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Article

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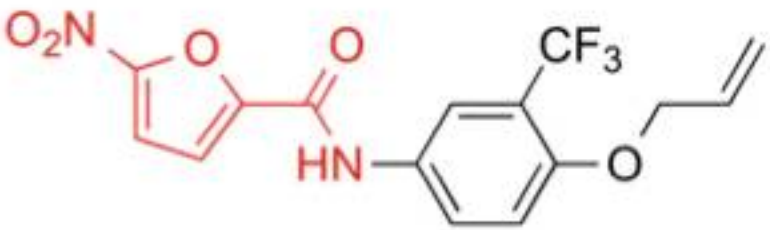
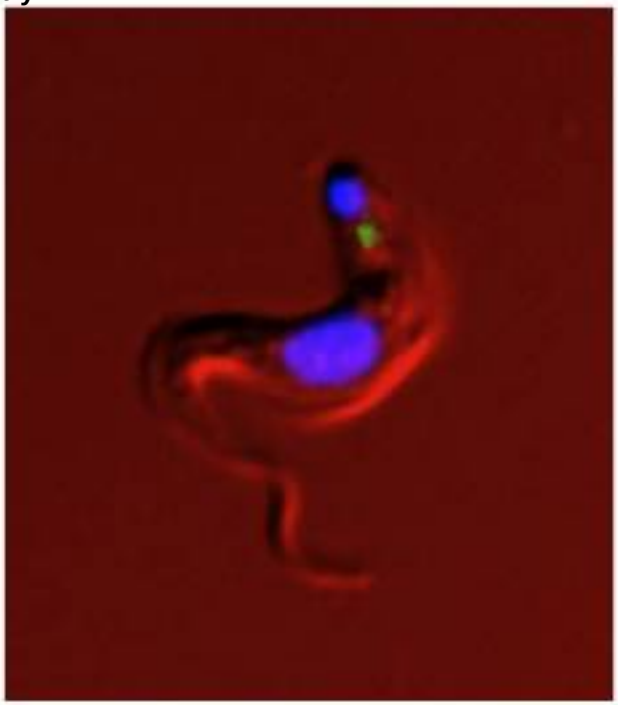


22s



	22s	Nifurtimox
T. brucei brucei	2.4 ± 0.3 nM	2397 ± 127 nM
T. brucei rhodesiense	2.9 ± 0.4 nM	1708 ± 197 nM

A class of 5-nitro-2-furancarboxylamides with potent trypanocidal activity against *Trypanosoma brucei in vitro*

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Running title: Potent nitro-furancarboxylamide analogues

ABSTRACT:

Recently the World Health Organization approved the nifurtimox-eflornithine combination therapy for the treatment of human African trypanosomiasis, renewing interest in nitro-heterocycles therapies for this and associated diseases. In this study we have synthesised a series of novel 5-nitro-2-furancarboxylamides that show potent trypanocidal activity, ~1000-fold more potent than nifurtimox against *in vitro Trypanosoma brucei* with very low cytotoxicity against human HeLa cells. More importantly, the most potent analog showed very limited cross-resistance to nifurtimox-resistant cells and vice-versa. This implies that our novel relatively ease to synthesise and therefore cheap, 5-nitro-2-furancarboxylamides are targeting a different, but still essential, biochemical process to those targeted by nifurtimox or its metabolites in the parasites. The significant increase in potency (smaller dose probably required) has the potential for greatly reducing unwanted side-effects and also reducing the likelihood of drug-resistance. Collectively these findings have important implications for the future therapeutic treatment of African sleeping sickness.

INTRODUCTION

Infectious diseases caused by parasitic protozoa affect ca. 15% of the global population and more than 65% of the population in the Third and developing world, yet current drug therapies for protozoan infections are woefully inadequate. As protozoan infections take their toll predominantly in the developing world, market forces are insufficient to promote the development of novel anti-protozoan drugs. In 2000, only ca. 0.1% of global investment in health research was spent on drug discovery for tropical diseases.

One such neglected parasitic disease is Human African Trypanosomiasis (HAT) or African sleeping sickness, which is caused by the protozoan parasite *Trypanosoma brucei*. The World Health Organization (WHO) estimates that HAT constitutes a serious health risk to 60 million people in sub-Saharan Africa, The WHO also estimates that there are <30 000 new cases per year in sub-Saharan Africa and at present an annual death toll of ~8,000.[1]

The related disease in cattle, cattle trypanosomiasis or Nagana, also represents a major health concern due to its devastating economic (estimated by the WHO to cause an annual economic loss of ~US$ 4 billion.), social and nutritional impact on African families. As such, the total burden of trypanosomiasis translates into 1,598,000 Disability-Adjusted Life Years (DALY). This is on a par with big killers such as tuberculosis and malaria.[2, 3]

Treatment of HAT is solely dependent upon a repertoire of four drugs: suramin (**1**), pentamidine (**2**), melarsoprol (**3**) and eflornithine (**4**) (Figure 1). These therapies are often toxic, difficult to administer and increasingly have an acquired drug resistance, highlighting the urgent need for new, more effective drug therapies. Developed before the 1950s, suramin (**1**) and melarsoprol (**3**) are used for chemotherapy of the early stage of the disease (*T. b. rhodesiense)*, as is pentamidine (**2**) (*T. b. gambiense)*. The arsenical melarsoprol (**3**) is extremely toxic, with death in ~ 6% of cases and treatment failure rates of as high as 30% in certain areas. Treatment of the second stage of the disease, where the parasites cross the

blood-brain-barrier and invade the central nervous system, is limited to melarsoprol (**3**) and eflornithine (**4**), the ornithine decarboxylase inhibitor (difluoromethylornithine).[4, 5]

Nifurtimox (**5**) (Figure 1), is often used to treat Chagas' disease, caused by *Trypanosoma cruzi,* and has been used as a monotherapy for melarsoprol-refractory HAT on compassionate grounds, despite its low efficacy and severe toxicity. The recent introduction of a nifurtimox-eflornithine combination therapy (NECT) by WHO in the Model Lists of Essential Medicines has been seen as highly advantageous in terms of cost, logistics, and human resources in areas of poverty. Patients given NECT, consisting of oral nifurtimox over 10 days with eflornithine (**4**) infusions for 7 days, were found to fair just as well as those given the eflornithine monotherapy, with cure rates of around 97%.[2]

The use of NECT has renewed general interest in the use of nitroheterocyclic compounds to treat a wide range of infectious disease, including tuberculosis and hepatitis C. [6-8] This increased awareness of the potential of nitroheterocyclic compounds has led to the reinvestigation of fexinidazole (**6**) (Figure 1), which is presently in clinical trials against both early- and late-stage African sleeping sickness.[6-8]

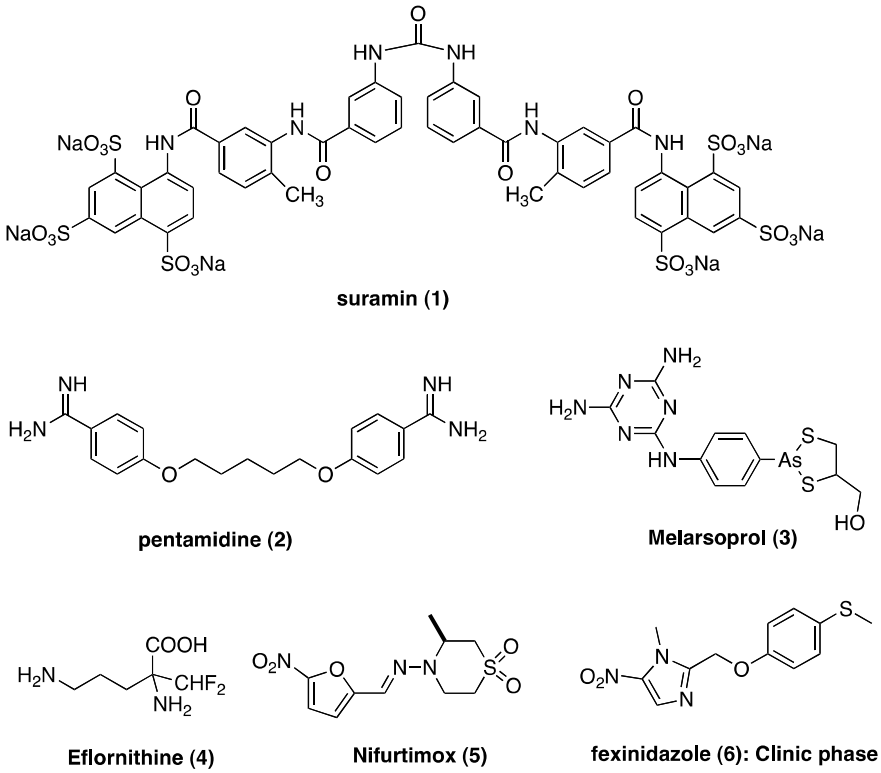


Figure 1. Structures of clinic drugs **1**-**5** for sleeping sickness and clinic phase I drug Fexinidazole (**6**).

Alarmingly, it has also been shown that *Trypanosoma brucei* nifurtimox-resistant cells show cross-resistance to other nitro-containing drugs, including fexinidazole, that are currently in clinical trials.[9, 10]. Several different types of studies on nitrohererocyclics have shed some light on this observed drug-resistance. Genome-wide RNAi screens of nifurtimox and benznidazole (another trypanocidal nitroheterocyclic drug) resistant *Trypanosoma brucei*, have identified that a decrease in nitroreductase activty is linked to nifurtimox-resistance.[9, 10] This was confirmed by showing that genetic deletion of one allele of the *T. brucei's* nitroreductase, led to nifurtimox-resistance. Likewise the overexpression of this nitroreductase, but not the alternative proposed prostaglandin F2α synthase and cytochrome P450 reductase, in *Trypanosoma brucei* resulted in increased susceptibility to nifurtimox.[11] Collectively, it is clear that this Type I *T. brucei* nitroreductase, a NAD(P)H dependent, flavin binding protein is involved in the reductive activation of nifurtimox. The nitroreductases mediate a series of two-electron reductions of the nitro group to a nitroso intermediate, then to a hydroxylamine and eventually to the

corresponding amine. However, the true function of the *T. brucei* mitochondrial nitroreductase is unknown, but its essentiality may explain why only a single allele deletion/mutation is associated with nifurtimox-resistance. [10, 11]

As well as the development of nifurtimox-resistance and its cross-resistance to other nitro-containing drugs, side-effects are also a major problem for nitro-containing drugs. For example, the adverse off-target side-effects with nifurtimox lead to treatment cessation in over 30% of patients with Chagas' disease.[12] The most common side-effects are anorexia, loss of weight, psychic alterations, excitability or sleepiness, digestive manifestations such as nausea or vomiting and occasionally intestinal colic and diarrhoea.[13] Various human enzymes have been identified to be capable of 5-nitrofuran reduction *in vitro*, in cells or tissues.[14-16] Recently we have elucidated in collaboration with the Patton laboratory a possible role of aldehyde dehydrogenase 2 (ALDH2) in the toxicity caused by 5-nitrofuran containing drugs such as nifurtimox.[17]

Whilst considerable effort has gone into the synthesis of nitro-aromatic containing compounds over the years[18-23], there remains several unexplored areas of chemical space. For example, in the context of nifurtimox most of the efforts have been focused on changing either the furan ring to other heterocycles (such as imidazole) or changing the substituent in the hydrazone motif,[18-23] with limited increases in activity compared to nifurtimox. Recently, two new types of typanocidal compounds, nitrobenzylphosphoramide mustards (for example see compound **7**, Figure 2)[24] and aziridinyl nitrobenzamides have been developed to target specifically *T. brucei* type I nitroreductase, the best having an EC_{50} ~ 1 μM against both *T. brucei* and *Trypanosoma cruzi*.[25, 26] Several 5-nitro-2-furancarboxylamide containing compounds have been reported to have potent antituberculosis and antimicrobial activity,[27-30] however, very few 5-nitro-2-furancarboxylamides are reported to have typanocidal activity, primarily low activity against *T. cruzi*.[31, 32] A nifurtimox analog (**8**) (Figure 2) showed trace activity against *T. cruzi* in a mouse model.[33] The best 5-nitro-2-furancarboxylamides identified thus far, **9** and **10,** have EC_{50} values of 42 nM and 262 nM respectively against *T. cruzi*

amastigotes (Figure 2) but have not been tested against *T. brucei*.[34] Several 5-nitro-2-furancarbohydrazides possess activity against *T. brucei* with the best, compound **11** having an EC_{50} of 0.13 μM, but low selectivity against human cells. [35]

In this study, we report that our recently discovered nitrofuran NFN1 (**12a**)[17] also shows promising activity against *T. brucei in vitro.* We have therefore synthesised a series of 5-nitro-2-furancarboxylamide analogs of **12a.** A lack of cross-resistance with nifurtimox and significantly increased (two to three orders of magnitude) trypanocidal activity has important implications for the future therapeutic use of these nitrofuran containing compounds in the treatment of HAT, instead of, or in combination with nifurtimox.

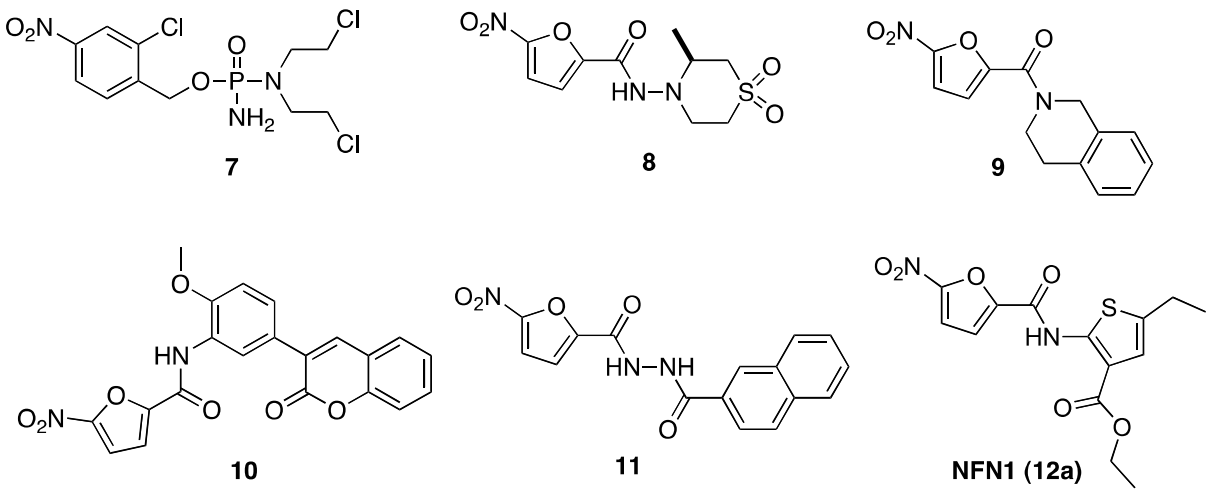
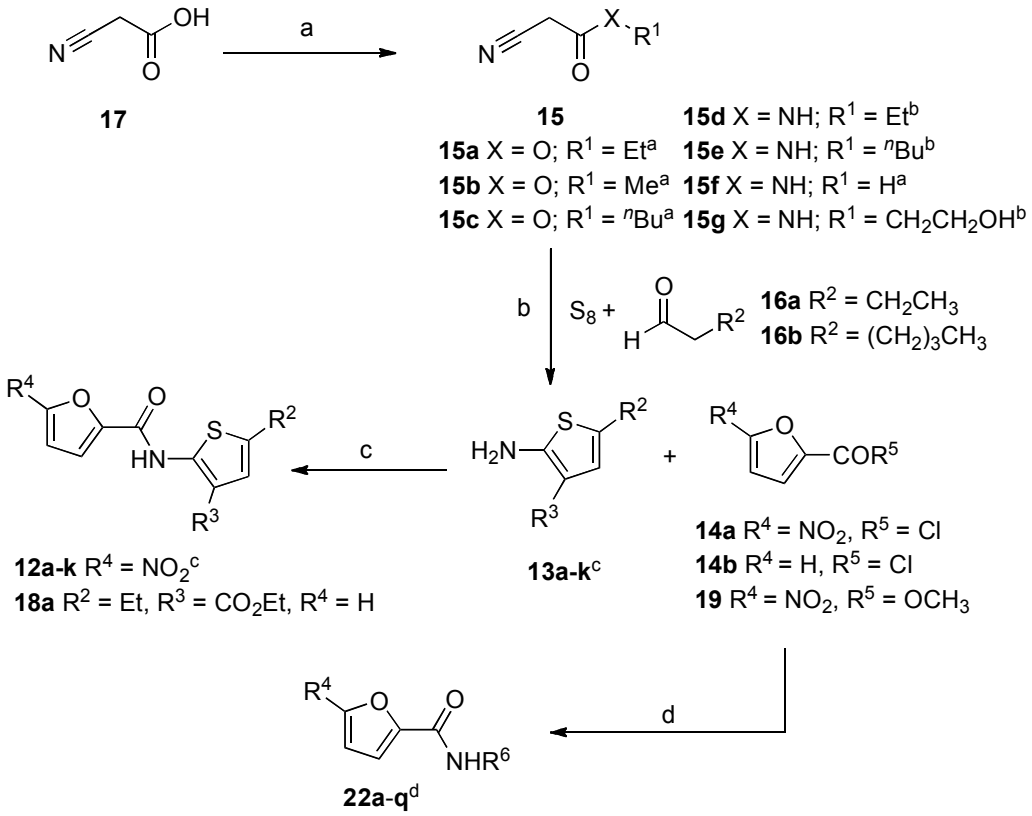


Figure 2. Structures of some reported bioactive nitroaromatic compounds **7**-**12a**.

Results and Discussion

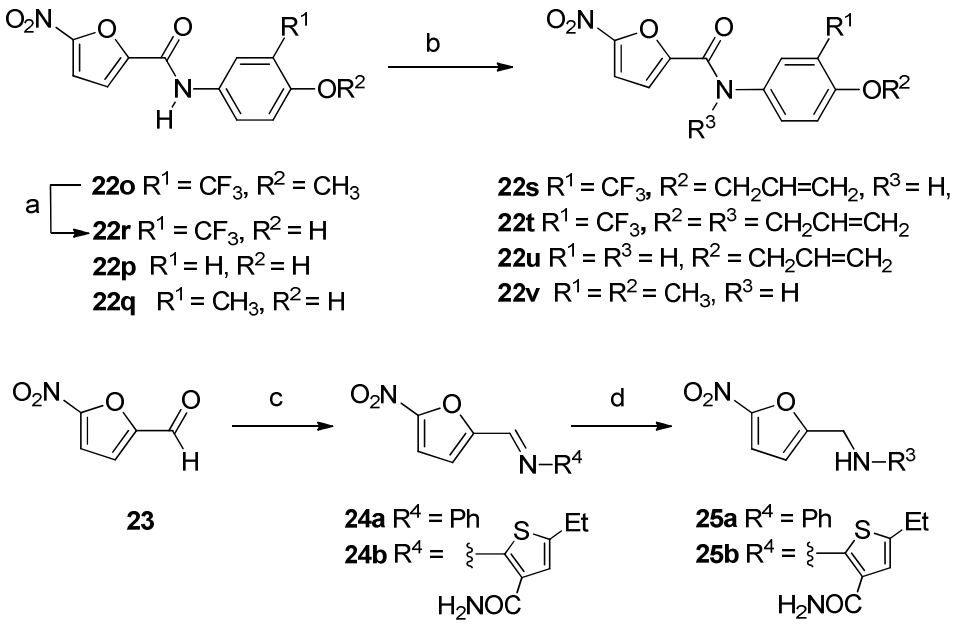
Chemistry

5-Nitrofurans **12a-k** were prepared by acylation of the corresponding 2-amino-thiophenes **13a-k** with 5-nitro-furancarboxylic acid chloride **14a** (Scheme 1, Table 1 for substituents). The required 2-amino-thiophenes **13a-h** were prepared using the Gewald multicomponent reaction with various combinations of the 2-cyanoacetyl esters or amides (**15a**-**15g**) with aldehydes (**16a** or **16b**) and elemental sulphur (see Table S1 in ESI for details).[36, 37] **13h** was *O*-silyl-protected before coupling to **14a**. This reaction gave **12h** directly as a result of *in situ* removal of the silyl-protecting group. Thiophene **13i** was prepared by hydrolysis of **13a** and **13j** was prepared by catalytic amination of 2-iodothiophene using CuI and L-proline.[38] **13k and 13l** (Table 2) were commercially available. Reaction of **13a** (R^2 = Et, R^3 = CO_2Et) with **14b** gave control compound **18a**. An analogous reaction of **14b** with aniline gave **18b** (Table 2). Furan ester **19** was commercially available.



Scheme 1. Synthetic route to 5-nitro-2-furancarboxylamide analogs **12a-k**: Reagents and conditions: (a) i) $(COCl)_2$, DCM/DMF, RT, 2 hrs; ii) R^1NH_2, Et_3N, DCM, 52% for **15d**; 49% (**15e**); 37% (**15g**); (b) S_8, **16a** or **16b**, Et_2NH, DMF, RT, o/n, 66% for **13a**; 59% (**13b**); 76% (**13c**); 53% (**13d**); 49% (**13e**); 46% (**13f**); 48% (**13g**); 46% (**13h**); for synthesis of **13i-j** see ESI; **13k** and **13l** were commercially available; (c) **13a-k**, Et_3N, DCM, RT with **14a**, 68% for **12a**; 62% (**12b**); 65% (**12c**); 58% (**12d**); 57% (**12e**); 57% (**12f**); 54% (**12g**); 47% (**12h**); 25% (**12i**); 41% (**12j**); 51% (**12k**); with **14b**, 56% for **18a**; (d) RNH_2, Et_3N, DCM, RT with **14a**, 65% for **22a**; 61% (**22b**); 72% (**22c**); 67% (**22d**); 37% (**22e**); 50% (**22f**); 45% (**22g**); 41% (**22h**); 66% (**22i**); 39% (**22j**); 51% (**22k**); 73% (**22l**); 51% (**22m**); 68% (**22n**); 67% (**22o**); 69% (**22p**); 48% (**22q**); with **14b** 73% for **18b** (see Table 2 for structure of **18b**) [a] **15a-c** and **15f** were commercially available; [b] **15d**, **15e** and**15g** were prepared from **17**; [c] for R^2 and R^3 substituents see Table 1; [c] for R^6 substituents see Tables 2 and 3.

Analogs **20a-c** and **21** (Table 2) based on **12g** but containing alternative nitroaromatic rings were prepared by coupling the corresponding acid chlorides with **13g** (see ESI for details). Similarly, reaction of **14a** with a range of amines and anilines led to the synthesis of **22a-22q** (Scheme 1). Demethylation of **22o** to give **22r** was followed by selective *O*-allylation to give **22s** (Scheme 2), the structure of which was confirmed by X-ray crystallographic analysis (data not shown). When a longer reaction time and an excess amount of allyl bromide was used in the *O*-allylation of **22r**, the diallylated analogue **22t** was formed (Scheme 2). Mono-*O*-allylation of **22p** gave **22u** (Scheme 2). Methylation of **22q** to give **22v** was achieved using MeI under basic conditions in moderate yield (Scheme 2). Conversion of 5-nitrofuran aldehyde **23** to imines **24a** or **24b** enabled the synthesis of the corresponding amines **25a** and **25b** using standard reductive amination conditions (Scheme 2).



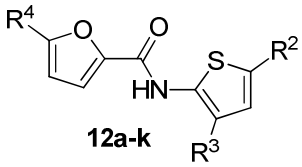
Scheme 2. Synthetic routes to Series 2 and Series 3 nitrofurancarboxylamides: Reagents and conditions: (a) pyridine hydrochloride, 160 ^{o}C, MW, 5 mins, 52%; (b) **22r** or **22p**, allyl bromide (2 or 10 equivs.), K_2CO_3, acetone, RT, 2 hrs for **22s** (75%), 24h for **22t** (55%); 24h for **22u** (72%); **22q**, MeI, K_2CO_3, acetone, 3h, for **22v** (51%); (c) aniline or **13g**, DCM, RT, 89% for **24a**, 63% for **24b**. (d) $NaBH_4$, DCM, RT, 79% for **25a**, 87% for **25b**.

Structure activity relationships

In comparison to nifurtimox, NFN1 was two orders of magnitude more potent with an EC_{50} of 31.3 ± 3.1 nM (Table 1, c.f. entries 1 and 2). The nitro group in NFN1 was found to be essential with no activity being observed up to the solubility limit of **18a** (entry 3). Removal, or worse, elongation of the alkyl substituent in NFN1 led to reduced activity (c.f. entry 2 and entries 4 and 5) and whilst removal of the ester group initially appeared to be tolerated in the R^2 = H series (c.f. entries 4 and 6), it was found that the methyl and *n*-butyl ester analogs (**12c** and **2d**) of NFN1 had similar activity to NFN1 (c.f. entry 2 and entries 7 and 8). Conversion of the ester groups in NFN1 and **12d** to the corresponding amides in **12e** and **12f** led to a significant loss in activity (c.f. entries 2 and 8 with entries 9 and 10 respectively). This observation, coupled with the decrease in activity associated with amide **12h** (entry 11), may be explained by the fact that hydrolysis of the ester groups in **12a-d** and **12k** in the parasite gives the

corresponding carboxylic acid-containing bioactive metabolites, something that is unlikely to occur for the amides **12e** and **12f**. However, acid **12i** showed much less activity with an EC_{50} of 959.0 ± 33.9 nM (entry 12), which may be due to a lack of cell permeability. The primary amide **12g** was found to be the most active thiophene-containing analog with an EC_{50} of 17.3 ± 2.4 nM (entry 13). Furthermore, analogs **12a-k** showed no significant toxicity against the human HeLa cell line even at a concentration above 20 μM. The highly selective toxicity of these analogs against the parasite suggests these nitrofuran analogs have real potential for therapeutic applications.

Table 1: SAR study of analogs 12a-12k.



12a-k

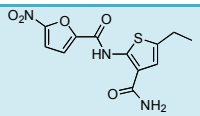
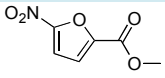
Entry	Compound	R^2	R^3	R^4	*T. brucei* EC_{50} (nM)	HeLa EC_{50} (μM)	Selectivity Index
1	Nifurtimox (**5**)[a]	-[a]	-[a]	-[a]	2400 ± 100	91.2±11.3	~38
2	NFN1 (**12a**)[b]	Et	CO_2Et	NO_2	31.3±3.1	>20	>640
3	**18a**	Et	CO_2Et	H	>20000	>20	N/A[c]
4	**12k**	H	CO_2Et	NO_2	72.3 ± 6.1	>20	>278
5	**12b**	^{n}Bu	CO_2Et	NO_2	125.3±6.0	>20	>160
6	**12j**	H	H	NO_2	62.6 ± 2.7	>20	>322
7	**12c**	Et	CO_2Me	NO_2	21.0±1.2	>20	>950
8	**12d**	Et	$CO_2{}^nBu$	NO_2	32.6±0.7	>20	>625
9	**12e**	Et	CONHEt	NO_2	147.0±10.1	>20	>136
10	**12f**	Et	$CONH^nBu$	NO_2	336.3±18.8	>20	>60
11	**12h**	Et	$CONH(CH_2)_2OH$	NO_2	79.6 ± 6.7	>20	>253
12	**12i**	Et	CO_2H	NO_2	959.0 ± 33.9	>20	N/A[c]

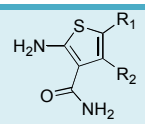
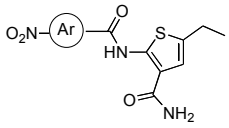
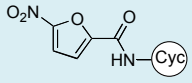
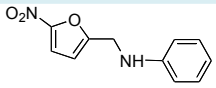
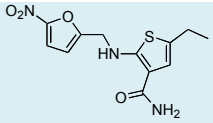
13	**12g**	Et	$CONH_2$	NO_2	17.3±2.4	>20	>1155

[a] for structure see Figure 1; [b] see reference[17]; [c] not applicable as essential no activity against *T.brucei* was observed for 12i.

Encouraged by the potent activity of **12g** (Table 1), it was decided to prepare more analogs based on this structure. Fragments **19** and **13g** along with **13l** were shown to be inactive against *T. brucei*, suggesting that both the 5-nitrofuran and thiophene fragments are required (Table 2, c.f. entry 1 and entries 2-4). Changing the nitrofuran motif in **12g** to either a nitrophenyl or nitropyrazole ring also lead to a 100-fold or more drop in activity (c.f. entry 1 and entries 5-8). Interestingly, the position of the nitro group in the nitrophenyl ring had an influence as **20b**, an analog with the nitro group in the *meta*-position was more activity than either **20a** (*ortho*-NO_2) or **20c** (*para*-NO_2). Studies next focused on replacing the thiophene motif in **12a-k**. By incorporating a cyclohexyl ring to give **22a**, a 10-fold decrease in activity was observed compared to **12g** (entry 9), whilst the phenyl (**22b**) and benzyl (**22c**) analogs were essentially equipotent with **12g** (c.f. entry 1 and entries 10 and 11). Incorporation of a nitrogen atom into the 2-position of the phenyl ring in **22c** to give **22d** led to a 10-fold drop in activity (entry 12). The nitrofuran group in **22b** was shown to be essential (c.f. entries 10 and 13) and the amide linkage in **22b** and **12g** was also shown to be important, as the amine analogs **25a** and **25b** showed a dramatic decrease in activity (entries 14 and 15).

Table 2: SAR study of Series 2 analogues

Structure	Substituent	Entry	Compound	*T. brucei* EC_{50} (µM)	HeLa EC_{50} (µM)	Selectivity Index
	-	**1**	**12g**	0.0173 ± 0.0024	>20	>1155
	-	**2**	**19**	67.0 ± 2.6	>20	N/A[a]

	R_1=Et, R_2= H	**3**	**13g**	32.6 ± 1.9	>20	N/A[a]
	R_1=H, R_2= Me	**4**	**13l**	65.1 ± 3.2	>20	N/A[a]
	*ortho*-Ph	**5**	**20a**	26.1 ± 2.5	>20	N/A[a]
	meta-Ph	**6**	**20b**	2.6 ± 0.2	>20	>8
	para-Ph	**7**	**20c**	>20	>20	N/A[a]
	Ortho-Pyrazole	**8**	**21**	>300	>20	N/A[a]
	Cyclohexyl	**9**	**22a**	0.2310 ± 0.0313	>20	>86
	Ph	**10**	**22b**	0.0281 ± 0.0015	>20	>710
	Bn	**11**	**22c**	0.0273 ± 0.0018	>20	>732
	CH_2-2-Py	**12**	**22d**	0.2910 ± 0.0315	>20	>68
	No NO_2, Ph	**13**	**18b**	>20	>20	N/A[a]
	-	**14**	**25a**	113.3 ± 14.7	>20	N/A[a]
	-	**15**	**25b**	5.3 ± 0.8	>20	>4

[a] not applicable as no activity or low activity against *T.brucei* was observed for **18b** and **25a**.

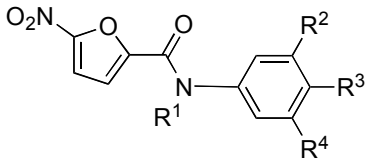
Based on the potent activity of **22b**, the effect of incorporating additional substituents into the phenyl ring was investigated (Table 3). Substitution at the *para*-(R^3) and *meta*-(R^4)-positions with either electron-withdrawing (entries 3 and 5-7) or electron-donating groups (entries 4 and 8) proved detrimental with the exception of the incorporation of the *meta*-CF_3 group in **22e** (EC_{50} of **22e** = 4.69 ± 0.14 nM, entry 2). Interestingly, incorporation of a *meta*-CF_3 into the phenyl ring of the benzyl analog **22c** (Table 2) gave analog **22l** which had an EC_{50} = 17.4 ± 3.0 nM, suggesting that the use of a phenyl substituent (as in **22e**) may be preferred to the benzyl substituent in **22l**. Combining the *meta*-CF_3 substituent with either an additional *meta*-methoxy- or a second *meta*-CF_3 substituent led to a decrease in activity (entries 9 and 11), whereas the *meta*-CF_3 group was able to override the effect of a *para*-methoxy substituent (c.f. entries 8 and 12). Incorporation of a *para*-hydroxy in both the *meta*-CF_3 and

unsubstituted series led to a significant reduction in activity (entries 13 and 14), but extending the *para*-alkoxy chain from methoxy in **22o** to allyloxy led to the most active compound prepared, analog **22s,** which had an EC_{50} against *T. brucei* of 2.4 ± 0.3 nM (entry 15). The positive role of the *para*-allyloxy substituent was also observed in the unsubstituted series (c.f. entries 8 and 16). The incorporation of an additional allyloxy-substituent on the amide nitrogen in **22s** led to a significant loss in activity (c.f. entries 15 and 17).

Interestingly, the lack of significant biological activity displayed by the 2-trifluoromethylphenol **22r**, e.g. EC_{50} value of 2.2 ± 0.1 μM *versus* 2.4 ± 0.3 nM for **22s** or 7.8 ± 0.3 nM for **22o** – might be due to the high instability of **22r** in the tested conditions, as the result of fluorine elimination generating a quinone methide, as previously reported.[39, 40] Masking the phenol as allyl or methyl ethers, e.g. like in **22s** or **22o**, respectively, restored the trypanocidal activity against *T. brucei*.

Finally, the special role played by the *meta*-CF_3 substituent, was further highlighted by a more than 5-fold drop in activity observed on its replacement by a *meta*-CH_3 substituent (c.f. entries 12 and 18). As was the case for analogs **12a-12k**, all the analogs shown in Tables 2 and 3 show low toxicity against the human HeLa cell line.

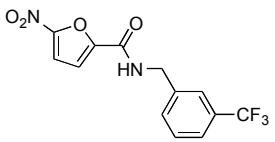
Table 3: SAR study of series 3 analogues



Entry	Compound	R^1	R^2	R^3	R^4	*T.brucei* EC_{50} (nM)	HeLa EC_{50} (μM)	Selectivity Index

1	**22b**	H	H	H	H	28.1 ± 1.5	>20	>710
2	**22e**	H	CF_3	H	H	4.69 ± 0.14	>20	>4454
3	**22f**	H	Br	H	H	103.8 ± 7.7	>20	>192
4	**22g**	H	OCH_3	H	H	58.6 ± 2.9	>20	>345
5	**22h**	H	H	CF_3	H	126.3 ± 10.2	>20	>158
6	**22i**	H	H	Cl	H	411.9 ± 41.6	>20	>48
7	**22j**	H	H	$COOCH_3$	H	680.0 ± 6.1	>20	>29
8	**22k**	H	H	OCH_3	H	76.5 ± 5.5	>20	>261
9	**22m**	H	CF_3	H	OCH_3	26.6 ± 2.6	>20	>752
10	**22l** [a]	-	-	-	-	17.4 ± 3.0	>20	>1149
11	**22n**	H	CF_3	H	CF_3	128.0 ± 5.1	>10	>78
12	**22o**	H	CF_3	OCH_3	H	7.8 ± 0.3	>20	>2565
13	**22r**	H	CF_3	OH	H	2200 ± 100	>20	>9
14	**22p**	H	H	OH	H	394.4 ± 21.0	>20	>50
15	**22s**	H	CF_3	*O*-allyl	H	2.4 ± 0.3	>20	>8330
16	**22u**	H	H	*O*-allyl	H	7.73 ± 0.72	>20	>258
17	**22t**	*O*-allyl	CF_3	*O*-allyl	H	2200 ± 100	>20	>9
18	**22v**	H	CH_3	OCH_3	H	38.0 ± 3.7	>20	>525

[a] structure of 5-Nitro-*N*-(3-(trifluoromethyl)benzyl)furan-2-carboxamide (**22l**)



Cross-Resistance Studies

The striking increase in potency of the 5-nitro-2-furancarboxylamide analogs compared to nifurtimox against bloodstream *T. brucei*, suggests they have a different or possibly additional mode of action to that of nifurtimox, i.e. in addition to the activation by the *T. brucei* nitroreductase. In order to investigate this possibility it was decided to generate drug resistant cell lines to nifurtimox and the most potent of the 5-nitro-2-furancarboxylamide analogs, **22s** (EC_{50} = 2.4 ± 0.3 nM). This would allow

subsequent cross-resistance studies to be conducted. Previous lab generated nifurtimox -resistant strains have shown that deletion or mutation of one of the alleles of the nitroreductase confers resistance [9, 10].

As with these previous studies nifurtimox -resistant parasites were generated easily by culturing bloodstream *T. brucei* in the continuous presence of nifurtimox. A stepwise increase in the concentrations of nifurtimox, initially starting at 1.5 μM, increasing to 2.5, 4, 5, 7.5, 10, 15, 20, 25, 30, 40, 45, 50 μM on days 2, 5, 9, 14, 23, 39, 55, 65, 76, 90, 109, 120 respectively was used (Fig 3A). At 143 days these nifurtimox -resistant parasites were cloned by serial dilution, while maintaining the 50 μM nifurtimox.

Compound **22s**-resistant parasites were initially harder to generate. Selection studies started at 1 nM of **22s**, increasing to 2, 3, 5, 7.5, 10, 15, 20, 25, 30, 35 40, 50 μM on days 2, 5, 9, 26, 36, 47, 58, 70, 76, 103, 130, 148 respectively (Fig. 3B). At 160 days the **22s**-resistant parasites were cloned by serial dilution, while maintaining a 50 nM concentration of **22s**. Both of the drug-resistant cell-lines were stable for 30 days in the absence of their respective drugs. The nifurtimox-resistant parasites had a doubling time of ~11.5 ± 0. 6 h compared to wild-type ~7.8 ± 0.4 h, while **22s**-resistant parasites had a doubling time of ~13.3 ± 1.1 h.

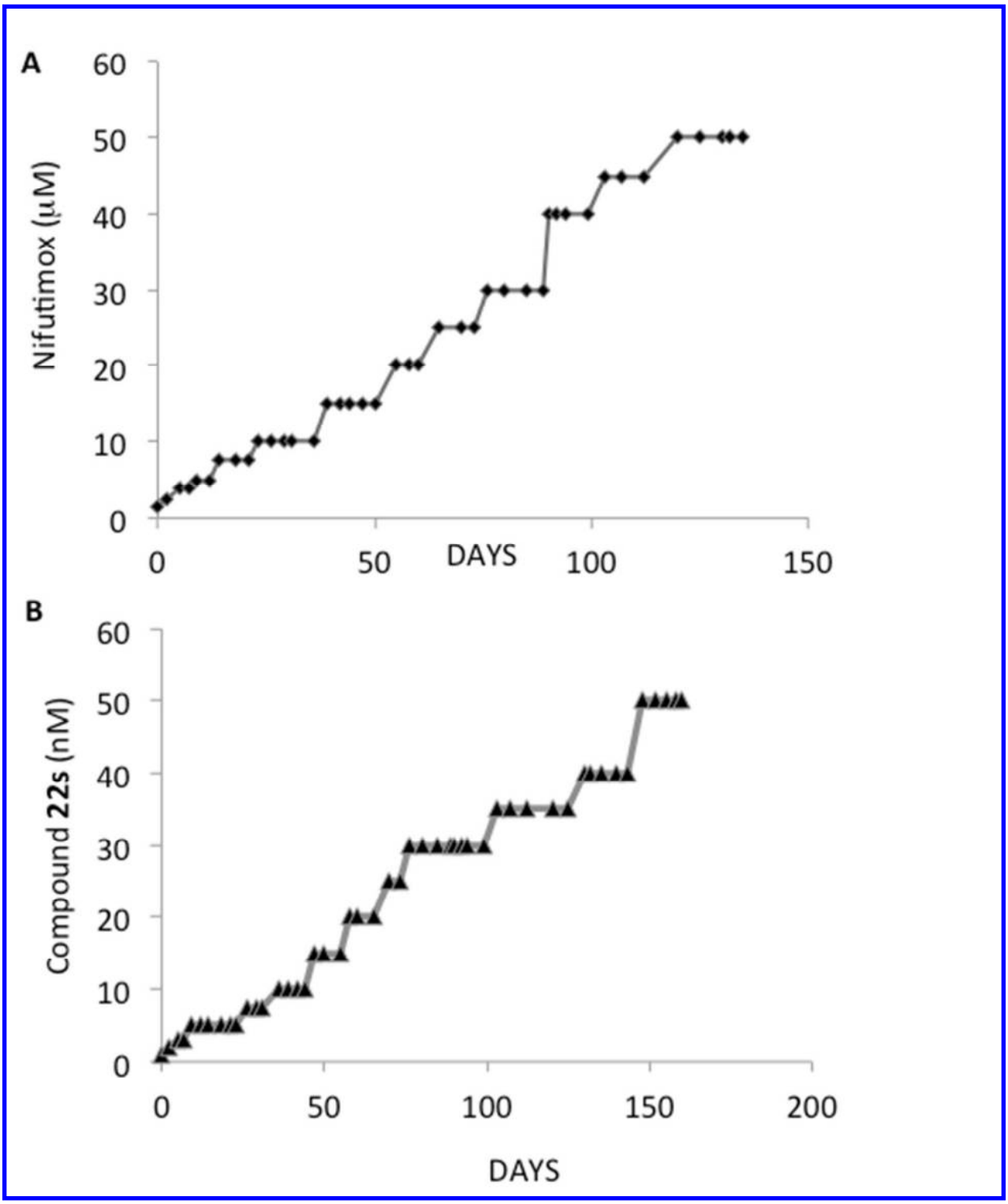


Figure. 3. Generation of nifurtimox and **22s** resistance cell-lines**:** Schematic representation of the generation of a (A) nifurtimox-resistant and (B) **22s**-resistant cell lines in *T. brucei*. Each point

represents the concentration at that time point when cells were checked and divided if required.

The sensitivities of the cloned nifurtimox-resistant and **22s**-resistant parasites to nifurtimox, **22s** and the diamidine pentamidine (**2**) were determined and compared to wild-type cells. The nifurtimox-resistant cells did not greatly alter (~2-fold increase) the sensitivity to pentamidine (**2**) compared to wild-type cells (Table 4), in accordance with previous lab generated nifurtimox-resistant cell-lines.[9,10] **22s**-Resistant parasites also showed very little alteration in the EC_{50} (1.1 ± 0.1 nM) of pentamidine (**2**), compared to wild-type cells (Table 4).

The nifurtimox-resistant cells were found to be 9-10-fold less sensitive to nifurtimox than wild-type cells, with EC_{50}s of 20.9 ± 1.7 and 2.1 ± 0.2 μM, respectively (Table 4), in accordance with previous findings.[9] **22s**-Resistant cells were found to be ~14-fold less sensitive to **22s** than wild-type cells, with EC_{50}s of 29.3 ± 2.0 and 2.4 ± 0 .3 nM, respectively (Table 4). Even at this level of resistance **22s** is still ~70 times more potent than nifurtimox in wild-type *T. brucei*.

To investigate cross-resistance between nifurtimox and **22s,** the EC_{50}s of the drug-resistant cell lines versus the alternative nitrofuran containing compounds were determined (Table 4). The results showed that nifurtimox and **22s** showed a low level of cross-resistance. Nifurtimox-resistant cells showed a ~3-fold increase in the EC_{50} of **22s**, whilst nifurtimox also showed ~3-fold increase in the EC_{50} against **22s**-resistant cells. These findings imply that these two nitrofuran compounds, nifurtimox and **22s**, are trypanocidal as a result of targeting primarily different biochemical process within the parasites. However, the low level of cross-resistance suggests there may be some minor overlap in mode of action. This issue is discussed in more detail later. These findings have important implications for the therapeutic use of this new generation of nitrofuran compounds as part of novel combination therapies or more importantly potentially replacing nifurtimox in a clinical setting.

Table 4: Cross-resistance studies, EC_{50}s of wild-type and cloned drug-resistant cell lines.

Cell Type	Nifurtimox (μM)[a]	Compound 22s (nM)	Pentamidine (nM)[b]
Wild-Type	2.1 ± 0.2	2.4 ± 0.3	1.0 ± 0.1
Nifurtimox Resistant	20.9 ± 1.7	7.8 ± 0.4	2.4 ± 0.2
Compound **22s** Resistant	7.3 ± 0.5	29.3 ± 2.0	1.1 ± 0.1

[a] **EC_{50}** literature value of nifurtimox in wild type *T. brucei* 2.4 ± 0.1 μM, and in nifurtimox resistant cells 20.1 ± 0.9 μM. [9]

[b] **EC_{50}** literature value of pentamidine (**2**) in wild type *T. brucei* 0.95 ± 0.02 nM, and in nifurtimox resistant cells 2.6 ± 0.1 nM. [9]

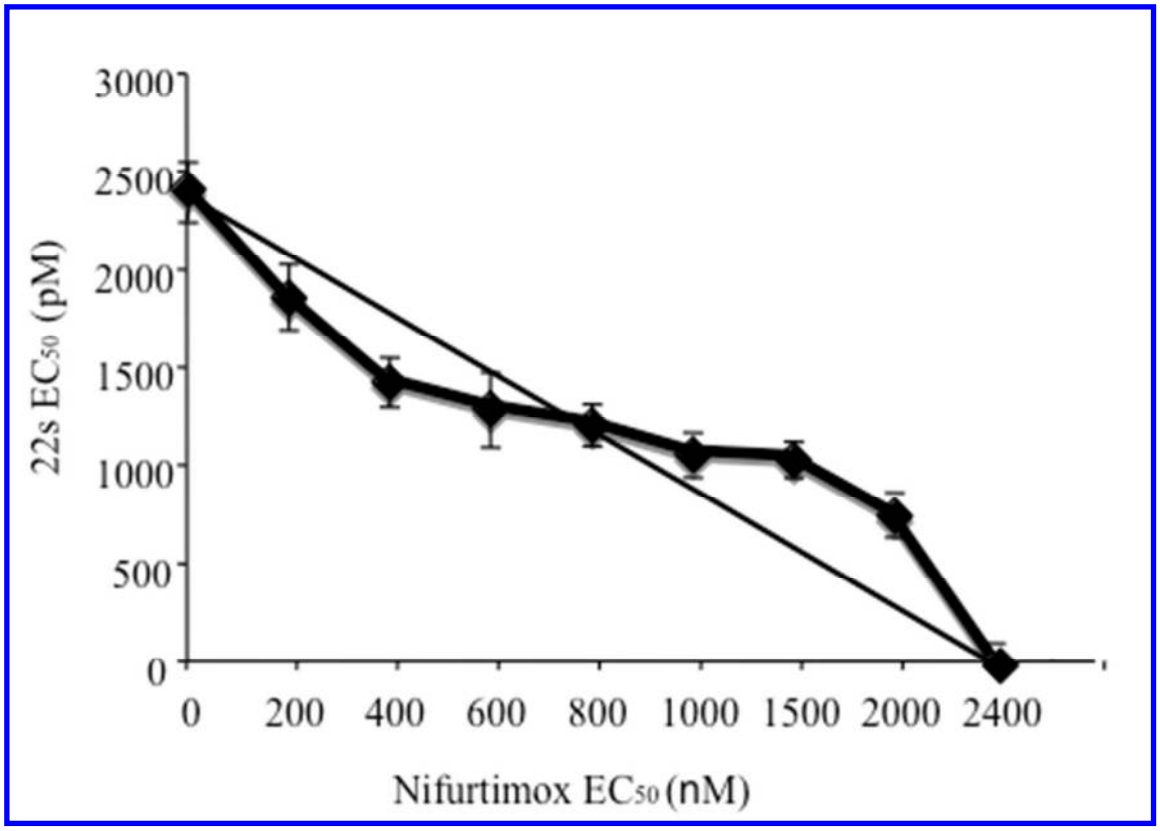


Figure. 4. Studies of possible synergy between nifurtimox (**5**) and **22s** in *T. brucei*.

As nifurtimox and 22s apparently act through primarily different modes of action, this suggests that their combined action against the parasite may be synergistic. As such a series of EC_{50}s were determined for **22s** in the presence of various concentrations of nifurtimox (Figure 4). At low concentrations of nifurtimox (<700 nM), synergy can be observed as the EC_{50}s are below the straight diagonal line that represents what would be expected for an additive trypanocidal effect of the two compounds. Above concentrations of 700 nM nifurtimox, there is a distinct lack of synergy, if anything competition occurs between the two inhibitors. This taken together with the observed low level of cross-resistance (Table 4) may indicate that nifurtimox at high concentrations is competing with the same biochemical process which is normally targeted by **22s**. This further highlights the possible multi-target nature for nifurtimox that has been suggested previously.[9-14] This type of information is relevant for the potential pharmacological applications of these novel nitro-furancarboxylamides as a combinational therapy, would likely lead to the use of lower nifurtimox doses.

Table 5: EC_{50}s of typical series 1 and series 2 analogs against *T. b. rhodesiense*

Entry	Compound	*T. brucei rhodesiense* EC_{50} (nM)
1	nifurtimox (**5**)[a]	1708 ± 197
2	NFN1 (**12a**)	44.2 ± 4.5
3	**12e**	201.8 ± 18.7
4	**12g**	23.1 ± 2.9
5	**22e**	9.4 ± 1.8
6	**22h**	159.0 ± 12.1
7	**22s**	2.9 ± 0.4
8	Melarsoprol (**3**)[b]	7.8 ± 0.9

[a] EC_{50} literature value of nifurtimox in *T. b. rhodesiense* 1.5 μM. [43]

[b] EC_{50} literature value of melarsoprol against *T. b. rhodesiense* 6 nM . [43]

It was decided to test some of the 5-nitro-2-furancarboxylamides from this study against cultured *T. b. rhodesiense*, one of the human-infective subspecies (Table 5). The potency of a selection of analogs against *T. b. rhodesiense* were similar to those observed for *T. b. brucei,* suggesting that these novel nitro-furans could be considered lead candidates for the next step towards a novel treatment for HAT, as well as the related animal disease, Nagana.

Conclusions

In this work, we designed and synthesised a series of nitrofuran-containing analogs that were tested for trypanocidal and cytotoxicity activity against cultured bloodstream form *T. b. brucei* and human HeLa cells respectively. The analog **22s** showed an Ec_{50} of 2.4 ± 0.3 nM, three orders of magnitude more potent than nifurtimox with a selectivity index >8000. SAR studies showed that all fragments of the compounds were required for activity. In addition, both the nitrofuran and amide functional groups and the amide NH were necessary. The thiophene ring in the starting analog **12a** can be replaced by a range of other substituents. However, in the thiophene series, an ester group was required and is likely converted to the corresponding carboxylic acid in the parasite. Several of our analogs were also tested against *T. b. rhodesiense* showing analogous activity to that in *T. b. brucei*. This exciting result demonstrates that this series of analogs is worthy of further study as a potential therapy for HAT.

Importantly the cross-resistance studies showed that the 5-nitro-2-furancarboxylamide analogs in this study have low levels of resistance to nifurtimox-resistant cells and vice versa, whilst showing no resistance to pentamidine (**2**). These results indicate that the trypanocidal mode-of-action of the 5-nitro-2-furancarboxylamide analogs in this study does not rely upon nitroreductase activation unlike

nifurtimox and other nitroheterocyclic compounds that are presently in clinical trials against HAT. However, the lack of expected synergy at high concentrations of nifurtimox suggests it may be interacting with the novel mode of action which is primarily targeted by our novel 5-nitro-2-furancarboxylamide analogs.

Our data suggests that these new 5-nitro-2-furancarboxylamide analogs have a potential therapeutic implication as a single reagent in regards to potency and selectivity. Also combination therapies can be considered due to their low cross-resistance to other trypanocidal drugs.

This idea is supported by our results that show there is a synergistic relationship between nifurtimox and **22s** at concentrations of nifurtimox of <700 nM (Figure 4). Whilst there remain many more challenges to the development of a HAT therapeutic than have not been addressed here (see Table S6 for a detailed discussion of drug-likeness parameters associated with our analogs), we believe that the novel mechanistic and high *in vitro* potency of the anti-trypanosomal compounds presented here renders them worthy of further study in the drug discovery context.

Experimental

BIOLOGY

Materials

All materials unless stated were purchased from either Sigma/Aldrich, or Invitrogen.

Trypanocidal Studies

The trypanocidal activity over a 72 hour period was determined using the Alamar Blue™ viability, as described previously[41]. Nifurtimox, pentamidine, melarsoprol and various novel nitrofuran analogues were tested against *Trypanosoma brucei brucei* bloodstream-form (strain 427) or drug-resistant strains were cultured at 37°C in HMI9 medium supplemented with 10% fetal calf serum and 2.5 $\mu g\,ml^{-1}$ G418 as described previously[42]. *T. brucei rhodesiense* (strain Z310) were cultured in a similar manner, but in the absence of G418. For the synergistic experiments, various concentrations of nifurtimox (0, 200, 400, 600, 800, 1000, 1500, 2000, 2400 nM) were used in conjunction with a serial dilution of **22s.** Experiments were conducted in replicates of four; the data was fitted using GraFit software to obtain EC_{50} ± standard deviations and slope factors.

Cytotoxicity Studies

Cytotoxic affects against HeLa cells were determined in a similar manner. Briefly, the cells were cultured in DMEM supplemented with 10% fetal calf serum and 2 mM L-Glutamine. Cells were plated at initial cell concentration of 2×10^4 cells/well and incubated with the compounds for ~65 hours prior to addition of Alamar Blue™ solution for a further 5 hours.

Generation of drug-resistant *T. brucei* cell lines.

Drug-resistant *T. brucei* cell lines were generated by sub-culturing bloodstream trypanosomes in the continuous presence of either nifurtimox or compound **22s.** Parasites were exposed to stepwise-

increased concentrations of drug, starting at appropriate sub-lethal concentrations, until they were routinely growing between 20-25 times their respective original ED_{50}s. After 150-160 days in culture, drug-resistant parasites were cloned by limiting dilution, and used for further study. Cell doubling times were determined in replicates of 4, over a 96 h period.

Chemistry

General. Chemicals and reagents were obtained from either Aldrich or Alfa-Aesar, except nifurtimox (**5**) from Bayer Argentina. All reactions involving moisture sensitive reagents were performed in oven dried glassware under a positive pressure of argon. Dichloromethane (DCM) was obtained dry from a solvent purification system (MBraun, SPS-800). Melting points were recorded in open capillaries using an Electrothermal 9100 melting point apparatus. Infrared spectra were recorded on a Perkin Elmer Spectrum GX FT-IR spectrometer using thin films on KBr (for solids) discs or Nujol (for liquids). Low resolution (LR) and high resolution (HR) electrospray mass spectral (ES-MS) analyses were acquired by electrospray ionisation (ESI) within the School of Chemistry, University of St Andrews. Low and high resolution ESI MS were carried out on a Micromass LCT spectrometer or at a high performance orthogonal acceleration reflecting TOF mass spectrometer, coupled to a Waters 2975 HPLC. Nuclear magnetic resonance (NMR) spectra were acquired either on a Bruker Avance 300 (^{1}H, 300.1 MHz; ^{13}C, 75.5 MHz) or on a Bruker Avance 400 (^{1}H, 400 MHz; ^{13}C, 100.1 MHz) spectrometer and in the deuterated solvent stated. ^{13}C NMR spectra were acquired using the PENDANT or DEPTQ pulse sequences.

For characterization of 2-aminothiophenes 13a-k, compound 20a-c, 21, intermediates and other known compounds please see supporting information (SI). The prepared analogues were analyzed by HPLC with purity above 95% (SI).

General Procedure for the synthesis of furancarboxyl amides 12a-k, 18a-b and 22a-q

The furoic acid chlorides **14a** and **14b** were prepared *in situ*: thionyl chloride (1.10 eq.) was added dropwise to a mixture of 5-nitrofuran-2-carboxylic acid or 2-furoic acid (1.10 eq.), triethylamine (1.50 eq.) in DCM (0.4 M) under a N_2 atmosphere. The reaction mixture was stirred at room temperature for 5 hours. Then crude **14a** or **14b** was added to another flask containing the corresponding amine or aniline (1.00 eq.) and triethylamine (2.00 eq.) in DCM (0.4 M). The reaction mixture was stirred at

room temperature for 5 hours. The solvent was then removed under reduced pressure and the crude reaction mixture was purified by column chromatography.

Details of the synthesis of furancarboxyl amides 12a, 12g, 22e, 22h and 22s are given here in the main text of the paper, the remainder (12b-12f, 12h-12k, 22a-22d, **22f, 22g**, **22i-22t** and **22u) are described in SI.**

Ethyl 5-ethyl-2-(5-nitrofuran-2-carboxamido)thiophene-3-carboxylate (12a): The general procedure was followed using 2-amiothiophene **13a** (3.99 g, 20.0 mmol). The crude reaction mixture was purified by column chromatography on silica gel (5:1, hexane/ethyl acetate) to afford the product as an orange solid (4.60 g, 13.6 mmol, 68%). Mp: 127-128 ^{o}C; IR (KBr) ν_{max} = 3120 (s) (NH), 2958 (m) (C-H), 1667 (s) (C=O), 1569 (s), 1531 (s) (NO_2), 1350 (s) (NO_2), 1280 (s) (C-O), 1266 (s) (C-O) cm^{-1}. ^{1}H NMR (400 MHz, d_6-Acetone): δ 11.75 (br. s, 1H), 7.58 (d, ^{3}J= 3.9 Hz, 1H), 7.42 (d, ^{3}J= 3.9 Hz, 1H), 6.85 (t, ^{4}J= 1.1 Hz, 1H), 4.28 (q, ^{3}J= 7.1 Hz, 2H), 2.67 (dq, ^{3}J= 7.5 Hz, ^{4}J= 1.1 Hz, 2H), 1.27 (t, ^{3}J= 7.1 Hz, 3H), 1.17 (t, ^{3}J= 7.5 Hz, 3H). ^{13}C NMR (100 MHz, d_6-Acetone): δ 165.8 (C), 153.6 (C), 153.1 (C), 147.4 (C), 145.6 (C), 139.2 (C), 120.6 (CH), 118.6 (CH), 115.0 (C), 113.8 (CH), 61.7 (CH_2), 23.2 (CH_2), 15.9 (CH_3), 14.6 (CH_3). LRMS (ES$^+$): m/z (%) 360.87 (100) [M+Na]$^+$; HRMS (ES$^+$): m/z calcd for $C_{14}H_{14}N_2O_6NaS$ [M+Na]$^+$: 361.0470; found 361.0469.

***N*-(3-carbamoyl-5-ethylthiophen-2-yl)-5-nitrofuran-2-carboxamide (12g):** The general procedure was followed using 2-amiothiophene **13g** (170 mg, 1.00 mmol). The crude reaction mixture was purified by column chromatography on silica gel (1:1, hexane/ethyl acetate) to afford the product as a yellow solid (167 mg, 0.54 mmol, 54%). Mp: (Dec.> 224 ^{o}C); IR (KBr) ν_{max} = 3476 (m), 3341 (m) (NH), 3211 (m), 3143 (m), 1659 (s) (C=O), 1591 (s), 1565 (s), 1534 (s) (NO_2), 1349 (s) (NO_2), 739 (m) cm^{-1}. ^{1}H NMR (400 MHz, d_6-Acetone): δ 7.56 (d, ^{3}J= 3.9 Hz, 1H), 7.36 (d, ^{3}J= 3.9 Hz, 1H), 7.05 (t, ^{4}J= 1.1 Hz, 1H), 6.80 (br. s, 1H), 2.66 (dq, ^{3}J= 7.5 Hz, ^{4}J= 1.1 Hz, 2H), 1.16 (t, ^{3}J= 7.5 Hz, 3H). ^{13}C

NMR (100 MHz, d_6-Acetone): δ 168.3 (C), 153.4 (C), 152.9 (C), 148.0 (C), 144.2 (C), 138.9 (C), 119.4 (CH), 117.9 (CH), 117.0 (C), 113.7 (CH), 23.4 (CH_2), 15.8 (CH_3). LRMS (ES^+): m/z (%) 331.91 (100) $[M+Na]^+$; HRMS (ES^+): m/z calcd for $C_{12}H_{11}N_3O_5NaS$ $[M+Na]^+$: 332.0317; found 332.0323.

5-nitro-*N*-(3-(trifluoromethyl)phenyl)furan-2-carboxamide (22e): The general procedure was followed using 3-(trifluoromethyl)aniline (622 μL, 5.00 mmol). The crude reaction mixture was purified by column chromatography on silica gel (2:1, hexane/ethyl acetate) to afford the product as a yellow solid (550 mg, 1.83 mmol, 37%). Mp: 188-189 ^oC; IR (KBr) ν_{max} = 3271 (m) (NH), 1668 (s) (C=O), 1496 (s) (NO_2), 1334 (s) (NO_2), 1266 (s), 1157 (s) (CF_3), 1119 (s) (CF_3) cm^{-1}. ^1H NMR (300 MHz, d_6-Acetone): δ 10.37 (br. s, 1H), 8.30 (s, 1H), 8.07 (d, ^3J= 8.3 Hz, 1H), 7.66 (d, ^3J= 3.8 Hz, 1H), 7.61 (d, ^3J= 7.9 Hz, 1H), 7.53-7.50 (m, 2H,). ^{13}C NMR (75 MHz, d_6-Acetone): δ 156.2 (C), 152.3 (C), 149.2 (C), 140.2 (C), 132.0 (C), 131.2 (CH), 127.3 (C), 125.2 (CH), 122.3 (CH), 118.3 (CH), 118.2 (CH), 114.1 (CH). LRMS (ES^+): m/z (%) 301.05 (100) $[M+H]^+$; HRMS (ES^+): m/z calcd for $C_{12}H_8N_2O_4F_3$ $[M+H]^+$: 301.0436; found 301.0428. Anal. Calcd for $C_{12}H_7F_3N_2O_4$: C, 48.01; H, 2.35; N, 9.33; found: C, 47.57; H, 2.27; N, 9.05.

5-Nitro-*N*-(4-(trifluoromethyl)phenyl)furan-2-carboxamide (22h): The general procedure was followed using 4-(trifluoromethyl)aniline (622 μL, 5.00 mmol). The crude reaction mixture was purified by column chromatography on silica gel (3:1, hexane/ethyl acetate) to afford the product a yellow solid (615 mg, 2.01 mmol, 41%). Mp: 235-236 ^oC; IR (KBr) ν_{max} = 3406 (m) (NH), 1697 (s) (C=O), 1538 (s), 1528 (s) (NO_2), 1323 (s) (NO_2), 1129 (s) (CF_3), 1115 (s) (CF_3), 1069 (s), 855 (s) cm^{-1}. ^1H NMR (300 MHz, d_6-Acetone): δ 10.29 (br. s, 1H), 7.94 (d, ^3J= 8.6 Hz, 2H), 7.61 (d, ^3J= 8.6 Hz, 2H), 7.54 (d, ^3J= 3.8 Hz, 1H), 7.40 (d, ^3J= 3.8 Hz, 1H). ^{13}C NMR (100 MHz, d_6-Acetone): δ 155.8 (C), 152.8 (C), 148.8 (C), 142.5 (C), 127.0 (2CH), 126.9 (C), 124.0 (C), 121.3 (2CH), 117.9 (CH), 113.7 (CH). LRMS (ES^+): m/z (%) 301.04 (100) $[M+H]^+$; HRMS (ES^+): m/z calcd for $C_{12}H_8N_2O_4F_3$ $[M+H]^+$: 301.0436; found 301.0432.

***N*-(4-(allyloxy)-3-(trifluoromethyl)phenyl)-5-nitrofuran-2- carboxamide (22s):** A mixture of **22r** (64 mg, 0.20 mmol, 1.00 eq.), K_2CO_3 (55 mg, 0.40 mmol, 2.00 eq.) and allyl bromide (0.03 mL, 0.40 mmol, 2.00 eq.) in acetone (5 mL) was stirred at room temperature for 3 hours. The reaction mixture was then diluted in ethyl acetate (20 mL), washed with water (10 mL), dried (Na_2SO_4) and concentrated under vacuum. The crude reaction mixture was purified by column chromatography (3:1, hexane/ethyl acetate) to afford the product as a yellow solid (53 mg, 0.15 mmol, 75%). Mp: 155-156 ^oC. IR (KBr) ν_{max} = 3350 (s), 3115 (m) (NH), 1681 (s) (C=O), 1611 (m), 1504 (s) (NO_2), 1331 (s) (NO_2), 1250 (s) (C-O), 1135 (s) (CF_3), 1113 (s) (CF_3) cm^{-1}. ^1H NMR (400 MHz, d_6-Acetone): δ 10.07 (br. s), 8.03 (d, ^4J= 2.5 Hz, 1H), 7.89 (dd, ^3J= 9.1 Hz, ^4J= 2.5 Hz, 1H), 7.52 (d, ^3J= 3.9 Hz, 1H), 7.35 (d, ^3J= 3.9 Hz, 1H,), 7.13 (d, ^3J= 9.1 Hz, 1H), 6.02-5.87 (m, 1H), 5.35 (d, ^3J= 17.5 Hz, 1H), 5.15 (d, ^3J= 10.7 Hz, 1H), 4.64-4.59 (m, 2H). ^{13}C NMR (100 MHz, d_6-Acetone): δ 155.5 (C), 153.9 (C), 152.6 (C), 149.1 (C), 133.8 (CH), 131.8 (C), 126.7 (CH), 125.9 (C), 123.2 (C), 120.3 (CH), 117.5 (CH), 117.4 (CH_2), 115.1 (CH), 113.7 (CH), 70.08 (CH_2). LRMS (ES^+): m/z (%) 378.91 (100) $[M+Na]^+$; HRMS (ES^+): m/z calcd for $C_{15}H_{11}N_2O_5NaF_3$ $[M+Na]^+$: 379.0518; found 379.0526.

ASSOCIATED CONTENT

Supporting Information

Supporting information available: [Experimental for 2-aminothiophenes **13a-k**, compound **20a-c**, **21**, intermediates and other known compounds and the unknown compounds **12b-12f, 12h-12k, 22a-22d**, **22f, 22g**, **22i-22t** and **22u**. HPLC purity analysis and calculated chemical properties] This material is available free of charge via the Internet at http://pubs.acs.org."

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Abbreviations

HAT, Human African Trypanosomiasis; WHO, World Health Organization; NECT, nifurtimox-eflornithine combination therapy; Disability-Adjusted Life Years, DALY

References

1. WHO, Trypanosomiasis, African. http://www.who.int/topics/trypanosomiasis_african/en/ **2012,** *(accessed: 10 August 2012)*.
2. Priotto, G.; Kasparian, S.; Mutombo, W.; Ngouama, D.; Ghorashian, S.; Arnold, U.; Ghabri, S.; Baudin, E.; Buard, V.; Kazadi-Kyanza, S.; Ilunga, M.; Mutangala, W.; Pohlig, G.; Schmid, C.; Karunakara, U.; Torreele, E.; Kande, V., Nifurtimox-eflornithine combination therapy for second-stage African Trypanosoma brucei gambiense trypanosomiasis: a multicentre, randomised, phase III, non-inferiority trial. *Lancet* **2009,** *374*, 56-64.
3. Steverding, D., The development of drugs for treatment of sleeping sickness: a historical review. *Parasites & vectors* **2010,** *3*, 15.
4. Delespaux, V.; de Koning, H. P., Drugs and drug resistance in African trypanosomiasis. *Drug resistance updates : reviews and commentaries in antimicrobial and anticancer chemotherapy* **2007,** *10*, 30-50.

5. Baker, N.; Alsford, S.; Horn, D., Genome-wide RNAi screens in African trypanosomes identify the nifurtimox activator NTR and the eflornithine transporter AAT6. *Mol Biochem Parasitol* **2011,** *176*, 55-57.

6. Barry, C. E., 3rd; Boshoff, H. I.; Dowd, C. S., Prospects for clinical introduction of nitroimidazole antibiotics for the treatment of tuberculosis. *Current pharmaceutical design* **2004,** *10*, 3239-3262.

7. Ginsberg, A. M.; Laurenzi, M. W.; Rouse, D. J.; Whitney, K. D.; Spigelman, M. K., Safety, tolerability, and pharmacokinetics of PA-824 in healthy subjects. *Antimicrobial agents and chemotherapy* **2009,** *53*, 3720-3725.

8. Nuermberger, E.; Tyagi, S.; Tasneen, R.; Williams, K. N.; Almeida, D.; Rosenthal, I.; Grosset, J. H., Powerful bactericidal and sterilizing activity of a regimen containing PA-824, moxifloxacin, and pyrazinamide in a murine model of tuberculosis. *Antimicrobial agents and chemotherapy* **2008,** *52*, 1522-1524.

9. Sokolova, A. Y.; Wyllie, S.; Patterson, S.; Oza, S. L.; Read, K. D.; Fairlamb, A. H., Cross-resistance to nitro drugs and implications for treatment of human African trypanosomiasis. *Antimicrobial agents and chemotherapy* **2010,** *54*, 2893-2900.

10. Wilkinson, S. R.; Taylor, M. C.; Horn, D.; Kelly, J. M.; Cheeseman, I., A mechanism for cross-resistance to nifurtimox and benznidazole in trypanosomes. *Proc Natl Acad Sci U S A* **2008,** *105*, 5022-5027.

11. Hall, B. S.; Bot, C.; Wilkinson, S. R., Nifurtimox activation by trypanosomal type I nitroreductases generates cytotoxic nitrile metabolites. *J Biol Chem* **2011,** *286*, 13088-13095.

12. Castro, J. A.; deMecca, M. M.; Bartel, L. C., Toxic Side Effects of Drugs Used to Treat Chagas' Disease (American Trypanosomiasis). *Human & Experimental Toxicology* **2006,** *25*, 471-479.

13. Rodriques Coura, J.; de Castro, S. L., A critical review on Chagas disease chemotherapy. *Memorias do Instituto Oswaldo Cruz* **2002,** *97*, 3-24.

14. Rao, D. N. R.; Mason, R. P., Generation of Nitro Radical-Anions of Some 5-Nitrofurans, 2-Nitrofurans and 5-Nitroimidazoles by Norepinephrine, Dopamine, and Serotonin - a Possible Mechanism for Neurotoxicity Caused by Nitroheterocyclic Drugs. *J Biol Chem* **1987,** *262*, 11731-11736.

15. Dubuisson, M. L. N.; De Wergifosse, B.; Kremers, P.; Marchand-Brynaert, J.; Trouet, A.; Rees, J. F., Protection against nitrofurantoin-induced oxidative stress by coelenterazine analogues and their oxidation products in rat hepatocytes. *Free Radical Res* **2001,** *34*, 285-296.

16. Rao, D. N.; Harman, L.; Motten, A.; Schreiber, J.; Mason, R. P., Generation of radical anions of nitrofurantoin, misonidazole, and metronidazole by ascorbate. *Archives of biochemistry and biophysics* **1987,** *255*, 419-427.

17. Zhou, L.; Ishizaki, H.; Spitzer, M.; Taylor, K. L.; Temperley, N. D.; Johnson, S. L.; Brear, P.; Gautier, P.; Zeng, Z.; Mitchell, A.; Narayan, V.; McNeil, E. M.; Melton, D. W.; Smith, T. K.; Tyers, M.; Westwood, N. J.; Patton, E. E., ALDH2 Mediates 5-Nitrofuran Activity in Multiple Species. *Chem Biol* **2012,** *19*, 883-892.

18. Bock, M.; Mayer, K. H.; Herlinge.H; Haberkor.A; Petersen, S., Structure-Activity Relationship of 4-(5'-Nitrofurfurylidene-Amino)-Tetrahydro-4h-1,4-Thiazine-1,1-Dioxides Active against Trypanosoma-Cruzi. *Arznei-Forschung* **1972,** *22*, 1564-1569.

19. Mester, B. E., J.; Claramunt, R. M.; Castanys, S.; Mascaro, M. L.; Osuna, A.; Vilaplana, M. J.; Molina, P., Activity against Trypanosoma cruzi of the new analogs of nifurtimox. *Archiv der Pharmazie* **1987,** *320*, 115-120.

20. Caracelli, I.; Stamato, F. M. L. G.; Mester, B.; Paulino, M.; Cerecetto, H., A new analogue of nifurtimox. *Acta Crystallogr C* **1996,** *52*, 1281-1282.

21. Pozas, R.; Carballo, J.; Castro, C.; Rubio, J., Synthesis and in vitro antitrypanosomal activity of novel Nifurtimox analogues. *Bioorg Med Chem Lett* **2005,** *15*, 1417-1421.

22. Arancibia, R.; Klahn, A. H.; Buono-Core, G. E.; Gutierrez-Puebla, E.; Monge, A.; Medina, M. E.; Olea-Azar, C.; Maya, J. D.; Godoy, F., Synthesis, characterization and anti-Trypanosoma cruzi evaluation of ferrocenyl and cyrhetrenyl imines derived from 5-nitrofurane. *J Organomet Chem* **2011,** *696*, 3238-3244.

23. Aguirre, G.; Boiani, L.; Cerecetto, H.; Fernandez, M.; Gonzalez, M.; Denicola, A.; Otero, L.; Gambino, D.; Rigol, C.; Olea-Azar, C.; Faundez, M., In vitro activity and mechanism of action against the protozoan parasite Trypanosoma cruzi of 5-nitrofuryl containing thiosemicarbazones. *Bioorgan Med Chem* **2004,** *12*, 4885-4893.

24. Hall, B. S.; Wu, X.; Hu, L.; Wilkinson, S. R., Exploiting the drug-activating properties of a novel trypanosomal nitroreductase. *Antimicrobial agents and chemotherapy* **2010,** *54*, 1193-1199.

25. Bot, C.; Hall, B. S.; Bashir, N.; Taylor, M. C.; Helsby, N. A.; Wilkinson, S. R., Trypanocidal activity of aziridinyl nitrobenzamide prodrugs. *Antimicrobial agents and chemotherapy* **2010,** *54*, 4246-4252.

26. Hu, L. Q.; Wu, X. H.; Han, J. Y.; Chen, L.; Vass, S. O.; Browne, P.; Hall, B. S.; Bot, C.; Gobalakrishnapillai, V.; Searle, P. F.; Knox, R. J.; Wilkinson, S. R., Synthesis and structure-activity relationships of nitrobenzyl phosphoramide mustards as nitroreductase-activated prodrugs. *Bioorg Med Chem Lett* **2011,** *21*, 3986-3991.

27. Tangallapally, R. P.; Yendapally, R.; Lee, R. E.; Hevener, K.; Jones, V. C.; Lenaerts, A. J. M.; McNeil, M. R.; Wang, Y. H.; Franzblau, S.; Lee, R. E., Synthesis and evaluation of nitrofuranylamides as novel antituberculosis agents. *J Med Chem* **2004,** *47*, 5276-5283.

28. Gupta, R. A.; Gupta, A. K.; Soni, L. K.; Kaskhedikar, S. G., Exploration of physicochemical properties and molecular modeling studies of furanylamide analogs as antituberculosis agents. *Qsar Comb Sci* **2007,** *26*, 897-907.

29. De La Fuente, R.; D Sonawane, N.; Arumainayagam, D.; Verkman, A. S., Small molecules with antimicrobial activity against E-coli and P-aeruginosa identified by high-throughput screening. *Brit J Pharmacol* **2006,** *149*, 551-559.

30. Tawari, N. R.; Degani, M. S., Pharmacophore Mapping and Electronic Feature Analysis for a Series of Nitroaromatic Compounds with Antitubercular Activity. *J Comput Chem* **2010,** *31*, 739-751.

31. Ancizu, S.; Moreno, E.; Torres, E.; Burguete, A.; Perez-Silanes, S.; Benitez, D.; Villar, R.; Solano, B.; Marin, A.; Aldana, I.; Cerecetto, H.; Gonzalez, M.; Monge, A., Heterocyclic-2-carboxylic Acid (3-Cyano-1,4-di-N-oxidequinoxalin-2-yl)amide Derivatives as Hits for the Development of Neglected Disease Drugs. *Molecules* **2009,** *14*, 2256-2272.

32. Ebringer, L. J., A.; Krkoska, P.; Kada, R.; Foltinova, P., New nitrofurans. Correlation study between chemical structures and biological activity *Advan. Antimicrob. Antineoplastic Chemother., Proc. Int. Congr. Chemother.,* **1972,** *7th*, 873-876.

33. Bock, M.; Haberkorn, A.; Herlinger, H.; Mayer, K. H.; Petersen, S., The structure-activity relationship of 4-(5'-nitrofurfurylidene-amino)-tetrahydro-4H-1,4-thiazine-1,1-dioxides active against Trypanosoma cruzi. *Arzneimittelforschung* **1972,** *22*, 1564-1569.

34. Byler, K. G.; Brito-Arias, M.; Marquez-Navarro, A.; Nogueda-Torres, B.; Torres-Bustillos, L. G.; Martinez-Mayorga, K., Identification of benzoylisoquinolines as potential anti-Chagas agents. *Bioorgan Med Chem* **2012,** *20*, 2587-2594.

35. Millet, R.; Maes, L.; Landry, V.; Sergheraert, C.; Davioud-Charvet, E., Antitrypanosomal activities and cytotoxicity of 5-nitro-2-furancarbohydrazides. *Bioorg Med Chem Lett* **2002,** *12*, 3601-3604.

36. Karl Gewald, E. S., Horst Böttcher, Heterocyclen aus CH-aciden Nitrilen, VIII. 2-Amino-thiophene aus methylenaktiven Nitrilen, Carbonylverbindungen und Schwefel. *Chemische Berichte* **1966,** *99*, 94-100.

37. Hesse, S.; Perspicace, E.; Kirsch, G., Microwave-assisted synthesis of 2-aminothiophene-3-carboxylic acid derivatives, 3H-thieno[2,3-d]pyrimidin-4-one and 4-chlorothieno[2,3-d]pyrimidine. *Tetrahedron Lett* **2007,** *48*, 5261-5264.

38. Kim, J.; Chang, S., Ammonium salts as an inexpensive and convenient nitrogen source in the Cu-catalyzed amination of aryl halides at room temperature. *Chem Commun* **2008**, 3052-3054.

39. Sakai TT, Santi DV. Hydrolysis of hydroxybenzotrifluorides and fluorinated uracil derivatives. A general mechanism for carbon-fluorine bond labilization. *J. Med. Chem.* 1973, 16, 1079-84.

40. Thompson DC, Perera K, London R. Spontaneous hydrolysis of 4-trifluoromethylphenol to a quinone methide and subsequent protein alkylation. *Chem. Biol. Interact.*, 2000, 126, 1-14.

41. Mikus, J., Steverding, D. A simple colorimetric method to screen drug cytotoxicity against *Leishmania* using the dye Alamar Blue *Parasitol Int.* **2000**, *48*, 265-269.

42. Martin, K.L. and T.K. Smith, The glycosylphosphatidylinositol (GPI) biosynthetic pathway of bloodstream-form Trypanosoma brucei is dependent on the de novo synthesis of inositol. *Mol Microbiol*. **2006** 61(1): p. 89-105.

43. Baliani, A; Bueno, GJ; Stewart, ML; Yardley, V; Brun, R; Barrett, MP; Gilbert, IH; *Design and Synthesis of a Series of Melamine-based Nitroheterocycles with Activity against Trypanosomatid Parasites.* J Med Chem, **2005**, 48 (17), 5570-5579.