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Thioether derivatives of 1,2,3-triazole-N-oxides: Synthesis, study of cytotoxicity and cellular nitric oxide (NO) production effect

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Abstract

In this work, a synthetic strategy based on the reaction of 5-bromo-2-(4-fluorophenyl)-4-methyl-2H-1,2,3-triazole with various thioaromatic derivatives were applied to afford a series of thioether derivatives of 1,2,3-triazole-N-oxides in yields of 52–80%. The obtained compounds were shown to be characterized by low cytotoxicity towards human embryonic kidney (HEK293) cells with IC50 values ranging from 128 to 76 µM and high toxicity towards malignant rhabdomyosarcoma cancer (Rd) cells with IC50 values ranging from 93 to 22 µM. These compounds were screened for the ability to induce nitric oxide production in cells via DAF-FM fluorescence. Several compounds demonstrated a twofold increase in nitric oxide levels relative to the control. Two leader molecules demonstrating the lowest cytotoxicity values and the effect on nitric oxide production in rhabdomyosarcoma cells were identified. Thus, the method could be considered as a convenient one for qualitative or semi-quantitative determination of nitric oxide in a screening process of large number of compounds to define lead molecules capable of influencing nitric oxide production.

Key findings

- A series of thioether derivatives of 1,2,3-triazole-N-oxides were synthesized
- The obtained compounds showed low toxicity in human embryonic kidney cells with IC_{50} values ranging from 128 to 76 µM.
- The obtained compounds have a promoting effect on the production of nitric oxide

Accompanying information

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Supplementary information
Supplementary materials: READ
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Sustainable Development Goals

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1. Introduction

Nitric oxide (NO) is a versatile signaling molecule involved in numerous physiological and pathophysiological processes, including neurotransmission, platelet aggregation inhibition [1], immune function, smooth muscle tone regulation, and memory formation [2,3]. Among its diverse functions, NO is best known as the primary endogenous vasodilator, constitutively secreted by the vascular endothelium to maintain myogenic vascular tone [4]. NO is synthesized from L-arginine by endothelial nitric oxide synthase (eNOS), requiring cofactors, such as flavin mononucleotide, flavin dinucleotide, tetrahydrobiopterin (BH4), Ca^{2+}-calmodulin, heme, NADPH, and molecular oxygen [5–7]. Dysregulation of NO production is implicated in cardiovascular pathologies, including hypertension, where NO deficiency contributes to chronic vasospasm, proliferation, and thrombosis. Consequently, NO represents a key therapeutic target, and the development of NO donors [8] and other NO-modulating agents remains a priority for improving NO bioavailability in cardiovascular

disease [9]. Thus, the modulation of nitric oxide (NO) levels through pharmacological agents represents a promising strategy for achieving vasodilatory effects and realizing potential endothelium-protective properties [10]. However, direct in vitro studies on primary endothelial cells are often challenging due to technical limitations, including their limited availability, phenotypic instability during culture, and rapid loss of endothelial markers upon passaging [11,12]. This justifies the use of alternative, more stable cell models for initial compound screening, e.g. cancer cells.

In this context, it is important to note that NO plays a complex and context-dependent role in cancer biology. Recent studies have highlighted the dichotomous nature of NO in malignancies: at low concentrations (10–200 nM), NO may promote tumor growth and proliferation, while it can induce apoptosis and inhibit tumor progression at high concentrations (>500 nM) [13,14]. Nitric oxide synthase (NOS) isoforms, particularly the constitutive forms (nNOS and eNOS), are frequently expressed in various tumor types, including sarcomas [15]. This concentration-dependent duality makes NO a potential target for anticancer strategies, and compounds that modulate NO production (either as donors or inhibitors) are of significant interest in oncological pharmacology. Currently, a wide range of (aza)heterocyclic architectures [16,17] is used for regulation and disease modeling in pharmacology and therapy for NO-related disorders (Figure 1). In particular, L-NAME [18–20] is an effective NO inhibitor, while folic acid and L-arginine [21] have an indirect promoting effect, when there is insufficient NO production in the body.

One of the most innovative and rapidly developing classes in medicinal chemistry are compounds containing an N-oxide group, which can be donors of NO and directly influence its regulation [22-26] along with antimicrobial, anxiolytic, neuroprotective, and other beneficial properties. Among the known and synthetically available compounds, mono- and di-N-oxides of azines, open-chain nitrones, imidazole-N-oxides of various structures, as well as 1,2,3-triazoles are currently considered as promising-oxide molecular systems [27–30] In addition, modification of these scaffolds with various functional groups, such as pyrroles, indoles, amines, aromatic derivatives, and thiophenols, allows the necessary parameters to be achieved in terms of specific activity and safety, lipophilicity, and bioavailability [31–36]. One of the most interesting classes of such heterocycles is 1,2,3-triazoles. The broad range of possible functionalization of this heterocyclic scaffold enables the creation of molecules with diverse functional blocks that contribute to various types of biological activity. Due to its lipophilic nature, the thiophenol moiety serves as a functional block that facilitates the penetration of compounds through the phospholipid bilayer, thereby improving cellular uptake [37,38].

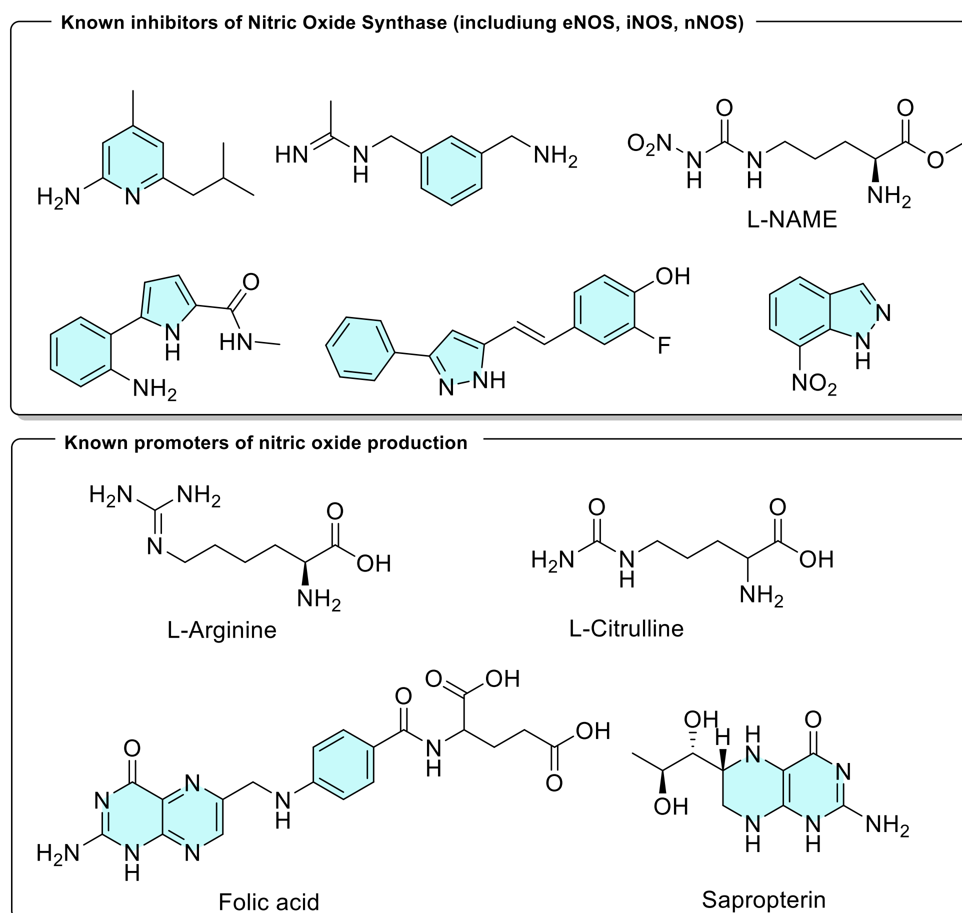

Figure 1 Known NO synthase inhibitors and NO production promoters

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The primary objective of this study was to synthesize a series of novel thioether derivatives of 1,2,3-triazole N-oxides and to evaluate their ability to modulate intracellular NO levels. For this initial screening, we selected the human rhabdomyosarcoma (Rd) cell line as a model system. This choice was based on two considerations: (I) as a continuous cell line, it provides a stable and reproducible platform for extensive preliminary testing, and (II) sarcoma cells have been reported to frequently express constitutive NOS isoforms [15], making them relevant for studying NO-modulating activity. We hypothesized that these compounds, at non-toxic concentrations, would significantly alter NO production in this cellular model, thereby demonstrating their potential as NO-modulating agents. The findings of this study could provide a basis for the further study of these compounds regarding applications, where NO modulation is therapeutically relevant.

2. Experimental part

2.1. General experimental methods

Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker Avance III (600 MHz). All ^{1}H NMR experiments were reported in δ units, parts per million (ppm), and were measured relative to a residual chloroform $CDCl_3$ (7.26 ppm) signal in the deuterated solvent. All ^{13}C NMR spectra were reported in ppm relative to $CDCl_3$ (77.16 ppm) and all spectra were obtained with ^{1}H decoupling. All coupling constants *J* were reported in Hertz (Hz). The following abbreviations were used to describe peak splitting patterns (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublet, m = multiplet, and br s = broad singlet). The mass spectra were recorded on a Shimadzu GCMS-QP2010 Ultra mass spectrometer (Shimadzu, Kyoto, Japan) with sample ionization by electron impact (EI). The elemental analysis was carried out on a Perkin Elmer Instrument equipped with a PE 2400 II CHN-analyzer. The course of the reactions was monitored by TCL on 0.25 mm silica gel plates (60F 254).

5-Bromo-2-(4-fluorophenyl)-4-methyl-2*H*-1,2,3-triazole 1-oxide were synthesized according to the literature procedure [39]. Thiophenol, 3-fluorothiophenol, 2-bromothiophenol, 4-hydroxythiophenol were purchased from commercial sources and used as received.

2.2. General method for the synthesis of 2-(4-fluorophenyl)-4-methyl-5-(phenylthio)-2*H*-1,2,3-triazole-1-oxides

5-Bromo-2-(4-fluorophenyl)-4-methyl-2*H*-1,2,3-triazole 1-oxide [39] (0.400 g, 1.5 mmol, 1 equiv), K_2CO_3 (0.406 g, 2.9 mmol, 2 equiv), the corresponding thiophenol (2.9 mmol, 2 equiv) were dissolved in DMSO (25 mL) and placed in a vial with a screw cap. The reaction was stirred for 24 hours at 110 °C. At the end of the reaction time, the reaction mixture was poured into H_2O (200 mL) and extracted with $CHCl_3$ (3 x 100 mL). The organic fraction washed with H_2O (3 x 50 mL) and dried over anhydrous Na_2SO_4. The solvent was evaporated and the product was purified by column chromatography (sorbent: SiO_2, eluent: hexane/EtOAc (8:2).

2-(4-Fluorophenyl)-4-methyl-5-(phenylthio)-2*H*-1,2,3-triazole 1-oxide (3a)

Light yellow powder. Yield 354 mg (80%). mp = 77-80 °C. R_f 0.7 (hexane/EtOAc: 9/1). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.99 – 7.97 (m, 2H), 7.43 – 7.37 (m, 2H), 7.33 – 7.30 (m, 2H), 7.28 – 7.27 (m, 1H) 7.23 – 7.20 (m, 2H), 2.39 (s, 3H) ppm. ^{19}F NMR (565 MHz, $CDCl_3$) δ -110.84 – (-110.89) (m, 1F) ppm. ^{13}C{^{1}H} NMR (151 MHz, $CDCl_3$) δ 162.4 (d, ^1J = 250.48 Hz), 146.6, 132.3, 131.5 (d, ^4J = 3.24 Hz), 129.8, 129.5, 127.8, 124.8 (d, ^3J = 7.89 Hz), 120.8, 116.2 (d, ^2J = 23.32 Hz), 12.4 ppm. IR (DRA): 3063, 2925, 1503, 1072, 838, 711, 685, 590, 508 cm^{-1}. MS (EI) m/z: [M]$^+$ Calcd for $C_{15}H_{12}FN_3OS$ 301; Found 301. Anal. Calcd for $C_{15}H_{12}FN_3OS$ C, 59.79; H, 4.01; F, 6.30; N, 13.94; O, 5.31; S, 10.64. Found: C, 59.80; H, 4.12; N, 14.12.

2-(4-Fluorophenyl)-5-((3-fluorophenyl)thio)-4-methyl-2*H*-1,2,3-triazole 1-oxide (3b)

Colourless powder. Yield 210 mg (60%). mp = 91-94 °C. R_f 0.7 (hexane/EtOAc: 9/1). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.99 – 7.97 (m, 2H), 7.28 – 7.27 (m, 1H), 7.24 – 7.21 (m, 2H), 7.14 – 7.12 (m, 1H), 7.06-7.01 (m, 1H), 6.98 – 6.94 (m, 1H), 2.40 (s, 3H) ppm. ^{19}F NMR (565 MHz, $CDCl_3$) δ -110.57 – (-110.62) (m, 1F), -110.86 – (-110.90) (m, 1F) ppm. ^{13}C{^{1}H} NMR (151 MHz, $CDCl_3$) δ 163.0 (d, ^1J = 249.89 Hz), 162.5 (d, ^1J = 249.76 Hz), 146.8, 134.6 (d, ^3J = 7.80 Hz), 131.5 (d, ^4J = 3.06 Hz), 130.9 (d, ^3J = 8.49 Hz), 124.9 (d, ^4J = 9.00 Hz) 124.8 (d, ^4J = 3.23 Hz), 119.9, 116.3 (d, ^2J = 23.06 Hz), 116.1 (d, ^2J = 23.56 Hz), 114.8 (d, ^2J = 21.89 Hz), 12.4 ppm. IR (DRA): 3060, 2950, 2848, 1927, 1599, 1288, 1115, 1080, 963, 635 cm^{-1}. MS (EI) m/z: [M]$^+$ Calcd for $C_{15}H_{11}F_2N_3OS$ 319; Found 319. Anal. Calcd for $C_{15}H_{11}F_2N_3OS$ C, 56.42; H, 3.47; F, 11.90; N, 13.16; O, 5.01; S, 10.04. Found: C, 56.63; H, 3.61; N, 13.38.

5-((2-Bromophenyl)thio)-2-(4-fluorophenyl)-4-methyl-2*H*-1,2,3-triazole 1-oxide (3c)

Light yellow powder. Yield 292 mg (70%). mp = 90-93 °C. R_f 0.7 (hexane/EtOAc: 9/1). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.96 – 7.94 (m, 2H), 7.41 (m, 1H), 7.21 – 7.19 (m, 3H), 7.11 – 7.08 (m, 1H), 7.03 – 7.00 (m, 1H), 2.40 (m, 3H) ppm. ^{19}F NMR (565 MHz, $CDCl_3$) δ -110.42 – (-110.46) (m, 1F) ppm.^{13}C{^{1}H} NMR (151 MHz, $CDCl_3$) δ 162.6 (d, ^1J = 251.96 Hz), 146.8, 133.6 133.5, 131.5 (d, ^4J = 3.22 Hz), 129.1, 128.3 (d, 22.42 Hz), 124.8 (d, ^3J = 8.81 Hz), 122.5, 119.2, 116.4, 116.2, 12.5 ppm. IR (DRA): 2956, 1513, 1466, 1414, 1261, 1172, 835, 806, 610, 515 cm^{-1}. MS (EI) m/z: [M]$^+$ Calcd for $C_{15}H_{11}BrFN_3OS$ 380; Found 300 [M-Br]$^+$. Anal. Calcd for $C_{15}H_{11}BrFN_3OS$ C, 47.38; H, 2.92; Br, 21.01;

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F, 5.90; N, 11.05; O, 4.21; S, 8.43. Found: C, 47.55; H, 3.12; N, 11.23.

2-(4-Fluorophenyl)-5-((4-hydroxyphenyl)thio)-4-methyl-2*H*-1,2,3-triazole 1-oxide (3d)

Yellow powder. Yield 180 mg (52%). mp = 135-138 °C. R_f 0.7 (hexane/EtOAc: 9/1). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.87 – 7.85 (m, 2H), 7.33 -7.31 (m, 2H), 7.18 – 7.15 (m, 2H), 6.66 – 6.63 (m, 2H), 6.55 (br s, 1H), 2.38 (s, 3H) ppm. ^{19}F NMR (565 MHz, $CDCl_3$) δ -110.31 – (-110.35) (m, 1F) ppm. ^{13}C{^{1}H} NMR (151 MHz, $CDCl_3$) δ 162.7 (d, ^1J = 250.89 Hz), 157.1, 146.2, 134.0, 131.2 (d, ^4J = 3.28 Hz), 125.4 (d, ^3J = 9.16 Hz), 123.0, 120.9, 116.8, 116.3 (d, ^2J = 23.22 Hz), 12.4 ppm. IR (DRA): 3387, 3192, 2922, 2660, 2351, 1897, 1581, 1385, 1148, 715 cm^{-1}. MS (EI) m/z: $[M]^+$ Calcd for $C_{15}H_{12}FN_3O_2S$ 317; Found 317. Anal. Calcd for $C_{15}H_{12}FN_3O_2S$ C, 56.77; H, 3.81; F, 5.99; N, 13.24; O, 10.08; S, 10.10. Found: C, 56.91; H, 3.99; N, 13.31.

2.3. Cell cultivation

The studies were conducted on cultured human embryonic kidney cells (HEK293, ATCC CRL 1573) and rhabdomyosarcoma cells (Rd, ATCC CCL-136) obtained from the Central Cell Culture Collection “Collection of Vertebrate Cell Cultures” (Institute of Cytology, Russian Academy of Sciences, St. Petersburg, Russia). The cells were cultured using DMEM/F-12 medium containing 10% fetal bovine serum (Biolot, Russia) at 37°C, 5% CO_2, and 98% humidity in a CB220 incubator (Binder, Germany). Subculturing with 0.25% trypsin solution (Biolot, Russia) was performed when the culture reached ≥90% confluence.

2.4. Assessment of viability

Compounds dissolved in DMSO were diluted with DMEM/F-12 culture medium containing 10% fetal bovine serum to the test concentrations: 1–128 μM for compounds **3a–c** and 2–256 μM for compound **3d**.

The cells were pre-seeded in 96-well plates at a seeding concentration of 4 × 103 cells per well. After 24 hours, the test compounds were added to the wells of the plate in a specified concentration range. The cells were then incubated for 72 hours, after which 20 μL (5 mg/mL) of MTT (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide) solution was added to each well. After 2.5 hours, the medium was removed from the wells and 200 μL of a 1:1 mixture of DMSO and isopropanol was added. The optical density was measured on a plate spectrophotometer at a wavelength of 570 nm (Victor Nivo™, PerkinElmer, USA).

2.5. Fluorescent staining

To determine the concentration of nitric oxide (NO), rhabdomyosarcoma (Rd) cells were double-stained with DAF-FM (Lumiprobe, Russia) and rhodamine B. The cells were pre-seeded in a 96-well plate at a seeding concentration of 4 × 103 cells per well. After 24 hours, the test compounds were added to the wells of the plate at a concentration equal to 0.1 of IC_{50}, after which they were incubated for 24 hours. Further, rhodamine B dye was added to the cells at a concentration of 0.5 mg/ml and incubated for 60 minutes (37°C, 5% CO_2, and 98% humidity), after which DAF-FM was added at a concentration of 10 μM and placed in a CO_2 incubator for 60 minutes (37°C, 5% CO_2, and 98% humidity). Then, the culture medium and serum in the test wells were replaced with fresh medium containing 0.5 mg/ml rhodamine B and incubated for another 30 minutes under the same conditions. Next, the wells were washed twice with D-PBS solution and microsporulation was performed using XDS-3FL4 (OPTIKA, Italy).

2.6. Statistical analysis

Statistical data processing was performed in RStudio (Version 2023.09.1© 2009-2023 RStudio, PBC) using the R package (version 4.3.2). The cytotoxicity index (IC_{50}) was calculated by constructing dose-response curves using the “drc” package [40]. Image analysis was performed using the “EBImage” package. Groups were compared using ANOVA. Welch's T-test for pairwise comparison of means with Bonferroni correction was also used for post-hoc analysis.

2.7. Image treatment

The images obtained after microscopy were analyzed using RStudio software (Version 2023.09.1© 2009-2023 RStudio, PBC), ImageJ (Version 1.53t), and Julia Programming Language (Version: 1.101.1).

3. Results and Discussion

The synthesis of desired thioether derivatives of 1,2,3-triazole-N-oxides was carried out by reacting 5-bromo-2-(4-fluorophenyl)-4-methyl-2*H*-1,2,3-triazole **1** with various thioaryl derivatives **2** in the presence of a base, yielding the target substitution product **3** (Scheme 1).

To determine the optimal conditions for the reaction, the parameters of the chemical transformation were evaluated, and the interaction between 5-bromo-2-(4-fluorophenyl)-4-methyl-2*H*-1,2,3-triazole 1-oxide **1** and benzenethiol **2a** was selected as the model reaction. It was found that a decrease in reaction temperature reduced the yield of the target product to trace amounts. Using K_2CO_3 as a base and DMSO as a solvent, it was possible to obtain a 31% yield of the substitution product (Table 1). However, it was found that using a twofold excess of the thioaryl derivative raised the product yield to 80%. At the same time, both the initial reaction components and the target reaction product were observed in the reaction mass allowing one to conclude that the addition of an excess of thioaryl derivatives in the reaction mass could lead to a shift in the equilibrium towards the formation of the substitution product.

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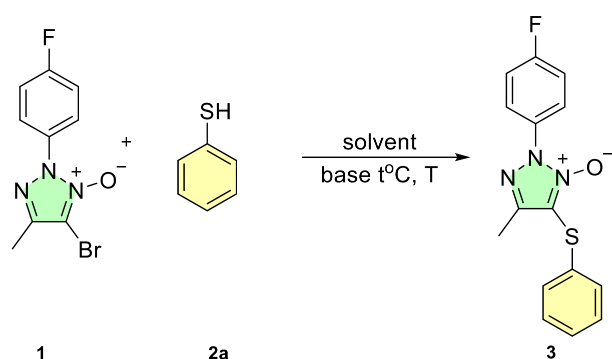
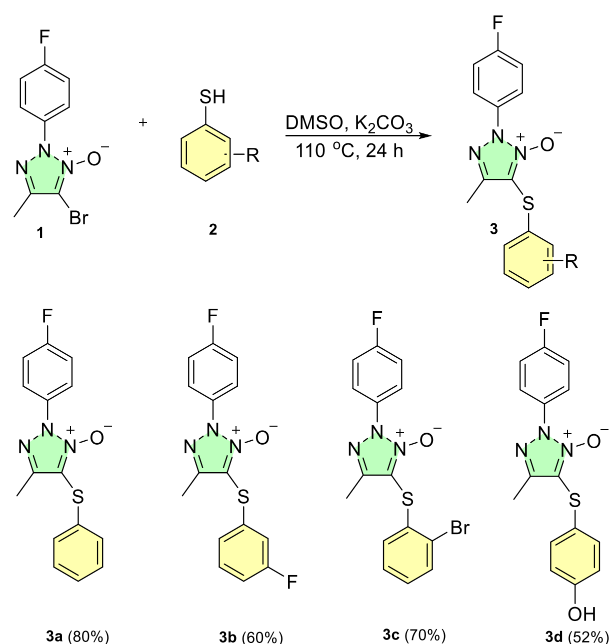


Scheme 1 Model reaction for optimizing reaction conditions



Scheme 2 Synthesis of thioether derivatives of 1,2,3-triazole-*N*-oxides

Table 1 Optimization of substitution reaction conditions

Entry	Base	Thiophenol equivalent	Solvent	t °C	Time h	Yield
1	K_2CO_3	2	$CHCl_3$	60	24	Traces
2	K_2CO_3	2	MeCN	80	24	35
3	K_2CO_3	2	DMSO	110	24	80
4	K_2CO_3	2	DMSO	110	12	45
5	K_2CO_3	2	DMSO	110	48	75
6	K_2CO_3	1	DMSO	110	24	31
8	K_3PO_4	1	DMSO	110	24	17
9	Et_3N	1	DMSO	110	24	7

Table 2 Cytotoxicity index (IC_{50} ± SE) of the investigated compounds on rhabdomyosarcoma (Rd) cells and human embryonic kidney (HEK-293) cells (μM)

Entry	Compound	Cell line	
		HEK-293	Rd
1	**3a**	126.59 ± 2.30	93.61 ± 5.70
2	**3b**	> 128	36.14 ± 3.55
3	**3c**	77.17 ± 10.27	22.29 ± 3.60
4	**3d**	124.77 ± 17.52	98.59 ± 9.39

Thus, four new thioether derivatives of 1,2,3-triazole-*N*-oxide containing various substituents (F, Br, OH) in their structure were obtained. It is worth noting that the yield of the target reaction products decreased, when various substituents were incorporated into the structure of the thioaromatic fragment, thus indicating a decrease in the reactivity of the SH group, which decreases in the order H>2-Br>3-F>4-OH. Thus, four new derivatives of 1,2,3-triazole-N-oxides were obtained with yields of 52-80% (Scheme 2).

All compounds obtained were evaluated for cytotoxicity against two cell lines: human embryonic kidney (HEK-293) cells and rhabdomyosarcoma tumor (Rd) cells. Based on the results of the MTT test, IC_{50} values were calculated (Table 2). The table and summary bar chart show that compound **3c** has the greatest activity against tumor cells, while sample **3b** also inhibits the viability of rhabdomyosarcoma (Rd). Substances **3d** and **3a** also exhibit activity, albeit to a lesser extent.

It is interesting to note that the IC_{50} value on tumor cells for all compounds is lower than for normal cells. From the dose-effect curves (Figure 2), one can conclude that **3a** and **3b** have some selectivity, since the toxic concentration for rhabdomyosarcoma remains safe for normal human embryonic kidney cells.

As mentioned in the introduction, the primary objective of this study (except synthesis) was to evaluate the ability of the compounds to modulate NO production at non-toxic concentrations, namely concentrations that do not affect cell viability. To determine appropriate non-toxic doses and to assess the baseline cytotoxic profile of the compounds, we performed an initial cytotoxicity screening on both normal (HEK-293) and tumor (rhabdomyosarcoma) cell lines. Based on the obtained IC_{50} values, subtoxic concentrations (ten times below IC_{50}) were selected for subsequent NO assays. For these experiments, the rhabdomyosarcoma (Rd) cell line was chosen as the model system, as it is better suited for NO-related studies due to its continuous growth characteristics and the reported expression of nitric oxide synthase isoforms in sarcoma cells [15,41].

To assess the effect of the studied azaheterocyclic compounds on nitric oxide production in cells, a double fluorescent staining method was used *in vitro*. Rhabdomyosarcoma cells, characterized by increased NOS expression [15,41], were staidness with the specific dye DAF-FM, which enables the visualization of intracellular nitric oxide (NO) levels. The results showed that samples **3a** and **3b** increased fluorescence intensity (and, presumably, NO production) more than two-fold, with statistically significant differences ($p < 0.01$) compared to the intact control group (Figure 3). This effect is also reflected in the micrographs obtained by fluorescence microscopy (Figure 4). Compound **3c** induced fluorescence to a lesser extent but still showed a statistically significant difference from the untreated group ($p < 0.05$). Meanwhile, compound **3d** increased the intracellular DAF-FM signal by more than 1.5 times; however, this result was not statistically significant compared to intact cells ($p > 0.05$) (Figure 3).

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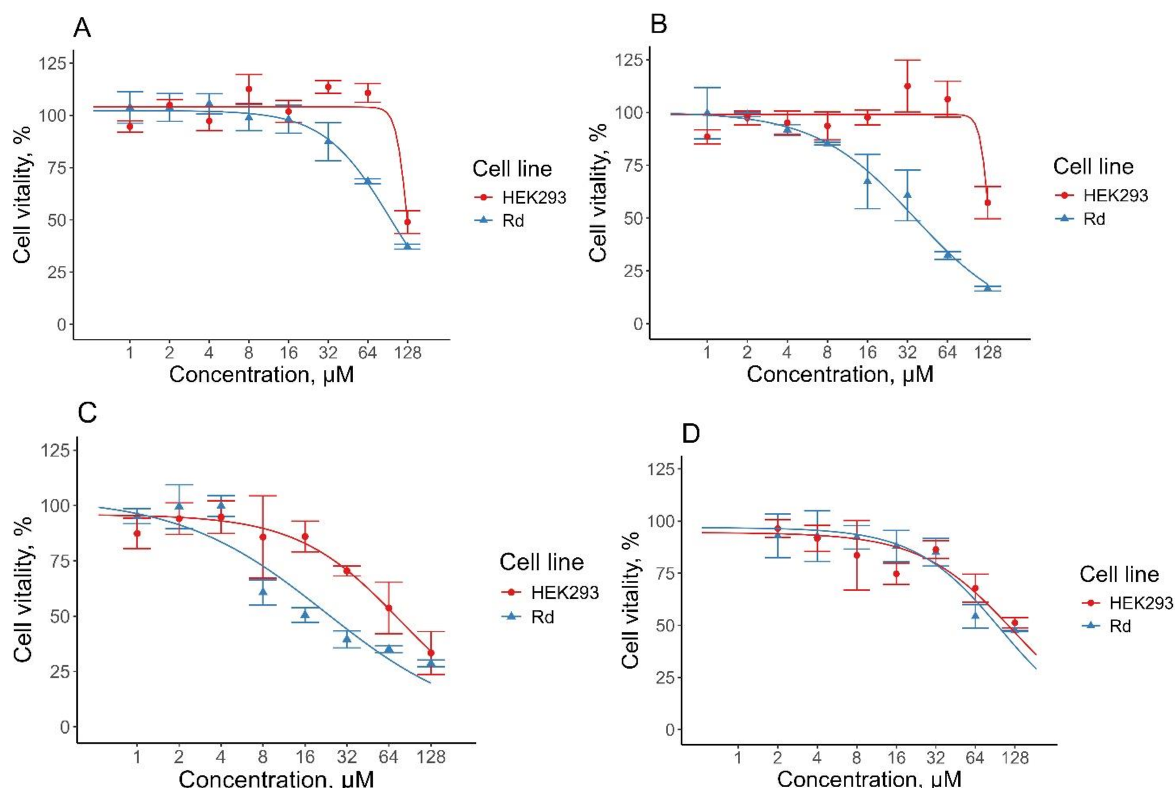

Figure 2 Cell growth inhibition curves under the influence of the compounds under study, mean ± SD, n = 3: A – sample 3a; B – sample 3b; C – sample 3c; D – sample 3d.

Notably, the compound concentrations used in this study were ten times lower than their respective IC_{50} values, representing subtoxic doses. This experimental design was specifically chosen to evaluate NO-modulating effects in the absence of significant cytotoxicity. Consequently, the observed changes in NO production should not be interpreted as directly correlating with or causing cytotoxic effects. Rather, these findings demonstrate that the compounds can modulate intracellular NO levels independently of toxicity, suggesting a specific pharmacological action on NO pathways.

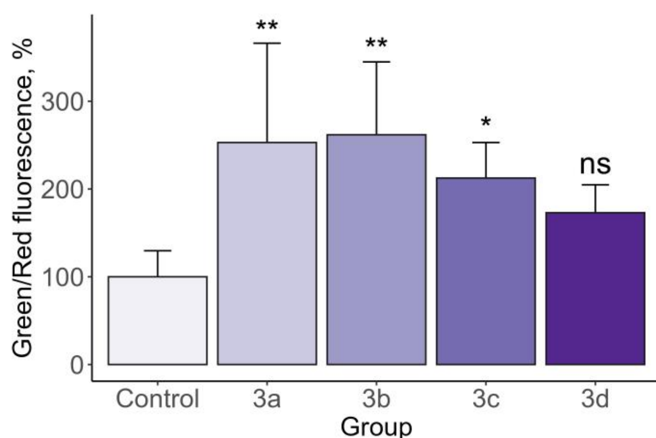

Figure 3 Percentage ratio of green (DAF-FM – NO) and red (rhodamine B – all cells) fluorescence, M ± SD, 100% is the ratio obtained on intact (untreated) rhabdomyosarcoma cells (Rd), n = 5, ns – no statistically significant differences from the control, * – $p < 0.05$, ** – $p < 0.01$.

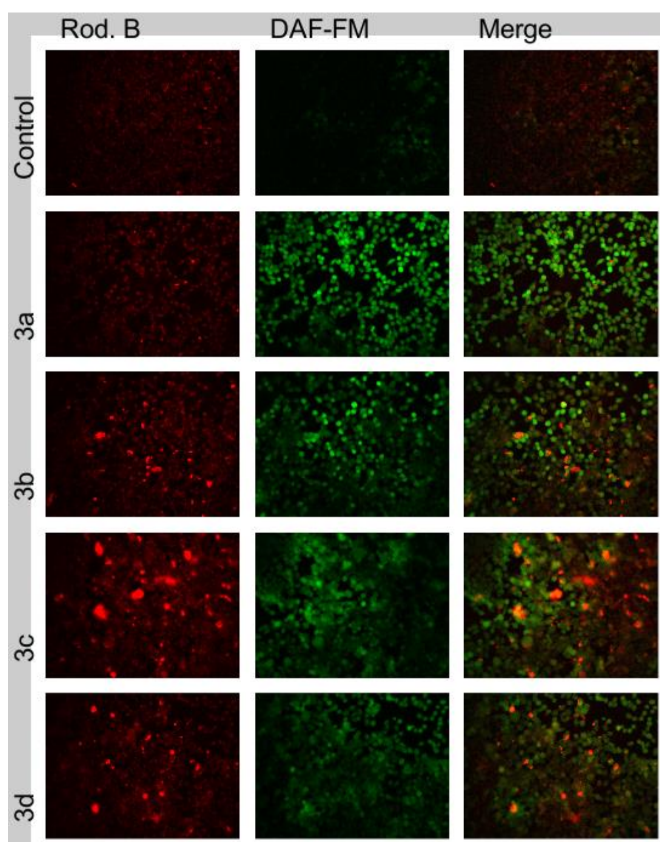

Figure 4 The result of staining rhabdomyosarcoma (Rd) cells with DAF-FM (10 μM) and rhodamine B (0.5 mg/ml): green fluorescence indicates nitric oxide (NO) content in cells, red indicates all viable cells. The microimages show the superimposition of green and red fluorescence layers, n = 5. Magnification 200x.

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It is important to note that while DAF-FM is a sensitive fluorescent probe for detecting NO, this method primarily provides qualitative or semi-quantitative data. It is valuable for indicating the absence of fluorescence in control samples and for observing relative changes in NO levels. Consequently, the fluorescence microscopy approach can be utilized in the initial screening phase to evaluate potential drug candidates with endothelium-protective or NO-modulating properties. However, since primary endothelial cells are difficult to culture in vitro and are not suitable for extensive preliminary experiments, our study was conducted on human rhabdomyosarcoma cells. This cell line was chosen as a convenient model for two main reasons: it is a continuous line that can be cultured indefinitely, and existing literature indicates that sarcoma cells often express constitutive nitric oxide synthase (NOS) isoforms, making them a relevant system for assessing NO-modulating activity [15]. Nonetheless, it is important to acknowledge that DAF-FM fluorescence is not entirely specific for NO and may be influenced by other reactive species, and thus these findings should be interpreted with appropriate caution.

Based on the data obtained on the effect on NO production in rhabdomyosarcoma cells, one can conclude that the presence of a fluorine atom in the structure of the molecule leads to an increase in the efficiency of nitric oxide production. On the other hand, incorporation of a bromine atom or a hydroxyl group into the structure has effect on reducing this indicator. In addition, the presence of a bromine atom in the molecule leads to an increase in the toxicity of this compound both to healthy cells and to rhabdomyosarcoma cells.

4. Limitations

This study has several limitations that should be considered when interpreting the findings. First, the experiments were conducted using a single cell line, namely human rhabdomyosarcoma. While this model was selected for its suitability for initial screening and its reported expression of NOS isoforms [15], the results may not be directly extrapolated to other cell types, particularly primary endothelial cells. Therefore, the findings should be regarded as preliminary and warrant further validation in relevant endothelial models.

Second, nitric oxide (NO) production was assessed using the fluorescent probe DAF-FM. Although this method is widely used for detecting intracellular NO, it is important to acknowledge its limitations: (i) DAF-FM fluorescence is primarily qualitative or semi-quantitative and does not provide absolute NO concentrations; (ii) the probe is not entirely specific for NO and may be influenced by other reactive nitrogen or oxygen species, such as peroxynitrite or reactive oxygen intermediates. Accordingly, the fluorescence data should be interpreted with appropriate caution, and complementary quantitative methods would be beneficial in future studies.

Third, as noted in the discussion, the compound concentrations used in this study were ten times lower than their respective IC_{50} values, representing subtoxic doses. This experimental design was specifically chosen to evaluate NO-modulating effects in the absence of significant cytotoxicity. Consequently, the observed changes in NO production should not be interpreted as directly correlating with or causing cytotoxic effects. Rather, these findings demonstrate that the compounds can modulate intracellular NO levels independently of toxicity, suggesting a specific pharmacological action on NO pathways. No causal relationship between NO modulation and cytotoxicity is established in the present work.

Fourth, experiments with nitric oxide synthase (NOS) inhibitors, such as L-NAME, were not within the scope of this initial screening study. While such controls would provide additional confirmation that the observed increase in DAF-FM fluorescence is attributable to NOS-derived NO production, their absence does not detract from the primary finding that the compounds modulate intracellular NO levels. Nevertheless, future studies incorporating NOS inhibitors would be valuable to further elucidate the precise mechanism of action.

Taken together, these limitations define the boundaries of the conclusions that can be drawn. The study provides initial evidence of NO-modulating effects in a cellular model, but further investigations—including quantitative NO measurements, additional cell types, and functional endothelial assays—are required to confirm the potential biological significance of these findings.

5. Conclusions

In summary, a method for obtaining thioether derivatives of 1,2,3-triazole-*N*-oxides in yields ranging from 52 to 80% has been developed. Cytotoxicity studies on human embryonic kidney (HEK-293) cells showed low toxicity with IC_{50} values ranging from 128 to 76 μM. On the other hand, the obtained derivatives are highly toxic to rhabdomyosarcoma cancer (Rd) cells, demonstrating IC_{50} values ranging from 93 to 22 μM. As a result of fluorescent staining of cells, it has been found that the obtained compounds have a promoting effect on the production of nitric oxide in cells, which increases up to two times. These data have suggested that these compounds could be of therapeutic effect for conditions involving cellular NO deficiency. The future studies are supposed to focus on a detailed analysis of the patterns identified in this work.

Supplementary materials

This manuscript contains supplementary materials, which are available on the corresponding online page. In particular, **Figures S1-S12**: ^{1}H, ^{13}C and ^{19}F NMR spectra, **Figures S13-S16**: IR spectra, **Figures S17-S20**: HRMS spectra of compounds **3a-d.**

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Spectral data were obtained in the Laboratory of Complex Research and Expert Evaluation of Organic Materials, Center for Collective Use of unique equipment of the Ural Federal University, https://ckp.urfu.ru.

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Conflict of interest

The authors declare no conflict of interest.

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Data available within the article or its supplementary materials

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