CHEMIJA. 2020. Vol. 31. No. 3. P. 170–177
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QSARs in prooxidant mammalian cell cytotoxicity of nitroaromatic compounds: the roles of compound lipophilicity and cytochrome P-450- and DT-diaphorase-catalyzed reactions

Aušra Nemeikaitė-Čėnienė[1],

Jonas Šarlauskas[2],

Violeta Jonušienė[3],

Lina Misevičienė[2],

Audronė Marozienė[2],

Aliaksei V. Yantsevich[4],

Narimantas Čėnas[2]*

[1] State Research Institute Centre for Innovative Medicine, 5 Santariškių Street, 08406 Vilnius, Lithuania

[2] Institute of Biochemistry of Vilnius University, 7 Saulėtekio Street, 10257 Vilnius, Lithuania

[3] Institute of Biosciences of Vilnius University, 7 Saulėtekio Street, 10257 Vilnius, Lithuania

[4] Institute of Bioorganic Chemistry, NAS of Belarus, Kuprevicha 5/2, BY-220072 Minsk, Belarus

Frequently, the aerobic mammalian cell cytotoxicity of nitroaromatic compounds ($ArNO_2$) increases with their single-electron reduction potential (E^1_7), thus reflecting the relationship between their enzymatic single-electron reduction rate and E^1_7. This shows that the main factor of $ArNO_2$ cytotoxicity is redox cycling and oxidative stress. In this work, we found that the reactivity of a series of nitrobenzenes, nitrofurans and nitrothiophenes towards single-electron transferring NADPH:cytochrome P-450 reductase and adrenodoxin reductase/adrenodoxin increases with their E^1_7. However, their cytotoxicity in mouse hepatoma MH22a and human colon carcinoma HCT-116 cells exhibited a poorly expressed dependence on E^1_7. The correlations were significantly improved after the introduction of compound octanol/water distribution coefficient at pH 7.0 (log D) as a second variable. This shows that the lipophilicity of $ArNO_2$ enhances their cytotoxicity. The inhibitors of cytochromes P-450, α-naphthoflavone, isoniazid and miconazole, and an inhibitor of DT-diaphorase, dicoumarol, in most cases decreased the cytotoxicity of several randomly chosen compounds. This shows that the observed cytotoxicity vs E^1_7 relationships in fact reflect the superposition of several cytotoxicity mechanisms.

Keywords: nitroaromatic compounds, cytotoxicity, oxidative stress, cytochrome P-450, DT-diaphorase

INTRODUCTION

Nitroaromatic compounds ($ArNO_2$) such as nitrobenzenes, nitrofurans, nitrothiophenes and nitroimidazoles are widely used as antimicrobial, antiparasitic, antifungal and anticancer agents. Besides, nitroaromatic explosives and pesticides comprise an important group of toxic environmental pollutants ([1–3], and references therein). The quantitative structure–activity relationships

* Corresponding author: Email: narimantas.cenas@bchi.vu.lt

(QSARs) of their cytotoxicity enable one to characterize their action mechanisms and provide the guidelines for the design of new compounds with desired properties.

The simplest form of QSARs describing the cytotoxicity of $ArNO_2$ under aerobic conditions is a negative dependence of cL_{50} (compound concentration causing 50% cell killing) on their electron-accepting potency, e.g. single-electron reduction potential, E^1_7. The frequently observed relationships $\Delta\log cL_{50}/\Delta E^1_7 \sim -10\ V^{-1}$ mirror the log (rate constant) vs E^1_7 relationships in single-electron reduction of nitroaromatics by flavoenzymes dehydrogenases–electrontransferases, e.g. NADPH:cytochrome P-450 reductase (P-450R) [4–8]. It could mean that the main cytotoxicity factor is the rate of formation of free radicals of nitroaromatics ($ArNO_2^{-\cdot}$). Further, their reoxidation with oxygen yields superoxide ($O_2^{-\cdot}$), H_2O_2 and hydroxyl radical ($OH^\cdot$), i.e. causes the oxidative stress [6, 9]. The presence of reactive substituents, e.g. aziridine or *N,N*-bis(2-chloroethyl)-amine group, may enhance the cytotoxicity of $ArNO_2$ above the limits predictable by their E^1_7 [7, 10].

However, the observed dependence of log cL_{50} on E^1_7 may result from the superposition of oxidative stress and other cytotoxicity factors. The cytotoxicity of $ArNO_2$ lacking bioreductively activated groups was modulated by the inhibitors of flavoenzyme DT-diaphorase (NAD(P)H: quinone oxidoreductase, NQO1) and cytochromes P-450 [8, 11–13]. NQO1 performs two(four)-electron reduction of $ArNO_2$ into DNA-alkylating hydroxylamines (ArNHOH) ([14, 15], and references therein), and cytochromes P-450 catalyze the oxidative denitration of $ArNO_2$ [16, 17].

Besides, ArNHOH and amines ($ArNH_2$) may be formed as the reaction byproducts due to the dismutation of $ArNO_2^{-\cdot}$ or due to a limited oxygen supply. However, it is unclear how do these processes contribute to cytotoxicity vs E^1_7 relationships. The data on the role of lipophilicity in the aerobic cytotoxicity of $ArNO_2$ are also equivocal [4, 5, 8, 13, 18]. This points to a need of more thorough characterization of the above factors.

In this work, we demonstrated that the cytotoxicity of a series of structurally diverse nitrofurans, nitrobenzenes and nitrothiophenes in two cell lines increased with their E^1_7, and possessed the prooxidant character. Further, we attempted to characterize the possible contribution of compound lipophilicity and NQO1- and cytochrome P-450-catalyzed processes to their cytotoxicity.

MATERIALS AND METHODS

Recombinant rat P-450R, bovine NADPH: adrenodoxin reductase (ADR) and adrenodoxin (ADX) were prepared as described in [19], their concentrations were determined according to ε_{456} = 21.4 $mM^{-1}\ cm^{-1}$, ε_{450} = 11.0 $mM^{-1}\ cm^{-1}$ and ε_{414} = 10.0 $mM^{-1}\ cm^{-1}$, respectively. NQO1 was prepared from rat liver according to Prochaska [20], its concentration was determined according to ε_{460} = 11.0 $mM^{-1}\ cm^{-1}$. Nitrothiophenes **1a–c** and vinylquinoline-substituted nitrofurans **2a–c** (Fig. 1) were synthesized as described in [21] and [22], respectively. The compound purity was characterized by IR and NMR spectrometry, melting point and elemental analysis. Other reagents were obtained from Sigma-Aldrich, and used as received.

The kinetic measurements were carried out spectrophotometrically using a PerkinElmer Lambda 25 spectrophotometer in the 0.1 M K-phosphate buffer (pH 7.0) containing 1 mM EDTA at 25°C. The enzyme activities determined according to the rate of reduction of 50 μM cytochrome *c* ($\Delta\varepsilon_{550}$ = 20 $mM^{-1}\ cm^{-1}$) at substrate concentrations indicated below were close to those reported previously [23]: 39 s^{-1} (P-450R, [NADPH] = 100 μM), 7.5 s^{-1} (ADR, [ADX] = 0.5 μM, [NADPH] = 50 μM), and 1750 s^{-1} (NQO1, [NADPH] = 150 μM, [menadione] = 10 μM). In this case, 0.01% Tween 20 and 0.25 mg/mL bovine serum albumin were added as NQO1 activators. The initial rates of enzymatic NADPH-dependent nitroreduction were determined according to $\Delta\varepsilon_{340}$ = 6.2 $mM^{-1}\ cm^{-1}$ after the subtraction of intrinsic NADPH oxidase activities of enzymes, 0.05 s^{-1} (P-450R), 0.1 s^{-1} (NQO1) and 0.11 s^{-1} (ADR + 0.5 μM ADX). The stock solutions of oxidants were prepared in DMSO (dilution factor 100). The values of turnover rate, k_{cat}, reflecting the maximal number of moles NADPH oxidized or oxidant reduced per mole of the enzyme active centre per second, and k_{cat}/K_m, the bimolecular rate constant (or catalytic efficiency constant), correspond to the inverse intercepts and slopes in Lineweaver–Burk coordinates, [E]/v vs 1/[oxidant]. These rate constants were obtained by fitting the experimental data to the parabolic expression using the SigmaPlot

2000 (Version 11.0, Systal Software). In some experiments, the NADPH regeneration system (20 µM NADPH, 10 mM glucose-6-phosphate and 0.3 mg/mL glucose-6-phosphate dehydrogenase) was used.

Murine hepatoma MH22a cells, obtained from the Institute of Cytology of the Russian Academy of Sciences (St. Petersburg, Russia), were grown and maintained at 37°C in DMEM medium, supplemented with 10% fetal bovine serum and antibiotics [23]. In the cytotoxicity experiments, 3.0×10^4/ml cells were seeded in 5-mL flasks in the absence or in the presence of compounds, and were grown for 24 h. The cell viability was determined by Trypan blue exclusion. In control experiments, the cell viability was 98.5–99.3%. Human colon adenocarcinoma cells HCT-116, obtained from ATCC (Manassas, VA, USA), were grown and maintained at 37°C in 5% CO_2 in the RPMI 1640 DMEM medium, supplemented with 10% fetal bovine serum, 2 mM L-glutamine and antibiotics [23]. In the cytotoxicity experiments, 1.0×10^5/ml cells were seeded in the absence or in the presence of compounds, and were grown for 48 h. Their viability was determined by staining with crystal violet. Stock solutions of compounds were prepared in DMSO. Its concentration in cultivation media did not exceed 0.2% and did not affect cell viability. The experiments were conducted in triplicate. The statistical analysis was performed using Statistica (Version 4.3, Statsoft). Octanol/water distribution coefficients at pH 7.0 (log *D*) were calculated using LogD Predictor (https://chemaxon.com).

RESULTS

In this work, we used a number of nitroaromatic compounds whose E^1_7 varied between –0.191 and –0.485 V (Table 1). The formulae of nontrivial compounds are given in Fig. 1. One may note that these compounds lack bioreductively activated or other reactive substituents. First, we studied their single-electron reduction with P-450R, which probably plays the most important role in redox cycling of

Table 1. **Single-electron reduction potentials of nitroaromatic compounds (E^1_7), their octanol/water distribution coefficients at pH 7.0 (log *D*), bimolecular reduction rate constants (k_{cat}/K_m) by NADPH:cytochrome P-450 reductase (P-450R) and adrenodoxin reductase/ adrenodoxin (ADR/ADX), and their concentrations causing 50% cell death (cL_{50}) or 50% cell growth inhibition (GI_{50})**

No.	Compound	E^1_7, V [21, 24, 25]	log *D*	k_{cat}/K_m, $M^{-1}s^{-1}$		cL_{50} (GI_{50}[a]), µM	
				P-450R	ADR/ADX	MH22a	HCT-116[a]
1.	Nitrobenzene	–0.485	1.91	$6.8 \pm 0.8 \times 10^2$	$3.4 \pm 0.2 \times 10^3$	1800 ± 200	>5000
2.	4-Nitrobenzoic acid	–0.425	–1.66	$2.3 \pm 0.2 \times 10^3$	$2.0 \pm 0.2 \times 10^3$	>6000	>6000
3.	2-Nitrothiophene	–0.390	1.86	$1.4 \pm 0.1 \times 10^4$	$4.2 \pm 0.3 \times 10^4$	341 ± 42	n.d.
4.	4-Nitroacetophenone	–0.355	1.47	$1.7 \pm 0.2 \times 10^4$	$3.2 \pm 0.3 \times 10^4$	239 ± 19	400 ± 80
5.	3,5-Dinitrobenzoic acid	–0.345	–1.79	$3.3 \pm 0.2 \times 10^4$	n.d.	910 ± 80	3000 ± 400
6.	1,3-Dinitrobenzene	–0.345	1.85	$4.9 \pm 0.2 \times 10^4$	$5.2 \pm 0.4 \times 10^4$	130 ± 14	350 ± 50
7.	4-Nitrobenzaldehyde	–0.325	1.63	$3.3 \pm 0.2 \times 10^4$	$1.7 \pm 0.3 \times 10^5$	200 ± 15	50 ± 6.0
8.	3,5-Dinitrobenzamide	–0.311	0.70	$6.6 \pm 0.3 \times 10^4$	n.d.	130 ± 15	100 ± 10
9.	Nitrothiophene **1a**	–0.305	1.07	$1.4 \pm 0.2 \times 10^5$	$4.1 \pm 0.6 \times 10^5$	82 ± 12	n.d.
10.	1,2-Dinitrobenzene	–0.287	1.85	$1.6 \pm 0.1 \times 10^5$	$1.8 \pm 0.2 \times 10^5$	25.4 ± 3.0	60 ± 10
11.	Nitrothiophene **1b**	–0.280	1.70	$2.2 \pm 0.2 \times 10^6$	$5.4 \pm 0.5 \times 10^5$	145 ± 30	20 ± 5.0
12.	Nitrothiophene **1c**	–0.260	1.26	$2.8 \pm 0.1 \times 10^5$	$4.0 \pm 0.5 \times 10^5$	42 ± 5.0	n.d.
13.	Nitrofurantoin	–0.255	–0.25	$9.1 \pm 1.4 \times 10^4$	$1.0 \pm 0.2 \times 10^6$	387 ± 25	60 ± 10
14.	Nifuroxime	–0.255	–0.34	$1.1 \pm 0.1 \times 10^5$	$1.0 \pm 0.1 \times 10^6$	40 ± 5.0	70 ± 10
15.	1,4-Dinitrobenzene	–0.255	1.85	$1.2 \pm 0.1 \times 10^6$	$2.0 \pm 0.2 \times 10^6$	12.0 ± 1.5	40 ± 7.0
16.	2,4,6-Trinitrotoluene	–0.253	2.31	$1.0 \pm 0.1 \times 10^5$	$7.3 \pm 0.2 \times 10^5$	17.4 ± 2.0	40 ± 8.0
17.	Nitrofuran **2a**	–0.225	0.27	n.d.	$8.7 \pm 0.7 \times 10^5$	120 ± 10	65 ± 5.0
18.	Nitrofuran **2b**	–0.225	2.64	$4.0 \pm 0.3 \times 10^5$	n.d.	3.4 ± 0,4	2.5 ± 0.3
19.	Nitrofuran **2e**	–0.225	2.45	$7.6 \pm 1.3 \times 10^5$	n.d.	13.6 ± 1.5	0.9 ± 0.2
20.	Tetryl	–0.191	1.38	$5.9 \pm 0.2 \times 10^6$	$8.9 \pm 1.0 \times 10^5$	7.0 ± 1.0	8.0 ± 1.5

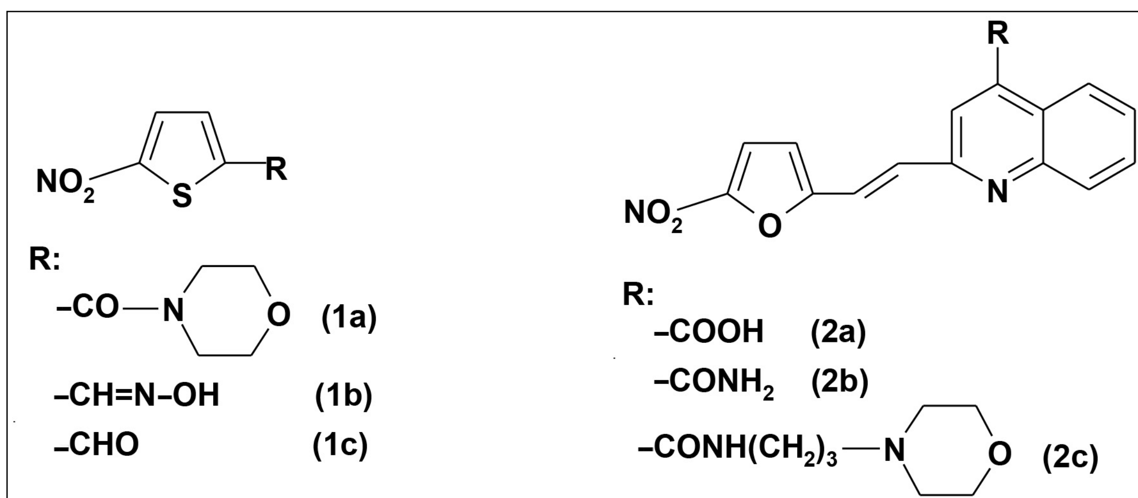

Fig. 1. Formulae of nontrivial nitrothiophenes (**1**) and nitrofurans (**2**) used in this work

$ArNO_2$ in the mammalian cell [26]. As an additional model reaction, we studied the reduction of $ArNO_2$ by Fe_2S_2 protein adrenodoxin (ADX). Flavoenzyme NADPH:adrenodoxin reductase (ADR) reduces nitroaromatics very slowly, and ADX stimulates the reaction providing an alternative more efficient electron-transfer pathway via ADX [27]. The bimolecular rate constants (k_{cat}/K_m) of reduction of $ArNO_2$ by P-450R and ADR/ADX are given in Table 1. For the most active oxidants of P-450R like tetryl, *p*-dinitrobenzene and nitrofurans (Table 1), the k_{cat} at their saturating concentrations were in a range of 18.0–19.0 s^{-1}, i.e. close to 50% of the rate of reduction of single-electron acceptor, cytochrome *c*. The k_{cat} for the same compounds in ADR/ADX-catalyzed reactions were in a range of 3.7–3.3 s^{-1}, which again was close to 50% of ADX-mediated cytochrome *c* reduction rate. In other cases, the reaction rates were almost proportional to the concentration of compounds up to the limits of their solubility. The data of Fig. 2a, b show that log k_{cat}/K_m of nitroaromatics increase with their E^1_7 values. This may be attributed

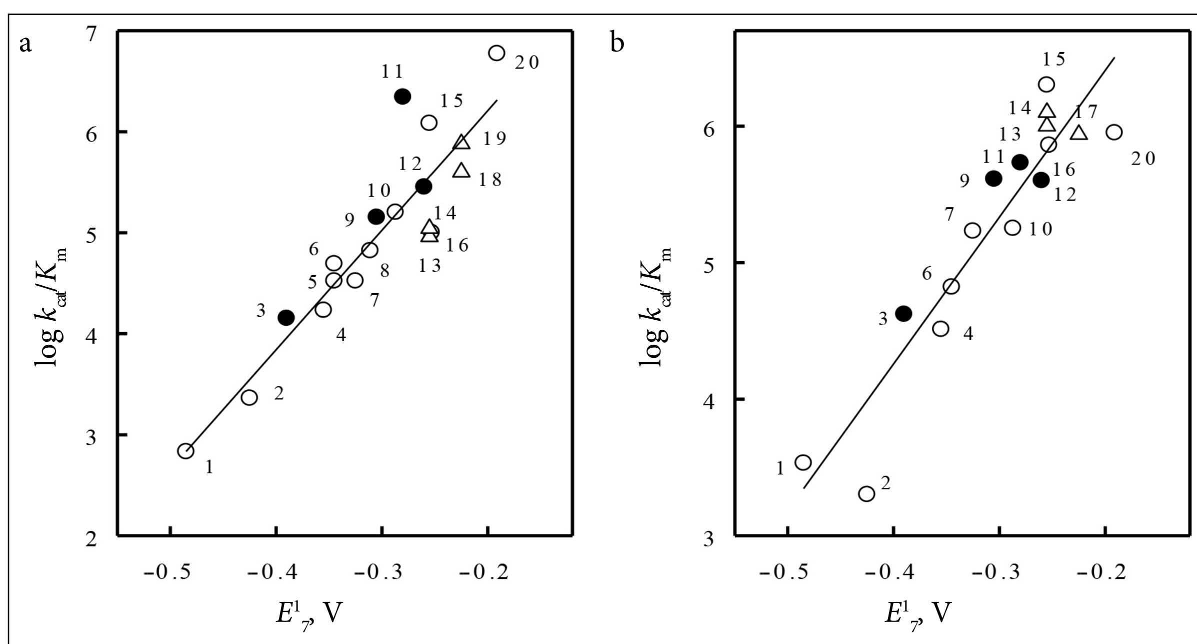

Fig. 2. Dependence of the reactivity (k_{cat}/K_m) of nitrobenzenes (o), nitrofurans (Δ) and nitrothiophenes (•) on their single-electron reduction potential (E^1_7) in P-450R- (a) and ADR/ADX-catalyzed reactions (b). The numbers of nitroaromatic compounds correspond to those in Table 1

to an 'outer-sphere' electron transfer mechanism of their reduction with a weak electronic coupling between the reactants and a relative lack of their structure specificity [6, 8].

Typically, NQO1 reduces nitroaromatics with low rates, their reactivity depending on E^1_7 and structural features in an ill-defined way ([15], and references therein). The reactivity of examined nitrobenzenes and nitrofurans was characterized previously [15]. Briefly, mononitrobenzenes and nitrofurans possessed k_{cat} = 0.05÷0.2 s^{-1} and k_{cat}/K_m of 25÷570 M^{-1} s^{-1}, dinitrobenzenes and 2,4,6-trinitrotoluene – k_{cat} = 0.2÷1.5 s^{-1} and k_{cat}/K_m = 670÷1600 M^{-1} s^{-1}, and tetryl possessed k_{cat} = 73 s^{-1} and k_{cat}/K_m = 2.6 × 10^5 M^{-1} s^{-1} [15]. In this work, the k_{cat} and k_{cat}/K_m of nitrothiophenes were obtained after the correction of NADPH oxidation rates for 340 nm absorbance changes due to nitrothiophene reduction. It was shown, using the NADPH regeneration system, that the latter did not exceed 15% total absorbance changes. Their k_{cat} and k_{cat}/K_m values were the following: 1.4 ± 0.1 s^{-1} and 3.2 ± 0.2 × 10^3 M^{-1} s^{-1} (2-nitrothiophene), ≤0.1 s^{-1} at saturating concentration (nitrothiophene **1a**), 11.1 ± 0.7 s^{-1} and 9.7 ± 0.8 × 10^4 M^{-1} s^{-1} (nitrothiophene **1b**), and 1.3 ± 0.2 s^{-1} and 1.7 ± 0.2 × 10^4 M^{-1} s^{-1} (nitrothiophene **1c**). Although nitrothiophenes were more reactive than nitrofurans, their reactivity was in line with the generally low nitroreductase activity of NQO1.

In cytotoxicity studies, we determined the cL_{50} values of nitroaromatics in murine hepatoma MH22a cells, and, for most of them, the concentrations for 50% of maximal inhibition (GI_{50}) of proliferation of human colon adenocarcinoma HCT-116 cells (Table 1). The cytotoxicity of several nitroaromatics in MH22a cells was decreased by desferrioxamine and the antioxidant *N,N*'-diphenyl-*p*-phenylene diamine (DPPD), and enhanced by 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), the latter inactivating glutathione reductase and depleting reduced glutathione [8, 23] (Table 2). This points to the prooxidant character of their cytotoxicity. In accordance with this, the cytotoxicity of nitroaromatics increased with E^1_7 with the coefficient $\Delta\log cL_{50}/\Delta E^1_7 = -9.12 \pm 1.47\ V^{-1}$ ($r^2 = 0.683$). This relatively scattered regression was significantly improved by the introduction of compound octanol/water distribution coefficient at pH 7.0 (log *D*, Table 1):

$$\log cL_{50} = -0.99 \pm 0.32 - (8.01 \pm 0.99)\ E^1_7 - (0.30 \pm 0.06) \log D,\ r^2 = 0.878. \quad (1)$$

GI_{50} of nitroaromatics in HCT-116 cells also decreased with their E^1_7 ($\Delta\log GI_{50}/\Delta E^1_7 = -11.88 \pm 1.74\ V^{-1}$, $r^2 = 0.756$). Again, the introduction of log *D* ignificantly improved the regression:

$$\log cL_{50} = -0.84 \pm 0.10 - (10.40 \pm 1.21)\ E^1_7 - (0.31 \pm 0.07) \log D,\ r^2 = 0.898. \quad (2)$$

Concerning the other enzymatic mechanisms possibly affecting the cytotoxicity of $ArNO_2$, we examined the effects of an inhibitor of NQO1, dicoumarol, and several inhibitors of cytochromes P-450 on the cytotoxicity of several randomly chosen compounds in MH22a cells (Table 3). In most cases, with a notable exception of tetryl and partly *p*-dinitrobenzene, the inhibitors decreased the cytotoxicity of $ArNO_2$ (Table 3). Interestingly, although cytochrome P-450-catalyzed oxidative denitration of nitrofurantoin in the cell-free system is most thoroughly documented [17], its inhibitors did not affect the cytotoxicity of nitrofurantoin in MH22a cells (data not shown).

Table 2. **Modulation of the cytotoxicity of nitroaromatic compounds in MH22a cells by *N.N'*-diphenyl-p-phenylene diamine (DPPD), desferrioxamine (DESF) and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), *n* = 3, *p* < 0.02***

Compound	Cell viability, %			
	No additions	Additions:		
		DPPD (2.0 μM)*	DESF (1.0 mM)*	BCNU (20 μM)*
p-Dinitrobenzene (12 μM)	50.5 ± 2.5	70.2 ± 5.2	75.3 ± 4.0	34.8 ± 2.7
2,4,6-Trinitrotoluene (35 μM)	35.8 ± 3.6	52.8 ± 5.0	55.3 ± 5.9	24.0 ± 2.7
Tetryl (15 μM)	37.1 ± 3.4	63.8 ± 4.7	65.7 ± 4.1	23.5 ± 3.2

Table 3. Modulation of the cytotoxicity of nitroaromatic compounds in MH22a cells by dicoumarol (DIC), α-naphthoflavone (α-NF), isoniazid (ISO) and miconazole (MIC), $n = 3$, $p < 0.05$*, $p < 0.02$, $p < 0.01$*****

Compound	Cell viability, %				
	No additions	Additions:			
		DIC (20 μM)	α-NF (5.0 μM)	ISO (1.0 mM)	MIC (5.0 μM)
Nifuroxime (60 μM)	37.0 ± 2.5	62.2 ± 8.2**	66.7 ± 9.3**	75.1 ± 7.3***	61.3 ± 5.3**
Nitrofuran 3e (25 μM)	37.9 ± 4.2	49.7 ± 4.2*	55.0 ± 4.9**	46.8 ± 4.2	49.8 ± 3.4*
Nitrothiophene (150 μM)	51.5 ± 3.4	71.1 ± 1.6**	74.5 ± 3.8**	71.5 ± 4.2**	77.8 ± 2.6**
p-Dinitrobenzene (18 μM)	34.2 ± 1.0	19.3 ± 5.9**	52.4 ± 6.3**	53.8 ± 6.8**	62.7 ± 10.7**
2,4,6-Trinitrotoluene (18 μM)	46.6 ± 5.2	44.7 ± 4.0	81.2 ± 4.2***	68.7 ± 3.1**	67.2 ± 3.5**
Tetryl (7.0 μM)	54.0 ± 5.6	33.9 ± 3.3**	65.7 ± 5.9	64.6 ± 4.3	29.0 ± 1.3***

DISCUSSION

Redox cycling is an intrinsic property of $ArNO_2$, being an important factor and a prognostic criterion for efficacy-to-safety ratio of existing and new nitroaromatic drugs [28]. In our opinion, the deviation from the limits predicted by the redox cycling activity could be instrumental in the characterization of additional mechanisms of cytotoxicity or therapeutic action of nitroaromatics.

Our data (Fig. 2a, b) demonstrate linear log k_{cat}/K_m vs E^1_7 dependences, which are typical of single-electron enzymatic reduction of $ArNO_2$ [6, 8]. They point to the absence of pronounced substrate specificity, including the previously uncharacterized oxidants, nitrothiophenes. In turn, the linear log cL_{50} (GI_{50}) vs E^1_7 relationships (Fig. 3a, b) taken together with the data on the antioxidant protection (Table 2) point to the predominantly prooxidant character of $ArNO_2$ cytotoxicity. The coefficients $\Delta\log cL_{50}/\Delta E^1_7$ in Eqs. 1 and 2 were similar to those obtained previously in V79 Chinese hamster cells, -8.37 ± 0.89 V^{-1} ([4], 168 h incubation), and FLK lamb kidney fibroblasts, -10.74 ± 1.19 V^{-1} ([8], 24 h). Importantly, the noticeable differences do not exist between the efficacy of nitrobenzenes, nitrofurans and nitrothiophenes (Fig. 3a, b). This fact rules out the manifestation of an additional

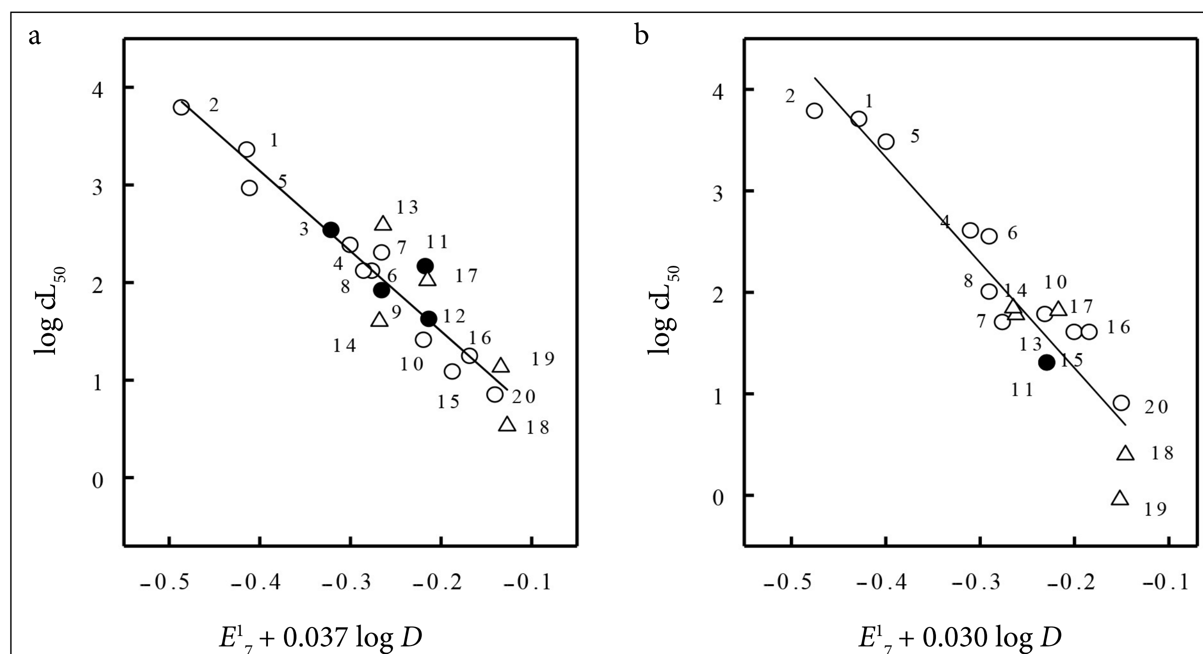

Fig. 3. Dependence of cytotoxicity (cL_{50}) or proliferation inhibition potency (GI_{50}) of nitrobenzenes (o), nitrofurans (Δ) and nitrothiophenes (•) on their single-electron reduction potential (E^1_7) and lipophilicity (log *D*) in MH22a (a) and HCT-116 cells (b) according to Eqs. 1 (a) and 2 (b). The numbers of nitroaromatic compounds correspond to those in Table 1

mechanism of cytotoxicity of nitrofurans, the formation of unsaturated open-chain nitriles [29]. However, our study clarifies the roles of several additional factors that modulate the prooxidant cytotoxicity of $ArNO_2$, which will be analysed below.

According to previous findings, the effects of lipophilicity were not evident in the action of $ArNO_2$ in primary rat hepatocytes [5] and primary mice splenocytes [13]. In V79 cells, this effect is poorly expressed, $\Delta \log cL_{50}/\Delta \log P = -0.14 \pm 0.09$ [4], where log *P* is an octanol/water partition coefficient. On the other hand, our data on the positive impact of log *D* on the cytotoxicity of nitroaromatics in two cell lines (Eqs. 1, 2) were close to those observed in FLK cells, $\Delta \log cL_{50}/\Delta \log P = -0.21 \pm 0.08$ [8], and L6 rat myoblasts, $\Delta \log cL_{50}/\Delta \log P = -0.388$ ([18], 72 h).

Evidently, the impact of $ArNO_2$ lipophilicity may depend on the cell type and experimental conditions, however, it should be taken into account in the analysis of QSARs of nitroaromatics.

NQO1 reduces $ArNO_2$ into DNA-alkylating hydroxylamines ([14], and references therein), therefore, it should contribute to their cytotoxicity. The reasons for an unexpected enhancement of cytotoxicity of tetryl and *p*-dinitrobenzene by dicoumarol (Table 3) are unclear, except the possible conversion of tetryl into less toxic *N*-methylpicramide by NQO1 [8]. The same effects were observed in FLK cells [8]. Interestingly, dicoumarol similarly affects the cytotoxicity of both weak and relatively active substrates of NQO1 (Tables 1, 3).

Cytochromes P-450 catalyze the denitration of heterocyclic compounds, nitrofurantoin and 5-nitro-1,2,4-triazol-3-one, with the formation of corresponding hydroxy derivatives [16,17]. The reaction intermediate, epoxide, reacts with thiol groups [17]. In our opinion, depending on the nature of the compound, this may either contribute to their toxicity (reactions with –SH groups of particular enzymes), either to detoxification (reaction with reduced glutathione). The data of Table 3 show that cytochromes P-450 are involved in the cytotoxicity of several nitrobenzenes as well. Currently, the data on their oxidative denitration are unavailable, thus, an alternative or parallel cytotoxicity mechanism could be the preventing of formation of amine products of polinitrobenzene reduction by their *N*-hydroxylation with formation of hydroxylamines [30].

CONCLUSIONS

A general conclusion based on current and previous studies is that in different mammalian cells and under different conditions, the aerobic cytotoxicity of nitroaromatics, which do not possess additional reactive substituents, similarly depends on their E^1_7 values. The dependence of cytotoxicity on compound lipophilicity may be more sensitive to the cell type and experimental conditions. These two factors may be important for the prediction of side-effects or estimation of therapeutic mechanisms of nitroaromatics. This study also shows that NQO1 and cytochromes P-450 exert equivocal effects on $ArNO_2$ cytotoxicity, which evidently do not significantly affect the observed QSARs. The elucidation of the roles of these enzymes warrants further studies.

ACKNOWLEDGEMENTS

This work was supported by the European Social Fund (Measure No. 09.33-LMT-K-712, Grant No. DOTSUT-34/09.3.3.-LMT-K712-01-0058/LSS-600000-58).

Received 8 May 2020
Accepted 19 May 2020

References

1. D. E. Rickert, *Drug Metab. Rev.*, **18**, 23 (1987).
2. V. Purohit, A. K. Basu, *Chem. Res. Toxicol.*, **13**, 673 (2000).
3. P. Kovacic, R. Somanathan, *J. Appl. Toxicol.*, **34**, 810 (2014).
4. G. E. Adams, E. D. Clarke, P. Gray, et al., *Int. J. Radiat. Biol.*, **35**, 151 (1979).
5. P. J. O'Brien, W. C. Wong, J. Silva, S. Khan, *Xenobiotica*, **20**, 945 (1990).
6. R. P. Mason, *Environ. Health Persp.*, **87**, 237 (1990).
7. V. Miškinienė, E. Sergedienė, A. Nemeikaitė, et al., *Cancer Lett.*, **146**, 217 (1999).
8. N. Čėnas, A. Nemeikaitė-Čėnienė, E. Sergedienė, et al., *Biochim. Biophys. Acta*, **1528**, 31 (2001).
9. P. Wardman, M. F. Dennis, S. A. Everett, et al., *Biochem. Soc. Symp.*, **61**, 171 (1995).
10. J. M. Walling, I. J. Stratford, G. E. Adams, *Int. J. Radiat. Biol.*, **52**, 31 (1987).
11. J. Šarlauskas, E. Dičkancaitė, A. Nemeikaitė, et al., *Arch. Biochem. Biophys.*, **346**, 219 (1997).
12. J. Pourahmad, S. Khan, P. J. O'Brien, *Adv. Exp. Med. Biol.*, **500**, 261 (2001).

13. V. Miliukienė, N. Čėnas, *Z. Naturforsch.*, **63c**, 519 (2008).
14. R. J. Knox, P. J. Burke, S. Chen, D. J. Kerr, *Curr. Pharm. Des.*, **9**, 2091 (2003).
15. L. Misevičienė, Ž. Anusevičius, J. Šarlauskas, N. Čėnas, *Acta Biochim. Pol.*, **53**, 569 (2006).
16. L. Le Campion, M. Delaforge, J. P. Noel, J. Ouazzani, *Eur. J. Biochem*., **248**, 401 (1997).
17. H. Li, D. Lin, Y. Peng, J. Zheng, *Xenobiotica,* **47**, 103 (2017).
18. M. V. Papadopolou, W. D. Bloomer, H. S. Rosenzweig, et al., *Eur. J. Med. Chem*., **117**, 179 (2016).
19. T. A. Pechurskaja, I. N. Hornastai, I. P. Grabovec, et al., *Biochem. Biophys. Res. Comm.*, **353**, 598 (2007).
20. H. J. Prochaska, *Arch. Biochem. Biophys.*, **267**, 529 (1988).
21. A. Breccia, F. Busi, E. Gattavechia, M. Tamba, *Radiat. Environ. Biophys*., **29**, 153 (1990).
22. E. Lukevits, T. V. Lapina, N. M. Sukhova, et al., *Pharm. Chem. J.*, **15**, 792 (1981).
23. A. Nemeikaitė-Čėnienė, J. Šarlauskas, V. Jonušienė, et al., *Int. J. Molec. Sci.*, **20**, 4602 (2019).
24. P. Wardman, *J. Phys. Chem. Ref. Data*, **18**, 1637 (1989).
25. A. Marozienė, M. Lesanavičius, E. Davioud-Charvet, et al., *Molecules*, **24**, 4509 (2019).
26. U. A. Boelsterli, H. K. Ho, S. Zhou, K. Y. Leow, *Curr. Drug Metab*., **7**, 715 (2006).
27. J. Marcinkevičienė, N. Čėnas, J. Kulys, et al., *Biomed. Biochim. Acta*, **49**, 167 (1990).
28. K. Nepali, H.-Y. Lee, J.-P. Liou, *J. Med. Chem*., **62**, 2851 (2019).
29. C. Bot, B. S. Hall, G. Alvarez, et al., *Antimicrob. Agents Chemother*., **57**, 1638 (2013).
30. M. Ohbushi, M. Miyata, D. Nagai, et al., *Drug Metab. Disp*., **25**, 1298 (2009).

Aušra Nemeikaitė-Čėnienė, Jonas Šarlauskas, Violeta Jonušienė, Lina Misevičienė, Audronė Marozienė, Aliaksei V. Yantsevich, Narimantas Čėnas

NITROAROMATINIŲ JUNGINIŲ PROOKSIDACINIO CITOTOKSIŠKUMO ŽINDUOLIŲ LĄSTELĖSE QSAR: JUNGINIŲ LIPOFILIŠKUMO IR CITOCHROMŲ P-450 BEI DT-DIAFORAZĖS KATALIZUOJAMŲ REAKCIJŲ VAIDMUO

Santrauka

Nitroaromatinių junginių ($ArNO_2$) citotoksiškumas žinduolių ląstelėse aerobinėmis sąlygomis dažnai didėja augant jų vienelektroninės redukcijos potencialui (E^1_7), taip atsispindi ryšys tarp jų E^1_7 ir vienelektroninės redukcijos fermentais greičio. Pagrindinis $ArNO_2$ citotoksiškumo veiksnys yra cikliniai redokso virsmai ir oksidacinis stresas. Nustatėme, kad eilės nitrobenzene, nitrofuranų ir nitrotiofenų reakcingumas vieną elektroną pernešančių NADPH:cytochromo P-450 reduktazės ir adrenodoksino reduktazės / adrenodoksino atžvilgiu didėja, didėjant jų E^1_7. Tačiau jų citotoksiškumas pelės hepatomos MH22a ir žmogaus gaubtinės žarnos karcinomos HCT-116 ląstelėse prastai koreliavo su E^1_7. Koreliacijos pagerėdavo antru kintamuoju naudojant junginio oktanolio / vandens pasiskirstymo koeficientą prie pH 7,0 (log *D*). Tai rodo, kad $ArNO_2$ lipofiliškumas didina jų citotoksiškumą. Citochromų P-450 inhibitoriai α-naftoflavonas, izoniazidas ir mikonazolas, taip pat DT-diaforazės inhibitorius dikumarolas dažniausiai didindavo kai kurių atsitiktinai parinktų junginių citotoksiškumą. Stebimos citotoksiškumo priklausomybės nuo E^1_7 faktiškai atspindi kelių citotoksiškumo mechanizmų atstojamąsias.