Synthesis and evaluation of the antitrypanosomal activity, ADME and DNA binding properties of benzothiazole amidoximes and arylimidamides

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Abstract

Novel 6-amidoxime and 6-arylimidamide benzothiazoles were synthesised to investigate their activity against *Trypanosoma brucei,* the causative agent of African trypanosomiasis. Benzothiazole amidoxime **12b**, with diethylaminoethyl and fluorine substituents on a phenoxymethylene linker exhibited pronounced (IC_{50} = 0.92 μM) and selective (SI = 18) antitrypanosomal activity. ADME profiling showed that the majority of compounds synthesised are metabolically stable and that arylimidamides have low membrane permeability, while amidoximes, including **12b**, have moderate to high permeability. Binding assays indicate that amidoxime **12b** binds to DNA/RNA by intercalation, whereas arylimidamide **29b** displays groove binding. **12b** was found to be a substrate of the P-glycoprotein efflux pump, a factor that may have limited its activity in a murine model of infection, despite the other favourable properties. Structural diversification of amidoxime **12b** will be explored to further optimize its activity and ADME properties.

Keywords: benzothiazole, amidoxime, arylimidamide, antitrypanosomal activity, ADME, DNA/RNA binding

1. **Introduction**

Human African Trypanosomiasis (HAT), also known as sleeping sickness, is a neglected tropical disease (NTD), caused by infection with subspecies of the parasitic protozoan *Trypanosoma brucei* [1–3]. Trypanosome infections of domestic livestock are also a major constraint on agricultural productivity throughout sub-Saharan Africa. Toxicity and resistance associated with the current drugs has complicated control and impeded progress towards the elimination of HAT as a public health problem by 2030 [4,5], although roll-out of fexinidazole as an oral treatment for stage 2 disease has been an important advance. Pentamidine (Figure 1), has been used as the first-line treatment against the hemolymphatic stage of HAT for more than 80 years [6].

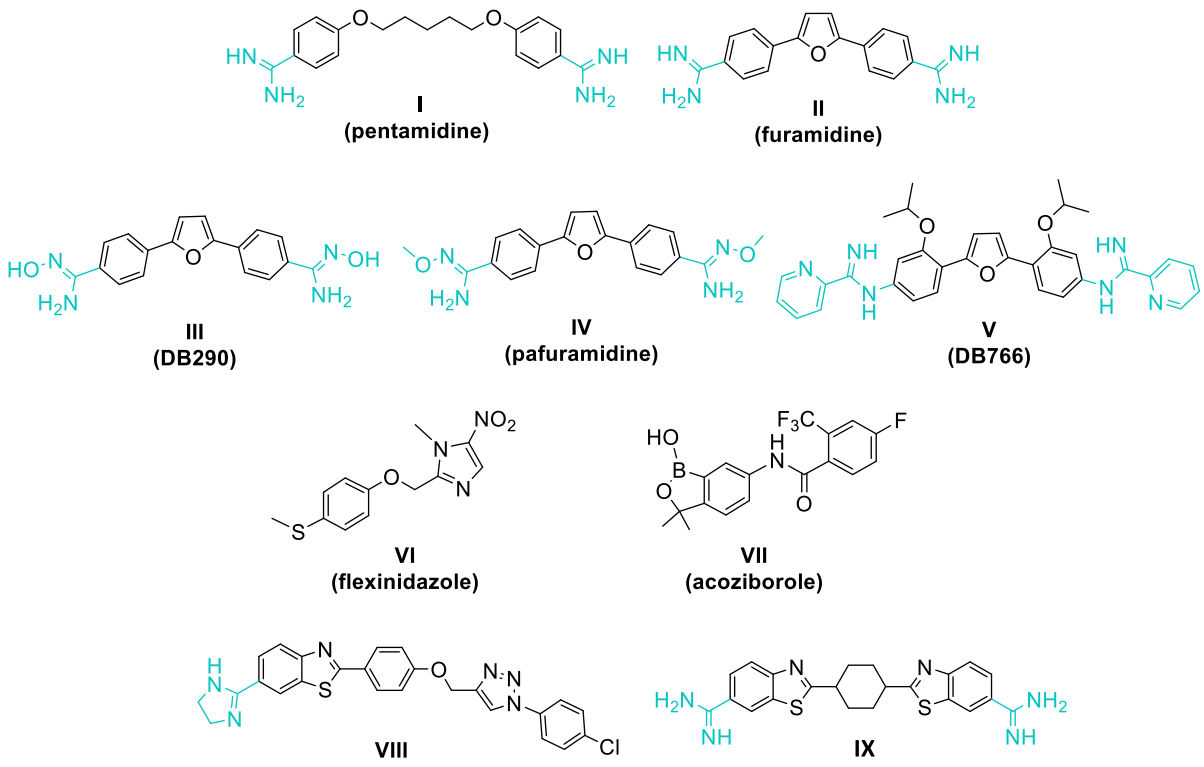
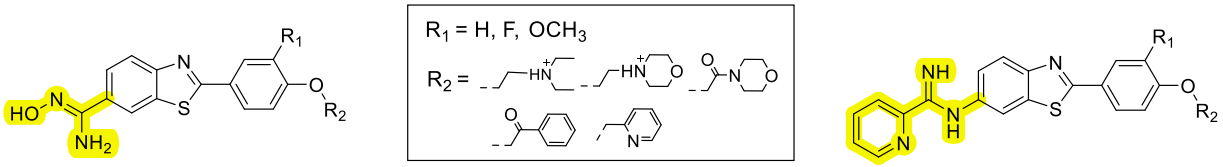


Figure 1. Drugs used to treat HAT (**I**–**VII**) and representative examples of benzothiazole amidines **VIII** and **IX** with potent antitrypanosomal activity.

Pentamidine **I** and similar aromatic diamidines have a high affinity for binding to double-stranded DNA, especially in the minor groove of AT-rich regions. It has been demonstrated that pentamidine **I** binds to kinetoplast DNA (kDNA) and inhibits topoisomerase activity, causing kDNA damage and interuption of the cell cycle [7–9]. Besides mitochondrial and nuclear DNA, other targets have been suggested for the antiparasitic action of dications, including microtubules, acidocalcisomes, and a range of enzymes [10]. A limitation of aromatic diamidines is that they can result in adverse effects and require

parenteral administration [10,11]. In addition, they have a restricted ability to cross the blood-brain barrier, an important consideration when treating stage 2 HAT, which occurs once parasites have accessed the central nervous system. To overcome the low oral bioavailability and absorption limitations of cationic diamidines, a prodrug strategy has been employed to enhance oral absorption [10]. The pK_a of amidine can be changed by modulating the amidine group by *N*-derivatization to form the amidoxime **III** (DB290) and pafuramidine **IV** (DB289), orally effective prodrugs of furamidine **II** [10,11]**.** Although pafuramidine **IV** was found to be effective against first-stage HAT in a Phase III trial, delayed renal toxicity led to discontinuation of its further development [12]. Another strategy for overcoming the low oral bioavailability of aromatic diamidines is through arylimidamides (AIAs), also known as reverse amidines, such as **V** (DB766) [13–15]. The superior activity of arylimidamides compared to amidine compounds has been found to be related to their lower pKa values and greater lipophilic character that contributed to their more efficient passage across the parasite cell membrane. There has been significant progress in the development of new therapeutics for HAT, with the orally active drug fexinidazole now approved and acoziborole in advanced, late-stage clinical trials [16–19].

Recently, studies on benzazoles and their amidino derivatives have been conducted to develop improved antitrypanosomal agents [20–37]. Our group reported that benzothiazole amidine **VIII** had good potency against *T. brucei* and bound to DNA through minor groove interactions [20]. Furthermore, within symmetric bis-6-amidino-benzothiazole derivatives, dicationic benzothiazole **IX** exhibited sub-nano molar *in vitro* potency against *T. brucei* as well as *in vivo* activity [25]. Based on the above, we have explored structural modifications that could improve the ADME and physicochemical properties of the benzothiazole derivatives and provide a compound suitable for *in vivo* study. Benzothiazole analogues containing 6-amidoxime and 6-arylimidamide in place of the amidine moiety, together with aromatic and aliphatic units at the phenoxymethylene attached to the C-2 of the benzothiazole core were designed and synthesised. Herein, we report the results of *in vitro* antitrypanosomal evaluations and ADME profiling of novel 6-amidoxime- **12a**–**14a**, **12b**–**15b**, **12c**, **13c** and **16c**, as well as 6-arylimidamide-substituted **31a**–**35a**, **31b**–**35b** and **31c**–**35c** benzothiazoles (Figure 2).



12a–14a, **12b–15b**, **12c**, **13c** and **16c** **31a–35a**, **31b–35b** and **31c–35c**

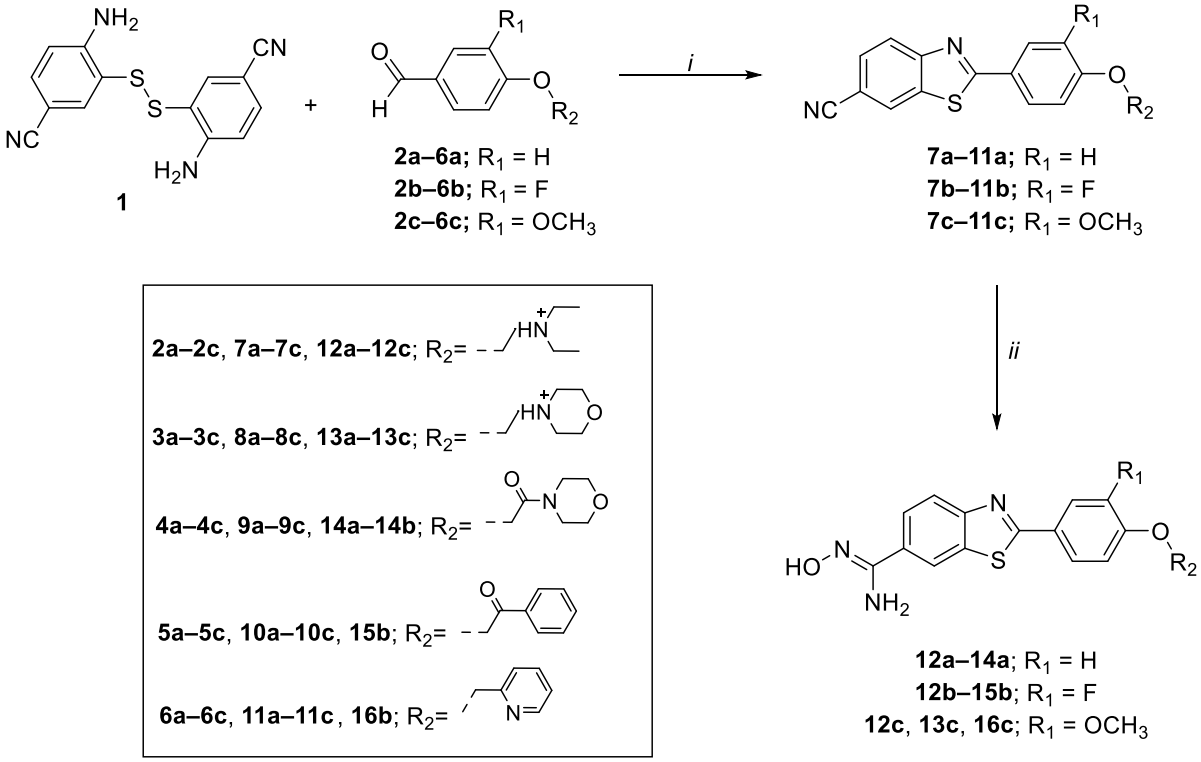
Figure 2. Designed and synthesised benzothiazole amidoximes and reverse amidines with aromatic and aliphatic units attached *via* phenoxymethylene.

Binding strength and mode of binding of small molecule DNA/RNA interactions were assessed using UV–Vis, fluorescence and circular dichroism (CD) spectroscopy, as well as thermal denaturation experiments in the case of the most potent antitrypanosomal compounds. The compound **12b** with selective antitrypanosomal activity and favourable ADME properties was subsequently submitted to *in vivo* evaluation.

2. Results and Discussion

2.1. Chemistry

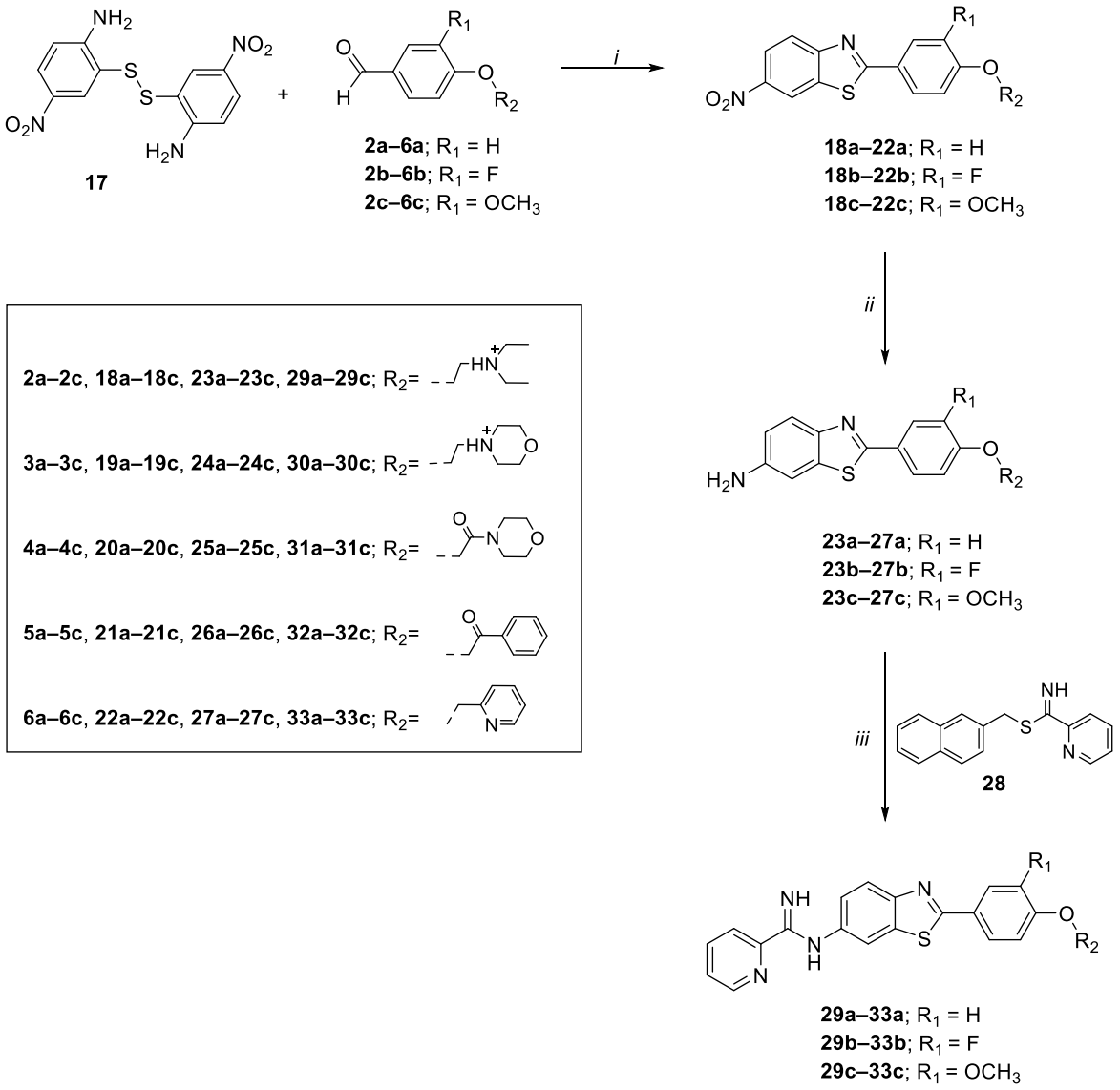
The novel amidoxime- and arylimidamide-substituted derivatives bearing benzothiazole nuclei were synthesised according to the experimental procedures presented in Schemes 1 and 2.



Scheme 1. Reagents and conditions: (*i*) glycerol, 165 °C, 30 min; (*ii*) ethanol, Et_3N, NH_2OH x HCl, 80 °C, 24 h.

For the preparation of aldehyde precursors **2a**–**6a**, **2b**–**6b**, **2c**–**6c** and disulfides **1** and **17**, efficient and environmentally benign synthetic protocols were applied using methods previously reported by our group [21,38]. Preparation of amidoxime benzothiazole analogues **12a**–**12c**, **13a–13c**, **14a**, **14b**, **15b** and **16c**, shown in Scheme 1, was achieved through a two-step synthetic route. In the first step, applying a green synthetic protocol, cyano-substituted benzothiazole analogues **7a**–**7c**, **8a**–**8c**, **9a**–**9a**, **10a**–**10c**, and **11a**–**11c** were prepared from bis(2-amino-5-cyanophenyl)disulfide **1** and aromatic aldehydes **2a**–**6a**, **2b**–**6b**, and **2c**–**6c** by a simple thermal reaction in glycerol [38], in moderate yields.

Next, the reaction of nitriles **7a**–**7c**, **8a**–**8c**, **9a**–**9a**, **10a**–**10c**, and **11a**–**11c** with hydroxylamine and triethylamine resulted in the desired amidoxime derivatives with diethylaminoethyl **12a**–**12c**, ethylmorpholine **13a**–**13c**, morpholinoyl **14a** and **14b**, benzoyl **15b** and pyridine **16c** substituents introduced to the phenoxymethylene linker of the amidoxime-substituted benzimidazoles.



Scheme 2. Reagents and conditions: (*i*) glycerol, 165 °C, 30 min; (*ii*) $SnCl_2 \times 2H_2O$, conc. HCl, methanol, reflux, 1 h; (*iii*) a) **28**, ethanol : acetonitrile = 3 : 1, 0 °C – rt, 24 h, b) HCl(g) abs. ethanol, rt, 12h.

For the preparation of benzothiazole arylimidamide (AIA) analogues **29a**–**33a**, **29b**–**33b** and **29c**–**33c** (Scheme 2), the synthesis of key amino intermediates was achieved starting with the condensation of bis(2-amino-5-nitrophenyl)disulfide **17** and the corresponding aromatic benzaldehydes **2a**–**6a**, **2b**–**6b** and **2c**–**6c** at 165 °C in glycerol within 30 min, to give nitro-substituted benzothiazole intermediates

18a–**22a**, **18b**–**22b** and **18c**–**22c**. The nitro group of benzothiazole intermediates was then reduced using tin(II) chloride dihydrate, generating key amino-substituted benzothiazoles **23a**–**27a**, **23b**–**27b** and **23c**–**27c**. The thioimidate **28** was prepared in two steps from picolinonitrile *via* the corresponding pyridine-2-carbothioamide, which was allowed to react with 2-(bromomethyl)naphthalene [39]. Finally, the reaction of 6-aminobenzothiazole intermediates with *S*-(2-naphthylmethyl)-2-pyridylthioimidate hydrobromide **28** produced, after salt formation, the benzothiazole arylimidamides (AIAs) **29a**–**33a**, **29b**–**33b** and **29c**–**33c** in moderate to high yields (44–95%).

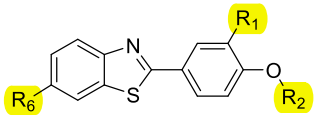
2.2. Antitrypanosomal activity and ADME profiling

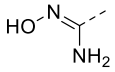
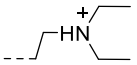
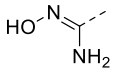
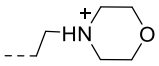
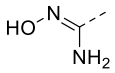
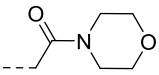
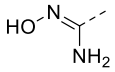
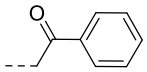
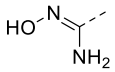
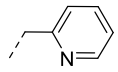
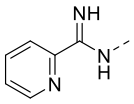
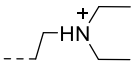
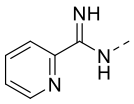
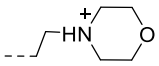
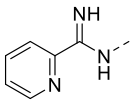
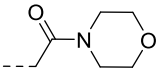
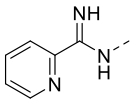
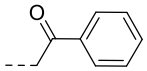
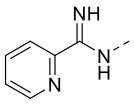
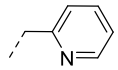
In vitro activity against bloodstream form *T. brucei* was evaluated for the benzothiazole amidoximes **12a–12c**, **13a–13c**, **14a**, **14b**, **15b** and **16c** and arylimidamides **29a–29c**, **30a–30c**, **31a–31c**, **32a–32c** and **33a–33c**, with fexinidazole as the reference drug (Table 1). Cytotoxicity was assessed using the HeLa cell line.

The effects of both types of substituent at the 6-position on the benzothiazole and varied aliphatic and aromatic right-hand residues were investigated (Figure 3). According to the results (Table 1), of the benzothiazole amidoximes tested **12a**–**12c**, **13a**–**13c**, **14a**, **14b**, **15b** and **16c**, compounds containing diethylaminoethyl (**12a**, **12b**) or benzoyl (**15b**) moieties on the phenoxymethylene linker showed good antitrypanosomal activity with IC_{50} values of 2.18 μM, 0.92 μM and 7.91 μM, respectively. Although these compounds were moderately cytotoxic to HeLa cells (IC_{50} ~20 μM), the selectivity indices (SI) of **12a** and **12b** (11 and 18, respectively) were both promising. The most active of these compounds, the 3-fluorophenyl containing benzothiazole **12b** had an IC_{90} value of 1.17 μM. Compounds **13a–13c**, **14a** and **14b**, which contain the morpholine moiety were not active against *T. brucei.*

We also found that the majority of compounds from the 6-arylimidamide-substituted (6-AIA) benzothiazole series displayed good antitrypanosomal activity. Similar to benzothiazole amidoximes, the introduction of the diethylaminoethyl in 6-arylimidamide-substituted benzothiazoles **29a**–**29c** was also associated with potent growth-inhibition of *T. brucei* (**29a**: IC_{50} = 1.49 μM; **29b**: IC_{50} = 0.88 μM; **29c**: IC_{50} = 1.12 μM), although cytotoxicity values meant that the SIs were unfavourable. Benzothiazole arylimidamides with benzoyl (**32a**–**32c)** and pyridine (**33a**–**33c)** moieties attached to a phenoxymethylene core showed good antitypanosomal activity with IC_{50} values in the range of 1.17–3.01 μM. However of these, only **32c** had an SI in the acceptable range (>150). In accord with benzothiazole amidoximes, benzothiazole arylimidamides with ethylmorpholine **30a**–**30c** and morpholinoyl **31a–31c** moieties had moderate or marginal potency against *T. brucei*, with exception of **30b**, which exhibited promising activity (IC_{50} = 1.85 μM)

Table 1. Antitrypanosomal activity[a] of benzothiazole amidoximes **12a–12c, 13a–13c, 14a–14b, 15b, 16c** and arylimidamides **29a–33a, 29b–33b, 29c–33c** against cultured bloodstream form *T. brucei.*



Cmpd	R_6	R_1	R_2	*T. brucei* IC_{50} (µM)	*T. brucei* IC_{90} (µM)	HeLa cells IC_{50} (µM)	SI[b] HeLa/*Tb* IC_{50}
12a		H		2.18 ± 0.05	3.41 ± 0.88	23.6 ± 1.9	11
12b		F		**0.92 ± 0.03**	**1.17 ± 0.03**	**16.6 ± 0.6**	**18**
12c		OMe		18.1 ± 0.4	31.1 ± 0.5	20.4 ± 1.9	1.1
13a		H		>250	>250	>250	-
13b		F		>240	>240	>240	-
13c		OMe		>23	>23	>23	-
14a		H		>24	>24	>240	-
14b		F		>23	>23	>230	-
15b		F		7.91 ± 0.42	11.0 ± 0.2	10.8 ± 0.3	1.4
16c		OMe		>240	>240	>240	-
29a		H		1.49 ± 0.08	2.59 ± 0.08	<2.0	-
29b		F		0.88 ± 0.07	1.70 ± 0.04	2.94 ± 0.26	3.3
29c		OMe		1.12 ± 0.11	1.68 ± 0.27	1.18 ± 0.12	1.1
30a		H		4.85 ± 0.20	16.4 ± 0.3	16.5 ± 0.8	3.4
30b		F		1.85 ± 0.45	8.99 ± 0.41	5.66 ± 0.70	3.1
30c		OMe		>19	>19	-	-
31a		H		>19	>19	-	-
31b		F		>19	>19	-	-
31c		OMe		10.3 ± 0.6	17.3 ± 0.3	16.3 ± 3.4	1.6
32a		H		2.50 ± 0.30	5.54 ± 0.18	3.56 ± 0.56	1.4
32b		F		1.70 ± 0.54	4.21 ± 0.19	0.89 ± 0.04	0.5
32c		OMe		1.17 ± 0.15	2.31 ± 0.70	>180	>150
33a		H		3.01 ± 0.23	5.36 ± 0.14	5.56 ± 0.59	1.8
33b		F		1.32 ± 0.39	4.49 ± 0.53	6.72 ± 0.25	5.1
33c		OMe		1.84 ± 0.18	2.81 ± 0.08	6.20 ± 0.12	3.4
FEX				2.40 ± 0.18	4.51 ± 0.57		

[a]*In vitro* activity against bloodstream form *T. brucei* was determined using microtiter plate assays (Experimental Section). Values are expressed as the concentration that inhibited growth by 50% (IC_{50}) and 90% (IC_{90}). The data are the mean of triplicate experiments ± SEM. [b]Selectivity index, SI = [IC_{50} HeLa cells]/[IC_{50} *T. brucei*]. FEX = fexinidazole.

Overall, it can be observed that benzothiazole analogues bearing an arylimidamide moiety show better antitrypanosomal activity compared to their amidoxime counterparts (Figure 3). Comparing the antitrypanosomal activity of amidoximes **12b** and **15b** to their arylimidamide counterparts **29b** and **32b**, it is apparent that benzothiazoles with an arylimidamide moiety have better antitrypanosomal activity. Assessment of the effect of R_3 substituents on the phenoxymethylene linker showed that among benzothiazoles with aliphatic moieties, compounds **12b**, **29b** and **30b**, with fluorine attached, exhibited better antitrypanosomal activities than their analogues with non-substituted and methoxy-substituted phenoxymethylene. Within benzothiazole arylimidamides with aromatic moieties, compounds **32c** and **33c** containing methoxy were the most active. Moreover, **32c** exhibited impressive selective activity (SI >150). Regarding the influence of R_2 substituents on the phenoxymethylene linker of benzothiazoles, analogues bearing diethylaminoethyl and benzoyl substituents showed the best antitrypanosomal activity, while analogues with ethylmorpholine and morpholinoyl substituents had no significant antitrypanosomal activity in line with their reduced basicity. Calculated PhysChem parameters are added in Table 2, together with estimation of potential for crossing the BBB (MPO [40] and BBB [41] scores). Crossing the BBB is important when treating stage 2 HAT, which occurs once parasites have accessed the central nervous system. Maximum value for the MPO score is 6, therefore compounds with an MPO score higher than 4.5 might have potential to cross the BBB. While the BBB score is more restrictive, it predicts that only compounds **12a-12c** have BBB-crossing potential.

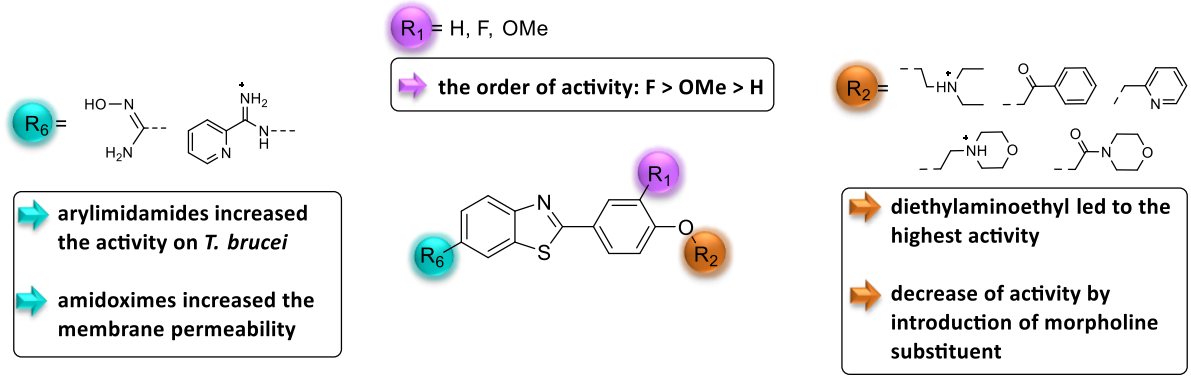
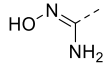
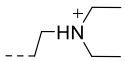
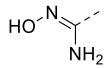
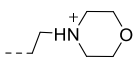
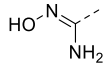
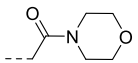
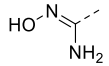
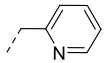
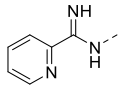
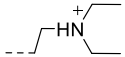
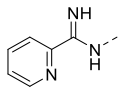
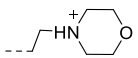
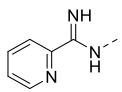
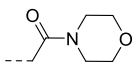
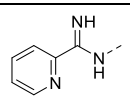
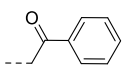
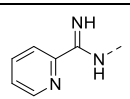
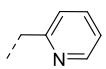


Figure 3. Insights into structure-activity relationship for antitrypanosomal activity of the amidoxime- and arylimidamide-substituted benzothiazoles.

Table 2. Measured metabolic stability in mouse liver microsomes (Pred *in vivo* hep CL) and apparent permeability (Papp) in MDCKII-hMDR1 cell assays from apical-to-basolateral (AB) and basolateral-to-apical (BA) side

					Without elacridar			With elacridar			PhysChem			BBB descriptors	
Cmpd	**R_6**	**R_1**	**R_2**	**Pred *in vivo* hep CL [% LBF]**	**P_{app} (AB) [x10^{-6} cm/s]**	**P_{app} (BA) [x10^{-6} cm/s]**	**Efflux ratio**	**P_{app} (AB) [x10^{-6} cm/s]**	**P_{app} (BA) [x10^{-6} cm/s]**	**Efflux ratio**	**logD**	**logP**	**pKa**	**MPO**	**BBB score**
12a		H		37	8.0	24.6	3.9	8.0	9.3	1.2	2.57	3.93	8.74	4.96	3.55
12b		F		47	5.0	18.6	3.8	5.0	6.4	1.4	3.08	4.27	8.56	4.92	3.56
12c		OMe		<30	2.0	42.4	21.9	5.5	8.0	1.5	2.68	4.02	8.72	4.65	3.15
13a		H		51	18.7	28.2	1.6	27.3	19.9	0.8	2.98	3.00	6.18	5.12	2.53
13b		F		78	24.7	24.0	1.4	34.3	22.3	0.7	3.36	3.38	6.10	4.99	2.96
13c		OMe		72	5.6	55.4	10.4	19.6	25.9	1.4	3.14	3.17	6.21	4.6	2.33
14a		H		<30	6.4	35.2	5.5	14.1	9.8	0.7	1.86	1.87	5.17	4.45	2.13
14b		F		30	2.3	6.8	2.9	11.4	7.4	0.7	2.27	2.27	5.04	4.32	2.07
16c		OMe		58	N/A	5.8	N/A	4.9	6.1	1.2	3.55	3.55	4.99	4.74	2.16
29a		H		63	<0.1	0.5	N/A	0.3	0.3	1.1	3.71	5.34	8.87	4.45	3.16
29b		F		67	<0.1	0.3	N/A	<0.1	0.2	N/A	3.95	5.40	8.70	4.41	3.16
29c		OMe		<30	<0.1	0.5	N/A	<0.1	0.2	N/A	3.46	5.07	8.85	4.25	2.74
30a		H		61	1.4	3.0	2.1	2.0	1.4	0.8	4.31	4.57	7.31	4.79	2.68
30b		F		69	0.3	2.6	9.8	1.5	1.6	1.1	4.43	4.67	7.26	4.66	2.68
30c		OMe		71	0.5	2.4	4.5	0.9	0.9	1.0	4.03	4.31	7.34	4.49	1.87
31a		H		33	1.1	3.9	3.4	2.5	1.6	0.6	3.33	3.56	7.23	4.34	1.8
31b		F		54	0.5	1.7	3.4	1.1	0.8	0.8	3.53	3.74	7.19	4.22	1.75
31c		OMe		54	6.2	18.1	2.9	5.8	9.1	1.5	3.10	3.34	7.26	3.85	1.58
32a		H		88	<0.1	<0.1	N/A	<0.1	<0.1	N/A	4.76	4.99	7.24	4.75	2.65
32b		F		90	<0.1	<0.1	N/A	<0.1	<0.1	N/A	4.89	5.10	7.20	4.62	2.65
32c		OMe		67	<0.1	<0.1	N/A	0.1	<0.1	N/A	4.58	4.82	7.26	4.3	2.24
33a		H		<30	<0.1	0.0	N/A	0.4	0.8	2.1	4.82	5.00	7.11	4.95	2.73
33b		F		<30	<0.1	0.1	N/A	0.4	0.3	1.0	4.96	5.12	7.07	4.82	2.72
33c		OMe		33	<0.1	0.8	N/A	0.6	1.6	2.5	4.68	4.87	7.13	4.63	2.31

Metabolic stability in mouse liver microsomes and permeability in the MDCKII-hMDR1 assay were determined for benzothiazole amidoximes and arylimidamides (Table 2). Metabolic studies showed that the majority of compounds had moderate (30–70% liver blood flow, LBF) to low clearance (< 30% LBF), proving to be metabolically stable, while benzothiazoles **13b**, **13c** and **30c** with ethylmorpholine, and **32a** and **32b** with benzoyl moieties, displayed high clearance (> 70% LBF) and reduced stability in microsomes.

The majority of compounds were characterised by low membrane permeability. Increase of permeability correlates with increased lipophilicity and lower basicity of RHS amines. The same properties however contribute to the increase in affinity for P-glycoprotein (Pgp) transporters. All benzothiazole arylimidamides had low permeability (P_{app}(AB) < 2 x 10^{-6} cm/s), except for methoxy-substituted benzothiazole **31c** (P_{app}(AB) = 6.2 x 10^{-6} cm/s). Amidoxime units in **12a**–**12c**, **13a**–**13c**, **14a** and **14b** increased the permeability. The highest permeability was observed for fluoro-substituted benzothiazole amidoxime **13b** (P_{app}(AB) = 24.7 x 10^{-6} cm/s), while the highest efflux ratio was found for methoxy-substituted benzothiazole amidoximes **12c** and **13c**. Based on the efflux ratios, with and without the Pgp-specific inhibitor elacridar, amidoximes **12a**–**12c**, **13c**, **14a** and **14b** and arylimidamides **30a**–**30c**, **31a**–**31c** are Pgp efflux pump substrates, with the methoxyphenyl moiety contributing significantly to efflux, and may be subject to absorption and excretion *via* this transporter.

2.3. DNA/RNA binding study

Four compounds **12a**, **12b**, **29b**, and **32c** with strong and selective antitrypanosomal activity were chosen for additional studies on their interactions with nucleic acids. The compounds were dissolved in DMSO at a concentration c = 5 × 10^{-3} mol dm^{-3}. The absorbance measurements of their buffered aqueous solutions showed a straight proportionality to their concentrations up to c = 2 × 10^{-5} mol dm^{-3}. This observation indicates that the compounds in this concentration range did not form assemblies through intermolecular stacking interactions (Figure 4).

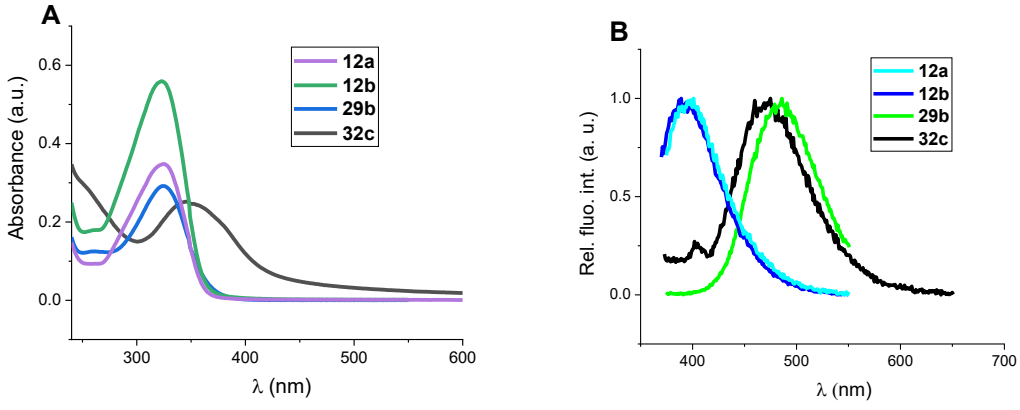


Figure 4**.** A) UV/Vis spectra of **12a**, **12b**, **29b** and **32c** (c = 1.0 × 10^{-5} mol dm^{-3}) at pH = 7.0, sodium cacodylate buffer, I = 0.05 M; B) Normalized emission spectra of **12a** (λ_{exc} = 325nm), **12b** (λ_{exc} = 323nm) **29b** (λ_{exc}= 326nm), and **32c** (λ_{exc}= 354nm) at concentration, c = 2.0 × 10^{-6} mol dm^{-3}; at pH = 7.0, Na cacodylate buffer, I = 0.05 mol dm^{-3}.

Figure 4 presents the absorption maxima and related molar extinction coefficients (ε) of **12a**, **12b**, **29b** and **32c** (Table S1 and Figure S1 in the Supporting Information). The fluorimetric measurements were carried out in a spectral range with no overlap between the excitation and emission spectra (Figure S2 in the Supporting Information).

Calf thymus (ct) DNA, which represents typical B-form DNA (58% AT, 42% GC base pairs), and AU homopoly-nucleotide (rArU), which acts as a model for A-helical structure (RNA), were used in the study with the tested compounds [42–44]. The binding study with RNA aimed to assess the preferable binding of compounds to DNA (recognition of polynucleotide conformation and/or bases) in comparison to RNA. Important parameters were obtained via thermal melting (T_m) experiments, such as the ΔT_m value, which indicates the difference between the T_m value of the free polynucleotide and the T_m of the complex obtained with a small molecule [45]. DNA can be significantly stabilized (positive ΔT_m values) or slightly destabilized (negative ΔT_m values) as a result of groove binding interactions. In contrast, intercalating small molecules are usually characterized by positive ΔT_m values. Selected compounds showed moderate (for **12b**, **29b**) or negligible (for **12a**, **32c**) stabilizing effects on DNA and RNA as shown in Table 3 and the SI (Table S2 and Figures S10-S11 in the Supporting Information). Among these compounds, **29b** had the strongest stabilizing effect on RNA (Figure 5), probably due to presence of two positively charged diethylaminoethyl chains at the phenoxymethylene and arylimidamide at C-6 of benzothiazole.

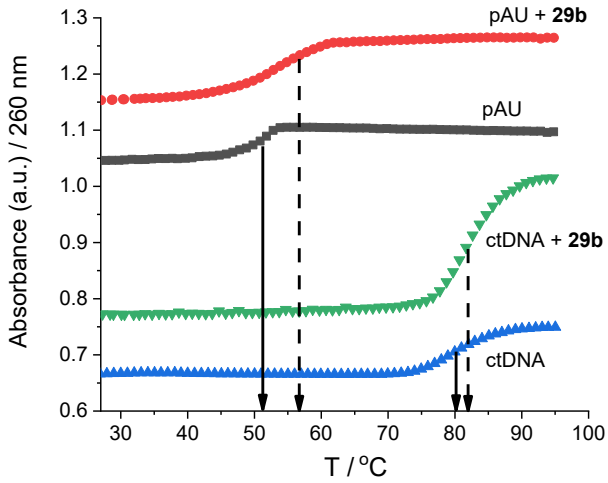


Figure 5**.** Melting curve of DNA and RNA upon addition, with ratio, r ([compound/[polynucleotide]) = 0.3 of **29b** at pH = 7.0 (buffer sodium cacodylate, I = 0.05 mol dm^{-3}).

The titrations with ctDNA and rArU led to a fluorescence decrease with **12a**, **12b** and **29b**, as depicted in Figure 6 (see also Figures S3–S9 in Supporting Information). The binding constants (K_a) for the complexes formed between the ligands and DNA/RNA were determined using the Scatchard equation [46,47], based on the fluorimetric titration data (Table 3). Notably, compounds **12a** and **12b**, which possess diethylaminoethyl chains on the phenoxy group and a hydroxy-substituted amidine, exhibited significant binding affinities to both DNA and RNA. **12a**, **12b** and **29b** bound better to DNA than to RNA (Table 3).

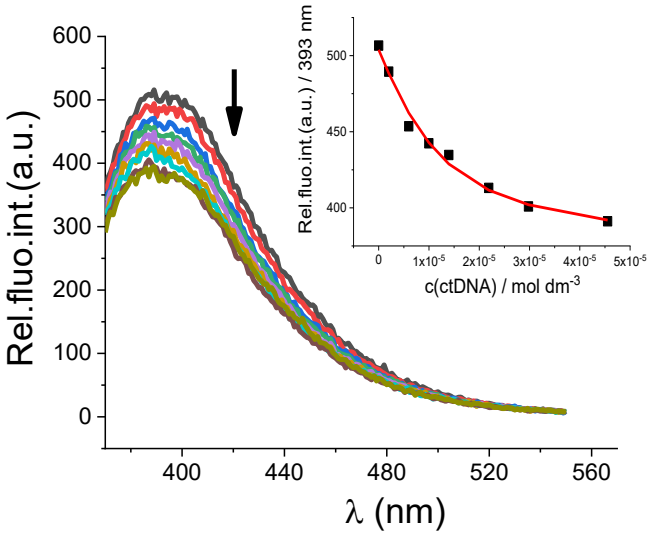


Figure 6**.** Changes in the fluorescence spectrum of compound **12b** (c = 1.0 × 10^{-6} mol dm^{-3}, λ_{exc}= 323 nm) upon titration with ctDNA (c = 2.0 × 10^{-6} – 6.1 × 10^{-5} mol dm^{-3}); Inset: Dependence of fluorescence of **12b** at λ_{em} = 393 nm on c(ctDNA); (buffer sodium cacodylate, pH = 7.0, I = 0.05 mol dm^{-3}).

Table 3**.** Binding constants ($\log K_a$)[a,b] for complexes of ligand-DNA/RNA calculated from the fluorescence titrations and ΔT_m[c] values (°C) of DNA/RNA upon addition of ratio[d] ***r*** = 0.3 of **12a**, **12b**, **29b** and **32c** at pH 7.0 (sodium cacodylate buffer, I = 0.05 mol dm^{-3}).

	ctDNA		rArU	
	logK_a	**ΔT_m / °C**	**logK_a**	**ΔT_m / °C**
12a	6.0	0.5	5.9	0.6
12b	6.3	1.2	5.7	0
29b	5.2	1.7	4.6	4.7

[a] Processing of titration data by means of the Scatchard equation [44,45] gave values of ratio n[bound ligand]/[polynucleotide from 0.05-0.15. For easier comparison all data were recalculated for fixed n = 0.1; correlation coefficients were > 0.99 for most of calculated K_a.

[b] Small fluorescence changes in titrations of **32c** with DNA prevented the accurate calculation of binding constants.

[c] Error in ΔT_m : ± 0.5°C.

[d] ***r*** = [compound] / [nucleotide phosphate].

Circular dichroism (CD) spectroscopy is a valuable tool for monitoring changes in nucleic acid conformation upon the interaction with small molecules. It provides insights into the interaction mode based on the relative orientation of the small molecule and the chiral axis of the nucleic acid [48,49]. Achiral small molecules like **12a**, **12b**, **29b** and **32c** can induce a CD spectrum known as Induced Circular Dichroism (ICD) when interacting with nucleic acids. In the spectral region beyond 300 nm, where DNA exhibits no absorption (as illustrated in Figure S12), informative data regarding binding can be extracted. Upon the addition of these compounds, a significant decrease in CD intensity was observed in most titrations with ctDNA at 275 nm and RNA at 260 nm (Figure 6 and Figure S12).

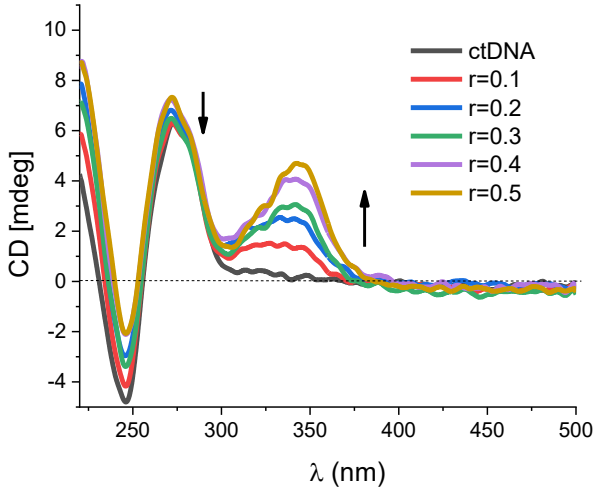


Figure 7**.** CD spectra of ctDNA (c = 3.0 × 10^{-5} mol dm^{-3}) treated with **29b** at different molar ratios ***r*** = [compound]/[nucleotide phosphate] indicated in the graph legend (pH = 7.0, buffer sodium cacodylate, *I* = 0.05 mol dm^{-3}).

Amidoximes **12a** and **12b** displayed the appearance of weak positive ICD bands (around 340 nm) with DNA as illustrated in Figure 7 and Figure S12. Such changes usually point to intercalation or partial intercalation with the transition moment of the ligand oriented perpendicular to the long axis of adjacent base pairs or groove binding with a loose orientation of the ligand with respect to the DNA axis [46,48]. The ICD bands were more evident for DNA compared to RNA in titrations of **12a** and **12b**. Compound **32c** induced negative ICD bands in the area from 300-380 nm, both with DNA and RNA. Weak negative ICD signals, resulting from the "parallel" orientation of the long axis of ligands to the long axis of adjacent base-pairs is an indication of intercalative binding [48]. Such changes indicate a groove binding or partial intercalation where the long axis of the aromatic core is positioned at some angle to the long axis of adjoining basepairs, abolishing positive and negative contributions [50]. The

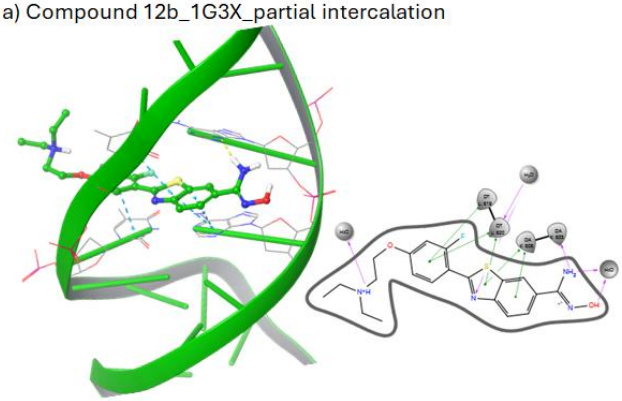
latter is additionally supported by the small binding affinity (<< 10 μM) and negligible thermal stabilization of DNA/RNA, observed for **32c**, which is not characteristic of classical intercalation.

Arylimidamide **29b** induced a strong positive ICD signal with ctDNA (centered at 344 nm) and bisignate negative (342 nm)-positive (311 nm) ICD spectra with RNA. Strong positive ICD strongly supports minor groove binding to DNA while bisignate ICD bands indicate the binding of **29b** in the form of a dimer within the RNA major groove.

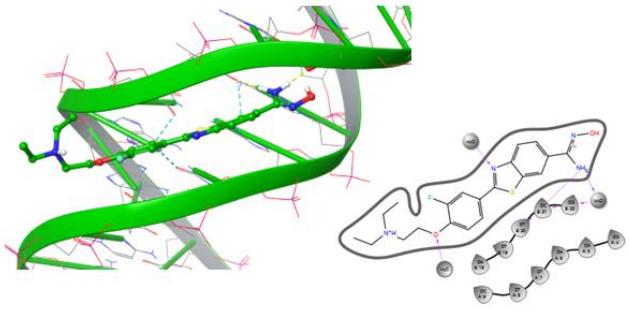
Overall, CD spectroscopy and thermal melting analysis along with the binding constants suggest that the benzothiazole amidoximes **12a** and **12b** bind to DNA and RNA by intercalation or partial intercalation. Conversely, the putative binding mode of benzothiazole arylimidamide **29b** to DNA and RNA involves binding within the groove.

Potential binding modes to DNA for compounds **12b** and **29b** were further explored by molecular docking and MD simulations. For compound **12b**, both intercalation and minor groove binding are investigated to support experimental binding studies. Compound **29b** was docked in the minor groove only, as indicated by experimental findings. As shown in the Figure 8a, compound **12b** intercalated efficiently into the DNA, forming an average 3-4 Pi-Pi stackings with the aromatic central part and one H-bond interaction of the oxime moiety that is stable over 80% of the simulation time.

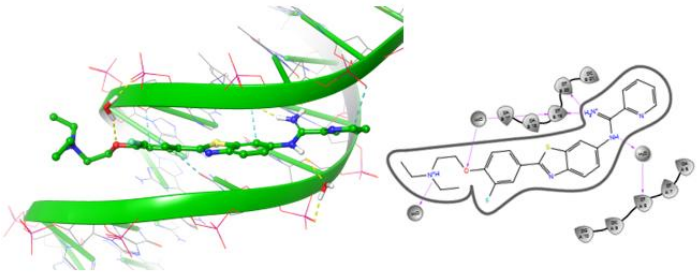
a) Compound 12b_1G3X_partial intercalation



b) Compound 12b_2B0K_partial minor groove binding



c) Compound 29b_2B0K_partial intercalation



d) Interaction distribution

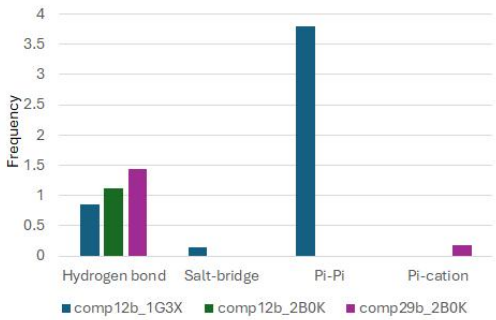


Figure 8. Summary of possible binding poses for: a) Compound **12b** with intercalation binding mode, b) Compound **12b** bound to minor groove, c) Compound **29b** bound to the minor groove, d) Distribution of interactions throughout the MD simulations.

Basic dimethyl amino groups form H-bonds with adjacent phosphate groups in docking, however this H-bond is lost throughout the simulation since solvatation is energetically more favourable. Binding of compound **12b** in the minor groove is shown in Figure 8b. Partial minor groove binding suggests a sub-optimal binding mode since compound **12b** lacks an appropriate radius of curvature to match the groove shape. On average, only 1 hydrogen bond remains stable throughout the MD simulation as shown in Figure 8d. Compound **29b** binds nicely to the minor groove in agreement with experimental findings (Figure 8c). It forms close to 1.5 hydrogen bonds throughout the MD simulations with lower contribution of Pi-cation interactions.

2.4. Evaluation of *in vivo* activity

Benzothiazole amidoxime **12b** that exhibited selective antitrypanosomal activity (IC_{50} = 0.92 μM, SI = 18), moderate metabolic stability (47% LBF) and membrane permeability (P_{app}(AB) = 5.0 x 10^{-6} cm/s) was selected for *in vivo* evaluation. BALB/c mice were infected with bioluminescent bloodstream form *T. brucei* strain GVR35-VSL2 [51] and treated with **12b** by the oral route twice daily for 4 days (50 mg/kg) (Materials and methods). There was no significant reduction in the parasite burden (Figure S83). P-glycoprotein activity in the intestinal epithelium, leading to excretion of **12b** back into the gut lumen, may be sufficient to keep the free blood concentration below the trypanocidal threshold. Future work will be aimed at designing **12b** analogues that do not act as substrates for this transporter.

3. Conclusions

A series of 6-carboximidamide **12a**–**14a**, **12b**–**15b**, **12c**, **13c** and **16c**, and 6-picolinimidamide benzothiazoles **31a**–**35a**, **31b**–**35b** and **31c**–**35c** with aromatic and aliphatic residues at the phenoxymethylene were synthesised and evaluated for antitrypanosomal activity.

The substituent at the C-3 and C-4 positions of the phenoxymethylene linker as well as at the C-6 of the benzothiazole core were found to influence activity such that the aliphatic moiety improved antitrypanosomal potency. Benzothiazoles **12b** and **29b,** containing both diethylaminoethyl and fluorine substituents on the phenoxymethylene linker, had the best antitrypanosomal activity (**12b**: IC_{50} = 0.92 μM; **29b**: IC_{50} = 0.88 μM). Amidoxime **12b** was more selective than arylimidamide **29b**, with a selectivity index value of 18. All benzimidazole arylimidamides were found to have low membrane

permeability, except for methoxy-substituted benzothiazole **31c**, while benzothiazole amidoximes displayed moderate to high permeability.

As regards mode of action, CD spectroscopy, thermal melting analysis and binding constants suggest that benzothiazole amidoximes **12a** and **12b** bind to DNA/RNA by intercalation, while benzothiazole arylimidamide **29b** demonstrates groove binding mode to DNA/RNA.

The ADME properties of the novel benzothiazole amidoximes were successfully modulated and **12b** showed favourable membrane permeability and metabolic stability. Enhanced uptake by the parasite compared to cultured human cells may explain the selective potency. However, treatment of infected mice did not result in curative outcomes. Therefore, further structural optimization is required to improve trypanocidal activity and selectivity without compromising the favourable ADME properties.

4. Materials and methods

4.1. General

For monitoring the progress of a reaction and for comparison purposes, thin layer chromatography (TLC) was performed on precoated Merck silica gel 60F-254 plates (Merck. Kenilworth. NJ. USA) using an appropriate solvent system, and the spots were detected under ultraviolet (UV) light (254 nm). For column chromatography 0.063-0.2 mm silica gel (Fluka. Seelze. Germany) was employed, and glass columns were slurry-packed under gravity. Elemental analyses for carbon, hydrogen, and nitrogen were performed on a Perkin-Elmer 2400 elemental analyser. Analyses are indicated as symbols of elements, and the analytical results obtained are within 0.4% of the theoretical value. Nuclear magnetic resonance (NMR) spectroscopic data for ^{1}H and ^{13}C nuclei were recorded at room temperature on a Bruker Avance spectrometer (Bruker, Billerca, MA, USA) 300 MHz and 600 MHz. All data were recorded in DMSO-d_6 at 298 K. Chemical shifts were referenced to the residual solvent signal of DMSO at δ 2.50 ppm for ^{1}H and δ 39.50 ppm for ^{13}C. Individual resonances were assigned based on their chemical shifts, signal intensities, multiplicity of resonances and H–H coupling constants. Melting points were recorded using Thermovar HT1BT1 (Reichert, Wien). All solvents and chemicals were purchased from commercial suppliers Aldrich (St. Louis, MO, USA) and Acros (Geel, Belgium).

4.2. Experimental

Compounds 4-(2-(diethylamino)ethoxy)benzaldehyde **2a** [21], 4-(2-(diethiylamino)ethoxy)-3-fluorobenzaldehyde **2b** [21], 4-(2-(dietihylamino)ethoxy)-3-methoxybenzaldehyde **2c** [21], 4-(2-morpholinoethoxy)benzaldehyde **3a** [21], 3-fluoro-4-(2-morpholinoethoxy)benzaldehyde **3b** [21], 3-

methoxy-4-(2-morpholinoethoxy)benzaldehyde **3c** [21], 4-(2-morpholino-2-oxoethoxy)benzaldehyde **4a** [21], 3-fluoro-4-(2-morpholino-2-oxoethoxy)benzaldehyde **4b** [21], 3-methoxy-4-(2-morpholino-2-oxoethoxy)benzaldehyde **4c** [21], 4-(2-oxo-2-phenylethoxy)benzaldehyde **5a** [21], 3-fluoro-4-(2-oxo-2-phenylethoxy)benzaldehyde **5b** [21], 3-methoxy-4-(2-oxo-2-phenylethoxy)benzaldehyde **5c** [21], 4-(piridin-2-yl-methoxy)benzaldehyde **6a** [21], 3-fluoro-4-(piridin-2-yl-methoxy)benzaldehyde **6b** [21], 3-methoxy-4-(piridin-2-yl-methoxy)benzaldehyde **6c** [21], bis(2-amino-5-cyanophenyl) disulfide **1** [38] and bis(2-amino-5-nitrophenyl) disulfide**17** [38], and S-(2-naphthylmethyl)-2-pyridylthioimidate hydrobromide **28** [39] were synthesized in accordance with procedures given in the literature.

4.2.1. General procedure for synthesis of cyano- (7a–11a, 7b–11b, 7c–11c) and nitro- (18a–22a, 18b–22b, 18c–22c) substituted benzothiazole derivatives

To a stirred suspension of the disulfide **1** (1 eq) or **17** (1eq) in glycerol (1.5–2.0 g) corresponding benzaldehyde **2a–6a, 2b–6b, 2c–6c** (1.75 eq) was added and heated at 160°C for 30 – 60 min. The reaction mixture was cooled below 100 °C, quenched with 75% ethanol and cooled in the refrigerator overnight. The resulting precipitate was collected by filtration, washed with diluted ethanol and air-dried giving target compounds.

2-(4-(2-(Diethylamino)ethoxy)phenyl)benzothiazole-6-carbonitrile (7a)

According to the above-mentioned general procedure from disulfide **1** (150 mg, 0.50 mmol) and benzaldehyde **2a** (194.7 mg, 0.88 mmol) compound **7a** was obtained as a pale yellow powder (225.1 mg, 72.7 %; m.p. 137–139 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 8.72 (1H, d, J = 1.2 Hz), 8.14 (1H, d, J = 8.5 Hz), 8.08 (2H, d, J = 8.8 Hz), 7.91 (1H, dd, J = 8.5, 1.6 Hz), 7.14 (2H, d, J = 8.8 Hz), 4.14 (2H, t, J = 5.9 Hz, OCH_2), 2.83 (2H, bs, NCH_2), 2.58 (4H, dd, J = 13.6, 6.7 Hz, NCH_2), 0.99 (6H, t, J = 7.1 Hz, CH_3). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 171.63, 161.79, 156.12, 134.84, 129.81, 129.42, 127.46, 124.68, 123.14, 118.85 (CN), 115.37, 107.00, 66.70 (OCH_2), 51.13 (NCH_2), 46.96 (NCH_2), 11.76 (CH_3).

2-(4-(2-(Diethylamino)ethoxy)-3-fluorophenyl)benzothiazole-6-carbonitrile (7b)

According to the above-mentioned general procedure from disulfide **1** (150 mg, 0.50 mmol) and benzaldehyde **2b** (210.6 mg, 0.88 mmol) compound **7b** was obtained as a yellow powder (107.3 mg, 33.0 %; m.p. 104–106 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 8.71 (1H, d, J = 1.2 Hz), 8.13 (1H, d, J = 8.5 Hz), 7.95 – 7.83 (3H, m), 7.35 (1H, t, J = 8.8 Hz), 4.19 (2H, t, J = 5.9 Hz, OCH_2), 2.84 (2H, t, J = 5.9 Hz, NCH_2), 2.57 (4H, q, J = 7.1 Hz, NCH_2), 0.98 (6H, t, J = 7.1 Hz, CH_3). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 170.41 (d, J_{CF} = 2.7 Hz), 155.82, 151.55 (d, J_{CF} = 245.9 Hz), 149.77 (d, J_{CF} = 10.6 Hz), 134.99, 129.81, 127.53, 124.89, 124.82 (d, J_{CF} = 3.5 Hz), 123.31, 118.75 (CN), 115.16 (d, J_{CF} = 1.6 Hz), 114.57 (d, J_{CF} = 20.2 Hz), 107.28, 67.75 (OCH_2), 50.97 (NCH_2), 47.00 (NCH_2), 11.76 (CH_3).

2-(4-(2-(Diethylamino)ethoxy)-3-methoxyphenyl)benzothiazole-6-carbonitrile (7c)

According to the above-mentioned general procedure from disulfide **1** (150 mg, 0.50 mmol) and benzaldehyde **2c** (221.2 mg, 0.88 mmol) compound **7c** was obtained as a brown powder (192.6 mg, 57.3 %; m.p. 112–115 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 8.71 (1H, d, *J* = 1.3 Hz), 8.15 (1H, d, *J* = 8.5 Hz), 7.91 (1H, dd, *J* = 8.5, 1.6 Hz), 7.71 – 7.61 (2H, m), 7.17 (1H, d, *J* = 9.0 Hz), 4.13 (2H, t, *J* = 6.0 Hz, OCH_2), 3.90 (3H, s, OCH_3), 2.87 (2H, s, NCH_2), 2.62 (4H, d, *J* = 6.5 Hz, NCH_2), 1.01 (6H, t, *J* = 7.1 Hz, CH_3). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 171.75, 156.03, 151.59, 149.26, 134.89, 129.77, 127.39, 124.80, 123.13, 121.61, 118.83 (CN), 112.96, 109.85, 107.02, 67.03 (OCH_2), 55.73 (OCH_3), 51.05 (NCH_2), 47.03 (NCH_2), 11.62 (CH_3).

2-(4-(2-Morpholinoethoxy)phenyl)benzothiazole-6-carbonitrile (8a)

According to the above-mentioned general procedure from disulfide **1** (150 mg, 0.50 mmol) and benzaldehyde **3a** (207.0 mg, 0.88 mmol) compound **8a** was obtained as a white powder (205.7 mg, 63.9 %; m.p. 159–164 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 8.72 (1H, d, *J* = 0.9 Hz), 8.14 (1H, d, *J* = 8.5 Hz), 8.08 (2H, d, *J* = 8.7 Hz), 7.92 (1H, dd, *J* = 8.5, 1.4 Hz), 7.15 (2H, d, *J* = 8.8 Hz), 4.21 (2H, t, *J* = 5.6 Hz, OCH_2), 3.59 (4H, t, *J* = 4.4 Hz, OCH_2), 2.73 (2H, t, *J* = 5.6 Hz, NCH_2). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 171.62, 161.72, 156.12, 134.85, 129.81, 129.40, 127.47, 124.74, 123.16, 118.85 (CN), 115.41, 107.01, 66.14 (OCH_2), 65.74 (OCH_2), 56.80 (NCH_2), 53.57(NCH_2).

2-(3-Fluoro-4-(2-morpholinoethoxy)phenyl)benzothiazole-6-carbonitrile (8b)

According to the above-mentioned general procedure from disulfide **1** (150 mg, 0.50 mmol) and benzaldehyde **3b** (222.9 mg, 0.88 mmol) compound **8b** was obtained as a pale yellow powder (211.3 mg, 62.6 %; m.p. 171–173 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 8.73 (1H, d, *J* = 1.1 Hz), 8.15 (1H, d, *J* = 8.5 Hz), 7.99 – 7.85 (3H, m), 7.39 (1H, t, *J* = 8.6 Hz), 4.28 (2H, t, *J* = 5.6 Hz, OCH_2), 3.58 (4H, t, *J* = 4.6 Hz, OCH_2), 2.76 (2H, t, *J* = 5.6 Hz, NCH_2). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 170.42 (d, J_{CF} = 2.7 Hz), 155.83, 151.57 (d, J_{CF} = 246.0 Hz), 149.69 (d, J_{CF} = 10.5 Hz), 135.02, 129.85, 127.58, 125.03, 124.90 (d, J_{CF} = 3.0 Hz), 123.35, 118.76 (CN), 115.33 (d, J_{CF} = 1.4 Hz), 114.64 (d, J_{CF} = 20.3 Hz), 107.32, 66.90 (OCH_2), 66.14 (OCH_2), 56.66 (NCH_2), 53.54 (NCH_2).

2-(3-Methoxy-4-(2-morpholinoethoxy)phenyl)benzothiazole-6-carbonitrile (8c)

According to the above-mentioned general procedure from disulfide **1** (150 mg, 0.50 mmol) and benzaldehyde **3c** (233.5 mg, 0.88 mmol) compound **8c** was obtained as a pale yellow powder (258.3mg, 74.2 %; m.p. 170–172 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 8.73 (1H, d, *J* = 1.2 Hz), 8.17 (1H, d, *J* = 8.5 Hz), 7.92 (1H, dd, *J* = 8.5, 1.6 Hz), 7.73 – 7.59 (2H, m), 7.19 (1H, d, *J* = 9.0 Hz), 4.20 (2H, t, *J* = 5.8 Hz, OCH_2), 3.90 (3H, s, OCH_3), 3.58 (4H, t, *J* = 4.6 Hz, OCH_2), 2.74 (2H, t, *J* = 5.8 Hz, NCH_2). ^{13}C NMR (151 MHz, DMSO) (δ/ppm) 171.76, 156.04, 151.58, 149.27, 134.91, 129.79, 127.42, 124.86, 123.15, 121.61, 118.84 (CN), 113.10, 109.87, 107.04, 66.35 (OCH_2), 66.15 (OCH_2), 56.80 (NCH_2), 55.73 (OCH_3), 53.63 (NCH_2).

2-(4-(2-Morpholino-2-oxoethoxy)phenyl)benzothiazole-6-carbonitrile (9a)

According to the above-mentioned general procedure from disulfide **1** (150 mg, 0.50 mmol) and benzaldehyde **4a** (219.3 mg, 0.88 mmol) compound **9a** was obtained as a pale yellow powder (197.7 mg, 59.2 %; m.p. 54–58 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 8.72 (1H, d, *J* = 1.2 Hz), 8.15 (1H, d, *J* = 8.5 Hz), 8.07 (2H, d, *J* = 8.8 Hz), 7.91 (1H, dd, *J* = 8.5, 1.6 Hz), 7.13 (2H, d, *J* = 8.9 Hz), 5.00 (2H, s, OCH_2), 3.69 – 3.54 (4H, m, NCH_2), 3.48 (4H, bs, OCH_2). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 171.59, 165.50 (C=O), 161.36, 156.11, 134.88, 129.79, 129.25, 127.46, 125.01, 123.17, 118.84 (CN), 115.55, 107.03, 66.00 (OCH_2), 65.96 (OCH_2), 65.69 (OCH_2), 44.60 (NCH_2), 41.58(NCH_2).

2-(3-Fluoro-4-(2-morpholino-2-oxoethoxy)phenyl)benzothiazole-6-carbonitrile (9b)

According to the above-mentioned general procedure from disulfide **1** (150 mg, 0.50 mmol) and benzaldehyde **4b** (235.2 mg, 0.88 mmol) compound **9b** was obtained as a white powder (130.2 mg, 37.2 %; m.p. 193–194 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 8.74 (1H, d, *J* = 1.2 Hz), 8.16 (1H, d, *J* = 8.5 Hz), 7.97 (1H, dd, *J* = 12.0, 2.1 Hz), 7.95 – 7.86 (2H, m), 7.27 (1H, t, *J* = 8.7 Hz), 5.13 (2H, s, OCH_2), 3.71 – 3.53 (4H, m, NCH_2), 3.47 (4H, bs, OCH_2). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 170.45 (d, J_{CF} = 2.6 Hz), 165.10 (C=O), 155.85, 151.47 (d, J_{CF} = 246.1 Hz), 149.39 (d, J_{CF} = 10.4 Hz), 135.06, 129.87, 127.61, 125.22 (d, J_{CF} = 7.1 Hz), 124.61 (d, J_{CF} = 3.0 Hz), 123.39, 118.77 (CN), 115.62 (d, J_{CF} = 1.4 Hz), 114.75 (d, J_{CF} = 20.3 Hz), 107.33, 66.06 (OCH_2), 66.00 (OCH_2), 65.90 (OCH_2), 44.52 (NCH_2), 41.59 (NCH_2).

2-(3-Methoxy-4-(2-morpholino-2-oxoethoxy)phenyl)benzothiazole-6-carbonitrile (9c)

According to the above-mentioned general procedure from disulfide **1** (150 mg, 0.50 mmol) and benzaldehyde **4c** (245.8 mg, 0.88 mmol) compound **9c** was obtained as a yellow powder (223.7 mg, 62.0 %; m.p. 197–201 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 8.72 (1H, d, *J* = 1.2 Hz), 8.16 (1H, d, *J* = 8.5 Hz), 7.91 (1H, dd, *J* = 8.5, 1.6 Hz), 7.73 – 7.60 (2H, m), 7.05 (1H, d, *J* = 8.4 Hz), 4.99 (2H, s, OCH_2), 3.92 (3H, s, OCH_3), 3.68 – 3.55 (4H, m, NCH_2), 3.48 (4H, bs, OCH_2). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 171.72, 165.47 (C=O), 156.03, 151.06, 149.19, 134.94, 129.79, 127.43, 125.18, 123.18, 121.33, 118.84 (CN), 113.43, 110.01, 107.06, 66.14 (OCH_2), 66.01 (OCH_2), 55.77 (OCH_3), 44.74 (NCH_2), 41.63 (NCH_2).

2-(4-(2-Oxo-2-phenylethoxy)phenyl)benzothiazole-6-carbonitrile (10a)

According to the above-mentioned general procedure from disulfide **1** (150 mg, 0.50 mmol) and benzaldehyde **5a** (211.4 mg, 0.88 mmol) compound **10a** was obtained as a white powder (170.5 mg, 52.3 %; m.p. 193–196 °C). 1H NMR (300 MHz, DMSO) (δ/ppm) 8.73 (1H, d, *J* = 1.2 Hz), 8.16 (1H, d, *J* = 8.5 Hz), 8.07 (4H, t, *J* = 9.0 Hz), 7.92 (1H, dd, *J* = 8.5, 1.6 Hz), 7.72 (1H, t, *J* = 7.4 Hz), 7.60 (2H, t, *J* = 7.5 Hz), 7.19 (2H, d, *J* = 8.9 Hz), 5.77 (2H, s, OCH_2). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 193.95 (C=O), 171.58, 161.25, 156.11, 134.89, 134.20, 133.90, 129.81, 129.33, 128.84, 127.87, 127.50, 125.08, 123.19, 118.85 (CN), 115.60, 107.04, 70.31 (OCH_2).

2-(3-Fluoro-4-(2-oxo-2-phenylethoxy)phenyl)benzothiazole-6-carbonitrile (10b)

According to the above-mentioned general procedure from disulfide **1** (150 mg, 0.50 mmol) and benzaldehyde **5b** (227.3 mg, 0.88 mmol) compound **10b** was obtained as a yellow powder (114.4 mg, 33.4 %; m.p. 204–205 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 8.74 (1H, d, *J* = 1.1 Hz), 8.17 (1H, d, *J* = 8.5 Hz), 8.09 – 7.96 (3H, m), 7.93 (1H, dd, *J* = 8.5, 1.5 Hz), 7.87 (1H, d, *J* = 8.7 Hz), 7.73 (1H, t, *J* = 7.4 Hz), 7.60 (2H, t, *J* = 7.5 Hz), 7.34 (1H, t, *J* = 8.7 Hz), 5.88 (2H, s, OCH_2). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 193.52 (C=O), 170.41 (d, J_{CF} = 2.9 Hz), 155.85, 151.47 (d, J_{CF} = 246.1 Hz), 149.23 (d, J_{CF} = 10.4 Hz), 135.07, 134.05, 133.98, 125.35 (d, J_{CF} = 6.8 Hz), 124.66 (d, J_{CF} = 3.0 Hz), 123.40, 118.77 (CN), 115.66, 114.89 (d, J_{CF} = 20.3 Hz), 107.30, 70.85 (OCH_2).

2-(3-Methoxy-4-(2-oxo-2-phenylethoxy)phenyl)benzothiazole-6-carbonitrile (10c)

According to the above-mentioned general procedure from disulfide **1** (150 mg, 0.50 mmol) and benzaldehyde **5c** (237.8 mg, 0.88 mmol) compound **10c** was obtained as a pale yellow powder (115.6 mg, 32.8 %; m.p. > 250 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 8.71 (1H, d, *J* = 1.2 Hz), 8.16 (1H, d, *J* = 8.5 Hz), 8.05 (2H, d, *J* = 7.2 Hz), 7.91 (1H, dd, *J* = 8.5, 1.6 Hz), 7.76 – 7.66 (2H, m), 7.66 – 7.55 (3H, m), 7.09 (1H, d, *J* = 8.5 Hz), 5.75 (2H, s, OCH_2), 3.94 (3H, s, OCH_3). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 193.93 (C=O), 171.71, 156.03, 150.97, 149.18, 134.94, 134.22, 133.87, 129.79, 128.83, 127.88, 127.43, 125.20, 123.19, 121.34, 118.84 (CN), 113.44, 110.12, 107.06, 70.57 (OCH2), 55.77 (OCH_3).

2-(4-(Pyridin-2-ylmethoxy)phenyl)benzothiazole-6-carbonitrile (11a)

According to the above-mentioned general procedure disulfide **1** (150 mg, 0.50 mmol) and benzaldehyde **6a** (187.6 mg, 0.88 mmol) compound **11a** was obtained as a pale yellow powder (173.1 mg, 57.2 %; m.p. 243–246 °C). ^{1}H NMR (600 MHz, DMSO) (δ/ppm) 8.67 (1H, d, *J* = 1.5 Hz), 8.58 (1H, d, *J* = 4.8 Hz), 8.13 (1H, d, *J* = 8.5 Hz), 8.08 (2H, d, *J* = 8.8 Hz), 7.87 (1H, dd, *J* = 8.4, 1.6 Hz), 7.83 (1H, td, *J* = 7.7, 1.8 Hz), 7.53 (1H, d, *J* = 7.8 Hz), 7.34 (1H, dd, *J* = 6.9, 4.9 Hz), 7.24 (2H, d, *J* = 8.9 Hz), 5.30 (2H, s). ^{13}C NMR (151 MHz, DMSO) (δ/ppm) 171.56, 161.36, 156.11, 156.04, 149.19, 137.06, 134.90, 129.83, 129.48, 127.52, 125.14, 123.21, 123.12, 121.80, 118.84 (CN), 115.74, 107.07, 70.53 (OCH_2).

2-(3-Fluoro-4-(pyridin-2-ylmethoxy)phenyl)benzothiazole-6-carbonitrile (11b)

According to the above-mentioned general procedure from disulfide **1** (150 mg, 0.50 mmol) and benzaldehyde **6b** (203.5 mg, 0.88 mmol) compound **11b** was obtained as a pale yellow powder (244.0 mg, 45.4 %; m.p. 222–225 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 8.77 (1H, d, *J* = 1.2 Hz), 8.62 (1H, d, *J* = 4.2 Hz), 8.18 (1H, d, *J* = 8.4 Hz), 8.03 (1H, dd, *J* = 11.9, 2.1 Hz), 7.94 (2H, dd, *J* = 8.5, 1.6 Hz), 7.89 (1H, td, *J* = 7.7, 1.7 Hz), 7.57 (1H, d, *J* = 7.8 Hz), 7.47 (1H, t, *J* = 8.6 Hz), 7.39 (1H, dd, *J* = 6.7, 5.0 Hz), 5.40 (2H, s, OCH_2).^{13}C NMR (75 MHz, DMSO) (δ/ppm) 151.03 (d, J_{CF} = 245.1 Hz), 149.26, 137.49, 137.17, 135.09, 129.96, 129.92, 127.68, 124.97 (d, J_{CF} = 3.9 Hz)., 123.44, 123.30, 121.90, 118.88 (CN), 115.78, 114.99, 107.39, 71.26 (OCH_2).

2-(3-Methoxy-4-(pyridin-2-ylmethoxy)phenyl)benzothiazole-6-carbonitrile (11c)

According to the above-mentioned method general procedure from disulfide **1** (150 mg, 0.50 mmol) and benzaldehyde **6c** (214.1 mg, 0.88 mmol) compound **11c** was obtained as a pale yellow powder (123.7 mg, 37.6 %; m.p. 210–212 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 8.74 (1H, d, *J* = 1.1 Hz), 8.61 (1H, d, *J* = 4.1 Hz), 8.18 (1H, d, *J* = 8.5 Hz), 7.93 (1H, dd, *J* = 8.5, 1.6 Hz), 7.87 (1H, td, *J* = 7.8, 1.7 Hz), 7.72 (1H, d, *J* = 2.0 Hz), 7.68 (1H, dd, *J* = 8.4, 2.0 Hz), 7.55 (1H, d, *J* = 7.8 Hz), 7.38 (1H, dd, *J* = 6.6, 5.0 Hz), 7.24 (1H, d, *J* = 8.5 Hz), 5.30 (2H, s, OCH_2), 3.94 (3H, s, OCH_3). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 171.72, 156.12, 156.03, 151.09, 149.40, 149.16, 137.07, 134.96, 129.82, 127.49, 125.27, 123.22, 123.12, 121.80, 121.56, 118.84 (CN), 113.51, 109.93, 107.10, 70.90 (OCH_2), 55.77 (OCH_3).

2-(4-(2-(Diethylamino)ethoxy)phenyl)-6-nitrobenzothiazole (18a)

According to the above-mentioned general procedure from disulfide **17** (85 mg, 0.25 mmol) and benzaldehyde **2a** (97.4 mg, 0.44 mmol) compound **18a** was obtained as a pale orange powder (144.3 mg, 88.3 %; m.p. 133–135 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 9.17 (1H, d, *J* = 2.3 Hz), 8.33 (1H, dd, *J* = 9.0, 2.4 Hz), 8.16 (1H, d, *J* = 9.0 Hz), 8.09 (2H, d, *J* = 8.8 Hz), 7.14 (2H, d, *J* = 8.8 Hz), 4.15 (2H, t, *J* = 6.0 Hz, OCH_2), 2.84 (2H, t, *J* = 5.7 Hz, NCH_2), 2.59 (4H, q, *J* = 7.1 Hz, NCH_2), 1.00 (6H, t, *J* = 7.1 Hz, CH_3). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 173.43, 161.91, 157.57, 144.06, 134.89, 129.50, 124.75, 122.61, 121.89, 119.29, 115.40, 66.68 (OCH_2), 51.12 (NCH_2), 46.97 (NCH_2), 11.72 (CH_3).

2-(4-(2-(Diethylamino)ethoxy)-3-fluorophenyl)-6-nitrobenzothiazole (18b)

According to the above-mentioned general procedure from disulfide **17** (85 mg, 0.25 mmol) and benzaldehyde **2b** (105.3 mg, 0.44 mmol) compound **18b** was obtained as an orange powder (149.7 mg, 87.4 %; m.p. 99–102 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 9.20 (1H, d, *J* = 2.2 Hz), 8.34 (1H, dd, *J* = 9.0, 2.3 Hz), 8.18 (1H, d, *J* = 9.0 Hz), 8.05 – 7.91 (2H, m), 7.43 (1H, t, *J* = 8.6 Hz), 4.43 (2H, s, OCH_2), 3.34 (2H, s, NCH_2), 3.02 (4H, d, *J* = 6.8 Hz, NCH_2), 1.17 (6H, t, *J* = 7.0 Hz, CH_3). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 172.10 (d, J_{CF} = 2.6 Hz), 157.22, 151.56 (d, J_{CF} = 246.5 Hz), 149.25 (d, J_{CF} = 10.7 Hz), 144.29, 135.12, 125.50 (d, J_{CF} = 6.9 Hz), 125.05 (d, *J* = 3.1 Hz), 122.92, 121.98, 119.45, 115.44 (d, J_{CF} = 1.2 Hz), 114.77 (d, J_{CF} = 20.1 Hz), 65.42 (OCH_2), 50.33 (NCH_2), 47.41 (NCH_2), 9.84 (CH_3).

2-(4-(2-(Diethylamino)ethoxy)-3-methoxyphenyl)-6-nitrobenzothiazole (18c)

According to the above-mentioned general procedure from disulfide **17** (85 mg, 0.25 mmol) and benzaldehyde **2c** (110.6 mg, 0.44 mmol) compound **18c** was obtained as an orange powder (162.0 mg, 97.7 %; m.p. 92–94 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 9.14 (1H, d, *J* = 2.3 Hz), 8.32 (1H, dd, *J* = 9.0, 2.4 Hz), 8.16 (1H, d, *J* = 9.0 Hz), 7.67 (2H, dd, *J* = 10.3, 2.1 Hz), 7.17 (1H, d, *J* = 8.2 Hz), 4.14 (2H, t, *J* = 6.0 Hz, OCH_2), 3.90 (3H, s, OCH_3), 2.89 (2H, t, *J* = 6.2 Hz, NCH_2), 2.63 (4H, dd, *J* = 14.4, 7.2 Hz, NCH_2), 1.02 (6H, t, *J* = 7.1 Hz, CH_3). ^{13}C NMR (151 MHz, DMSO) (δ/ppm) 173.71, 157.66, 151.90, 149.44, 144.23, 135.12, 125.05, 122.78, 122.04, 121.92, 119.39, 113.15, 110.04, 67.18 (OCH_2), 55.92 (OCH_3), 51.20 (NCH_2), 47.23 (NCH_2), 11.76 (CH_3).

2-(4-(2-Morpholinoethoxy)phenyl)-6-nitrobenzothiazole (19a)

According to the above-mentioned general procedure from disulfide **17** (85 mg, 0.25 mmol) and benzaldehyde **3a** (103.5 mg, 0.44 mmol) compound **19a** was obtained as an orange powder (159.7 mg, 94.2 %; m.p. 147–150 °C). ^{1}H NMR (600 MHz, DMSO) (δ/ppm) 9.10 (1H, d, *J* = 2.3 Hz), 8.30 (1H, dd, *J* = 8.9, 2.3 Hz), 8.13 (1H, d, *J* = 8.9 Hz), 8.06 (2H, d, *J* = 8.8 Hz), 7.13 (2H, d, *J* = 8.8 Hz), 4.20 (2H, t, *J* = 5.6 Hz, OCH_2), 3.57 (4H, t, *J* = 4.8 Hz, OCH_2), 2.72 (2H, t, *J* = 5.7 Hz, NCH_2). ^{13}C NMR (151 MHz, DMSO) (δ/ppm) 173.44, 161.86, 157.58, 144.09, 134.91, 129.51, 124.85, 122.64, 121.93, 119.34, 115.46, 66.13 (OCH_2), 65.76 (OCH_2), 56.80 (NCH_2), 53.56 (NCH_2).

2-(3-Fluoro-4-(2-morpholinoethoxy)phenyl)-6-nitrobenzothiazole (19b)

According to the above-mentioned general procedure from disulfide **17** (85 mg, 0.25 mmol) and benzaldehyde **3b** (111.4 mg, 0.44 mmol) compound **19b** was obtained as a yellow powder (165.3 mg, 83.2 %; m.p. 145–148 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 9.15 (1H, d, *J* = 2.2 Hz), 8.31 (1H, dd, *J* = 9.0, 2.3 Hz), 8.14 (1H, d, *J* = 9.0 Hz), 7.92 (2H, dd, *J* = 14.5, 4.6 Hz), 7.37 (1H, t, *J* = 8.8 Hz), 4.28 (2H, t, *J* = 5.6 Hz, OCH_2), 3.59 (4H, t, *J* = 4.6 Hz, OCH_2), 2.76 (2H, t, *J* = 5.6 Hz, NCH_2). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 172.15 (d, J_{CF} = 2.9 Hz), 157.22, 151.54 (d, J_{CF} = 246.1 Hz), 149.80 (d, J_{CF} = 10.7 Hz), 144.20, 135.05, 125.06 (d, J_{CF} = 3.2 Hz), 124.98 (d, J_{CF} = 2.7 Hz), 122.83, 121.90, 119.35, 115.28 (d, J_{CF} = 1.6 Hz), 114.63 (d, J_{CF} = 20.3 Hz), 66.91 (OCH_2), 66.14 (OCH_2), 56.65 (NCH_2), 53.54 (NCH_2).

2-(3-Methoxy-4-(2-morpholinoethoxy)phenyl)-6-nitrobenzothiazole (19c)

According to the above-mentioned general procedure from disulfide **17** (85 mg, 0.25 mmol) and benzaldehyde **3c** (116.7 mg, 0.44 mmol) compound **19c** was obtained as an orange powder (143.1 mg, 78.3 %; m.p. 207–210 °C). ^{1}H NMR (600 MHz, DMSO) (δ/ppm) 9.06 (1H, d, *J* = 2.4 Hz), 8.30 (1H, dd, *J* = 8.9, 2.4 Hz), 8.14 (1H, d, *J* = 8.9 Hz), 7.68 (2H, dd, *J* = 7.2, 1.9 Hz), 7.18 (1H, d, *J* = 9.0 Hz), 4.21 (2H, t, *J* = 5.8 Hz, OCH_2), 3.90 (3H, s, OCH_3), 3.58 (4H, t, *J* = 4.7 Hz, OCH_2), 2.75 (2H, t, *J* = 5.8 Hz, NCH_2), 2.50 (4H, t, *J* = 4.7 Hz, NCH2). ^{13}C NMR (151 MHz, DMSO) (δ/ppm) 173.44, 157.55, 152.17, 149.86, 144.42, 135.10, 125.42, 122.68, 121.83, 121.76, 118.91, 114.16, 111.35, 67.05(OCH2), 66.26 (OCH_2), 56.91 (NCH_2), 56.27 (OCH_3), 53.63 (NCH_2).

2-(4-(2-Morpholino-2-oxoethoxy)phenyl)-6-nitrobenzothiazole (20a)

According to the above-mentioned general procedure from disulfide **17** (85 mg, 0.25 mmol) and benzaldehyde **4a** (109.7 mg, 0.44 mmol) compound **20a** was obtained as a yellow powder (148.7 mg, 84.6 %; m.p. 203–205 °C). 1H NMR (300 MHz, DMSO) (δ/ppm) 9.15 (1H, d, *J* = 2.1 Hz), 8.32 (1H, dd, *J* = 9.0, 2.2 Hz), 8.15 (1H, d, *J* = 9.0 Hz), 8.07 (2H, d, *J* = 8.7 Hz), 7.13 (2H, d, *J* = 8.7 Hz), 5.01 (2H, s, OCH_2), 3.64 (2H, bs, NCH_2), 3.59 (2H, bs, NCH_2), 3.48 (4H, bs, OCH_2). 13C NMR (151 MHz, DMSO) (δ/ppm) 173.41, 165.49 (C=O), 161.51, 157.56, 144.09, 134.94, 129.35, 125.08, 122.66, 121.91, 119.33, 115.60, 66.02 (OCH_2), 65.95 (OCH_2), 65.69 (OCH_2), 44.60 (NCH_2), 41.59 (NCH_2).

2-(3-Fluoro-4-(2-morpholino-2-oxoethoxy)phenyl)-6-nitrobenzothiazole (20b)

According to the above-mentioned general procedure from disulfide **17** (85 mg, 0.25 mmol) and benzaldehyde **4b** (117.6 mg, 0.44 mmol) compound **20b** was obtained as a yellow powder (125.1 mg, 68.2 %; m.p. 206–208 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 9.18 (1H, d, *J* = 2.3 Hz), 8.33 (1H, dd, *J* = 9.0, 2.3 Hz), 8.17 (1H, d, *J* = 9.0 Hz), 7.98 (1H, dd, *J* = 11.9, 2.0 Hz), 7.90 (1H, d, *J* = 8.7 Hz), 7.27 (1H, t, *J* = 8.6 Hz), 5.13 (2H, s, OCH_2), 3.65 (2H, bs, NCH_2), 3.59 (2H, d, *J* = 6.0 Hz, NCH_2), 3.47 (4H, bs, OCH_2). ^{13}C NMR (151 MHz, DMSO) (δ/ppm) 172.20 (d, J_{CF} = 2.4 Hz), 165.09 (C=O), 157.26, 151.47 (d, J_{CF} = 246.1 Hz), 149.53 (d, J_{CF} = 10.5 Hz), 144.26, 135.12, 125.26 (d, J_{CF} = 6.9 Hz), 124.71 (d, J_{CF} = 2.8 Hz), 122.89, 121.94, 119.41, 115.63, 114.77 (d, J_{CF} = 20.2 Hz), 66.07 (OCH_2), 66.01 (OCH_2), 65.90 (OCH_2), 44.52 (NCH_2), 41.60 (NCH_2).

2-(3-Methoxy-4-(2-morpholino-2-oxoethoxy)phenyl)-6-nitrobenzothiazole (20c)

According to the above-mentioned general procedure from disulfide **17** (85 mg, 0.25 mmol) and benzaldehyde **4c** (122.9 mg, 0.44 mmol) compound **20c** was obtained as a yellow powder (118.6 mg, 62.8 %; m.p. 214–216 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 9.17 (1H, d, *J* = 2.3 Hz), 8.33 (1H, dd, *J* = 9.0, 2.4 Hz), 8.18 (1H, d, *J* = 9.0 Hz), 7.74 – 7.62 (2H, m), 7.06 (1H, d, *J* = 8.2 Hz), 5.00 (2H, s, OCH_2), 3.93 (3H ,s, OCH_3), 3.58 (2H, bs, NCH_2), 3.48 (2H, bs, NCH_2), 3.38 (4H, bs, OCH_2). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 173.52, 165.46 (C=O), 157.48, 151.22, 149.21, 144.11, 135.00, 125.24, 122.67, 121.90, 121.47, 119.27, 113.45, 110.05, 66.14 (OCH_2), 66.01 (OCH_2), 55.78 (OCH_3), 44.74 (NCH_2), 41.63 (NCH_2).

2-(4-(2-Oxo-2-phenylethoxy)phenyl)-6-nitrobenzothizole (21a)

According to the above-mentioned general procedure from disulfide **17** (85 mg, 0.25 mmol) and benzaldehyde **5a** (105.7 mg, 0 44 mmol) compound **21a** was obtained as a yellow powder (163.8 mg, 95.4 %; m.p. 216–218°C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 9.19 (1H, d, *J* = 1.9 Hz), 8.35 (1H, dd, *J* = 8.9, 2.0 Hz), 8.18 (1H, d, *J* = 8.9 Hz), 8.11 (2H, d, *J* = 8.7 Hz), 8.05 (2H, d, *J* = 7.6 Hz), 7.72 (1H, t, *J* = 7.5 Hz), 7.60 (2H, t, *J* = 7.4 Hz), 7.20 (2H, d, *J* = 8.7 Hz), 5.78 (2H, s, OCH_2). ^{13}C NMR (151 MHz, DMSO) (δ/ppm) 193.95 (C=O), 173.40, 161.40, 157.57, 154.29, 144.12, 134.96, 134.20, 133.91, 129.44, 128.85, 127.88, 125.16, 122.69, 121.93, 119.36, 115.65, 70.33 (OCH_2).

2-(3-Fluoro-4-(2-oxo-2-phenylethoxy)phenyl)-6-nitrobenzothizole (21b)

According to the above-mentioned general procedure from disulfide **17** (85 mg, 0.25 mmol) and benzaldehyde **5b** (113.6 mg, 0.44 mmol) compound **21b** was obtained as a yellow powder (148.2 mg, 82.5 %; m.p. 218–220 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 9.21 (1H, s), 8.35 (1H, d, *J* = 8.5 Hz), 8.20 (1H, d, *J* = 8.8 Hz), 8.04 (3H, d, *J* = 7.5 Hz), 7.90 (1H, d, *J* = 7.9 Hz), 7.72 (1H, d, *J* = 6.7 Hz), 7.62 (2H, d, *J* = 6.7 Hz), 7.35 (1H, t, *J* = 8.3 Hz), 5.89 (2H, s, OCH_2). ^{13}C NMR (151 MHz, DMSO) (δ/ppm) 193.47 (C=O), 172.16, 157.24, 151.46 (d, J_{CF} = 246.2 Hz), 149.36 (d, J_{CF} = 10.2 Hz), 144.24, 135.11, 134.03, 133.99,

128.85, 127.88, 125.37 (d, J_{CF} = 6.9 Hz), 124.75 (d, J_{CF} = 2.7 Hz), 122.88, 121.93, 119.40, 115.64, 114.89 (d, J_{CF} = 20.3 Hz), 70.86 (OCH_2).

2-(3-Methoxy-4-(2-oxo-2-phenylethoxy)phenyl)-6-nitrobenzothizole (21c)

According to the above-mentioned general procedure from disulfide **17** (85 mg, 0.25 mmol) and benzaldehyde **5c** (118.9 mg, 0.44 mmol) compound **21c** was obtained as a yellow powder (172.3 mg, 93.2 %; m.p. 194–196 °C). 1H NMR (300 MHz, DMSO) (δ/ppm) 9.17 (1H, s), 8.33 (1H, d, *J* = 8.2 Hz), 8.19 (1H, d, *J* = 8.6 Hz), 8.05 (2H, d, *J* = 6.9 Hz), 7.81 – 7.49 (5H, m), 7.10 (1H, d, *J* = 8.0 Hz), 5.76 (2H, s, OCH_2), 3.95 (3H, s, OCH_3). ^{13}C NMR (151 MHz, DMSO) (δ/ppm) 194.03 (C=O), 173.39, 157.50, 151.37, 149.52, 144.31, 135.08, 134.50, 133.71, 128.76, 127.87, 125.64, 122.71, 121.80, 121.52, 119.05, 114.08, 111.02, 70.96 (OCH_2), 56.11 (OCH_3).

2-(4-(Pyridin-2-ylmethoxy)phenyl)-6-nitrobenzothiazole (22a)

According to the above-mentioned general procedure from disulfide **17** (85 mg, 0.25 mmol) and benzaldehyde **6a** (93.8 mg, 0.44 mmol) compound **22a** was obtained as a yellow powder (145.1 mg, 90.8 %; m.p. 239–242°C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 9.19 (d, *J* = 2.3 Hz, 1H), 8.61 (1H, d, *J* = 4.5 Hz), 8.35 (1H, dd, *J* = 9.0, 2.4 Hz), 8.18 (1H, d, *J* = 9.0 Hz), 8.13 (2H, d, *J* = 8.8 Hz), 7.87 (1H, dd, *J* = 8.5, 6.9 Hz), 7.56 (1H, d, *J* = 7.9 Hz), 7.38 (1H, dd, *J* = 7.0, 5.2 Hz), 7.26 (2H, d, *J* = 8.8 Hz), 5.33 (2H, s, OCH_2). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 161.49, 154.93, 149.16, 137.12, 134.96, 131.27, 129.58, 128.75, 123.15, 122.90, 122.70, 122.19, 121.83, 118.33, 115.78, 70.52 (OCH_2).

2-(3-Fluoro-4-(pyridin-2-ylmethoxy)phenyl)-6-nitrobenzothiazole (22b)

According to the above-mentioned general procedure from disulfide **17** (85 mg, 0.25 mmol) and benzaldehyde **6b** (101.7 mg, 0.44 mmol) compound **22b** was obtained as a yellow powder (131.9 mg, 78.6 %; m.p. 240–242 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 9.23 (1H, d, *J* = 2.2 Hz), 8.62 (1H, d, *J* = 4.4 Hz), 8.36 (1H, dd, *J* = 8.9, 2.4 Hz), 8.21 (1H, d, *J* = 9.0 Hz), 8.05 (1H, dd, *J* = 11.8, 1.9 Hz), 7.97 (1H, d, *J* = 8.8 Hz), 7.89 (1H, td, *J* = 7.7, 1.5 Hz), 7.57 (1H, d, *J* = 7.8 Hz), 7.48 (1H, t, *J* = 8.6 Hz), 7.40 (1H, dd, *J* = 7.2, 4.3 Hz), 5.41 (2H, s, OCH_2). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 172.21, 157.27, 155.51, 151.71 (d, J_{CF} = 244.9 Hz), 149.27, 149.06, 144.34, 137.17, 135.17, 125.09 (d, J_{CF} = 3.0 Hz), 123.05, 121.91, 120.66, 119.52, 115.81, 114.90 (d, J_{CF} = 20.0 Hz), 71.26 (OCH_2).

2-(3-Methoxy-4-(pyridin-2-ylmethoxy)phenyl)-6-nitrobenzothiazole (22c)

According to the above-mentioned general procedure from disulfide **17** (85 mg, 0.25 mmol) and benzaldehyde **6c** (107.0 mg, 0.44 mmol) compound **22c** was obtained as a yellow powder (160.7 mg, 92.9 %; m.p. 204–206 °C). ^{1}H NMR (600 MHz, DMSO) (δ/ppm) 9.18 (1H, d, *J* = 2.4 Hz), 8.60 (1H, d, *J* = 4.3 Hz), 8.34 (1H, dd, *J* = 9.0, 2.4 Hz), 8.19 (1H, d, *J* = 9.0 Hz), 7.87 (1H, td, *J* = 7.7, 1.7 Hz), 7.72 (1H, d, *J* = 2.0 Hz), 7.69 (1H, dd, *J* = 8.4, 2.1 Hz), 7.55 (1H, d, *J* = 7.8 Hz), 7.38 (1H, dd, *J* = 6.9, 5.0 Hz), 7.25 (1H, d, *J* = 8.4 Hz), 5.30 (2H ,s, OCH_2), 3.94 (3H ,s, OCH_3). ^{13}C NMR (151 MHz, DMSO) (δ/ppm) 173.50, 157.47,

156.09, 151.24, 149.40, 149.17, 144.14, 137.07, 135.02, 125.32, 123.13, 122.70, 121.92, 121.81, 121.68, 119.31, 113.51, 109.95, 70.91 (OCH_2), 55.78 (OCH_3).

4.2.2. General procedure for synthesis of target 6-carboximidamide benzothiazole derivatives 12a–12c, 13a–13c, 14a, 14b, 15b and 16c

Corresponding cyano derivatives **7a–11a, 7b–11b, 7c–11c** were suspended in dry ethanol, and the suspension was heated at 75 °C. Triethylamine (Et_3N) (1 eq) and $NH_2OH \times HCl$ (2 eq) were added and the reaction mixture was stirred for 24 h at 80 °C. After cooling, the mixture was diluted with water and the precipitate filtered off. If necessary, precipitate was purified by column chromatography with dichloromethane : methanol = 10 : 1 as eluent, or trituration in hot ethanol : methanol = 10 : 1 mL solvent mixture.

(*Z*)-2-(4-(2-(Diethylamino)ethoxy)phenyl)-*N*'-hydroxybenzothiazole-6-carboximidamide hydrochloride (12a)

According to the above-mentioned general procedure from intermediate **7a** (100 mg, 0.28 mmol) and $NH_2OH \times HCl$ (19.5 mg, 0.56 mmol) compound **12a** was obtained as a beige powder (38.9 mg, 36.1 %; m.p. 219–221 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 10.62 (1H, s, OH), 9.82 (1H, s), 8.39 (1H, s), 8.09 (2H, d, *J* = 8.3 Hz), 7.99 (1H, d, *J* = 8.5 Hz), 7.86 (1H, d, *J* = 8.5 Hz), 7.19 (2H, d, *J* = 8.4 Hz), 6.00 (2H, s, NH_2), 4.50 (2H, bs, OCH_2), 3.53 (2H, bs, NCH_2), 3.21 (4H, d, *J* = 6.3 Hz, NCH_2), 1.27 (6H, t, *J* = 6.9 Hz, CH_3). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 167.59, 160.08, 153.93, 150.53 ($C(NH_2)$=NOH), 134.22, 130.28, 128.95, 126.08, 124.23, 121.93, 119.01, 115.42, 62.68 (OCH_2), 49.59 (NCH_2), 46.96 (NCH_2), 8.51 (CH_3). Anal. calcd. for $C_{20}H_{24}N_4O_2S$ (Mr = 384.50): C 62.48, H 6.29, N 14.57; found: C 62.34, H 6.30, N 14.54%.

(*Z*)-2-(4-(2-(Diethylamino)ethoxy)-3-fluorophenyl)-*N*'-hydroxybenzothiazole-6-carboximidamide hydrochloride (12b)

According to the above-mentioned general procedure from intermediate **7b** (100 mg, 0.27 mmol) and $NH_2OH \times HCl$ (18.8 mg, 0.54 mmol) compound **12b** was obtained as a beige powder (42.4 mg, 39.0 %; m.p. 219–221°C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 10.72 (1H, s, OH), 9.85 (1H, s), 8.41 (1H, s), 8.00 (2H, t, *J* = 10.4 Hz), 7.93 (1H, d, *J* = 9.0 Hz), 7.88 (1H, d, *J* = 8.5 Hz), 7.43 (1H, t, *J* = 8.6 Hz), 6.04 (2H, s, NH_2), 4.59 (2H, bs, OCH_2), 3.58 (2H, bs, NCH_2), 3.24 (4H, d, *J* = 7.0 Hz, NCH_2), 1.28 (6H, t, *J* = 7.2 Hz, CH_3). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 166.43 (d, J_{CF} = 2.6 Hz), 153.73, 151.56 (d, J_{CF} = 246.0 Hz), 150.53 ($C(NH_2)$=NOH), 149.93, 148.09 (d, J_{CF} = 10.7 Hz), 134.45, 130.50, 126.45 (d, J_{CF} = 6.8 Hz), 124.38, 122.13, 119.11, 115.44, 114.43 (d, J_{CF} = 20.0 Hz)., 63.90 (OCH_2), 49.44 (NCH_2), 47.19 (NCH_2), 8.54 (CH_3). Anal. calcd. for $C_{20}H_{23}FN_4O_2S$ (Mr = 402.49): C 59.68, H 5.76, N 13.92; found: C 59.61, H 5.78, N 13.88%.

(*Z*)-2-(4-(2-(Diethylamino)ethoxy)-3-methoxyphenyl)-*N*'-hydroxybenzothiazole-6-carboximidamide hydrochloride (12c)

According to the above-mentioned general procedure from intermediate **7c** (100 mg, 0.26 mmol) and NH_2OH×HCl (18.1 mg, 0.52 mmol) compound **12c** was obtained as a beige powder (40.2 mg, 37.3 %; m.p. 148–150°C). ^{1}H NMR (600 MHz, DMSO) (δ/ppm) 9.80 (1H, s, OH), 8.38 (1H, t, *J* = 4.3 Hz), 8.00 (1H, d, *J* = 8.6 Hz), 7.86 (1H, dd, *J* = 8.6, 1.7 Hz), 7.69 (1H, dd, *J* = 6.9, 2.0 Hz), 7.64 (1H, dt, *J* = 8.4, 4.2 Hz), 7.20 (1H, t, *J* = 8.1 Hz), 5.96 (2H, s, NH_2), 4.38 (2H, bs, OCH_2), 3.92 (3H, s, OCH_3), 3.36 (4H, s, NCH_2), 3.06 (2H, bs, NCH_2), 1.29 – 1.12 (6H, m, CH_3). ^{13}C NMR (151 MHz, DMSO) (δ/ppm) 167.76, 153.84, 150.42 ($C(NH_2)$=NOH), 149.33, 134.28, 130.41, 124.18, 121.90, 120.87, 118.90, 113.39, 109.71, 62.91 (OCH_2), 56.31 (OCH_3), 49.82 (NCH_2), 47.19 (NCH_2), 8.74 (CH_3). Anal. calcd. for $C_{21}H_{26}N_4O_3S$ (Mr = 414.52): C 60.85, H 6.32, N 13.52; found: C 60.74, H 6.36, N 13.44%.

(*Z*)-2-(4-(2-Morpholinoethoxy)phenyl)-*N*'-hydroxybenzothiazole-6-carboximidamide (13a)

According to the above-mentioned general procedure from intermediate **8a** (100 mg, 0.27 mmol) and NH_2OH×HCl (18.8 mg,0.54 mmol) compound **13a** was obtained as a beige powder (66.6 mg, 61.9 %; m.p. 216–219 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 9.79 (1H, s, OH), 8.37 (1H, s), 8.03 (2H, d, *J* = 8.6 Hz), 7.98 (1H, d, *J* = 8.6 Hz), 7.85 (1H, d, *J* = 8.5 Hz), 7.13 (2H, d, *J* = 8.7 Hz), 5.95 (2H, s, NH_2), 4.19 (2H. t, *J* = 5.5 Hz, OCH_2), 3.65 – 3.51 (4H, m, OCH_2), 2.73 (2H, t, *J* = 5.4 Hz, NCH_2). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 167.71, 161.06, 153.94, 150.42 ($C(NH_2)$=NOH), 134.17, 130.30, 128.87, 125.38, 124.15, 121.85, 118.90, 115.26, 66.12 (OCH_2), 65.61 (OCH_2), 56.83 (NCH_2), 53.56 (NCH_2). Anal. calcd. for $C_{20}H_{22}N_4O_3S$ (Mr = 398.48): 60.28, H 5.57, N 14.06; found: C 60.25, H 5.60, N 14.00%.

(*Z*)-2-(3-Fluoro-4-(2-morpholinoethoxy)phenyl)-*N*'-hydroxybenzothiazole-6-carboximidamide hydrochloride (13b)

According to the above-mentioned general procedure from intermediate **8b** (100 mg, 0.26 mmol) and NH_2OH×HCl (18.1 mg, 0.52 mmol) compound **13b** was obtained as a beige powder (68.8 mg, 63.5 %; m.p. 223–225°C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 11.84 (1H, bs, NH), 10.00 (1H, s, OH), 8.43 (1H, s), 8.02 (1H, t, *J* = 8.0 Hz), 7.97 (1H, d, *J* = 11.9 Hz), 7.94 – 7.82 (2H, m), 7.42 (1H, t, *J* = 8.6 Hz), 6.33 (2H, s, NH_2), 4.59 (2H, s, OCH_2), 3.86 (4H, OCH_2), 3.20 (4H, NCH_2). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 166.76, 153.95, 151.59 (d, J_{CF} = 246.0 Hz), 151.32 ($C(NH_2)$=NOH), 148.26 (d, J_{CF} = 10.8 Hz), 134.46, 129.73, 126.29 (d, J_{CF} = 6.8 Hz), 124.54, 124.39 (d, J_{CF} = 2.9 Hz), 122.19, 119.46, 115.51, 114.43 (d, J_{CF} = 20.0 Hz), 64.50 (OCH_2), 63.78 (OCH_2), 55.02 (NCH_2), 52.08 (NCH_2). Anal. calcd. for $C_{20}H_{21}FN_4O_3S$ x HCl (Mr = 452.93): 53.04, H 4.90, N 12.37; found: C 53.10, H 4.86, N 12.31 %.

(*Z*)-2-(3-Methoxy-4-(2-morpholinoethoxy)phenyl)-*N*'-hydroxybenzothiazole-6-carboximidamide hydrochloride (13c)

According to the above-mentioned general procedure from intermediate **8c** (100 mg, 0.25 mmol) and NH_2OH×HCl (17.4 mg, 0.50 mmol) compound **13c** was obtained as a beige powder (70.7 mg, 65.9 %; m.p. 199–202°C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 12.28 (1H, s, NH), 10.57 (1H, s, OH), 8.49 (1H,

s), 8.10 (1H, d, *J* = 8.6 Hz), 7.86 (1H, d, *J* = 8.6 Hz), 7.69 (2H, d, *J* = 8.2 Hz), 7.42 (2H, bs, NH_2), 7.24 (1H, d, *J* = 8.1 Hz), 4.59 (2H, s, OCH_2), 3.93 (7H, s, OCH_3, OCH_2), 3.58 (2H, s, NCH_2), 3.40 (4H, bs, NCH_2). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 169.12, 154.92, 150.03 (C(NH_2)=NOH), 149.43, 134.43, 126.26, 125.13, 122.24, 121.08, 120.79, 113.89, 109.89, 63.77 (OCH_2), 63.19 (OCH_2), 55.89 (OCH_3), 54.63 (NCH_2), 51.81 (NCH_2). Anal. calcd. for $C_{21}H_{24}N_4O_4S$ x HCl (Mr = 464.96): 54.25, H 5.42, N 12.05; found: C 54.16, H 5.46, N 11.98%.

(*Z*)-2-(4-(2-Morpholino-2-oxoethoxy)phenyl)-*N*'-hydroxybenzothiazole-6-carboximidamide (14a)

According to the above-mentioned general procedure from intermediate **9a** (100 mg, 0.26 mmol) and NH_2OH×HCl (18.1 mg, 0.52 mmol) compound **14a** was obtained as a beige powder (50.9 mg, 47.4 %; m.p. 244–246 °C). ^{1}H NMR (300 MHz, DMSO) (δppm) 9.79 (1H, s, OH), 8.37 (1H, s), 8.03 (2H, d, *J* = 8.7 Hz), 7.99 (1H, d, *J* = 8.6 Hz), 7.85 (1H, d, *J* = 8.6 Hz), 7.11 (2H, d, *J* = 8.8 Hz), 5.94 (2H, s, NH_2), 4.99 (2H, s, OCH_2), 3.64 (2H, s, OCH_2), 3.59 (2H, s, OCH_2), 3.48 (4H, bs, NCH_2). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 167.69, 165.58, 160.70, 153.93, 150.42 (C(NH_2)=NOH), 134.21, 130.33, 128.73, 125.66, 124.16, 121.88, 118.91, 115.42, 66.02 (OCH_2), 65.95 (OCH_2), 65.65 (OCH_2), 44.59 (NCH_2), 41.57 (NCH_2). Anal. calcd. for $C_{20}H_{20}N_4O_4S$ (Mr = 412.46): 58.24, H 4.89, N 13.58; found: C 58.18, H 4.91, N 13.54%.

(*Z*)-2-(3-Fluoro-4-(2-morpholino-2-oxoethoxy)phenyl)-*N*'-hydroxybenzothiazole-6-carboximidamide (14b)

According to the above-mentioned general procedure from intermediate **9b** (100 mg, 0.25 mmol) and NH_2OH×HCl (17.4 mg, 0.50 mmol) compound **14b** was obtained as a beige powder (74.5 mg, 69.2 %; m.p. 248–250°C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 9.81 (1H, OH), 8.39 (1H, s), 7.99 (1H, t, *J* = 8.5 Hz), 7.96 – 7.91 (1H, m), 7.90 – 7.81 (2H, m), 7.26 (1H, t, *J* = 8.7 Hz), 5.96 (2H, s, NH_2), 5.12 (2H, s, OCH_2), 3.65 (2H, s, OCH_2), 3.59 (2H, s, OCH_2), 3.47 (4H, s, NCH_2). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 166.52 (d, J_{CF} = 2.3 Hz), 165.18, 153.74, 151.50 (d, J_{CF} = 245.9 Hz), 150.40 (C(NH_2)=NOH), 148.72 (d, J_{CF} = 10.5 Hz), 134.43, 130.60, 125.95 (d, J_{CF} = 7.0 Hz), 124.29, 124.01 (d, J_{CF} = 2.4 Hz), 122.08, 118.99, 115.57, 114.36 (d, J_{CF} = 20.6 Hz), 66.07 (OCH_2), 66.00 (OCH_2), 65.92(OCH_2), 44.54 (NCH_2), 41.59 (NCH_2). Anal. calcd. for $C_{20}H_{19}FN_4O_4S$ (Mr = 430.45): 55.81, H 4.45, N 13.02; found: C 55.74, H 4.48, N 12.95%.

(*Z*)-2-(3-Fluoro-4-(2-oxo-2-phenylethoxy)phenyl)-*N*'-hydroxybenzothiazole-6-carboximidamide (15b)

According to the above-mentioned general procedure from intermediate **10b** (100 mg, 0.26 mmol) and NH_2OH×HCl (18.1 mg, 0.52 mmol) compound **15b** was obtained as a beige powder (77.3 mg, 70.5 %; m.p. 206–208°C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 9.84 (1H, s, OH), 8.75 (1H, s), 8.15 (1H, d, *J* = 8.5 Hz), 7.93 (3H, t, *J* = 7.6 Hz), 7.73 – 7.64 (2H, m), 7.48 – 7.37 (4H, m), 6.00 (2H, s, NH_2), 5.47 (2H, s, OCH_2). ^{13}C NMR (151 MHz, DMSO) (δ/ppm) 170.34, 155.81, 151.54 (d, J_{CF} = 246.6 Hz), 152.01, 150.23 (C(NH_2)=NOH), 148.95 (d, J_{CF} = 10.6 Hz), 135.08, 133.79, 129.87, 129.04, 128.46, 128.34, 128.06,

127.63, 126.33, 125.55 (d, J_{CF} = 6.9 Hz), 124.94 (d, J_{CF} = 3.0 Hz), 123.41, 118.76, 115.16, 114.78 (d, J_{CF} = 20.1 Hz), 59.82 (OCH_2). Anal. calcd. for $C_{22}H_{16}FN_3O_3S$ (Mr = 421.44): 62.70, H 3.83, N 9.97; found: C 62.56, H 3.86, N 9.91%.

(*Z*)-2-(3-Methoxy-4-(pyridin-2-ylmethoxy)phenyl)-*N*'-hydroxybenzothiazole-6-carboximidamide (16c)

According to the above-mentioned general procedure from intermediate **11c** (100 mg, 0.27 mmol) and NH_2OH×HCl (18.8 mg, mmol) compound **16c** was obtained as a beige powder (64.3 mg, 58.5 %; m.p. 231–234°C). ^{1}H NMR (600 MHz, DMSO) (δ/ppm) 9.79 (1H, s, OH), 8.74 (1H, s), 8.61 (1H, d, *J* = 3.1 Hz), 8.18 (1H, d, *J* = 8.4 Hz), 7.93 (1H, d, *J* = 8.3 Hz), 7.88 (1H, t, *J* = 7.3 Hz), 7.72 (1H, s), 7.68 (1H, d, *J* = 8.1 Hz), 7.56 (1H, d, *J* = 7.6 Hz), 7.42 – 7.36 (1H, m), 7.24 (1H, d, *J* = 8.3 Hz), 5.95 (2H, s, NH_2), 5.30 (2H ,s, OCH_2), 3.94 (3H, s, OCH_3). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 170.89, 159.09, 156.30, 153.96, 150.56 ($C(NH_2)$=NOH), 149.47, 146.31, 140.88, 134.60, 126.24, 125.88, 124.53, 123.49, 123.04, 122.65, 121.89, 121.42, 113.90, 110.03, 68.98 (OCH_2), 55.83 (OCH_3). Anal. calcd. for $C_{21}H_{18}N_4O_3S$ (Mr = 406.46): 62.06, H 4.46, N 13.78; found: C 61.93, H 4.51, N 13.84%.

4.2.3. General procedure for synthesis of amino derivatives 23a–27a, 23b–27b, 23c–27c

To a solution of 6-nitrobenzothiazole derivatives **18a**–**22a, 18b**–**22b, 18b**–**22b** (1 eq) in methanol (10 mL), $SnCl_2$×$2H_2O$ (6 eq) and HCl (5 mL) were added. The reaction mixture was stirred at 100 °C for 1 hour. After completion of the reaction, cold water was added to the reaction mixture. Obtained residue was basidified with 1M NaOH to pH = 10 and the aqeous layer was extracted with EtOAc (3×20 mL). The combined organic layer was removed under reduced pressure and purified by column chromatography with dichloromethane : methanol = 10 : 1.

2-(4-(2-(Diethylamino)ethoxy)phenyl)benzothiazol-6-amine (23a)

According to the above-mentioned general procedure by reduction of nitro analogue **18a** (130 mg, 0.35 mmol) compound **23a** was obtained as a brown powder (109.0 mg, 91.2 %, m.p. 101–104 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 7.87 (2H, d, *J* = 8.8 Hz), 7.64 (1H, d, *J* = 8.7 Hz), 7.13 – 6.99 (3H, m), 6.78 (1H, dd, *J* = 8.7, 2.1 Hz), 5.42 (2H, s, NH_2), 4.08 (2H, t, *J* = 6.1 Hz, OCH_2), 2.79 (2H, t, *J* = 6.0 Hz, NCH_2), 2.55 (4H, dd, *J* = 14.2, 7.1 Hz, NCH_2), 0.98 (6H, t, *J* = 7.1 Hz, CH_3). ^{13}C NMR (151 MHz, DMSO) (δ/ppm) 160.40, 160.14, 147.10, 145.23, 135.97, 127.95, 126.12, 122.75, 115.04, 114.85, 103.84, 66.59 (OCH_2), 51.21 (NCH2), 46.95 (NCH_2), 11.84 (CH_3).

2-(4-(2-(Diethylamino)ethoxy)-3-fluorophenyl)benzothiazol-6-amine (23b)

According to the above-mentioned general procedure by reduction of nitro analogue **18b** (200 mg, 0.51 mmol) compound **23b** was obtained as a yellow powder (154.4 mg, 84.2 %, m.p. 97–99 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 7.76 (1H, dd, *J* = 12.2, 2.0 Hz), 7.68 (2H, t, *J* = 8.8 Hz), 7.31 (1H, t, *J* = 8.7 Hz), 7.08 (1H, d, *J* = 2.0 Hz), 6.79 (1H, dd, *J* = 8.7, 2.1 Hz), 5.48 (2H, s, NH_2), 4.18 (2H, t, *J* = 5.9 Hz,

OCH_2), 2.86 (2H, t, *J* = 5.8 Hz, NCH_2), 2.60 (4H, q, *J* = 7.1 Hz, NCH_2), 0.99 (6H, t, *J* = 7.1 Hz, CH_3). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 171.97, 159.06, 151.65 (d, J_{CF} = 245.1 Hz), 148.00 (d, J_{CF} = 10.8 Hz), 147.39, 145.02, 136.24, 126.56 (d, J_{CF} = 6.7 Hz), 123.23 (d, J_{CF} = 3.1 Hz), 122.98, 115.18 (d, J_{CF} = 1.8 Hz), 115.06, 113.45 (d, J_{CF} = 20.1 Hz), 103.70, 67.50 (OCH_2), 50.99 (NCH_2), 47.01 (NCH_2), 11.66 (CH_3).

2-(4-(2-(Diethylamino)ethoxy)-3-methoxyphenyl)benzothiazol-6-amine (23c)

According to the above-mentioned general procedure by reduction of nitro analogue **18c** (200 mg, 0.49 mmol) compound **23c** was obtained as a brown oil (143.1 mg, 78.6 %). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 7.66 (1H, d, *J* = 8.7 Hz), 7.53 (1H, d, *J* = 1.9 Hz), 7.44 (1H, dd, *J* = 8.3, 1.9 Hz), 7.09 (2H, d, *J* = 8.0 Hz), 6.78 (1H, dd, *J* = 8.7, 2.1 Hz,), 5.44 (2H, s, NH_2), 4.09 (2H, t, *J* = 6.0 Hz, OCH_2), 3.86 (3H, s, OCH_3), 2.86 (2H, s, NCH_2), 2.61 (4H, d, *J* = 6.8 Hz, NCH_2), 1.00 (6H, t, *J* = 7.1 Hz, CH_3). ^{13}C NMR (151 MHz, DMSO) δ 160.53, 149.86, 149.20, 147.14, 145.14, 136.05, 126.41, 122.77, 119.81, 114.88, 113.05, 109.15, 103.80, 66.94 (OCH_2), 55.63 (OCH_3), 51.11 (NCH_2), 47.03 (NCH_2), 11.61 (CH_3).

2-(4-(2-Morpholinoethoxy)phenyl)benzothiazol-6-amine (24a)

According to the above-mentioned general procedure by reduction of nitro analogue **19a** (164 mg, 0.43 mmol) compound **24a** was obtained as a yellow powder (119.3 mg, 78.1 %, m.p. 162–165 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 7.88 (2H, d, *J* = 8.7 Hz), 7.66 (1H, t, *J* = 8.9 Hz), 7.07 (3H, dd, *J* = 5.3, 3.1 Hz), 6.77 (1H, dd, *J* = 8.7, 2.1 Hz), 5.43 (2H, s, NH_2), 4.16 (2H, t, *J* = 5.7 Hz, OCH_2), 3.66 – 3.50 (4H, m, OCH_2), 2.71 (2H, t, *J* = 5.6 Hz, NCH_2), 2.47 (4H, bs, NCH_2). ^{13}C NMR (151 MHz, DMSO) (δ/ppm) 160.87, 160.56, 147.63, 145.71, 136.48, 128.45, 126.70, 123.27, 115.58, 115.37, 104.33, 66.64 (OCH_2), 66.04 (OCH_2), 57.39 (NCH_2), 54.08 (NCH_2).

2-(3-Fluoro-4-(2-morpholinoethoxy)phenyl)benzothiazol-6-amine (24b)

According to the above-mentioned general procedure by reduction of nitro analogue **19b** (300 mg, 0.74 mmol) compound **24b** was obtained as a beige powder (212.4 mg, 76.9 %, m.p. 139–142 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 7.76 (1H, dd, *J* = 12.2, 2.0 Hz), 7.68 (2H, t, *J* = 8.8 Hz), 7.32 (1H, t, *J* = 8.7 Hz), 7.08 (1H, d, *J* = 2.0 Hz), 6.79 (1H, dd, *J* = 8.7, 2.1 Hz), 5.48 (2H, s, NH_2), 4.24 (2H, t, *J* = 5.6 Hz, OCH_2), 3.65 – 3.52 (4H, m, OCH_2), 2.74 (2H, t, *J* = 5.6 Hz, NCH_2), 2.49 (4H, d, *J* = 6.4 Hz, NCH_2). ^{13}C NMR (151 MHz, DMSO) (δ/ppm) 159.03, 151.66 (d, J_{CF} = 245.1 Hz), 147.95 (d, J_{CF} = 10.7 Hz), 147.40, 145.02, 136.25, 126.65 (d, J_{CF} = 6.8 Hz), 123.23 (d, J_{CF} = 2.9 Hz), 123.00, 115.32, 115.07, 113.47 (d, J_{CF} = 20.1 Hz), 103.70, 66.79 (OCH_2), 66.15 (OCH_2), 56.73 (NCH_2), 53.55 (NCH_2).

2-(3-Methoxy-4-(2-morpholinoethoxy)phenyl)benzothiazol-6-amine (24c)

According to the above-mentioned general procedure by reduction of nitro analogue **19c** (200 mg, 0.48 mmol) compound **24c** was obtained as a brown oil (139.2 mg, 75.7 %). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 7.66 (1H, d, *J* = 8.7 Hz), 7.53 (1H, d, *J* = 1.9 Hz), 7.42 (1H, dt, *J* = 12.8, 6.4 Hz), 7.14 – 7.05 (2H, m), 6.77 (1H, dd, *J* = 8.7, 2.1 Hz), 5.43 (2H, s, NH_2), 4.14 (2H, t, *J* = 5.8 Hz, OCH_2), 3.86 (3H, s, OCH_3),

3.65 – 3.53 (4H, m, OCH_2), 2.72 (2H, t, *J* = 5.8 Hz, NCH_2), 2.47 (4H, bs, NCH_2). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 160.53, 149.86, 147.15, 145.14, 136.06, 126.47, 122.78, 119.80, 114.88, 113.18, 109.17, 103.80, 66.28 (OCH_2), 66.15 (OCH_2), 56.89 (NCH_2), 55.62 (OCH_3), 53.63 (NCH_2).

2-(4-(2-Morpholino-2-oxoethoxy)phenyl)benzothiazol-6-amine (25a)

According to the above-mentioned general procedure by reduction of nitro analogue **20a** (200 mg, 0.47 mmol) compound **25a** was obtained as a yellow powder (114.9 mg, 61.2 %, m.p. 205–208 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 7.88 (2H, d, *J* = 8.8 Hz), 7.65 (1H, d, *J* = 8.7 Hz), 7.08 (1H, d, *J* = 2.0 Hz), 7.05 (2H, d, *J* = 8.9 Hz), 6.78 (1H, dd, *J* = 8.7, 2.1 Hz), 5.43 (2H, s, NH2), 4.94 (2H, s, OCH2), 3.69 – 3.53 (4H, m, NCH_2), 3.48 (4H, bs, OCH_2). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 165.69 (C=O), 160.33, 159.66, 147.12, 145.22, 136.02, 127.81, 126.51, 122.79, 115.24, 114.89, 103.82, 65.99(OCH_2), 65.72 (OCH_2), 44.65 (NCH_2), 41.58 (NCH_2).

2-(3-Fluoro-4-(2-morpholino-2-oxoethoxy)phenyl)benzothiazol-6-amine (25b)

According to the above-mentioned general procedure by reduction of nitro analogue **20b** (400 mg, 0.96 mmol) compound **25b** was obtained as a yellow powder (147.3 mg, 39.6 %, m.p. 216–219 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 7.77 (1H, dd, *J* = 12.2, 2.1 Hz), 7.67 (2H, d, *J* = 8.7 Hz), 7.20 (1H, t, *J* = 8.7 Hz), 7.08 (1H, d, *J* = 2.1 Hz), 6.79 (1H, dd, *J* = 8.7, 2.2 Hz), 5.48 (2H, s, NH_2), 5.06 (2H, s, OCH_2), 3.61 (4H, d, *J* = 14.9 Hz, NCH_2), 3.46 (4H, bs, OCH_2). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 165.29 (C=O), 159.00 (d, J_{CF} = 2.7 Hz), 151.52 (d, J_{CF} = 245.2 Hz), 147.60 (d, J_{CF} = 10.6 Hz), 147.40, 145.02, 136.29, 126.87 (d, J_{CF} = 7.0 Hz), 123.01, 122.95 (d, J_{CF} = 3.2 Hz), 115.49 (d, J_{CF} = 1.2 Hz), 115.08, 113.54 (d, J_{CF} = 20.0 Hz), 103.69, 66.10 (OCH_2), 66.00 (OCH_2), 44.55 (NCH_2), 41.59 (NCH_2).

2-(3-Methoxy-4-(2-morpholino-2-oxoethoxy)phenyl)benzothiazol-6-amine (25c)

According to the above-mentioned general procedure by reduction of nitro analogue **20c** (256 mg, 0.64 mmol) compound **25c** was obtained as a yellow powder (117.0 mg, 72.3 %, m.p. 93–96 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 7.66 (1H, d, *J* = 8.7 Hz), 7.55 (1H, d, *J* = 1.9 Hz), 7.41 (1H, dd, *J* = 8.3, 2.0 Hz), 7.08 (1H, d, *J* = 2.0 Hz), 6.99 (1H, d, *J* = 8.5 Hz), 6.77 (1H, dd, *J* = 8.7, 2.1 Hz), 5.44 (2H, s, NH_2), 4.91 (2H, s, OCH2), 3.89 (3H, s, OCH_3), 3.59 (4H, d, *J* = 9.5 Hz, NCH_2), 3.47 (4H, bs, OCH_2). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 165.60 (C=O), 160.37, 149.23, 149.05, 147.09, 145.06, 136.03, 126.75, 122.74, 119.46, 114.83, 113.41, 109.19, 103.70, 66.26 (OCH_2), 65.95 (OCH_2), 55.59 (OCH_3), 44.73 (NCH_2), 41.55 (NCH_2).

2-(4-(2-Oxo-2-phenylethoxy)phenyl)benzothiazole-6-amine (26a)

According to the above-mentioned general procedure by reduction of nitro analogue **21a** (185 mg, 0.47 mmol) compound **26a** was obtained as a yellow powder (106.3 mg, 62.7 %, m.p. 202–204 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 8.05 (2H, d, *J* = 7.2 Hz), 7.88 (2H, d, *J* = 8.8 Hz), 7.71 (1H, t, *J* = 7.4 Hz), 7.66 (1H, d, *J* = 8.7 Hz), 7.59 (2H, t, *J* = 7.5 Hz), 7.11 (2H, d, *J* = 8.9 Hz), 7.08 (1H, d, *J* = 2.0 Hz), 6.78 (1H,

dd, *J* = 8.7, 2.1 Hz), 5.70 (2H, s, OCH_2), 5.43 (2H, s, NH_2). ^{13}C NMR (151 MHz, DMSO) (δ/ppm) 194.18 (C=O), 160.31, 159.57, 147.13, 145.22, 136.03, 134.27, 133.84, 128.83, 127.86, 126.55, 122.80, 115.27, 114.89, 103.83, 70.23 (OCH_2).

2-(3-Fluoro-4-(2-oxo-2-phenylethoxy)phenyl)benzothiazole-6-amine (26b)

According to the above-mentioned general procedure by reduction of nitro analogue **21b** (300 mg, 0.74 mmol) compound **26b** was obtained as a brown powder (168.9 mg, 60.0 %, m.p. 186–189 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 8.04 (2H, d, *J* = 7.3 Hz,), 7.81 (1H, dd, *J* = 12.2, 2.0 Hz), 7.76 – 7.54 (5H, m), 7.26 (1H, t, *J* = 8.7 Hz), 7.09 (1H, d, *J* = 2.0 Hz), 6.80 (1H, dd, *J* = 8.7, 2.1 Hz), 5.82 (2H, s, OCH_2), 5.49 (2H, s, NH2). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 193.74 (C=O), 158.98 (d, J_{CF} = 2.7 Hz), 151.52 (d, J_{CF} = 245.2 Hz), 147.54, 147.41, 145.03, 136.30, 134.11, 133.93, 128.84, 127.86, 126.97 (d, J_{CF} = 6.8 Hz), 123.02, 122.98, 115.50 (d, J_{CF} = 1.0 Hz), 115.09, 113.66 (d, J_{CF} = 20.1 Hz), 103.70, 70.80 (OCH_2).

2-(3-Methoxy-4-(2-oxo-2-phenylethoxy)phenyl)benzothiazole-6-amine (26c)

According to the above-mentioned general procedure by reduction of nitro analogue **21c** (210 mg, 0.50 mmol) compound **26c** was obtained as a yellow powder (125.0 mg, 64.0 %, m.p. 175–178 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 8.04 (2H, d, *J* = 7.2 Hz), 7.75 – 7.63 (2H, m), 7.63 – 7.53 (3H, m), 7.39 (1H, dd, *J* = 8.4, 1.9 Hz), 7.06 (1H, t, *J* = 5.4 Hz), 7.01 (1H, d, *J* = 8.5 Hz), 6.78 (1H, dd, *J* = 8.7, 2.1 Hz), 5.68 (2H, s, OCH_2), 5.44 (2H, s, NH_2), 3.91 (3H, s, OCH_3). ^{13}C NMR (151 MHz, DMSO) (δ/ppm) 194.19 (C=O), 160.44, 149.24, 149.09, 147.17, 145.13, 136.10, 134.29, 133.82, 128.82, 127.87, 126.79, 122.82, 119.54, 114.90, 113.43, 109.35, 103.78, 70.60 (OCH_2), 55.66 (OCH_3).

2-(4-(Pyridin-2-ylmethoxy)phenyl)benzothiazole-6-amine (27a)

According to the above-mentioned general procedure by reduction of nitro analogue **22a** (250 mg, 0.69 mmol) compound **27a** was obtained as a yellow powder (120.2 mg, 52.4 %, m.p. 195–198 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 8.60 (1H, d, *J* = 4.2 Hz), 7.90 (2H, d, *J* = 8.8 Hz), 7.84 (1H, dd, *J* = 7.7, 1.7 Hz), 7.65 (1H, d, *J* = 8.7 Hz), 7.54 (1H, d, *J* = 7.8 Hz), 7.36 (1H, dd, *J* = 6.8, 5.1 Hz), 7.16 (2H, d, *J* = 8.8 Hz), 7.08 (1H, d, *J* = 2.0 Hz), 6.78 (1H, dd, *J* = 8.7, 2.1 Hz), 5.43 (2H, s, NH_2), 5.27 (2H, s, OCH_2). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 160.26, 159.70, 156.29, 149.15, 147.14, 145.21, 137.01, 136.03, 128.00, 126.59, 123.04, 122.80, 121.71, 115.40, 114.89, 103.81, 70.41 (OCH_2).

2-(3-Fluoro-4-(pyridin-2-ylmethoxy)phenyl)benzothiazole-6-amine (27b)

According to the above-mentioned general procedure by reduction of nitro analogue **22b** (200 mg, 0.52 mmol) compound **27b** was obtained as a beige powder (108.0 mg, 58.6 %, m.p. 195–198 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 8.61 (1H, d, *J* = 4.3 Hz), 7.87 (1H, td, *J* = 7.7, 1.5 Hz), 7.81 (1H, dd, *J* = 12.2, 1.9 Hz), 7.68 (2H, t, *J* = 7.9 Hz), 7.56 (1H, d, *J* = 7.8 Hz), 7.37 (2H, dd, *J* = 10.3, 6.9 Hz), 7.09 (1H, d, *J* = 1.8 Hz), 6.79 (1H, dd, *J* = 8.7, 2.0 Hz), 5.49 (2H, s, NH_2), 5.35 (2H, s, OCH_2). ^{13}C NMR (75 MHz, DMSO) δ 158.92 (d, J_{CF} = 2.9 Hz), 155.76, 151.72 (d, J_{CF} = 245.3 Hz), 149.21, 47.57 (d, J_{CF} = 10.8 Hz), 147.43,

145.01, 137.12, 136.30, 127.05 (d, J_{CF} = 6.9 Hz), 123.22, 123.03, 121.82, 115.65 (d, J_{CF} = 1.7 Hz), 115.09, 113.61 (d, J_{CF} = 19.9 Hz), 103.68, 71.20 (OCH_2).

2-(3-Methoxy-4-(pyridin-2-ylmethoxy)phenyl)benzothiazole-6-amine (27c)

According to the above-mentioned general procedure by reduction of nitro analogue **22c** (666 mg, 1.69 mmol) compound **27c** was obtained as a yellow powder (252.7 mg, 41.1 %, m.p. 142–145 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 8.60 (1H, d, *J* = 4.2 Hz), 7.86 (1H, td, *J* = 7.7, 1.7 Hz), 7.67 (1H, d, *J* = 8.7 Hz), 7.58 (1H, d, *J* = 1.9 Hz), 7.54 (1H, d, *J* = 7.8 Hz), 7.43 (1H, dd, *J* = 8.4, 1.9 Hz), 7.36 (1H, dd, *J* = 6.8, 5.1 Hz), 7.15 (1H, d, *J* = 8.5 Hz), 7.08 (1H, d, *J* = 2.0 Hz), 6.78 (1H, dd, *J* = 8.7, 2.1 Hz), 5.44 (2H, s, NH_2), 5.25 (2H, s, OCH_2), 3.91 (3H, s, OCH_3). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 160.42, 156.38, 149.38, 149.30, 149.10, 147.18, 145.13, 137.01, 136.11, 126.84, 123.03, 122.82, 121.73, 119.71, 114.90, 113.49, 109.17, 103.78, 70.89 (OCH_2), 55.65 (OCH_3).

4.2.4. General procedure for synthesis of target 6-picolinimidamide benzothiazole derivatives 29a–32a, 29b–32b, 29c–32c

Dry EtOH (3 mL) was added to a solution of amino benzothiazole analogues **25a–29a, 25b–29b** and **25c–29c** (1 eq) in dry ACN (1 mL), and the solution was chilled briefly in an ice water bath. S-(2-Naphthylmethyl)thiobenzimidate hydrobromide **30** (2 eq) was then added, and the mixture was stirred overnight at room-temperature. The resulting solution was concentrated and purified by column chromatography with CH_2CL_2 : CH_3OH = 10 : 1. The obtained solid was dissolved in HCl saturated EtOH (5 mL) and stirred for 12 h. Addition of ether resulted in precipitation of product. Solid was collected by filtration, washed with anhydrous ether, and dried under vacuum.

***N*-(2-(4-(2-(Diethylamino)ethoxy)phenyl)benzothiazol-6-yl)picolinimidamide dihydrochloride (29a)**

Using the above-mentioned procedure from **23a** (100 mg, 0.29 mmol) and compound **28** (208.4 mg, 0.58 mmol) compound **29a** was obtained as a brown powder (71.2 mg, 47.4 %, m.p. 78–81 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 12.09 (1H, s, NH), 11.10 (1H, s, NH), 10.23 (1H, s, NH), 9.38 (1H, s, NH), 8.92 (1H, d, *J* = 4.1 Hz), 8.59 (1H, d, *J* = 7.9 Hz), 8.31 (1H, s), 8.24 (1H, t, *J* = 7.5 Hz), 8.18 (1H, d, *J* = 8.7 Hz), 8.12 (2H, d, *J* = 8.6 Hz), 7.87 (1H, dd, *J* = 7.3, 4.8 Hz), 7.59 (1H, t, *J* = 10.7 Hz), 7.22 (2H, d, *J* = 8.7 Hz), 4.54 (2H, d, *J* = 4.6 Hz, OCH_2), 3.54 (2H, d, *J* = 4.0 Hz, NCH_2), 3.22 (4H, bs, NCH_2), 1.29 (6H, t, *J* = 7.1 Hz, CH_3). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 168.50, 160.27, 159.82, 153.35, 149.79, 144.35, 138.35, 135.27, 131.29, 129.07, 128.62, 125.89, 124.96, 124.20, 123.51, 120.35, 115.53, 62.72 (OCH_2), 49.47 (NCH_2), 46.91 (NCH_2), 8.40 (CH_3). Anal. calc. for $C_{25}H_{27}N_5OS$ x 2HCl (Mr = 518.50): C 57.91, H 5.64, N 13.51; found: C 57.81, H 5.71, N 13.48 %.

***N*-(2-(4-(2-(Diethylamino)ethoxy)3-fluorophenyl)benzothiazol-6-yl)picolinimidamide dihydrochloride (29b)**

Using the above-mentioned procedure from **23b** (100 mg, 0.28 mmol) and compound **28** (201.2 mg, 0.56 mmol) compound **29b** was obtained as a beige powder (94.1 mg, 62.6 %, m.p. 209–212 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 12.15 (1H, s, NH), 11.33 (1H, s, NH), 10.26 (1H, s, NH), 9.41 (1H, s, NH), 8.91 (1H, d, *J* = 4.1 Hz), 8.63 (1H, d, *J* = 7.9 Hz), 8.33 (1H, d, *J* = 1.3 Hz), 8.27 – 8.15 (2H, m), 8.05 – 7.93 (2H, m), 7.87 (1H, dd, *J* = 7.4, 4.9 Hz), 7.62 (1H, dd, *J* = 8.7, 1.6 Hz), 7.45 (1H, t, *J* = 8.6 Hz), 4.65 (2H, t, *J* = 4.6 Hz, OCH_2), 3.57 (2H, d, *J* = 4.4 Hz, NCH_2), 3.32 – 3.15 (4H, m, NCH_2), 1.29 (6H, t, *J* = 7.2 Hz, CH_3). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 167.33, 159.78, 153.11, 151.62 (d, J_{CF} = 246.3 Hz), 149.77, 148.27 (d, J_{CF} = 10.9 Hz), 144.32, 138.33, 135.47, 131.59, 128.62, 126.24 (d, J_{CF} = 6.9 Hz), 125.12, 124.52 (d, J_{CF} = 3.1 Hz), 124.29, 123.72, 120.46, 115.55, 114.52 (d, J_{CF} = 19.7 Hz), 63.96 (OCH_2), 49.27 (NCH_2), 47.06 (NCH_2), 8.42 (CH_3). Anal. calc. for $C_{25}H_{26}FN_5OS$ x 2HCl (Mr = 536.49): C 55.97, H 5.26, N 13.05; found: C 55.92, H 5.32, N 13.00 %.

***N*-(2-(4-(2-(Diethylamino)ethoxy)3-methoxyphenyl)benzothiazol-6-yl)picolinimidamide dihydrochloride (29c)**

Using the above-mentioned procedure from **23c** (100 mg, 0.27 mmol) and compound **28** (194.0 mg, 0.54 mmol) compound **29c** was obtained as a yellow powder (123.0 mg, 83.1 %, m.p. 188–191 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 12.11 (1H, s, NH), 11.18 (1H, s, NH), 10.23 (1H, s, NH), 9.40 (1H ,s, NH), 8.91 (1H, d, *J* = 4.2 Hz), 8.61 (1h, d, *J* = 8.0 Hz), 8.31 (1H, d, *J* = 1.5 Hz), 8.23 (1H, dd, *J* = 13.3, 4.1 Hz), 8.19 (1H, d, *J* = 8.7 Hz), 7.87 (1H, dd, *J* = 7.4, 4.9 Hz), 7.69 – 7.56 (2H, m), 7.41 (1H, s, *J* = 7.5 Hz), 7.26 – 7.21 (1H, m), 4.54 (2H, d, *J* = 4.8 Hz, OCH_2), 3.93 (3H, s, OCH_3), 3.54 (2H, d, *J* = 4.5 Hz, NCH_2), 3.31 – 3.15 (4H, m, NCH_2), 1.29 (6H, t, *J* = 7.2 Hz, CH_3). ^{13}C NMR (151 MHz, DMSO) (δ/ppm) 168.63, 159.78, 153.25, 150.04, 149.78, 149.43, 144.34, 138.34, 135.33, 131.34, 128.62, 126.22, 124.97, 124.25, 123.52, 121.02, 120.33, 113.67, 109.78, 63.60 (OCH_2), 55.93 (OCH_3), 49.28 (NCH_2), 47.06 (NCH_2), 8.45 (CH_3). Anal. calc. for $C_{26}H_{29}N_5O_2S$ x 2HCl (Mr = 548.53): C 56.93, H 5.70, N 12.77; found: C 56.84, H 5.74, N 12.62 %.

***N*-(2-(4-(2-Morpholinoethoxy)phenyl)benzothiazol-6-yl)picolinimidamide dihydrochloride (30a)**

Using the above-mentioned procedure from **24a** (100 mg, 0.28 mmol) and compound **28** (201.2 mg, 0-56 mmol) compound **30a** was obtained as a yellow powder (81.9 mg, 58.9 %, m.p. 164–167 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 12.15 (1H, s, NH), 11.98 (1H, s, NH), 10.26 (1H, s, NH), 9.40 (1H, s, NH), 8.92 (1H, d, *J* = 4.2 Hz), 8.65 (1H, d, *J* = 7.9 Hz), 8.32 (1H, s), 8.24 (1H, t, *J* = 7.3 Hz), 8.20 – 8.15 (1H, m), 8.12 (2H, d, *J* = 8.7 Hz), 7.88 (1H, dd, *J* = 7.4, 4.8 Hz), 7.61 (1H ,d, *J* = 8.7 Hz), 7.23 (2H, d, *J* = 8.8 Hz), 4.62 (2H, s, OCH_2), 3.94 (4H, q, *J* = 11.6 Hz, OCH_2), 3.62 (2H, s, NCH_2), 3.52 (2H, d, *J* = 12.1 Hz, NCH_2), 3.27 (2H, s, NCH_2). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 168.49, 160.21, 159.80, 153.34, 149.78, 144.33, 138.34, 135.27, 131.28, 129.05, 128.62, 125.89, 124.97, 124.25, 123.50, 120.36, 115.59,

63.05 (OCH_2), 62.62 (OCH_2), 54.58 (NCH_2), 51.59 (NCH_2). Anal. calc. for $C_{25}H_{25}N_5O_2S$ x 2HCl (Mr = 532.48): C 56.39, H 5.11, N 13.15; found: C 56.31, H 5.15, N 13.09 %.

***N*-(2-(3-Fluoro-4-(2-morpholinoethoxy)phenyl)benzothiazol-6-yl)picolinimidamide dihydrochloride (30b)**

Using the above-mentioned procedure from **24b** (100 mg, 0.27 mmol) and compound **28** (194.0 mg, 0.54 mmol) compound **30b** was obtained as a beige powder (89.6 mg, 64.6 %, m.p. 212–216 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 12.11 (2H, bs, NH), 10.24 (1H, s, NH), 9.40 (1H, s, NH), 8.91 (1H, d, *J* = 4.1 Hz), 8.60 (1H, d, *J* = 7.9 Hz), 8.33 (1H, d, *J* = 1.4 Hz), 8.22 (2H, dd, *J* = 18.1, 7.8 Hz), 8.05 – 7.92 (2H, m), 7.87 (1H, dd, *J* = 7.3, 4.9 Hz), 7.66 – 7.58 (1H ,m), 7.46 (1H, t, *J* = 8.6 Hz), 4.77 – 4.64 (2H, m, OCH_2), 4.04 – 3.81 (4H, m, *J* = 23.6, 11.8 Hz, OCH_2), 3.64 (2H, s, NCH_2), 3.52 (2H, d, *J* = 11.8 Hz, NCH_2), 3.26 (2H, d, *J* = 10.9 Hz, NCH_2). ^{13}C NMR (151 MHz, DMSO) (δ/ppm) 167.32, 159.81, 153.12, 151.66 (d, J_{CF} = 246.3 Hz), 149.83, 149.79, 148.19 (d, JC_F = 10.9 Hz), 144.33, 138.35, 138.26, 135.48, 131.58, 128.63, 128.49, 126.32 (d, J_{CF} = 6.9 Hz), 125.13, 124.52 (d, J_{CF} = 3.0 Hz), 124.23, 123.74, 123.41, 120.47, 115.72, 114.56 (d, J_{CF} = 20.1 Hz)., 64.01 (OCH_2), 63.11 (OCH_2), 54.53 (NCH_2), 51.69 (NCH_2). Anal. calc. for $C_{25}H_{24}FN_5O_2S$ x 2HCl (Mr = 550.47): C 54.55, H 4.76, N 12.72; found: C 54.48, H 4.83, N 12.64 %.

***N*-(2-(3-Methoxy-4-(2-morpholinoethoxy)phenyl)benzothiazol-6-yl)picolinimidamide dihydrochloride (30c)**

Using the above-mentioned procedure from **24c** (100 mg, 0.26 mmol) and compound **28** (186.8 mg, 0.52 mmol) compound **30c** was obtained as a brown powder (71.4 mg, 52.2 %, m.p. 173–176 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 12.12 (1H, s, NH), 12.05 (1H, s, NH), 10.24 (1H ,s, NH), 9.40 (1H, s, NH), 8.91 (1H ,d, *J* = 4.3 Hz), 8.62 (1H, d, *J* = 7.9 Hz), 8.31 (1H, d, *J* = 1.5 Hz), 8.27 – 8.14 (2H, m), 7.87 (1H, dd, *J* = 7.4, 4.9 Hz), 7.71 (1H, s), 7.65 (1H, dd, *J* = 18.1, 1.8 Hz), 7.59 (1H, s), 7.29 – 7.20 (1H ,m), 4.60 (2H, d, *J* = 4.8 Hz, OCH_2), 4.01 – 3.83 (7H, m, OCH_2, OCH_3), 3.65 – 3.49 (4H, m, NCH_2), 3.26 (2H, dd, *J* = 18.2, 7.6 Hz, NCH_2). ^{13}C NMR (151 MHz, DMSO) (δ/ppm) 168.61, 159.78, 153.24, 149.97, 149.77, 149.49, 144.33, 138.34, 135.34, 131.35, 128.61, 126.34, 124.98, 124.27, 123.53, 121.01, 120.33, 114.01, 109.85, 63.76 (OCH_2), 63.10 (OCH_2), 55.93 (OCH_3), 54.60 (NCH_2), 51.79 (NCH_2). Anal. calc. for $C_{26}H_{27}N_5O_3S$ x 2HCl (Mr = 562.51): C 55.52, H 5.20, N 12.45; found: C 55.46, H 5.27, N 12.37 %.

***N*-(2-(4-(2-Morpholino-2-oxoethoxy)phenyl)benzothiazol-6-yl)picolinimidamide hydrochloride (31a)**

Using the above-mentioned procedure from **25a** (100 mg, 0.27 mmol) and compound **28** (194.0 mg, 0.54 mmol) compound **31a** was obtained as a yellow powder (52.2 mg, 37.9 %, m.p. 156–158 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 12.07 (1H, s, NH), 10.21 (1H, s, NH), 9.37 (1H, s, NH), 8.92 (1H ,d, *J* = 4.2 Hz), 8.58 (1H, d, *J* = 7.9 Hz), 8.30 (1H, d, *J* = 1.3 Hz), 8.24 (1H, t, *J* = 7.4 Hz), 8.17 (1H, d, *J* = 8.6 Hz), 8.07 (2H, d, *J* = 8.8 Hz), 7.87 (1H, dd, *J* = 7.3, 4.9 Hz), 7.60 (1H, dd, *J* = 8.7, 1.6 Hz), 7.14 (2H, d, *J* = 8.8 Hz), 5.01 (2H, s, OCH_2), 3.64 (2H, s, NCH_2), 3.59 (2H, s, NCH_2), 3.48 (4H, s, OCH_2). ^{13}C NMR (75 MHz,

DMSO) (δ/ppm) 168.64, 165.59 (C=O), 160.92, 159.83, 153.39, 149.79, 144.35, 138.35, 135.26, 131.21, 128.87, 128.61, 125.46, 124.92, 124.17, 123.47, 120.32, 115.52, 65.99 (OCH_2), 65.68 (OCH_2), 44.63 (NCH_2), 41.59 (NCH_2). Anal. calc. for $C_{25}H_{23}N_5O_3S$ x HCl (Mr = 510.01): C 58.88, H 4.74, N 13.73; found: C 58.74, H 4.69, N 13.62 %.

***N*-(2-(3-Fluoro-4-(2-morpholino-2-oxoethoxy)phenyl)benzothiazol-6-yl)picolinimidamide hydrochloride (31b)**

Using the above-mentioned procedure from **25b** (100 mg, 0.26 mmol) and compound **28** (186.8 mg, 0.52 mmol) compound **31b** was obtained as a white powder (117.6 mg, 85.7 %, m.p. 172–175 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 12.12 (1H, s, NH), 10.25 (1H, s, NH), 9.39 (1h, s, NH), 8.92 (1H, d, *J* = 4.2 Hz), 8.61 (1H, d, *J* = 7.9 Hz), 8.33 (1H, d, *J* = 1.5 Hz), 8.22 (2H, dd, *J* = 18.7, 8.2 Hz), 7.98 (1H, dd, *J* = 11.9, 2.0 Hz), 7.87 (2H, dd, *J* = 7.3, 5.5 Hz), 7.62 (1H, dd, *J* = 8.7, 1.7 Hz), 7.37 – 7.24 (1H, m), 5.14 (2h, s, OCH_2), 3.65 (2H, s, NCH_2), 3.59 (2H, s, NCH_2), 3.48 (4H, s, OCH_2). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 167.45, 165.19 (C=O), 159.80, 151.53 (d, J_{CF} = 245.9 Hz), 149.90, 148.94 (d, J_{CF} = 10.4 Hz), 144.33, 138.34, 135.45, 131.50, 128.62, 125.69 (d, J_{CF} = 7.0 Hz), 125.08, 124.23, 124.18 (d, J_{CF} = 3.0 Hz), 123.67, 120.43, 115.68 (d, J_{CF} = 1.2 Hz), 114.44 (d, J_{CF} = 20.4 Hz), 66.06 (OCH_2), 65.99 (OCH_2), 65.92 (OCH_2), 44.51 (NCH_2), 41.59 (NCH_2). Anal. calc. for $C_{25}H_{22}FN_5O_3S$ x HCl (Mr = 527.99): C 56.87, H 4.39, N 13.26; found: C 56.73, H 4.43, N 13.15 %.

***N*-(2-(3-Methoxy-4-(2-morpholino-2-oxoethoxy)phenyl)benzothiazol-6-yl)picolinimidamide hydrochloride (31c)**

Using the above-mentioned procedure from **25c** (100 mg, 0.25 mmol) and compound **28** (179.6 mg, 0.50 mmol) compound **31c** was obtained as a yellow powder (107.6 mg, 79.7 %, m.p. 185–188 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 12.11 (1H, s, NH), 10.24 (1H, s, NH), 9.39 (1H, s, NH), 8.92 (1H, d, *J* = 4.2 Hz), 8.61 (1H, d, *J* = 7.9 Hz), 8.30 (1H, d, *J* = 1.4 Hz), 8.24 (1H, t, *J* = 8.4 Hz), 8.18 (1H, d, *J* = 8.7 Hz), 7.87 (1H, dd, *J* = 7.4, 4.8 Hz), 7.69 (1H, d, *J* = 1.8 Hz), 7.62 (2H, t, *J* = 9.1 Hz), 7.07 (1H, d, *J* = 8.5 Hz), 5.00 (2H, s, OCH_2), 3.93 (3H, s, OCH_3), 3.62 (2H, d, *J* = 8.6 Hz, NCH_2), 3.59 (2H, s, NCH_2), 3.55 – 3.40 (4H, m, OCH_2). ^{13}C NMR (151 MHz, DMSO) (δ/ppm) 169.30, 166.06 (C=O), 160.32, 153.82, 151.11, 150.32, 149.72, 144.87, 138.89, 135.83, 131.75, 129.14, 126.13, 125.46, 124.67, 124.00, 121.37, 120.83, 113.97, 110.27, 66.62 (OCH_2), 66.55 (OCH_2), 66.50 (OCH_2), 56.29 (OCH_3), 45.24 (NCH_2), 42.12 (NCH_2). Anal. calc. for $C_{25}H_{23}N_5O_3S$ x HCl (Mr = 540.03): C 57.83, H 4.85, N 12.97; found: C 57.72, H 4.89, N 12.88 %.

***N*-(2-(4-(2-Oxo-2-phenylethoxy)phenyl)benzothiazol-6-yl)picolinimidamide hydrochloride (32a)**

Using the above-mentioned procedure from **26a** (100 mg, 0.28 mmol) and compound **28** (201.2 mg, 0.56 mmol) compound **32a** was obtained as a yellow powder (124.1 mg, 88.5 %, m.p. 198–201 °C). ^{1}H NMR (300 MHz, DMSO) (δ/ppm) 12.09 (1H, s, NH), 10.22 (1H, s, NH), 9.37 (1H, s, NH), 8.91 (1H, d, *J* =

4.1 Hz), 8.59 (1H, d, *J* = 8.0 Hz), 8.30 (1H, d, *J* = 1.5 Hz), 8.24 (1H, t, *J* = 7.8 Hz), 8.17 (1H, d, *J* = 8.6 Hz), 8.06 (4H, dd, *J* = 8.0, 2.8 Hz), 7.87 (1H, dd, *J* = 7.3, 4.9 Hz), 7.73 (1H, t, *J* = 7.4 Hz), 7.60 (3H, t, *J* = 7.4 Hz), 7.19 (2H, d, *J* = 8.9 Hz), 5.77 (2H, s, OCH_2). ^{13}C NMR (151 MHz, DMSO) (δ/ppm) 194.58 (C=O), 169.10, 161.29, 160.27, 153.85, 150.27, 144.82, 138.84, 135.74, 134.69, 134.43, 131.75, 129.44, 129.37, 129.12, 128.39, 126.00, 125.45, 124.77, 123.96, 120.85, 116.05, 70.81 (OCH_2). Anal. calc. for $C_{27}H_{20}N_4O_2S$ x HCl (Mr = 501.00): C 64.73, H 4.23, N 11.18; found: C 64.64, H 4.28, N 11.13 %.

***N*-(2-(3-Fluoro-4-(2-oxo-2-phenylethoxy)phenyl)benzothiazol-6-yl)picolinimidamide hydrochloride (32b)**

Using the above-mentioned procedure from **26b** (100 mg, 0.26 mmol) and compound **28** (186.8 mg, 0.52 mmol) compound **32b** was obtained as a yellow powder (128.8 mg, 95.5 %, m.p. 191–194 °C). ^1H NMR (300 MHz, DMSO) (δ/ppm) 12.09 (1H, s, NH), 10.22 (1H, s, NH), 9.38 (1H, s, NH), 8.91 (1H, d, *J* = 4.2 Hz), 8.58 (1H, d, *J* = 8.0 Hz), 8.32 (1H, d, *J* = 1.5 Hz), 8.27 – 8.16 (2H, m), 8.08 – 7.96 (3H, m), 7.90 – 7.83 (2H, m), 7.73 (1H, t, *J* = 7.4 Hz), 7.60 (3H, t, *J* = 7.5 Hz), 7.35 (1H, t, *J* = 8.7 Hz), 5.89 (2H, s, OCH_2). ^{13}C NMR (151 MHz, DMSO) (δ/ppm) 193.63 (C=O), 167.45, 159.82, 153.16, 151.53 (d, J_{CF} = 245.9 Hz), 149.78, 148.78 (d, J_{CF} = 10.4 Hz), 144.33, 138.35, 135.46, 134.05, 133.99, 131.49, 128.86, 128.61, 127.88, 125.82 (d, J_{CF} = 6.8 Hz), 125.08, 124.22 (d, J_{CF} = 2.9 Hz), 124.18, 123.69, 120.43, 115.68, 114.58 (d, J_{CF} = 20.2 Hz), 70.87 (OCH_2). Anal. calc. for $C_{27}H_{19}FN_4O_2S$ x HCl (Mr = 518.99): C 62.49, H 3.88, N 10.80; found: C 62.37, H 3.92, N 10.84 %.

***N*-(2-(3-Methoxy-4-(2-oxo-2-phenylethoxy)phenyl)benzothiazol-6-yl)picolinimidamide hydrochloride (32c)**

Using the above-mentioned procedure from **26c** (100 mg, 0.26 mmol) and compound **28** (186.8 mg, 0.52 mmol) compound **32c** was obtained as a yellow powder (77.5 mg, 56.1 %, m.p. 158-161 °C). ^1H NMR (300 MHz, DMSO) (δ/ppm) 12.06 (1H, s, NH), 10.21 (1H, s, NH), 9.38 (1H, s, NH), 8.91 (1H, d, *J* = 4.2 Hz), 8.57 (1H, d, *J* = 7.9 Hz), 8.29 (1H, d, *J* = 1.5 Hz), 8.28 – 8.21 (1H, m), 8.19 (1H, d, *J* = 8.7 Hz), 8.05 (2H, d, *J* = 7.2 Hz), 7.87 (1H, dd, *J* = 7.4, 4.9 Hz), 7.76 – 7.68 (2H, m), 7.59 (4H, dd, *J* = 8.8, 5.9 Hz), 7.09 (1H, d, *J* = 8.6 Hz), 5.76 (2H, s, OCH_2), 3.93 (3H, d, *J* = 12.6 Hz, OCH_3). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 194.05 (C=O), 168.76, 159.81, 153.30, 150.52, 149.79, 149.20, 144.35, 138.35, 135.31, 134.23, 133.89, 131.24, 128.84, 128.62, 127.88, 125.67, 124.93, 124.16, 123.48, 120.86, 120.30, 113.48, 109.93, 70.59 (OCH_2), 55.80 (OCH_3). Anal. calc. for $C_{28}H_{22}N_4O_3S$ x HCl (Mr = 531.03): C 63.33, H 4.37, N 10.55; found: C 63.27, H 4.41, N 10.48 %.

***N*-(2-(4-(Pyridin-2-ylmethoxy)phenyl)benzothiazol-6-yl)picolinimidamide hydrochloride (33a)**

Using the above-mentioned procedure from **27a** (100 mg, 0.30 mmol) and compound **28** (215.6 mg, 0.60 mmol) compound **33a** was obtained as a white powder (67.0 mg, 50.8 %, m.p. 235–238 °C). ^1H NMR (300 MHz, DMSO) (δ/ppm) 12.14 (1H, s, NH)), 10.26 (1H, s, NH), 9.40 (1H, s, NH), 8.91 (2H, t, *J* =

4.9 Hz), 8.63 (1H, d, *J* = 7.9 Hz), 8.48 (1H, t, *J* = 7.8 Hz), 8.32 (1H, d, *J* = 1.6 Hz), 8.24 (1H, dd, *J* = 8.4, 7.4 Hz), 8.18 (1H, d, *J* = 8.8 Hz), 8.14 (2H, d, *J* = 8.8 Hz), 8.07 (1H, d, *J* = 7.9 Hz), 7.95 – 7.84 (2H, m), 7.61 (1H, dd, *J* = 8.6, 1.7 Hz), 7.32 (2H, d, *J* = 8.9 Hz), 5.63 (2H, s, OCH_2). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 168.39, 160.12, 159.78, 153.31, 151.97, 149.77, 144.33, 144.20, 143.70, 138.33, 135.29, 131.34, 129.14, 128.61, 126.21, 125.74, 124.98, 124.87, 124.27, 123.53, 120.37, 115.79, 66.58 (OCH_2). Anal. calc. for $C_{25}H_{19}N_5OS$ x HCl (Mr = 473.98): C 63.35, H 4.25, N 14.78; found: 19. 58.74, H 4.31, N 14.69 %.

***N*-(2-(3-Fluoro-4-(pyridin-2-ylmethoxy)phenyl)benzothiazol-6-yl)picolinimidamide hydrochloride (33b)**

Using the above-mentioned procedure from **27b** (100 mg, 0.28 mmol) and compound **28** (201.2 mg, 0.56 mmol) compound **33b** was obtained as a yellow powder (71.7 mg, 52.1 %, m.p. 223–226 °C). ^1H NMR (300 MHz, DMSO) (δ/ppm) 12.16 (1H, s, NH), 10.27 (1H, s, NH), 9.42 (1H, s, NH), 8.92 (1H, d, *J* = 4.2 Hz), 8.85 (1H, d, *J* = 4.7 Hz), 8.64 (1H, d, *J* = 7.9 Hz), 8.40 – 8.31 (2H, m), 8.28 – 8.16 (2H, m), 8.04 (1H, dd, *J* = 11.8, 2.0 Hz), 7.97 (2H, d, *J* = 7.6 Hz), 7.87 (1H, dd, *J* = 7.4, 5.0 Hz), 7.85 – 7.77 (1H, m), 7.63 (1H, dd, *J* = 8.7, 1.6 Hz), 7.52 (1H, t, *J* = 8.6 Hz), 5.63 (2H, s, OCH_2). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 167.26, 159.78, 153.10, 152.30, 151.72 (d, J_{CF} = 246.5 Hz), 149.77, 148.30 (d, J_{CF} = 10.8 Hz), 145.03, 144.32, 142.78, 138.34, 135.49, 131.61, 128.62, 126.52 (d, J_{CF} = 6.8 Hz), 125.38, 125.13, 124.53 (d, J_{CF} = 2.7 Hz), 124.39, 124.29, 123.74, 120.46, 115.99, 114.71 (d, J_{CF} = 19.9 Hz), 68.27 (OCH_2). Anal. calc. for $C_{25}H_{18}FN_5OS$ x HCl (Mr = 491.97): C 61.04, H 3.89, N 14.24; found: C 60.99, H 3.94, N 14.17 %.

***N*-(2-(3-Methoxy-4-(pyridin-2-ylmethoxy)phenyl)benzothiazol-6-yl)picolinimidamide hydrochloride (33c)**

Using the above-mentioned procedure from **27c** (100 mg, 0.27 mmol) and compound **28** (194.0 mg, 0.54 mmol) compound **33c** was obtained as an off-white powder (60.4 mg, 44.4 %, m.p. 231–234 °c). ^1H NMR (300 MHz, DMSO) (δ/ppm) 12.09 (1H, s, NH), 10.23 (1H, s, NH), 9.40 (1H, s, NH), 8.92 (1H, d, *J* = 4.2 Hz), 8.86 (1H, d, *J* = 4.8 Hz), 8.59 (1H, d, *J* = 7.9 Hz), 8.39 (1H, dt, *J* = 7.8, 3.9 Hz), 8.31 (1H, d, *J* = 1.5 Hz), 8.23 (2H, dd, *J* = 17.6, 8.2 Hz), 7.97 (1H, d, *J* = 7.9 Hz), 7.91 – 7.80 (2H, m), 7.75 (1H, d, *J* = 1.8 Hz), 7.68 (1H, dd, *J* = 8.4, 1.8 Hz), 7.61 (1H, dd, *J* = 8.7, 1.7 Hz), 7.30 (1H, d, *J* = 8.5 Hz), 5.54 (2H, s, OCH_2), 3.96 (3H, s, OCH_3). ^{13}C NMR (75 MHz, DMSO) (δ/ppm) 168.56, 159.79, 153.25, 152.68, 149.95, 149.79, 149.54, 144.49, 144.35, 143.26, 138.35, 135.36, 131.36, 128.61, 126.54, 125.39, 124.98, 124.52, 124.20, 123.56, 120.98, 120.33, 114.22, 109.95, 67.79 (OCH_2), 55.86 (OCH_3). Anal. calc. for $C_{26}H_{21}N_5O_2S$ x HCl (Mr = 504.01): C 61.96, H 4.40, N 13.90; found: C 61.92, H 4.44, N 13.83 %.

4.3. Antitrypanosomal and cytotoxicity screening

To assess trypanocidal activity, *in vitro* assays were carried out using bloodstream form *T. b. brucei* 221a (MiTat1.2a) as previously described [25], using a 96-well microtiter plate format (200 µL volumes). Experiments were initiated at 2.5×10^4 parasites/mL, compounds added and the plates

incubated at 37 °C for 48 hours. Resazurin (20 µL at 0.125 mg/mL) was then added, and the plates incubated for a further 16 hours. Fluorescence was determined using a BMG FLUOstar Omega plate reader (excitation 545 nm, emission 590 nm), and the data analyzed using GraphPad Prism 9.0 software. The values are presented as IC_{50} ± SEM and were derived from three independent replicates. For cytotoxicity assays, HeLa cells were seeded into microtiter plates at 1×10^4 /mL in 200 µL DMEM medium with 10% FBS, and test compounds added. Plates were incubated for 5 days at 37°C and 20 µL resazurin then added to each well. After a further 6 hours, fluorescence was determined as outlined above.

4.4. Binding with nucleic acids

The UV/Vis spectra were recorded on a Varian Cary 100 Bio spectrophotometer (Agilent, Santa Clara, CA, USA), CD spectra on a *J*ASCO *J*815 spectrophotometer (ABL&E Handels GmbH, Wien, Austria), and fluorescence spectra on a Varian Cary Eclipse spectrophotometer (Agilent, Santa Clara, CA, USA) at 25°C using appropriate 1 cm path quartz cuvettes. Calf thymus DNA, ctDNA, and rArU were purchased from Sigma-Aldrich (St. Louis, MI, USA) and dissolved in Na-cacodylate buffer, I = 0.05 mol dm^{-3}, pH = 7.0. The ctDNA was additionally sonicated and filtered through a 0.45 mm filter [52]. DNA and RNA concentration was determined spectroscopically as the concentration of phosphates [53]. Spectrophotometric titrations were performed at pH = 7.0 (I = 0.05 mol dm^{-3}, sodium cacodylate buffer) by adding portions of polynucleotide solution into the solution of the studied compound for fluorimetric experiments, and CD experiments were performed by adding portions of the compound stock solution into the solution of a polynucleotide. In fluorimetric experiments, an excitation wavelength of $\lambda_{exc} \geq 300$ nm was used to avoid the inner filter effect caused by increasing absorbance of the polynucleotide. Emission was collected in the range λ_{em}= 380–600 nm. Values for K_a were obtained by processing titration data using the Scatchard equation (Table 3). Most of them had satisfactory correlation coefficients (> 0.99). Thermal melting curves for DNA/RNA and their complexes with studied compounds were determined as previously described by following the absorption change at 260 nm as a function of temperature. The absorbance of the ligands was subtracted from every curve and the absorbance scale was normalized. T_m values are the midpoints of the transition curves determined from the maximum of the first derivative and checked graphically by the tangent method. The ΔT_m values were calculated by subtracting the T_m of the free nucleic acid from the T_m of the complex. Every ΔT_m value reported here was the average of at least two measurements. The error in ΔT_m is ± 0.5°C.

4.5. ADME

4.5.1. MDCKII-MDR1 permeability assay

MDCKII-hMDR1 cells were obtained from Solvo Biotechnology, Hungary. DMEM, fetal bovine serum, Glutamax-100, Antibiotic/Antimycotic, DMSO, Dulbecco's phosphate buffer saline, MEM non-essential amino acids were purchased from Sigma (St. Louis, MO, USA). Bi-directional permeability and P-glycoprotein substrate assessment were investigated in Madin-Darby canine epithelial cells that over-expressed the human MDR1 gene (MDCKII-MDR1), coding for P-glycoprotein. Experimental procedures, as well as cell culture conditions, were the same as previously described [54]. Briefly, compounds (10 µM, 1% DMSO v/v) in duplicate were incubated at 37 °C for 60 min with cell monolayers on 96-well Millicell inserts (Millipore, Burlington, MA, USA), without and with the P-glycoprotein inhibitor Elacridar (2 µM, International Laboratory, USA). Inhibition of P-glycoprotein was verified by amprenavir (Moravek Biochemicals Inc, Brea, CA, USA), a known P-gp substrate, while cellmonolayer integrity was checked by Lucifer yellow (Sigma, St. Louis, MO, USA). Compound concentrations were measured by LC-MS/MS and Lucifer yellow was measured on an Infinite F500 (Tecan, Männedorf, CH), using excitation of 485 nm and emission of 530 nm.

4.5.2. Metabolic stability

Mouse liver microsomes were obtained from Corning Life Sciences (Corning, USA). DMSO, nicotinamide adenine dinucleotide phosphate (NADP), glucose-6-phosphate, glucose-6-phosphate dehydrogenase, magnesium chloride, propranolol, caffeine, diclofenac, phosphate buffer saline (PBS) were purchased from Sigma (St. Louis, MO, USA). Acetonitrile (ACN) and methanol (MeOH) were obtained from Merck (Darmstadt, Germany). Testosterone was purchased from Steraloids (Newport, RI, USA). Metabolic stability was assessed in mouse liver microsomes. Compounds (final concentration of 1 µM, 0.03% DMSO v/v) were incubated in duplicate in phosphate buffer (50 mM, pH 7.4) at 37°C together with mouse liver microsomes in the absence and presence of the NADPH cofactor (0.5 mM nicotinamide adenine dinucleotide phosphate, 5 mM glucose-6-phosphate, 1.5 U/mL glucose-6-phosphate dehydrogenase and 0.5 mM magnesium chloride). Incubation and sampling was performed on a Freedom EVO 200 (Tecan, Männedorf, CH) at 0.3, 10, 20, 30, 45 and 60 min. The reaction was quenched using 3 volumes of a mixture of ACN/MeOH (2 : 1) containing internal standard (diclofenac), centrifuged and supernatants were analysed using LC-MS/MS.

Metabolic activity of microsomes was verified by simultaneous analysis of several controls including testosterone, propranolol and caffeine. The *in vitro* half-life ($t_{1/2}$) was calculated using GraphPad Prism non-linear regression of % parent compound remaining versus time. *In vitro* clearance, expressed as µL/min/mg, was estimated from the *in vitro* half-life ($t_{1/2}$), and normalized for the protein amount in the incubation mixture and assuming 52.5 mg of protein per gram of liver and

using constant values for mouse liver weight/body weight [87.5 g/kg] and mouse liver blood flow (LBF) [131 ml/min/kg].

4.5.3. LC-MS/MS analysis

All ADME samples were quantified using tandem mass spectrometry coupled to liquid chromatography. Samples were analysed on a Sciex API4000 or API4500 Triple Quadrupole Mass Spectrometer (Sciex, Division of MDS Inc., Toronto, Canada) coupled to a Shimadzu Nexera X2 UHPLC frontend (Kyoto, Japan). Samples were injected onto a UHPLC column (HALO2 C18, 2.1x20 mm, 2 µm; Luna Omega 1.6 µm Polar C18 100A, 30x2.1 mm; Waters Acquity UPLC BEH C18 1.7 µm, 2.1x50 mm) and eluted with a gradient at 50 °C. The mobile phase was composed of acetonitrile/water mixture (9/1, with 0.1 % formic acid) and 0.1 % formic acid in deionized water. The flow rate was 0.7 mL/min and under gradient conditions, leading to a total run time of 1–2 min. Positive ion mode with turbo spray and an ion source temperature of 600 °C were utilized for mass spectrometric detection. Quantitation was performed using multiple reaction monitoring (MRM) at the specific transitions for each compound.

4.6. Computational methods

Available X-ray structures of DNA complexes with small ligands were downloaded from the Protein data bank [55]. Crystal structure of the DB921-D(CGCGAATTCGCG)2 complex (pdb:2B0K) was used for the docking into minor groove while the intercalation of acridine-peptide drug in the crystal structure of [d(CGCGAATTCGCG)] (pdb:1G3X) was used for the intercalation studies. Compounds **12b** and **29b** were first docked using Glide [56] docking protocol with extra precision (XP) within Schrödinger suite of software. Molecular dynamic simulations were performed using Desmond software [57] within the Schroedinger package. Simulations were carried out at the room temperature for 100 ns. *In silico* ADME properties as well as structural parameters were calculated by SimulationPlus software [58].

4.7. *In vivo* imaging

For screening, we used the pleomorphic *T. b. brucei* GVR35-VSL2 strain that expresses a red-shifted luciferase transgene (*PpyRE9h*) [52]. Infections, were initiated by i.p. inoculation. Mice were maintained in individually ventilated cages, under specific pathogen-free conditions, with a 12-hour light/dark cycle, and given food and water *ad libitum*. Experiments were performed under UK Home Office project license P9AEE04E4, with consent of the LSHTM Animal Welfare and Ethical Review Board (AWERB). All protocols and procedures were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986 (ASPA). For *in vivo* bioluminescence imaging, mice were injected with 150 mg/kg d-luciferin i.p., anaesthetized using 2.5 % (v/v) isoflurane in oxygen for 2-3 min [25], and then imaged using an IVIS Spectrum (Revvity, Hopkinton, MA, USA). Exposure times varied from 1 sec to 5 min, depending on signal

intensity. After imaging, mice were revived and returned to cages. To estimate parasite burden, regions of interest were drawn using Living Image 4.7.3 to quantify bioluminescence expressed as total flux (photons/second; p/s). The detection threshold was established from uninfected mice.

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