH] + (k_{-2} + k_3)/k_2[\text{aromatic nitrocompound}]\},$$

where k_{cat} equals k_3 and the apparent Michaelis constant $K_{m(NADPH)}$ for NADPH oxidation equals k_3/k_1. The $K_{m(\text{aromatic nitrocompound})}$ and the $k_{cat}/K_{m(\text{aromatic nitrocompound})}$ ratio for the reduction of aromatic nitro compound equal $(k_{-2} + k_3)/k_2$ and $k_2k_3/(k_{-2} + k_3)$, respectively. Since in the nitroreductase reaction of DT-diaphorase the $K_{m(NADPH)}$ values for NADPH are too low to be evaluated experimentally, we calculated the tentative $K_{m(NADPH)}$ values using the determined value of k_{cat} for the reactions of aromatic nitrocompounds (Table 3) and the k_1 value determined for NADPH oxidation in the quinone reductase reaction of DT-diaphorase ($k_1 = 3.3 \times 10^7$ M^{-1} s^{-1}).

Our data indicate that the low values of the apparent K_m for NADPH in the nitroreductase reactions of DT-diaphora-

Table 1. **Data on reactivity towards DT-diaphorase for compounds 2 b–h**

Compound	k_{cat} (s^{-1})	$K^a_{m\ (NADPH)}$ (μM)	$K_{m(\text{nitroaromatic compound})}$ (μM)	$k_{cat}/K_{m\ (\text{nitroaromatic compound})}$ (M^{-1} s^{-1})
2a	0.13	4.0	232.10	5.60×10^2
2b	0.13	4.0	135.14	9.62×10^2
2c	0.07	2.0	89.97	7.78×10^2
2d	0.19	6.0	275.36	6.90×10^2
2e	0.03	0.9	272.73	1.10×10^2
2f	0.07	2.0	211.48	3.31×10^2

[s] $K_{m(NADPH)}$ values are calculated from $K_{m(NADPH)} = k_{cat}/k_1$, assuming that k_1 for NADPH oxidation in the menadione reduction reaction equals 3.3×10^7 M^{-1} s^{-1}.

Table 2. **Experimental, physico-chemical and spectral data on compounds 2 a–i**

Compound	Reaction time and yield	M.p. (°C)	IR: ν_{OH} (cm^{-1})	^{1}H-NMR (δ, TMS, in DMSO-D_6): CH_2O, CH_3O	OH	5, 6-H	4, 7-H	ArH	=CH
2a	2 h, 76%	138–140	3139	s 5.13	s 3.91		m 7.35–8.30		s 9.35
2b	2 h, 50%	202–203	3270	s 4.91	m 3.72–4.24	m 3.72–4.24	m 7.61–8.38		s 9.60
2c	2 h, 61%	222–223	3232	d 4.91	t 5.72		m 7.09–8.46		s 9.40
2d	2 h, 54%	209–210	3194	s 5.14	s 4.46		m 7.28–8.41		s 9.52
2e	12 h, 83%	213–214	3230	d 4.87	t 5.59		m 7.23–8.64	s 9.26	
2f	8 h, 89%	219–220	3187	d 4.87	t 5.73	m 7.22–7.38	m 7.54–7.84	d 7.95, 8.41	s 9.34
2g	18 h, 53%	249–250	3248	d 4.91	t 5.63	m 7.28–7.49	m 7.89–8.09	s 7.73, 7.79	s 9.61
2h	24 h, 63%	236–237	3222	d 4.87, s 6.37	t 5.62	m 7.25–7.43	m 7.86–8.14	s 7.76, 7.82	s 9.51
2i	24 h, 58%	238–239	3188	d 4.85, s 4.48	t 5.62	m 7.39–7.48	m 7.86–8.12	s 7.71, 7.79	s 9.53

[a] Shoulder, [b] Singlet, [c] Multiplet, [d] Triplet (J = 6 Hz), [e] Quartet (J = 6 Hz).

Table 3. **Elemental analysis data on compounds 2 a–i and 3–5**

Compound	Formula	Found, %			Calculated, %		
		C	H	N	C	H	N
2a	$C_{15}H_{13}N_3O$	72.04	5.43	17.02	71.7	5.21	16.72
2b	$C_{15}H_{12}N_4O_3$	60.89	4.08	18.91	60.81	4.08	18.91
2c	$C_{15}H_{12}N_4O_3$	60.50	4.25	19.20	60.81	4.08	18.91
2d	$C_{15}H_{12}N_4O_3$	61.13	4.41	18.64	60.81	4.08	18.91
2e	$C_{17}H_{16}N_4O_5$	57.37	5.08	15.77	57.3	4.53	15.72
2f	$C_{17}H_{16}N_4O_5$	57.19	4.71	15.87	57.3	4.53	15.72
2g	$C_{16}H_{11}N_4O_4$	58.94	4.96	16.77	58.89	4.32	17.17
2h	$C_{17}H_{14}N_4O_5$	57.38	4.15	15.68	57.63	3.98	15.81
2i	$C_{16}H_{11}N_4O_5$	56.63	3.80	16.20	56.47	3.55	16.46
3	$C_{15}H_{12}N_3Cl$	67.52	5.16	15.60	66.79	4.48	15.58
4	$C_{19}H_{20}N_4O$	70.85	6.88	17.59	71.23	6.29	17.49
5	$C_{12}H_{16}N_4O$	62.30	7.17	24.25	62.05	6.94	24.12

se are influenced by the low k_{cat} or k_{cat}/k_1 ratio values.

The postulated structure of the newly synthesized compounds and **2 a–i** and **3–5** is in agreement with their IR, ^{1}H NMR spectral (Table 2) and elemental analysis (Table 3) data.

EXPERIMENTAL

Chemistry

Melting points were determined in open capillaries and are uncorrected. The UV spectra were recorded on a Lambda 20 (Perkin–Elmer, Sweden), IR spectra on a FT-IR Spectrum BX (Perkin–Elmer, Sweden) in nujol and ^{1}H-NMR spectra on a BS-587A (80 MHz, Tesla, Czechoslovakia) with TMS as an internal standart. Chemical shifts (δ) are reported in ppm, coupling constants (J) are given in Hz. All new compounds were analyzed for C, H and N and the results were in an acceptable range.

1-Arylmethylideneaminobenzo[d]imidazol-2-ylmethanols (2 a–i). A mixture of 1 g (0.6 mmol) 1-aminobenzo[d]imidazol-2-ylmethanol (**1**), 1 drop of piperidine, 0.6 mmol of a corresponding aldehyde and 20 ml 2-propanol was refluxed 2 (**2 a–d**), 8 (**2e**), 16 (**2g**) or 24 (**2 f**, **h**, **i**) h and cooled. The obtained solids were filtred off and recrystallized from toluene (**2a**), 2-propanol (**2b**), acetone (**2e**) or dimethylformamide (**2 c**,**d**, **f-i**). Experimental, physico-chemical and spectral data for compounds **2 a–i** are given in Table 2.

2-Chloromethylbenzo[d]imidazolyl-1-phenylmethanimine (3). A mixture of 0.3 g (0.12 mmol) of 1-phenylmethylideneaminobenzo[d]imidazol-2-ylmethanol (**2a**) and 20 ml thionyl chloride was heated for 1 h at 50–60 °C. The excess of thionyl chloride was evaporated *in vacuo*. The residue was triturated with ethanol. The obtained crystalls were filtered off, washed with toluene and recrystallized from ethanol. Yield 0.28 g (86%), m. p. 147.5–148 °C. ^{1}H BMR (δ, ppm, $CDCl_3$): 5.21 (2H, s, CH_2), 9.25 (1H, s, CH), 7.4–8.05 (4H, m, ArH).

2-Morpholinomethylbenzo[d]imidazolyl-1-phenylmethanimine (4). A solution of 0.27 g (0.1 mmol) of 2-chloromethylbenzo[d]imidazolyl-1-phenylmethanimine (**3**) and 0.17g (0.2 mmol) morpholine in 30 ml ethanol was heated at 50–60 °C for 8 h and evaporated

in vacuo. The residue was dissolved in trichloromethane, washed with water, dried and evaporated *in vacuo*. The obtained solid product was triturated with octane and recrystallized from octane. Yield 0.23 g (72%), m. p. 60–61 °C. ^{1}H NMR (δ, ppm, $CDCl_3$): 2.53–2.81 (4H, m, $N(CH_2)_2$), 3.58–3.85 (4H, m, $O(CH_2)_2$), 3.99 (2H, s, CH_2), 9.1 (1H, s, CH), 7.2–7.98 (4H, m, ArH).

2-Morpholinomethylbenzo[d]imidazolyl-1-amine (5). A mixture of 0.09 g (0.028 mmol) 2-morpholinomethylbenzo[d]imidazolyl-1-phenylmetanimine (**4**) and a solution of 0.8 g (2 mmol) sodium hydroxide in 20 ml water was refluxed for 7 h and evaporated *in vacuo*. The obtained crystalls were washed with water, dried and recrystallized from toluene. Yield 0.06 g (92%), m. p. 188–189 °C. IR (ν, cm^{-1}): 3298, 3234 (NH_2). ^{1}H NMR (δ, ppm, $CDCl_3$): 2.42–2.66 (4H, m, $N(CH_2)_2$), 3.61–3.82 (4H, m, $O(CH_2)_2$), 3.88 (2H, s, CH_2), 5.20 (2H, s, NH_2), 7.11–7.78 (4H, m, ArH.).

Enzymatic assays

Rat liver DT-diaphorase (NAD(P)H:quinone oxidoreductase, EC 1.6.99.2) was prepared as described in [10] and the enzyme concentration was determined spectrophotometrically using $\Delta\varepsilon_{460}$ = 11 mM^{-1} cm^{-1} [6, 7]. The activity of DT-diaphorase was monitored according to the increase in absorbance of reduced cytochrome c at 550 nm as described [8], in the presence of 10 μM menadione (2-methyl-1,4-naphthoquinone), 50 μM cytochrome c and 200 μM NADPH. The enzyme activity was equal to 2000 s^{-1}. The reduction rates of aromatic nitrocompounds 2 a–h by rat liver DT-diaphorase were monitored spectrophotometrically following NADPH oxidation ($\Delta\varepsilon_{340}$ = = 6.2 mM^{-1} cm^{-1}). All kinetic measurements were carried out in 0.1 M potassium phosphate pH 7.0 containing 1 mM EDTA at 25 °C. Tween-20 (final concentration 0.01%) and bovine serum albumin (final concentration 0.25 mg/ml) were used as enzyme activators [8]. The kinetic parameters, the catalytic constant (k_{cat}) and the Michaelis constant ($K_{m\ (aromatic\ nitrocompound)}$) were determined by fitting the data to the Michaelis–Menten equation. k_{cat} is the number of NADPH molecules oxidized by the single active center of the enzyme per second. The rates obtained were corrected for the intrinsic NADPH-oxidase activity of the enzyme. The nitroaromatic compound inhibition constants ($K_{inhib.}$) *vs* NADPH : quinone reductase reaction of DT-diaphorase were obtained from the equation:

$$v/v_{inhib} = (K_{inhib.} + [\text{nitroaromatic compound}])/K_{inhib.},$$

where v and $v_{inhib.}$ are the initial rates of menadione reduction in the absence and presence of an aromatic nitrocompound, respectively.

CONCLUSIONS

A series of new nitro substituted 1-arylmethylideneaminobenzo[*d*]imidazol-2-ylmethanols were synthesized and investigated for their reactivity towards the enzyme DT-diaphorase. Some changes in the hydroxy group of 1-aminobenzo[*d*]imidazol-2-ylmethanol were induced.

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1-AMINOBENZO[*D*]IMIDAZOL-2-ILMETANOLIO DARINIŲ SINTEZĖ IR JŲ REAKTYVUMAS DT-DIAFORAZĖS ATŽVILGIU

S a n t r a u k a

Nauji 1-arilmetilidenaminobenzo[*d*]imidazol-2-ilmetanolio dariniai buvo susintetinti kondensuojant 1-aminobenzo[*d*]imidazol-2-ilmetanolį su įvairiais aromatiniais nitrogrupę turinčiais aldehidais. Kinetiniais metodais buvo ištirtas gautų nitrodarinių reaktyvumas fermento DT-diaforazės, redukuojančios nitrojunginius iki hidroksilaminų, atžvilgiu. Atlikti kai kurie 1-aminobenzo[*d*]imidazol-2-ilmetanolio hidroksigrupės kitimai.