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Estimation of single-electron reduction potentials (E^1_7) of nitroaromatic compounds according to the kinetics of their single-electron reduction by flavoenzymes

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Because of the instability of nitroaromatic anion-radicals, the single-electron reduction potentials of nitroaromatic compounds are usually obtained by means of pulse-radiolysis and flash-photolysis. Here we present an alternative method of the estimation of single-electron reduction potentials of nitroaromatic compounds at pH 7.0 (E^1_7), based on the linear log rate constant *vs.* E^1_7 dependences in their single-electron reduction by flavoenzymes electrontransferases. The geometric averages of the bimolecular steady-state rate constants of the reduction of nitroaromatics by flavocytochrome b_2, ferredoxin: $NADP^+$ reductase, or NADPH: cytochrome P-450 reductase were used as the correlation parameters. The differences between the directly determined E^1_7 for a number of nitroaromatic compounds and their calculated values did not exceed 35 mV. This approach enabled us to characterize the E^1_7 values of 36 previously uncharacterized nitroaromatic compounds, including important antitumour and antiparasitic agents and explosives.

Key words: nitroaromatic compounds, single-electron reduction potential, flavoenzymes

Abbreviations: $ArNO_2$, aromatic nitrocompound; $ArNO_2^{-\cdot}$, nitroaromatic anion-radical; CYT B_2, flavocytochrome b_2; E^1_7, singe-electron reduction potential; $E^1_{7(calc.)}$, calculated single-electron reduction potential; FAD, flavinadenindinucleotide; FMN, flavinmononucleotide; FNR, ferredoxin:$NADP^+$ reductase; k_{cat}, catalytic constant; k_{cat} / K_m, bimolecular rate constant in steady-state enzymatic reactions; NADPH, reduced nicotinamide adenine dinucleotide phosphate; P, product; P-450R, NADPH:cytochrome P-450 reductase; S, substrate

INTRODUCTION

Nitroaromatic compounds are widely used in the industry, military activities, pharmacy, and agriculture. The toxicity and/or therapeutic activity of nitroaromatic compounds ($ArNO_2$) is frequently caused by their single-electron enzymatic reduction to anion-radicals ($ArNO_2^{-\cdot}$), the latter further undergoing redox cycling, i.e. the reoxidation by O_2 with the formation of parent compound and superoxide ($O_2^{-\cdot}$) (Fig. 1), which subsequently forms toxic hydrogen peroxide (H_2O_2) and hydroxyl ($OH\cdot$) [1]. In this case, the mammalian cell toxicity or antiparasitic activity of nitroaromatics may increase upon an increase in their single-electron reduction potential at pH 7.0 (E^1_7), i.e. the potential of $ArNO_2/ArNO_2^{-\cdot}$ redox couple [2, 3]. Thus, E^1_7 of nitroaromatic compounds is an impor-

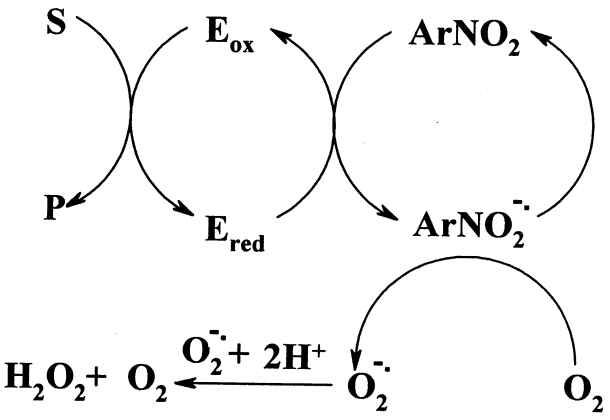

Fig. 1. Enzymatic redox cycling of nitroaromatic compounds ($ArNO_2$). E_{ox}, E_{red}, S, and P stand for oxidized and reduced enzyme, substrate, and product, respectively

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tant parameter having an impact on their biological activity.

Nitroaromatic anion-radicals are very unstable, their dismutation rate constants range from 10^4 to 10^8 $M^{-1}s^{-1}$ [3]. The electrochemical reduction of nitroaromatic compounds in aqueous media proceeds irreversibly with the formation of hydroxylamines ([4] and references therein) and does not reflect the energetics of single-electron reduction. For this reason, the E^1_7 values of nitroaromatics are obtained only by means of pulse-radiolysis or flash-photolysis, monitoring the equilibrium of transiently formed $ArNO_2^{-\cdot}$ with the redox indicators [3].

An alternative but yet unexploited approach to obtain the unavailable E^1_7 of nitroaromatics may be the use of the linear log rate constant *vs.* E^1_7 dependences in their single-electron reduction by flavoenzymes electrontransferases [5]. These reactions follow an 'outer-sphere' electron-transfer model and are relatively insensitive to the particular structures of nitroaromatics. In this paper, we demonstrate that this approach enables us to obtain the E^1_7 of nitroaromatic compounds which fairly agree with their directly determined values. Further, we characterized the previously unavailable E^1_7 values for a number of important nitroaromatic compounds.

EXPERIMENTAL

Model nitroaromatic compounds (nitrobenzene, *p*-nitrobenzyl alcohol, *p*-nitrobenzoic acid, *p*-nitroacetophenone, 3,5-dinitrobenzoic acid, 3,5-dinitrobenzamide, *p*-nitrobenzaldehyde, nitracrine, nifuroxime, nitrofurantoin, and *o*-, *m*-, and *p*-dinitrobenzenenes) were obtained from Sigma and used without further purificiation. The formulae of nontrivial nitroaromatic compounds are given in Figs. 2, 3. The methods of their synthesis are listed consecutively: 2,4,6-trinitrotoluene (TNT) and its amino- and hydroxylamino derivatives [6, 7], tetryl and *N*-methylpicramide [8], pentryl (2,4,6-trinitrophenyl-*N*-nitraminoethylnitrate) and dipentryl [9, 10], 5-[bis(2,2'-chloroethyl)amino]-2,4-dinitrobenzoic acid amide (SN-23682), 5-(aziridin-1-yl)-2,4-dinitrobenzamide (CB-1954) and its derivatives [11, 12], nitrofuranes (derivatives of 2-(5'-nitrofurylvinyl)-quinoline-4-carbonic acid [13], nitrobenzimidazoles and nitrobenzimidazolones [14, 15], 1,3,6,8-tetranitrocarbazole (TNC) [16], 5-nitro-1,2,4-triazol-3-one (NTO) and 5-nitro-1,2,4-triazol-3-amine (ANTA) [17], 4,6-dinitrobenzofuroxan (DNBF), and 5,7-diamino-4,6-dinitrobenzofuroxan (CL-14) [18, 19]. The purity of the compounds was determined using melting points, TLC, NMR, IR, and elemental analysis.

Flavocytochrome b_2 (L-lactate:cytochrome *c* reductase, EC 1.1.2.3) from *Saccharomyces cerevisiae* (CYT B_2) and NADPH:cytochrome P-450 reductase (EC 1.6.4.2) from pig liver (P-450R) were prepared by the

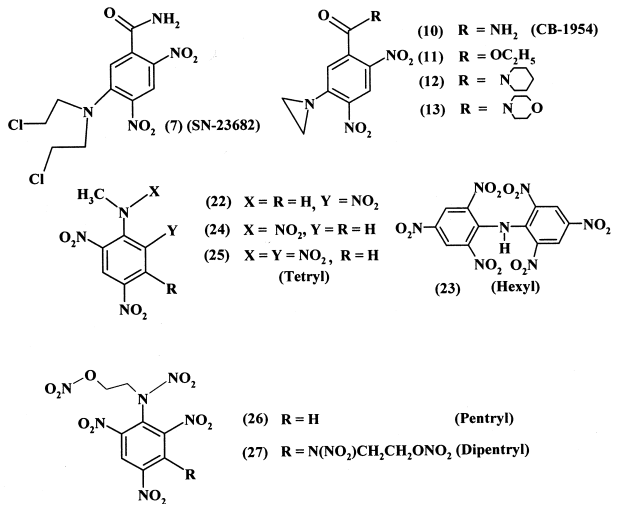

Fig. 2. Structural formulae of nitrobenzene derivatives studied in this work

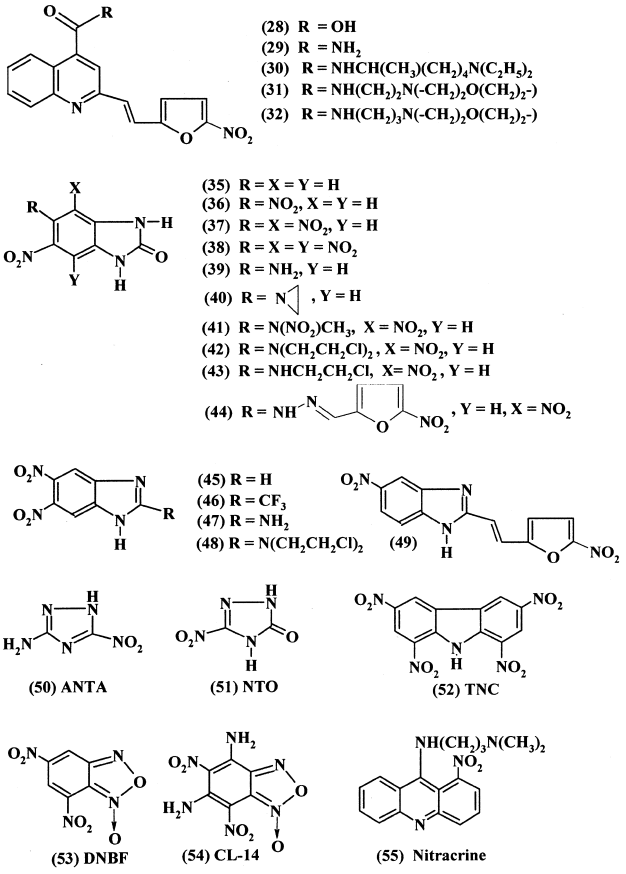

Fig. 3. Structural formulae of nitroheteterocyclic compounds studied in this work

described methods [20, 21]. Ferredoxin: $NADP^+$ reductase (EC 1.18.1.2) from *Anabaena* (FNR) prepared as described in [22] was a generous gift of Dr. M. Martinez-Julvez and Professor C. Gomez-Moreno (Zaragoza University, Spain). Enzyme concentrations were determined spectrophotometrically using a Hitachi 557 spectrophotometer according to ε_{423} = 183 mM^{-1} cm^{-1} (CYT B_2, reduced form (in the presence

Table. **Single-electron reduction potentials (E^1_7) of nitroaromatic compounds; bimolecular rate constants (k_{cat} / K_m) of their reduction by flavocytochrome b_2 (CYT B_2), ferredoxin: $NADP^+$ reductase (FNR), NADPH:cytochrome P-450 reductase (P-450 R); $E^1_{7(calc.)}$ calculated according to Eqs. (1–3), and its mean values obtained from the data of three correlations**

No.	Compound	E^1_7 [a] (V)	k_{cat} / K_m (M^{-1} s^{-1})[b]			$E^1_{7(calc.)}$ (V)			
			CYT B_2	FNR	P-450R	Eq. (1)	Eq. (2)	Eq. (3)	Mean
	Nitrobenzenes								
1	2,4-Diamino-6-nitrotoluene	–0.502[c]		2.1×10^2	2.0×10^3		–0.467		
2	Nitrobenzene	–0.485	1.0×10^1	1.1×10^1	2.8×10^3	–0.507	–0.519	–0.470	–0.499 ± 0.025
3	4-Nitrobenzyl alcohol	–0.475		1.4×10^2	1.2×10^4		–0.440		
4	4-Amino-2,6-dinitrotoluene	–0.449[c]		3.4×10^2	2.5×10^3		–0.453		
5	4-Hydroxylamino-2,6-dinitrotoluene			2.1×10^2	1.3×10^4		–0.429		
6	4-Nitrobenzoic acid	–0.425	5.0×10^1	2.5×10^2	5.6×10^3	–0.422	–0.443	–0.427	–0.431 ± 0.011
7	5-[bis(2,2'-chloroethyl) amino]-2,4-dinitrobenzoic acid amide (SN-23682)	–0.425	5.0×10^1[d]	1.0×10^3[d]		–0.398			
8	2-Amino-4,6-dinitrotoluene	–0.417[c]		6.2×10^2	6.1×10^3		–0.423		
9	2-Hydroxylamino-4,6-dinitrotoluene			3.1×10^3	4.5×10^4		–0.351		
10	5-(aziridin-1-yl)-2,4-dinitrobenzoic acid amide (CB-1954)	–0.385	1.4×10^2[d]	4.0×10^3[d]		–0.354			
11	5-(aziridin-1-yl)-2,4-r dinitrobenzoic acid ethyl este		6.5×10^2[d]	1.2×10^4[d]		–0.308			
12	5-(aziridin-1-yl)-2,4-dinitrobenzoic acid *N*-morpholide		1.8×10^2[d]	6.0×10^2[d]		–0.384			
13	5-(aziridin-1-yl)-2,4-dinitrobenzoic acid *N*-piperidide		4.0×10^2[d]	1.3×10^3[d]		–0.356			
14	4-Nitroacetophenone	–0.355	2.5×10^2	1.0×10^3	9.4×10^4	–0.369	–0.359	–0.345	–0.358 ± 0.013
15	1,3-Dinitrobenzene	–0.348		2.5×10^3	3.3×10^5		–0.316		
16	3,5-Dinitrobenzoic acid	–0.344		1.1×10^3	3.4×10^5		–0.332		
17	3,5-Dinitro-benzamide		8.5×10^2	1.2×10^4	3.0×10^5	–0.303	–0.287	–0.301	–0.297 ± 0.009
18	4-Nitrobenzaldehyde	–0.325	7.3×10^2	8.3×10^3	1.3×10^4	–0.312	–0.356	–0.362	–0.343 ± 0.024
19	1,2-Dinitrobenzene	–0.287		2.2×10^3	5.4×10^5		–0.309		
20	1,4-Dinitrobenzene	–0.257		3.0×10^4	2.3×10^6		–0.228		
21	2,4,6-Trinitrotoluene (TNT)	–0.253[c]		1.1×10^4	1.7×10^6		–0.254		
22	N-Methylpicramide			2.6×10^4	2.0×10^6		–0.233		
23	2,2',4,4',6,6'-Hexanitro-diphenylamine			1.6×10^5	9.4×10^6		–0.167		
24	2,4-Dinitrophenyl-N-methylnitramine			2.5×10^5	3.8×10^6		–0.176		

Table continued

No.	Compound	E^1_7 [a] (V)	k_{cat} / K_m (M^{-1} s^{-1})[b]			$E^1_{7(calc.)}$ (V)			
			CYT B_2	FNR	P-450R	Eq. (1)	Eq. (2)	Eq. (3)	Mean
25	2,4,6-Tinitrophenyl-N-methylnitramine (Tetryl)			1.1×10^5	2.3×10^7		–0.156		
26	2,4,6-Trinitrophenyl-*N*-nitramino-ethylnitrate (Pentryl)			5.5×10^5	1.3×10^7		–0.136		
27	2,4,6-Trinitrophenyl-1,3-bis (*N*-nitramino-ethylnitrate) (Dipentryl)			1.2×10^{5d}	4.0×10^{6d}		–0.189		
	Nitrofuranes								
28	2-(5'-Nitrofurylvinyl)-quinoline-4-carbonic acid		3.2×10^{3d}	2.5×10^{5d}		–0.225			
29	2-(5'-Nitrofurylvinyl)-quinoline-4-carbonic acid amide		6.5×10^{3d}	1.0×10^{5d}		–0.229			
30	2-(5'-Nitrofurylvinyl)-quinoline-4-carbonic acid diethylamino-1-methyl-but-1-ylamide	–0.225[e]	1.5×10^4	6.0×10^4	1.5×10^6	–0.223	–0.223	–0.218	–0.221 ± 0.003
31	2-(5'-Nitrofurylvinyl)-quinoline-4-carbonic acid 2-morpholino-*N*-eth-1-ylamide		1.7×10^{3d}	1.1×10^{5d}		–0.251			
32	2-(5'-Nitrofurylvinyl)-quinoline-4-carbonic acid 3-morpholino-*N*-prop-1-ylamide		5.5×10^{3d}	1.6×10^{5d}		–0.223			
33	Nifuroxim	–0.255	3.7×10^3	7.5×10^3	5.0×10^5	–0.285	–0.286	–0.265	–0.279 ± 0.012
34	Nitrofurantoin	–0.255	5.9×10^3	1.5×10^4	4.9×10^5	–0.264	–0.272	–0.256	–0.264 ± 0.008
	Nitrobenzimidazoles								
35	5-Nitrobenzimidazolone		2.0×10^2	$6. \times 10^2$		–0.380			
36	5,6-Dinitrobenzimidazolone		3.2×10^3	1.9×10^4		–0.271			
37	4,5,6-Trinitrobenzimi-dazolone		1.5×10^4	1.0×10^5	2.6×10^{6d}	–0.214	–0.201	–0.208	–0.208 ± 0.006
38	4,5,6,7-Tetranitrobenzimi-dazolone		3.2×10^4	3.1×10^5	8.1×10^{5d}	–0.180	–0.202	–0.216	–0.199 ± 0.018
39	5-Amino-4,6-dinitrobenzi-midazolone		5.3×10^3	2.7×10^4		–0.256			
40	5-Aziridinyl-4,6-dinitro-benzimidazolone		2.1×10^3	2.8×10^3		–0.313			
41	5-(*N* –Methylnitramino)-4,6-dinitrobenzimidazolone		1.0×10^4	1.0×10^5		–0.221			
42	5-[Bis(2,2'-chloroethyl)-amino]-4,6-dinitrobenzimi-dazolone		5.6×10^2	1.2×10^3		–0.351			
43	5-(2-Chloroethyl)amino-4,6-dinitrobenzimidazolone		8.2×10^2	3.2×10^3		–0.327			
44	5-Nitrofurfurylidenehydrazino-4,6-dinitrobenzimidazolone		1.4×10^3	1.8×10^4		–0.287			
45	5,6-Dinitrobenzimidazole		1.5×10^3	7.3×10^3		–0.301			
46	2-(Trifluoromethyl)-5,6-dinitrobenzimidazole		2.8×10^3	8.0×10^3		–0.289			

No.	Compound	E^1_7 [a] (V)	k_{cat} / K_m (M^{-1} s^{-1})[b]			$E^1_{7(calc.)}$ (V)			
			CYT B_2	FNR	P-450R	Eq. (1)	Eq. (2)	Eq. (3)	Mean
47	2-Amino-5,6-dinitrobenzimidazole		2.3×10^3	5.0×10^3		–0.301			
48	2-[Bis(2,2'-chloroethyl)-amino]-5,6-dinitrobenzimidazole		1.2×10^3	7.6×10^3		–0.305			
49	2-Nitrofurfurilvinylidene-5-nitrobenzimidazole		1.6×10^3	6.0×10^5		–0.222			
	Miscellaneous nitroheterocycles								
50	3-Nitro-1,2,4-triazolone (NTO)			5.5×10^1	9.0×10^2		–0.509		
51	5-Amino-3-nitro-1,2,4-triazole (ANTA)			2.6×10^2	1.7×10^3		–0.466		
52	1,3,5,8-Tetranitrocarbazole (TNC)			7.3×10^5	2.6×10^7		–0.116		
53	4,6-Dinitrobenzofuroxane (DNBF)			3.8×10^4	3.9×10^5		–0.258		
54	5,7-Diamino-4,6-dinitrobenzofuroxane (CL-14)			7.4×10^4[d]	2.2×10^5[d]		–0.257		
55	Nitracrine	–0.303	3.7×10^3		1.7×10^5			–0.285	

[a]Obtained from Ref. [28] unless indicated otherwise. [b]From our previous papers [5, 24, 30–34] unless indicated otherwise. Standard error ≤ 10%. [c]From Ref. [35]. [d]Present work [e]From Ref. [26]

10 mM L-lactate)), ε_{459} = 9.4 mM^{-1} cm^{-1} (FNR, oxidized form), and ε_{460} = 22 mM^{-1} cm^{-1} (P-450R, oxidized form). All the kinetic experiments were carried out in 0.1 M K-phosphate buffer solution (pH 7.0) containing 1 mM EDTA at 25 °C. The rates of nitroreductase activities of FNR and P-450R were monitored following NADPH oxidation ($\Delta\varepsilon_{340}$ = 6.2 mM^{-1} cm^{-1}, concentration of NADPH, 100 μM (FNR) or 50 μM (P-450R)) in the presence of enzyme and nitroaromatic compound. The rate of nitroreductase reaction of CYT B_2 was monitored according to a decrease in O_2 concentration, using a Clark electrode in the presence of 10 mM L-lactate, due to the reoxidation of the nitroradical formed [5, 23] (Fig. 1). The kinetic parameters of the reactions, the catalytic constant (k_{cat}) and the bimolecular rate constant (k_{cat} / K_m) correspond to the reciprocal intercepts and slopes in coordinates [E] / *v*, 1 / [$ArNO_2$], where [E] is the enzyme concentration, *v* is the reaction velocity, K_m is the Michaelis constant of nitroaromatic compound, i.e. the compound concentration corresponding to the half-maximal reaction rate (k_{cat} / *2*). k_{cat} is expressed as a number of molecules of substrate (NADPH or L-lactate) by the active center of the enzyme (FMN of CYT B_2 and P-450R or FAD of FNR) per 1 s. The calculation of the reaction kinetic parameters and statistical analysis were performed using Statistica (version 4.3, StatSoft, 1993).

RESULTS AND DISCUSSION

The linear log k_{cat} / K_m *vs.* E^1_7 relationships in the single-electron reduction of nitroaromatic compounds by flavoenzymes NADPH: cytochrome P-450 reductase (P-450R) and ferredoxin: $NADP^+$ reductase (FNR) were first observed by Orna and Mason [24] and later by us in these and other enzymatic systems and using a larger number of structurally different nitroaromatic compounds [5, 25, 26]. The slopes in the correlations, $\Delta\log k_{cat}/K_m / \Delta E^1_7 \sim 10$ V^{-1}, were in line with an outer-sphere single-electron transfer model [27], i.e. the weak electronic coupling between the reactants and little specificity for their particular structure. In Table, we present the k_{cat} / K_m values for model nitroaromatic compounds with available E^1_7 values and for a number of compounds with unavailable E^1_7 values (Fig. 1) obtained in our previous and current studies using three model single-electron transferring enzymes, P-450R, FNR, and flavocytochrome b_2 (CYT B_2). The k_{cat} values of the above compounds show little sensitivity on their reduction potential, therefore they were not presented.

To calculate the unavailable E^1_7 values of nitroaromatic compounds from the linear log k_{cat} / K_m *vs.* E^1_7 relationships, one should minimize the effects of possible specific interactions of the compounds with the particular enzyme. Although an excellent linear k_{cat} / K_m *vs.* E^1_7 correlation was obtained for the reactivity of CYT B_2 with a limited number of compounds (r^2 = 0.9785, n = 10), the other two enzymes were characterized by poorer correlations, r^2 = 0.8146 (FNR, n = 18), and r^2 = 0.8441 (P-450R, n = 17) (Table). For this reason, the geometric averages of log k_{cat} / K_m of nitroaromatics towards two enzymes were used as the correlation parameters. Using the k_{cat} / K_m of nitroaromatics with the avai-

lable E^1_7 values (Table), we obtained the following correlations:

$E^1_{7(calc.)}$ (V) = –0.5904 ± 0.0231 + (0.0821 ± 0.0074) (0.5 log k_{cat}/K_m (FNR) + (0.5 log k_{cat}/K_m (CYT B_2)), (n = 9, r^2 = 0.9461) (1)

$E^1_{7(calc.)}$ (V) = –0.7242 ± 0.0266 + (0.0916 ± 0.0064) (0.5 log k_{cat}/K_m (FNR) + 0.5 log k_{cat}/K_m (P-450R)), (n = 16, r^2 = 0.9362) (2)

and

$E^1_{7(calc.)}$ (V) = –0.6592 ± 0.0279 + (0.0852 ± 0.0070) (0.5 log k_{cat}/K_m (P-450R) + 0.5 log k_{cat}/K_m (CYT B_2)) (n = 8, r^2 = 0.9615) (3)

The $E^1_{7(calc.)}$ for model compounds calculated according to Eqs. (1–3) fairly agree with their experimentally determined E^1_7 values. Calculations according to a single correlation give the difference between E^1_7 and $E^1_{7(calc.)}$ not exceeding 35 mV, with an average difference of ±18 mV (Table). Using the mean $E^1_{7(calc.)}$ value obtained from the data of the three correlations (Table), this difference is further reduced to ±11 mV. For comparison, the experimental error in the E^1_7 determination by pulse-radiolysis is ±8–15 mV [28].

Equations (1–3) enabled us to characterize the previously unavailable E^1_7 values for 36 nitrobenzenes, nitrofuranes, nitrobenzimidazoles, and miscellaneous nitroheterocycles (Table). Some of these compounds are of considerable importance, *e.g.*, the derivatives of CB-1954 (compounds 11–13) exhibit antitumour activity [11, 12], whereas nitrobenzimidazoles (compounds 35–49) and vinylquinoline-substituted nitrofuranes (compounds 28, 29, 31, 32) show antiparasitic activity [26]. Other substances such as nitrophenyl-*N*-nitramines (compounds 24–27) and nitroheterocyclic compounds 50–54 are used as explosives and rocket fuel components [9, 10, 17, 19]. The obtained $E^1_{7(calc.)}$ values for compounds 23–37, 37, 38, and 52 are above –0.225 V (Table), i.e. outside the range of E^1_7 of model compounds. However, the high electron-accepting potency of tetryl, pentryl, and TNC (compounds 25, 26, 52) is in line with the data of quantum-mechanical calculations, which show that these compounds possess more negative enthalpies of single-electron reduction than TNT (compound 21) [29]. On the other hand, the similar $E^1_{7(calc.)}$ values for NTO, ANTA, and nitrobenzene (compounds 50, 51, 2) correlate with their similar enthalpies of single-electron reduction [29, 30]. Thus, the obtained $E^1_{7(calc.)}$ values should be considered as realistic, with an error of determination not exceeding 35 mV, *i.e.* the same as for the model nitroaromatic compounds (Table). This is also supported by a small variation between $E^1_{7(calc.)}$ values in a series of homologous compounds with presumably similar electron-accepting potency, *e.g.*, derivatives of CB-1954 (compounds 10, 12, 13) or a series of vinylquinoline-substituted nitrofuranes (compounds 28–32).

CONCLUSIONS

The linear log rate constant *vs.* E^1_7 relationships in single-electron reduction of nitroaromatic compounds by flavoenzymes appeared to be a useful tool for the estimation of the previously unavailable E^1_7 values of important nitroaromatic compounds, including antitumour and antiparasitic agents, and explosives. The estimated $E^1_{7(calc.)}$ values seem to be realistic, with an error not exceeding 35 mV, and may be useful in the further studies of therapeutic activity and/or cytotoxicity of the above compounds. In our opinion, the described approach may be extended to the studies of other groups of redox active organic compounds, *e.g.*, quinones and quinine imines, whose redox reactions follow the outer-sphere electron-transfer model.

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NITROAROMATINIŲ JUNGINIŲ VIENELEKTRONINĖS REDUKCIJOS POTENCIALŲ (E^1_7) NUSTATYMAS PAGAL JŲ VIENELEKTRONINĖS REDUKCIJOS FLAVININIAIS FERMENTAIS KINETIKĄ

S a n t r a u k a

Kadangi nitroaromatinių junginių anijonradikalai yra nestabilūs, junginių vienelektroninės redukcijos potencialai (E^1_7) daugiausia nustatomi naudojant impulsinę radiolizę arba fotolizę. Šiame darbe pateikiamas alternatyvus nitroaromatinių junginių E^1_7 verčių nustatymo metodas, pagrįstas reakcijos greičio konstantų logaritmo ir E^1_7 tiesinėmis koreliacijomis junginių vienelektroninėje redukcijoje flavininėmis elektrontransferazėmis. Tiesinių koreliacijų parametrai buvo nitroaromatinių junginių redukcijos flavocitochromu b_2, ferredoksin:$NADP^+$ reduktaze ir NADPH:citochromo P-450 reduktaze bimolekulinių greičio konstantų geometriniai vidurkiai. Šiuo metodu apskaičiuotų junginių E^1_7 ir jų eksperimentiškai nustatytų verčių skirtumai buvo ne didesni kaip 35 mV. Šis metodas įgalino įvertinti 36 iki šiol nenustatytus nitroaromatinių junginių, tarp jų priešnavikinių ir antiparazitinių junginių bei sprogmenų, vienelektroninės redukcijos potencialus.