European Journal of Medicinal Chemistry 127 (2017) 22–40

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Synthesis, biological characterisation and structure activity relationships of aromatic bisamidines active against *Plasmodium falciparum*

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ARTICLE INFO

Article history:
Received 21 October 2016
Received in revised form
19 December 2016
Accepted 20 December 2016
Available online 23 December 2016

Keywords:
Plasmodium falciparum
Bisamidine
Furamidine
Cytotoxicity

ABSTRACT

Malaria is one of the most significant tropical diseases and remains a major challenge due to the lack of a broadly effective vaccine and parasite resistance to current drugs. This means there is a need for new drug candidates with novel modes of action. Aromatic bisamidines, such as furamidine (DB75), were initially developed as anti-*Trypanosoma* agents however as clinical trials with furamidine highlighted potential side effects they were not pursued further in that setting. Despite apparent cytotoxicity liabilities the potency of furamidine against *Plasmodium falciparum* makes it a promising scaffold for the development of new anti-*Plasmodium* agents with improved selectivity. In this study a bisamidine compound series based on furamidine was synthesized by introducing modifications at the furan core structure and terminal amidine groups. The activity of the derived compounds was tested *in vitro* against drug sensitive and resistant *P. falciparum* lines and a human cell line (HEK293 cells) to generate anti-*Plasmodium* structure-activity relationships and to provide preliminary selectivity data.

1. Introduction

Malaria is an infectious hematologic disease that is caused by different *Plasmodium* species. Infection with *Plasmodium falciparum* can lead to severe symptoms, with >200 million clinical cases and ~450,000 malaria-related deaths occurring annually [1]. Clinical manifestations of malaria are induced by the asexual stages of the parasite that develop inside red blood cells [2] and it is this lifecycle stage that is the primary target of most treatment drugs. Over the past two decades, there has been significant reduction in the number of malaria cases and, since 2000, malaria-related deaths have halved [1,3]. Despite these gains, the emergence of drug resistant *P. falciparum* parasites and reduced clinical efficacy of current drugs, including the gold standard artemisinin combination therapies (ACTs) is a major obstacle and is fueling the discovery of new alternatives [4].

To address the need for new treatment options for malaria, new chemical entities with different modes of action to currently used antimalarial agents are needed. Among bis-tertiary amines, bis-quaternary ammonium salts and bis-2-aminopyridinium salts also aliphatic guanidines and aliphatic amidines were subjects of investigation to antimalarial activity [5]. Bisamidines are well known for their wide range of biological activities including antimicrobial, anti-inflammatory and anticancer effects [6]. The dibenzenecarboximidamide derivative, pentamidine, is used clinically for the treatment of leishmaniasis, trypanosomiasis and pneumocystis pneumonia [7]. Pentamidine is thought to act through blocking replication of parasite kinetoplast DNA in *Leishmania* parasites [8]. Due to a lack of alternatives, pentamidine is still used despite its potential life-threatening side effects. Investigation of pentamidine alternatives resulted in the discovery of the furan derivative furamidine (also known as **DB75**; Fig. 1). Furamidine is a fluorescent structural analogue of pentamidine with activity against *trypanosoma sp*, *Pneumocystis carinii, Cryptosporidium parvum* [9–12], and *in vitro* cultured asexual intraerythrocytic stage *P. falciparum* K1 parasites (IC_{50} 15.5 nM) [13], but with low oral

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http://dx.doi.org/10.1016/j.ejmech.2016.12.041
0223-5234/

bioavailability (c_{max} = 0.008 μmol/L, $t_{1/2}$ = 107 h, F = 0.2% after *p.o.* administration to mice) [14]. While the mode of action of furamidine is not fully understood, this compound has been localized to the nucleus and mitochondria of tumor cells [15,16] and to the nucleus in *P. falciparum* [17]. Furamidine has also been shown to bind to the minor groove of DNA at 5′-AATT-3' [18,19]. Different 2,5-bis(4-guanylphenyl)furans, substituted on position 3 and/or 4 of the furane ring including the 3,4-dimethylfuran derivative have been synthesized and tested in mice [20]. Several compounds showed good activity against *Trypanosoma rhodesiense* but were not able to cure mice infected with *P. berghei* [20,21]. Pafuramidine (also known as **DB289**), developed by Boykin et al. [22], is an orally active *O*-methylamidoxime prodrug of furamidine and has enhanced bioavailability [23,24]. Pharmacokinetics, absorption, distribution, biotransformation, excretion and conversion of pafuramidine to furamidine has been investigated in rat and cynomolgus monkey, showing that oral doses were well absorbed and effectively converted (c_{max} (male) = 26.1 ng/mL, $t_{1/2}$ (male) = 0.9 h, F (male) = 11% after *p.o.* administration of ^{14}C-pafuramidine to rats) [25]. In a recent human African *trypanosomiasis* (HAT) trial comparing efficacy versus standard treatment with pentamidine, pafuramidine was well tolerated [26]. In a clinical trial in Thailand in *P. vivax* and *P. falciparum* malaria patients, monotherapy with pafuramidine (100 mg, twice daily for 5 days) led to a cure rate of >95% [27]. However, nephron- and hepatotoxicity observed in a Phase 3 trial for first stage HAT [28] have compromised further use of this compound. Related analogues of furamidine, including amidines in which the guanyl function was incorporated into a heterocycle, have been already described but showed no increased antimalarial activity when tested against *P. berghei* in mice [20,21]. The “masked” amidines also generally exhibited lower antitrypanosomal activity than their guanyl counterparts [20]. While some symmetrical and non-symmetrical amidines have been synthesized and have been shown to have potent *in vitro* antiparasitic activity (e.g. *P. falciparum* K1 IC_{50} 10–140 nM) [29,30], the selectivity of these compounds for parasites versus normal human cells was not reported [31]. Here we describe the synthesis and biological characterization of a set of novel aromatic bisamidines based on furamidine and dimethylfuramidine to determine their potential as antimalarial agents. This work includes the synthesis of novel *N*-substituted dimethylfuramidine derivatives, 3,4-bisalkoxymethylenfuramidines, *N*-substituted 3-acetamidefuramidines, 3-chlorofuramidine derivatives, as well as a set of urea and guanidine based bisamidines. The growth inhibitory effects of the compounds were tested against the chloroquine sensitive *P. falciparum* 3D7 line [32] and the multi-drug resistant Dd2 line [33]. To assess the general toxicity of the compounds, a cytotoxicity assay on human epithelial kidney cell line HEK293 was performed and data were compared to that obtained for *P. falciparum*.

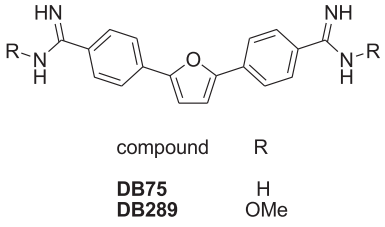


Fig. 1. Structures of reported furamidine derivatives - furamidine **DB75** and pafuramidine **DB289**.

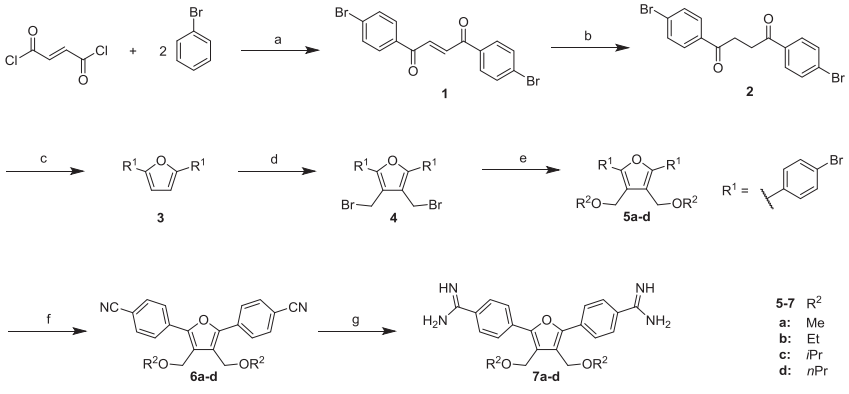
2. Chemistry

2.1. Synthesis of 3,4-bisalkoxymethylenfuramidines

The strategy for the synthesis of 3,4-bisalkoxymethylenfuramidines was developed based on a modified procedure of *Stephen* et al. [34]. As first step, a Friedel-Crafts-acylation reaction of bromobenzene using fumarylchloride was performed [35]. Reduction of compound **1** [36] yielded the saturated 1,4-diketon **2** [37] which was converted to the desired compound **3** [36] by cyclic dehydration using phosphoric acid. Modification of position 3 and 4 of the furan core structure was realized by introducing two bromomethylene groups (**4**) [20] followed by treatment with sodium alcoholate in THF yielding compounds **5a-d**. Finally, a two-step procedure was used to convert the aryl bromides into the final amidine functions. The cyano groups were introduced by a palladium-catalyzed coupling reaction [38]. Final treatment of the cyano groups in **6a-d** with LIHMDS and ethanolic hydrochloric acid gave the final compounds **7a-d** which were purified by RP-18 chromatography developed by Bakunov et al. [39]. The final compounds were handled as formate salts (Scheme 1).

2.2. Synthesis of N-substituted 3-acetamidefuramidines

The synthetic route for obtaining 3-acetamidefuramidines (**14a-y**) was based on the 1,4-addition reaction of dimethyl malonate to compound **1** (2.1.) [40]. Compound **9** represents the key compound to attain asymmetric substituted furan derivatives. Cyclization of the substituted diketone **9** using phosphoric acid in acetic



Scheme 1. *Reagents and conditions*: (a) $AlCl_3$, 0 → 60 °C, 48 h, HCl, H_2O; (b) $SnCl_2$, EtOH, AcOH, reflux, 20 min; (c) cat. H^+, AcO_2, reflux, 10 min; (d) paraformaldehyde, HBr, HOAc, RT, 48 h; (e) Na, R^2OH, 0 °C→reflux, 5 h; (f) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 80 °C, 24 h; (g) LIHMDS, THF, 0 °C→RT, 48 h, HCl, EtOH, 0 °C→RT, 24 h.

anhydride gave compound **10** according to the described procedure (2.1.). Ester hydrolysis of the dimethyl malonate residue and the spontaneous decarboxylation yielded compound **11**. The conversion of the arylbromides to the cyano group was then performed as previously described. Various substituents at the 3-position of the furan moiety were introduced by amide synthesis using a common PyBOP based procedure [41]. Treatment with ethanolic hydrochloric acid and ethanolic ammonia, also known as Pinner-method, afforded the desired bisamidines **14a-y** [42] (Scheme 2). An overview of the synthesized *N*-substituted 3-acetamidefuramidines is given in Table 2.

2.3. Synthesis of 3-chlorofuramidines

For synthesis of the 3-chlorofuramidines **20a** and **b**, compound **1** (2.1.) had to be cyclisized by introduction of the chloro-substituent at position 3. Therefore phosphorus pentachloride was heated with educt **1** without solvent to get the furan derivative **16** [36]. After introduction of a bromomethylene group the crude **17** [20] was etherified with the corresponding alkoxides to get the compounds **18a,b** which were converted *via* **19a,b** into the corresponding amidines **20a,b** (Scheme 3). An overview of the synthesized 3-chlorofuramidines is given in Table 3.

2.4. Synthesis of guanidine and urea based bisamidines

For the synthesis of guanidine based bisamidines, a three-step procedure was developed which started with an addition reaction of 4-cyanophenylisocyanate and 4-aminobenzonitrile to get compound **21** [43]. Afterwards the yielded urea derivative was converted to the corresponding guanidine by treatment with triphenylphosphine bromide and the respective amine [44]. Here, the dehydration of the urea compound gives a reactive carbodiimide *in situ* which reacts with various amines to the corresponding guanidines **22a-d**. Finally, the Pinner-procedure was applied on compounds **22a-d** yielding the desired bisamidines **23a-d**. The desired urea based bisamidine derivative **24**, was obtained from compound **21** using the Pinner-method (Scheme 4) [45]. An overview of the synthesized guanidine and urea based bisamidines is given in Table 4.

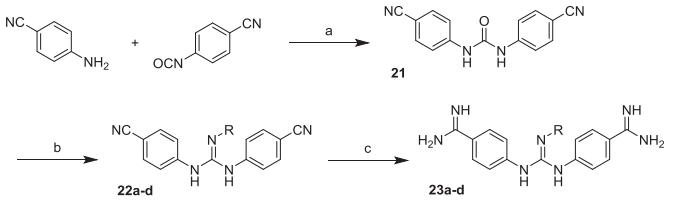
2.5. Synthesis of N-substituted dimethylfuramidine derivatives

For the synthesis of dimethylfuramidine derivatives, a method described by Anbazhagan et al. [46] was used. Starting with **8** [20], hydroxylamine was first added to obtain the *N*-hydroxycarbimidamide derivative **25**. To convert the oxime into the methoxy derivative, methylation of **25** was achieved using dimethyl sulfate to get **26**.

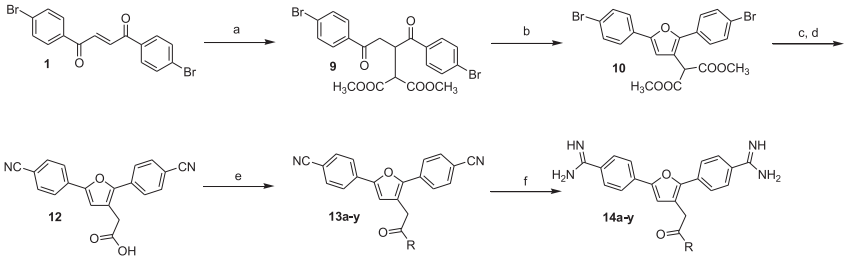
The starting point for the synthesis of the *N*-alkyl substituted dimethylfuramidine derivatives was **8**. According to the Pinner-method, the corresponding amine derivatives were used to obtain the *N*-alkylated bisamidines **27a-d** (Scheme 5) [20,47]. An overview of the synthesized *N*-substituted dimethylfuramidine compounds is given in Table 5.

3. Results and discussion

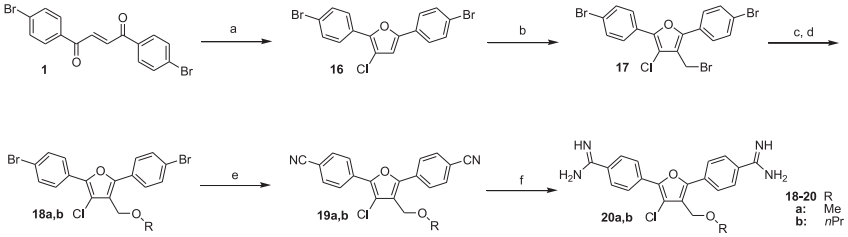
In 1977, the bisamidine compound furamidine (**DB75**) was developed as an anti-*Trypanosoma* agent (IC_{50} 4.5 nM *T. brucei rhodesiense*; IC_{50} 23.3 μM *T. cruzi*) and exhibited promising antiparasitic activity against a broad spectrum of microorganisms including *Giardia lamblia* (IC_{50} 0.2 μM), *P. falciparum* (IC_{50} 15.5 nM) and *Leishmania* spp. (IC_{50} 2.8 μM) [10–15]. Furamidine and its



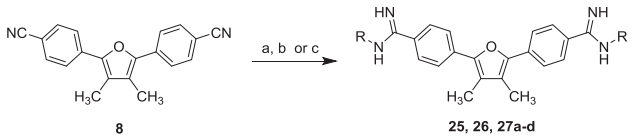
Scheme 4. *Reagents and conditions*: (a) CH_2Cl_2, DIPEA, overnight, RT; (b) Ph_3PBr_2, $CHCl_3$, DIPEA, H_2NR, 0 °C→reflux, 1,5 h; (c) HCl, EtOH, 7 d, RT, NH_3, EtOH, 7 d, RT. R groups are shown in Table 4.



Scheme 2. *Reagents and conditions*: (a) dimethylmalonate, toluene, DBU, RT, 30 min; (b) cat. H^+, AcO_2, reflux, 10 min; (c) K_2CO_3, MeOH, H_2O, reflux, 12 h, (d) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 80 °C, 24 h; (e) PyBOP, THF, 10 min, RT, HNR_2, DIPEA, overnight, RT; (f) HCl, EtOH, 7 d, RT, NH_3, EtOH, 7 d, RT. R groups are shown in Table 2.



Scheme 3. *Reagents and conditions*: (a) PCl_5, 3 h, 40 °C; (b), HBr, HOAc, RT, 5 d; (c) Na, ROH, 0 °C→reflux, 5 h; (d) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 80 °C, 24 h; (e) HCl, EtOH, 7 d, RT, NH_3, EtOH, 7 d, RT.



Scheme 5. *Reagents and conditions*: (a) DMSO, *t*-BuOK, overnight, 0 °C→RT; (b) dioxane, NaOH, overnight, RT; (c) HCl, EtOH, 7 d, RT, corresp. amine, EtOH, 7 d, RT. R groups ae shown in Table 5.

analogues exhibit very low oral bioavailability due to their cationic nature and as a result are not effective if administered orally. In efforts to circumvent this limitation, various prodrug strategies for bisamidines have been explored [22,27]. Subsequently, the derivative pafuramidine (**DB289**) was subjected to clinical trials against human African trypanosomes, malaria parasites and *P. jiroveci pneumonia*. After oral administration and absorption, pafuramidine is converted to furamidine, which results in excellent antimicrobial activity [29]. However, the emergence of liver and kidney toxicity led to their discontinuation. Because of this, optimized furamidine derivatives with anti-*Plasmodium* activity, reduced toxicity and improved pharmacokinetics are required if this class of compounds is to be pursued for malaria or other parasitic diseases.

To begin this optimization, the influence of modifications to the furan core structure of these compounds on *in vitro* anti-Plasmodium activity and general toxicity were assessed by synthesizing a set of 44 compounds. The impact of hydrophobic substituents at position 3 and 4 of the furan ring was assessed by introducing several dialkoxy groups (3,4-bisalkoxymethylenfuramidines **7a-d**). Furthermore, *N*-substituted 3-acetamidefuramidines **14a-y** were synthesized using a 7-step synthetic procedure to produce asymmetric substituted furan derivatives. The nature of the amide substituents was varied including hydrophobic, polar and basic substituents to obtain a chemically diverse set of compounds. As further asymmetric derivatives, 3-chloro-4-alkoxymethylenfuramidines **20a** and **20b** were synthesized. Moreover, a set of guanidine (**23a-d**) and urea (**24**) based bisamidines was additionally synthesized to evaluate the influence of the linker structure. The unsubstituted guanidine (Table 4, R = H) has been reported to be active against *Trypanosoma congolense* and *Babesia rodhaini* [48]. The urea derivative **24** has been reported to inhibit human serine protease [49] and HRgpA and RgpB gingipains, enzymes which are involved in the pathogenesis of gingivitis and periodontal disease [50].

For biological characterization, we concentrated on testing anti-*Plasmodium* activity and, to exclude possible toxic effects, activity against a human cell line. The *in vitro* anti-*Plasmodium* growth inhibitory effects were tested against *P. falciparum* drug-sensitive (3D7) and drug-resistant (Dd2) lines. Growth inhibition of the compounds was initially tested against these lines at 10 μM in at least two independent assays. Hit compounds (≥50% inhibition at 10 μM) were then tested in dose response assays to determine 50% growth inhibition values (IC_{50}). Overall, none of the compounds displayed greater activity against *P. falciparum* than furamidine (**7e**; Table 1), although some have low nM IC_{50}s, as discussed below. Hit compounds were assessed for *in vitro* cytotoxicity using a human epithelial kidney cell line (HEK293). All compounds exhibited low or no cytotoxicity at the concentrations tested (HEK293 IC_{50} > 33 μM).

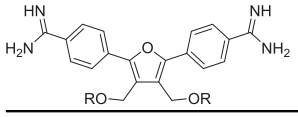
Together with the reference structure furamidine (**7e;** Table 1), the most potent anti-*Plasmodium* activity was seen for the *N*-substituted dimethylfuramidine compounds **27a-d** (IC_{50} 0.02–0.15 μM; Table 5). Compounds **27a-d** also showed high selectivity for the parasite versus HEK293 cells (SI 220–8131; Table 5), with **27b** being less toxic than furamidine **7e** (HEK295 IC_{50} 309.01 μM versus 178.04 μM, respectively) and more parasite-selective (SI 3433–8131; Table 5). The next most active class of compounds were the 3,4-bisalkoxymethylenfuramidines (**7a-d**), however *P. falciparum* IC_{50}s were around 1 μM, at least an order of magnitude lower than the *N*-substituted dimethylfuramidine compounds **27a-d.** The best from this series were the unbranched alkoxy derivatives **7a, b** and **d** which have IC_{50} values in the high nM range against Dd2 (900 nM, 850 nM and 310 nM, respectively; Table 1). Introducing more bulky substituents (i.e. isopropoxy, **7c**) resulted in reduced inhibitory activity against both *P. falciparum* strains.

In case of the 3-acetamidefuramidines a significant drop in inhibitory activity against *P. falciparum* was observed (Table 2). Of note, the introduction of a basic group containing side chains (e.g. **14k-u**) resulted in a complete loss of activity. Only derivatives with hydrophobic substituents such as **14d-14g** showed inhibitory activity around 1 μM against both lines. Compound **15,** having an ester side chain instead of an amide and a small ethyl rest, showed good inhibition against *P. falciparum* 3D7 with an IC_{50} of 220 nM, however was less active against Dd2.

As alternative to the furan linker, urea and guanidine derivatives were synthesized (Table 4). While the guanidine derivatives (**23a-d**) showed a noticeable loss in activity, the urea derivative **24** showed comparable activity and selectivity for 3D7 as the furan derivatives (IC_{50} 3D7 46 nM, SI 5783, Table 4), whereas the inhibition against Dd2 is reduced.

To analyze the further *in vivo* potential of the inhibitors we calculated several physicochemical properties and predicted pharmacokinetic parameters (Table 6). For predicting the properties PreADMET (https://preadmet.bmdrc.kr/admetox/) and PROTOX (http://tox.charite.de/tox/) web services were used. For

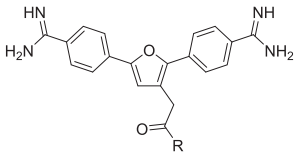
Table 1
Biological activity of compounds **7a-d**, furamidine **(7e)** [20], and positive controls chloroquine and SAHA.



Cpd. no.	R	*P. falciparum* IC_{50} (μM)		HEK293 IC_{50} (μM)	SI[a]	
		Dd2	3D7		Dd2	3D7
7a	Me	0.90 ± 0.27	1.04 ± 0.09	>50	>56	>48
7b	Et	0.85 ± 0.22	1.19 ± 0.05	>50	>59	>42
7c	*i*Pr	1.20 ± 0.20	1.08 ± 0.06	>50	>42	>46
7d	*n*Pr	0.31 ± 0.09	0.39 ± 0.04	>50	>128	>161
7e (furamidine)		0.05 ± 0.02	0.011 ± 0.006	178.04 ± 3.68	3560	16182
chloroquine		0.09 ± 0.01	0.02 ± 0.0025	n.d.	n.d.	n.d.
SAHA		0.26 ± 0.04	n.d.	n.d.	n.d.	n.d.

[a] SI – selectivity index; mammalian cell IC_{50}/*P. falciparum* IC_{50}.

Table 2
Biological activity of *N*-substituted 3-acetamidefuramidines compounds **14a-y**, **15**, and positive controls chloroquine and SAHA.



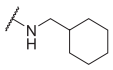
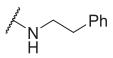
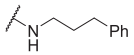
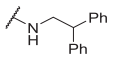
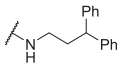
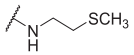
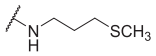
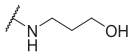
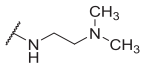
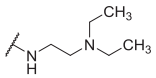
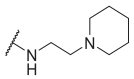
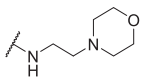
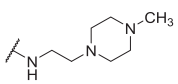
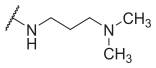
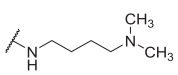
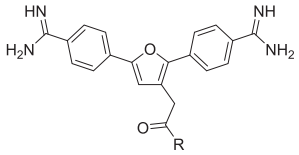
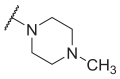
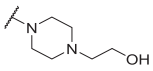
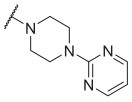
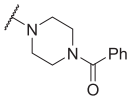
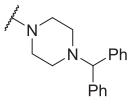
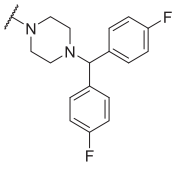
Cpd. no.	R	*P. falciparum* IC_{50} (μM)		HEK293 IC_{50} (μM)	SI[a]	
		Dd2	3D7		Dd2	3D7
14a		3.07 ± 1.17	5.41 ± 0.08	>50	>16	>9
14b		3.83 ± 0.65	5.76 ± 0.46	>50	>13	>8
14c		2.42 ± 0.97	n.d.	>50	>20	n.d.
14d		1.19 ± 0.36	1.18 ± 0.07	>50	>42	>42
14e		1.67 ± 0.31	0.77 ± 0.06	>50	>30	>65
14f		1.43 ± 0.25	1.39 ± 0.22	>50	>35	>36
14g		1.47 ± 0.15	1.40 ± 0.86	>50	>34	>36
14h		5.48 ± 2.88	6.09 ± 0.56	>50	>10	>8
14i		5.43 ± 2.50	6.61 ± 0.70	>50	>10	>7
14j		22.25 ± 11.24	>10	>50	>3	n.d.
14k		>10	>10	>50	n.d.	n.d.
14l		>10	>10	>50	n.d.	n.d.
14m		>10	>10	>50	n.d.	n.d.
14n		>10	>10	>50	n.d.	n.d.
14o		n.d.	>10	>50	n.d.	n.d.
14p		>10	>10	>50	n.d.	n.d.
14q		>10	>10	>50	n.d.	n.d.
14r		n.d.	5.13 ± 0.37	>50	n.d.	>9

Table 2 (*continued*)



Cpd. no.	R	*P. falciparum* IC_{50} (μM)		HEK293 IC_{50} (μM)	SI[a]	
		Dd2	3D7		Dd2	3D7
14s		>10	>10	>50	n.d.	n.d.
14t		>10	>10	>50	n.d.	n.d.
14u		>10	>10	>50	n.d.	n.d.
14v		17.37 ± 7.05	7.54 ± 0.71	>50	>3	>7
14w		>10	>10	>50	n.d.	n.d.
14x		5.33 ± 0.70	1.43 ± 0.59	>50	>10	>35
14y		6.63 ± 2.25	1.70 ± 0.10	>50	>8	>30
15		0.86 ± 0.27	0.22 ± 0.001	232.04 ± 1.87	270	1055
chloroquine		0.09 ± 0.01	0.02 ± 0.0025	n.d.	n.d.	n.d.
SAHA		0.26 ± 0.04	n.d.	n.d.	n.d.	n.d.

[a] SI – selectivity index; mammalian cell IC_{50}/*P. falciparum* IC_{50}.

comparison the experimentally derived IC_{50} values on HEK293 cells are included in Table 6. The *in silico* pharmacokinetic data (e.g. Caco2 apparent permeability (Papp nm/s) and human intestinal absorption (HIA %) as well as physicochemical data (e.g. TPSA and consensus logP) showed that compounds **20a, 24 and 27** have similar or better predicted permeability and oral bioavailability compared to the reference furamidine (**7e**) [20]. Compound **20a** and **27b** are classified as well absorbed compounds (HIA 70–100%) [51].

In addition the predicted human toxicity (LD_{50}) using the PROTOX approach [52] as well as the experimentally measured cytotoxicity against HEK293 cells of the most potent compounds (e.g. **20a, 24** and **27b**) was found to be lower compared to furamidine (**7e**) or dimethylfuramidine (**27d**).

4. Conclusions

The current work including the synthesis of a wide number of bisamidine derivatives and the assessment of their activities against two strains of *P. falciparum* and toxicity to human cells shed light on the structural requirements for activity and selectivity. Our work has uncovered novel aromatic bisamidines with promising activities against drug-sensitive and drug-resistant lines of *P. falciparum*, some with high selectivity for the parasite versus human HEK293 cells. The most promising compounds, bisamidines **20a**, and **27b** represent promising structures for further *in vivo* and tox investigation and optimization as anti-*Plasmodium* agents.

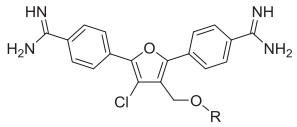
5. Experimental protocols

5.1. Chemistry

5.1.1. General

All materials and reagents were purchased from Sigma–Aldrich Co. Ltd. and Carbolution Chemicals. All of the solvents were analytically pure and dried before use. Thin-layer chromatography was carried out on aluminum sheets coated with silica gel 60 F254 (Merck, Darmstadt, Germany). For column chromatography under

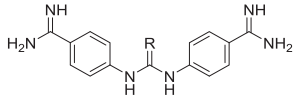
Table 3
Biological activity of 3-chlorofuramidine derivatives **20a**, **b**, and positive controls chloroquine and SAHA.

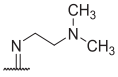
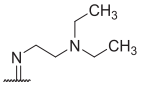
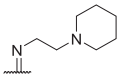


Cpd. no.	R	P. falciparum IC_{50} (μM)		HEK293 IC_{50} (μM)	SI[a]	
		Dd2	3D7		Dd2	3D7
20a	Me	0.09 ± 0.01	0.16 ± 0.01	209.00 ± 2.16	2322	1306
20b	*n*Pr	0.14 ± 0.03	0.09 ± 0.02	1% viability at 50 μM	n.d.	n.d.
chloroquine		0.09 ± 0.01	0.02 ± 0.0025	n.d.	n.d.	n.d.
SAHA		0.26 ± 0.04	n.d.	n.d.	n.d.	n.d.

[a] SI – selectivity index; mammalian cell IC_{50}/*P. falciparum* IC_{50}.

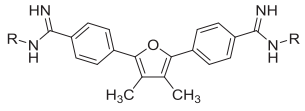
Table 4
Biological activity of guanidine and urea based bisamidine derivatives **23a-d**, **24** [45], and positive controls chloroquine and SAHA.

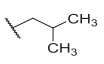


Cpd. no.	R	*P. falciparum* IC_{50} (μM)		HEK293 IC_{50} (μM)	SI[a]	
		Dd2	3D7		Dd2	3D7
23a		>10	>10	>50	n.d.	n.d.
23b		>10	>10	>50	n.d.	n.d.
23c		>10	>10	>50	n.d.	n.d.
23d		>10	>10	>50	n.d.	n.d.
24	O	0.21 ± 0.14	0.046 ± 0.003	266.04 ± 3.18	429	5783
chloroquine		0.09 ± 0.01	0.02 ± 0.0025	n.d.	n.d.	n.d.
SAHA		0.26 ± 0.04	n.d.	n.d.	n.d.	n.d.

[a] SI – selectivity index; mammalian cell IC_{50}/*P. falciparum* IC_{50}.

Table 5
Biological activity of *N*-substituted dimethylfuramidine derivatives **25**, **26**, **27a-d** [20,47], and positive controls chloroquine and SAHA.



Cpd. no.	R	*P. falciparum* IC_{50} (μM)		HEK293 IC_{50} (μM)	SI[a]	
		Dd2	3D7		Dd2	3D7
25	OH	7.15 ± 1.48	>1	>50	>10	n.d.
26	OCH_3	10.60 ± 0.99	>1	>50	>5	n.d.
27a		0.03 ± 0.01	0.02 ± 0.004	33.29 ± 2.61	1109	1664
27b		0.09 ± 0.03	0.038 ± 0.005	309.01 ± 0.05	3433	8131
27c		0.15 ± 0.05	0.038 ± 0.010	33.06 ± 1.98	220	870
27d	H	0.06 ± 0.01	0.033 ± 0.015	74.04 ± 1.34	1233	2242
chloroquine		0.09 ± 0.01	0.02 ± 0.0025	n.d.	n.d.	n.d.
SAHA		0.261 ± 0.04	n.d.	n.d.	n.d.	n.d.

[a] SI – selectivity index; mammalian cell IC_{50}/*P. falciparum* IC_{50}.

Table 6
In silico ADME and Tox prediction for furamidine **(7e)** and the most potent compounds. For predicting the properties PreADMET (https://preadmet.bmdrc.kr/admetox/) and PROTOX (http://tox.charite.de/tox/) web applications were used. For comparison the experimentally derived IC_{50} values on HEK293 cells are shown in the last row.

	Furamidine (7e)	20a	24	27b	27d
BBB	0.05	0.16	0.03	1.33	0.06
Caco2 Papp nm/s	0.48	6.56	21.11	17.69	0.48
HIA %	64.48	78.98	67.53	90.73	84.99
CYP 2C19 inhibition	Non	Non	Non	Non	Non
CYP 2C9 inhibition	Non	Non	Non	Non	Non
CYP 2D6 inhibition	Inhibitor	Inhibitor	Non	Inhibitor	Inhibitor
CYP 3A4 inhibition	Non	Non	Non	Non	Non
P-gp inhibition	Non	Non	Non	Non	Non
hERG inhibition	medium risk	medium risk	medium risk	medium risk	medium risk
Plasma protein binding %	15.7	70.0	28.9	72.7	27.9
Buffer solubility mg/L	1350.9	61365.9	1506.3	976.2	3912.1
Water solubility	soluble	soluble	very soluble	moderately	moderately
TPSA Å^2	116.36	122.11	144.35	88.38	116.36
Consensus logP	1.75	3.01	0.34	4.35	2.36
MDDR like rule		Drug-like		Drug-like	
Rule of five	Suitable	Suitable	Suitable	Suitable	Suitable
WDI like rule	Failed	In 90% cutoff	Failed	In 90% cutoff	Failed
LD_{50} mg	165	206	600	165	165
HEK293 IC_{50} μM	178.04 ± 3.68	209.00 ± 2.16	266.04 ± 3.18	309.01 ± 0.05	74.04 ± 1.34

normal pressure, silica gel 60 (0.036–0.200 mm) was used. Purification of the final compounds was carried out by preparative RP-HPLC (Shimadzu, Kyoto, Japan; LC-10AD, SIL-HT auto sampler). A prepacked 7.8–300 mm XTerra RP-18 (7 lm) column from Waters (Milford, MA, USA) was used and the UV–vis detector SPD-M10A VP PDA was set to 254 nm. A shallow gradient over 45 min from water containing 0.1% TFA to 95% methanol was run and the eluate collected and fractionated. Final compounds were confirmed to be of >95% purity based on HPLC. The purity was measured by UV absorbance at 254 nm. The HPLC system consisted of an XTerra RP18 column (3.5 μm 3.9 × 100 mm) from the manufacturer Waters (Milford, MA, USA), two LC-10AD pumps, an SPD-M10A VP PDA detector, and a SIL-HT autosampler, all from the manufacturer Shimadzu (Kyoto, Japan). The mobile phase was in all cases a gradient of methanol/water (starting at 95% water and going to 5% water). Mass spectrometry analyses were performed with a Finnigan MAT 710C mass spectrometer (Thermo Separation Products, San Jose, CA, USA) for the ESI-MS spectra and with an LTQ (linear ion trap)-Orbitrap XL hybrid mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) for the HRMS-ESI (high-resolution mass spectrometry) spectra. For the HRMS analyses, the signals for the isotopes with the highest prevalence (^{35}Cl, ^{79}Br) were given and calculated. ^1H and ^{13}C NMR spectra were recorded on Varian Gemini 2000 and Varian Inova 500 spectrometers using deuterated chloroform ($CDCl_3$) and deuterated DMSO ($(CD_3)_2SO$) as solvents. Chemical shifts are referenced to the residual solvent signals. The following abbreviations for solvents and reagents were used: ethyl acetate (EtOAc), methanol (MeOH), tetrahydrofuran (THF), chloroform ($CHCl_3$), water (H_2O).

The spectroscopic data of compounds **15**, **20a** and **27a-d** are provided in the Supplementary Material.

*5.1.2. (E)-1,4-Bis(4-bromophenyl)but-2-en-1,4-dione **(1)** [36]*

To a suspension of $AlCl_3$ (0.24 mol) and bromobenzene (100 mL) fumaryl chloride (0.1 mol) was given dropwise at 0 °C. The mixture was stirred for 48 h at 60 °C. After cooling to RT, the crude mixture was poured into ice water/HCl (750 mL) and extracted with DCM (3 times 500 mL). The organic phase was washed two times with 2 M NaOH, dried over Na_2SO_4, filtered, and evaporated. The crude product was recrystallized in $CHCl_3$ to give **3** (65% yield) as a light yellow solid. ^1H–NMR ($CDCl_3$, 400 MHz), δ (ppm): 7.94 (2H, s, RC**H**C**H**R); 7.90 (4H, m, Ar–**H**); 7.66 (4H, m, Ar–**H**). ^{13}C–NMR ($CDCl_3$, 125 MHz), δ (ppm):188.52, 135.50, 134.85, 132.30, 130.29, 129.39. EI-MS: *m/z*: 392, 394, 396 $[M]^+$.

*5.1.3. 1,4-Bis(4-bromophenyl)butan-1,4-dione **(2)** [37]*

$SnCl_2{\cdot}2H_2O$ (17.9 mmol) was given to a suspension of **1** (5.1 mmol) in AcOH (67 mL) and EtOH (67 mL). The mixture was heated under reflux for 20 min giving a pale yellow solution. After cooling to RT the mixture was evaporated. The crude product was filtered, and washed with diethyl ether to get **2** (94%) as a white solid. ^1H NMR ($CDCl_3$, 400 MHz), δ (ppm): 7.87 (4H, m, Ar–**H**), 7.60 (4H, m, Ar–**H**), 3.39 (4H, s, RC**H**$_2$C**H**$_2$R). ^{13}C NMR ($CDCl_3$, 125 MHz), δ (ppm):197.47, 135.40, 131.94, 129.63, 128.40, 32.44. EI-MS: *m/z*: 394, 396, 398 $[M]^+$.

*5.1.4. 2,5-Bis(4-bromophenyl)furan **(3)** [36]*

Phosphoric acid (65 μl) in acetic anhydride (1 mL) was given to a boiling solution of **2** (1 mmol) and acetic anhydride (6.5 mL) and heated for 10 min. After cooling to RT the solid was filtered and purified by column chromatography (eluent: heptane/$CHCl_3$) to give the furan derivative **3** as light yellow crystals (84% yield). ^1H NMR ($CDCl_3$, 400 MHz), δ (ppm): 7.57 (4H, d, $J = 8.6$ Hz, Ar–**H**); 7.50 (4H, d, $J = 8.6$ Hz, Ar–**H**); 6.72 (2H, s, furan–). ^{13}C NMR ($CDCl_3$, 125 MHz), δ (ppm): 152.66, 131.89, 129.43, 125.22, 121.29, 107.89. EI-MS: *m/z*: 376, 378, 380 $[M]^+$.

*5.1.5. 3,4-Bis(bromomethyl)-2,5-bis(4-bromophenyl)furan **(4)** [20]*

A suspension of **3** (0.02 mol), paraformaldehyde (0.1 mol) and 30% HBr in AcOH (80 g) was stirred for 48 h at RT. The solid was filtered off, washed with water and recrystallized in acetone to give **4** (83% yield) as white crystals. ^1H NMR ($CDCl_3$, 400 MHz), δ (ppm): 7.61 (8H, m, Ar–); 4.62 (4H, s, C**H**$_2$). ^{13}C NMR ($CDCl_3$, 125 MHz), δ (ppm): 150.41; 132.44; 128.33; 128.02; 123.04; 119.35; 23.22. EI-MS: *m/z*: 562, 564, 566 $[M]^+$.

*5.1.6. General method for Williamson-ether-synthesis to get **5a-d***

Sodium (4.3 mmol) was dissolved and stirred at 0 °C in 1 mL of the corresponding alcohol. Afterwards **4** (1.77 mmol) was dissolved in 10 mL of the corresponding alcohol and given dropwise to the alcoholate solution. The mixture was heated to reflux for 5 h. After cooling down to RT, water was given to the mixture. The formed

solid was filtered off and purified by column chromatography (eluent: $CHCl_3$) to give **5a-e**.

*5.1.6.1. 2,5-Bis(4-bromophenyl)-3,4-bis(methoxymethyl)furan **(5a)**.* White solid (85% yield). ^{1}H NMR (500 MHz, $CDCl_3$), δ (ppm): 7.60–7.54 (m, 8H, Ar–**H**), 4.45 (s, 4H, furan–C**H**$_2$), 3.45 (s, 6H, C**H**$_3$). ^{13}C NMR (126 MHz, $CDCl_3$), δ (ppm): 150.62, 131.88, 129.20, 127.90, 122.19, 120.17, 64.25, 58.10. EI-MS: *m/z*: 464, 466, 468 $[M]^+$.

*5.1.6.2. 2,5-Bis(4-bromophenyl)-3,4-bis(ethoxymethyl)furan **(5b)**.* White solid (85% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63–7.59 (m, 4H, Ar–**H**), 7.59–7.55 (m, 4H, Ar–**H**), 4.52 (s, 4H, furan–C**H**$_2$), 3.64 (q, J = 7.0 Hz, 4H, C**H**$_2$CH$_3$), 1.29 (t, J = 7.0 Hz, 6H, CH$_2$C**H**$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 150.62, 131.99, 129.48, 128.10, 122.25, 120.56, 66.04, 62.55, 15.44. EI-MS: *m/z*: 492, 494, 496 $[M]^+$.

*5.1.6.3. 2,5-Bis(4-bromophenyl)-3,4-bis[(1-methylethoxy)methyl] furan **(5c)**.* White solid (57% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64–7.59 (m, 4H, Ar–**H**), 7.59–7.54 (m, 4H, Ar–**H**), 4.49 (s, 4H, furan–C**H**$_2$), 3.80 (hept, J = 6.1 Hz, 2H, C**H**(CH$_3$)$_2$), 1.28 (d, J = 6.1 Hz, 12H, CH(C**H**$_3$)$_2$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 150.65, 131.94, 129.55, 128.12, 122.19, 120.69, 71.65, 60.24, 22.33. EI-MS: *m/z*: 520, 522, 524 $[M]^+$.

*5.1.6.4. 2,5-Bis(4-bromophenyl)-3,4-bis(propoxymethyl)furan **(5d)**.* White solid (94% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.64–7.60 (m, 4H, Ar–**H**), 7.59–7.55 (m, 4H, Ar–**H**), 4.50 (s, 4H, furan–C**H**$_2$), 3.54 (t, J = 6.6 Hz, 4H, C**H**$_2$CH$_2$CH$_3$), 1.73–1.63 (m, 4H, CH$_2$C**H**$_2$CH$_3$), 0.98 (t, J = 7.4 Hz, 6H, CH$_2$CH$_2$C**H**$_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 150.63, 131.97, 129.48, 128.07, 122.21, 120.62, 72.53, 62.75, 23.14, 10.98. EI-MS: *m/z*: 520, 522, 524 $[M]^+$.

*5.1.7. General method for cyanization of phenylbromides to get **6a-d***

A suspension of the corresponding phenylbromide derivative (**5a-d)** (1 mmol), $Zn(CN)_2$ (2.8 mmol), $Pd(Ph_3P)_4$ (0.1 mmol) and DMF (3.6 mL) was heated at 80 °C for 12 h under argon. After cooling to RT the mixture was evaporated and the residue dissolved in DCM/H_2O (75:25). The organic layer was separated, washed two times with water, dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography (eluent: $CHCl_3$/MeOH) to give **6a-d** as white solids.

*5.1.7.1. 4,4'-[3,4-Bis(methoxymethyl)furan-2,5-diyl]bisbenzonitrile **(6a)**.* White solid (19% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90–7.83 (m, 4H, Ar–**H**), 7.78–7.71 (m, 4H, Ar–**H**), 4.50 (s, 4H, furan–C**H**$_2$), 3.50 (s, 6H, C**H**$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.67, 150.59, 134.03, 132.73, 126.82, 122.87, 118.81, 111.68, 64.01, 58.46. EI-MS: *m/z*: 358 $[M]^+$.

*5.1.7.2. 4,4'-[3,4-Bis(ethoxymethyl)furan-2,5-diyl]bisbenzonitrile **(6b)**.* White solid (25% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.89–7.84 (m, 4H, Ar–**H**), 7.76–7.71 (m, 4H, Ar–**H**), 4.55 (s, 4H, furan–C**H**$_2$), 3.67 (q, J = 7.0 Hz, 4H, C**H**$_2$CH$_3$), 1.30 (t, J = 7.0 Hz, 6H, CH$_2$C**H**$_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 150.44, 134.14, 132.68, 126.81, 123.12, 118.84, 111.57, 66.30, 62.14, 15.39. EI-MS: *m/z*: 386 $[M]^+$.

*5.1.7.3. 4,4'-{3,4-Bis[(1-methylethoxy)methyl]furan-2,5-diyl}bisbenzonitrile **(6c)**.* White solid (19% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.90–7.85 (m, 4H, Ar–**H**), 7.75–7.70 (m, 4H, Ar–**H**), 4.53 (s, 4H, furan–C**H**$_2$), 3.82 (hept, J = 6.1 Hz, 2H, C**H**(CH$_3$)$_2$), 1.29 (d, J = 6.1 Hz, 12H, CH(C**H**$_3$)$_2$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 150.40, 134.17, 132.59, 126.77, 123.24, 118.81, 111.47, 71.92, 59.76, 22.24. EI-MS: *m/z*: 414 $[M]^+$.

*5.1.7.4. 4,4'-[3,4-Bis(propoxymethyl)furan-2,5-diyl]bisbenzonitrile **(6d)**.* White solid (32% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91–7.86 (m, 4H, Ar–**H**), 7.76–7.71 (m, 4H, Ar–**H**), 4.54 (s, 4H, furan–C**H**$_2$), 3.57 (t, J = 6.6 Hz, 4H, C**H**$_2$CH$_2$CH$_3$), 1.74–1.63 (m, 4H, CH$_2$C**H**$_2$CH$_3$), 0.98 (t, J = 7.4 Hz, 3H, CH$_2$CH$_2$C**H**$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 150.32, 134.00, 132.52, 126.63, 123.03, 118.69, 111.39, 72.57, 62.18, 22.95, 10.77. EI-MS: *m/z*: 414 $[M]^+$.

*5.1.8. General method for the amidination of benzonitriles to get **7a-d***

To a suspension of the corresponding bisbenzonitriles **6a-d** (1 mmol) in THF (8 mL) LiHMDs (5 mmol, 1 M in THF) was given dropwise under argon, and the reaction mixture was stirred for 48 h at RT. Afterwards the mixture was cooled to 0 °C, ethanolic HCl solution (15 mL) was added and stirred for another 24 h. After dilution with diethyl ether, the precipitate was separated by centrifugation and purified by preparative HPLC (eluent: MeOH/ammonium formate solution) to get **7a-d** as formate salts.

*5.1.8.1. 4,4'-[3,4-Bis(methoxymethyl)furan-2,5-diyl]bis(benzenecarboximidamide) formate **(7a)**.* Light yellow solid (46% yield). ^{1}H NMR (500 MHz, DMSO) δ 8.43, (s, 2H, formate), 8.02 (d, J = 8.7 Hz, 4H, Ar–**H**), 7.98 (d, J = 8.7 Hz, 4H, Ar–**H**), 4.54 (s, 4H, furan–C**H**$_2$), 3.39 (s, 6H, C**H**$_3$). ^{13}C NMR (126 MHz, DMSO) δ 165.84, 150.08, 134.27, 129.07, 128.35, 126.52, 123.23, 63.46, 58.01. HR-MS: 393.1925 [calculated for $C_{22}H_{25}N_4O_3^+$: 393.1921].

*5.1.8.2. 4,4'-[3,4-Bis(ethoxymethyl)furan-2,5-diyl]bis(benzenecarboxyimidamide) formate **(7b)**.* Light yellow solid (61% yield). ^{1}H NMR (500 MHz, DMSO) δ 8.46 (s, 2H, formate), 8.03 (d, J = 8.7 Hz, 4H, Ar-**H**), 7.96 (d, J = 8.7 Hz, 4H, Ar-**H**), 4.57 (s, 4H, furan-C**H**$_2$), 3.62 (q, J = 7.0 Hz, 4H, C**H**$_2$CH$_3$), 1.19 (t, J = 7.0 Hz, 6H, CH$_2$C**H**$_3$). ^{13}C NMR (126 MHz, DMSO) δ 167.74, 165.51, 149.49, 133.77, 128.43, 128.22, 126.08, 122.93, 65.09, 61.11, 15.07. HR-MS: *m/z*: 211.1155 [calculated for $C_{24}H_{30}N_4O_3^{2+}$: 211.1154].

*5.1.8.3. 4,4'-{3,4-Bis[(1-methylethoxy)methyl]furan-2,5-diyl}bis(benzenecarboximidamide) formate **(7c)**.* Light yellow solid (37% yield). ^{1}H NMR (400 MHz, DMSO) δ 8.42 (s, 2H, formate), 8.09–7.99 (m, 8H, Ar–**H**), 4.54 (s, 4H, furan–C**H**$_2$), 3.80 (hept, J = 6.1 Hz, 2H, C**H**(CH$_3$)$_2$), 1.21 (d, J = 6.1 Hz, 12H, CH(C**H**$_3$)$_2$). ^{13}C NMR (101 MHz, DMSO) δ 167.29, 164.99, 149.46, 134.12, 128.82, 127.13, 126.03, 123.31, 70.79, 58.90, 21.98. HR-MS: *m/z*: 449.2545 [calculated for $C_{26}H_{33}N_4O_3^+$: 449.2547].

*5.1.8.4. 4,4'-[3,4-Bis(propoxymethyl)furan-2,5-diyl]bis(benzenecarboximidamide) formate **(7d)**.* Light yellow solid (29% yield). ^{1}H NMR (400 MHz, DMSO) δ 8.44 (s, 2.5H, formate), 8.04 (d, J = 8.4 Hz, 4H, Ar-**H**), 7.97 (d, J = 8.4 Hz, 4H, Ar-**H**), 4.57 (s, 4H, furan-C**H**$_2$), 3.53 (t, J = 6.4 Hz, 4H, C**H**$_2$CH$_2$CH$_3$), 1.58 (m, 4H, CH$_2$C**H**$_2$CH$_3$), 0.91 (t, J = 7.4 Hz, 6H, CH$_2$CH$_2$C**H**$_3$). ^{13}C NMR (101 MHz, DMSO) δ 167.30, 165.52, 149.57, 133.77, 128.43, 128.17, 126.06, 122.94, 71.42, 61.35, 22.45, 10.68. HR-MS: *m/z*: 449.2544 [calculated for $C_{26}H_{33}N_4O_3^+$: 449.2547].

*5.1.8.5. 4,4'-Furan-2,5-diyl)bis(benzenecarboximidamide) hydrochloride **(7e)**.* Resynthesis of furamidine (**DB75**) was realized according to the reported procedure [20].

Light yellow solid (58% yield). ^{1}H NMR (400 MHz, DMSO) δ 9.59 (s, 4H, **H**$_2$NR), 9.39 (s, 4H, **H**$_2$NR), 8.11 (d, J = 8.6 Hz, 4H, Ar–**H**), 8.01 (d, J = 8.4 Hz, 4H, Ar–**H**), 7.46 (s, 2H, furan–**H**). ^{13}C NMR (101 MHz, DMSO) δ 164.90, 152.46, 134.24, 128.98, 126.40, 123.83, 111.74. HR-MS: *m/z*: 305.1393 [calculated for $C_{18}H_{17}N_4O^+$: 305.1397].

*5.1.9. 4,4'-(3,4-Dimethylfuran-2,5-diyl)bisbenzonitrile **(8)** [20]*

Synthesis of compound **8** as starting material for *N*-substituted analogues of **NSC 305836** was performed according to the reported procedures [47].

*5.1.10. Dimethyl-2-[(1,4-bis(4-bromophenyl))-1,4-diketobut-2-yl] malonate **(9)***

To a suspension of **1** (16 mmol) and dimethylmalonate (7.26 mL) in toluene (50 mL) DBU (157 μl) was given. The mixture was stirred at RT until the educt was completely converted (ca. 30 min; TLC control). The crude mixture was evaporated and purified by column chromatography (eluent: EtOAc) to get **9** as a colorless oil (89% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (d, J = 8.5 Hz, 2H, Ar–**H**), 7.75 (d, J = 8.4 Hz, 2H, Ar–**H**), 7.62 (d, J = 8.4 Hz, 2H, Ar–**H**), 7.58 (d, J = 8.4 Hz, 2H, Ar–**H**), 4.79 (ddd, J = 8.6, 6.7, 5.7 Hz, 1H, C**H**RCH$_2$), 3.90 (d, J = 8.7 Hz, 1H, C**H**R), 3.68 (s, 3H, C**H**$_3$), 3.62 (s, 3H, C**H**$_3$), 3.51 (dd, J = 18.1, 5.6 Hz, 1H, CHRC**H**$_2$), 3.36 (dd, J = 18.1, 5.6 Hz, 1H, CHRC**H**$_2$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 199.44, 195.84, 168.56, 168.35, 134.86, 134.64, 132.23, 132.17, 130.42, 129.77, 129.01, 128.90, 53.02, 52.99, 52.97, 40.66, 39.27.

*5.1.11. Dimethyl-2-[2,5-bis(4-bromophenyl)furan-3-yl]malonate **(10)***

Cyclization of **9** was achieved according to the method described in 5.3. to get **10** as light yellow solid (87% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63–7.56 (m, 4H, Ar–**H**), 7.56–7.49 (m, 4H, Ar–**H**), 6.99 (s, 1H, furan–**H**), 4.84 (s, 1H, C**H**), 3.81 (s, 6H, C**H**$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.12, 152.37, 150.32, 132.27, 132.03, 129.22, 128.91, 128.50, 125.56, 122.82, 121.82, 115.68, 108.95, 53.30, 49.04.

*5.1.12. 2-[2,5-Bis(4-bromophenyl)furan-3-yl]acetic acid **(11)***

6 mmol **10** was given to a mixture of 37.5 mL MeOH and 12.5 mL saturated K_2CO_3-solution and the reaction mixture was heated for 12 h. After cooling down to RT, the crude mixture was acidified with HCl and extracted (3 times with EtOAc). The organic layer was dried over Na_2SO_4 and evaporated to get **11** as a white solid (>95% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61–7.48 (m, 8H, Ar–**H**), 6.78 (s, 1H, furan–**H**), 3.70 (s, 2H, furan–C**H**$_2$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.10, 152.12, 149.56, 132.17, 132.07, 129.38, 129.24, 127.74, 125.48, 122.18, 121.76, 115.61, 110.08, 31.74.

*5.1.13. 2-[2,5-Bis(4-cyanophenyl)furan-3-yl]acetic acid **(12)***

Cyanisation of **11** was achieved according to the method described in 5.6. to get **12** as a light yellow solid (73% yield). Cave! Nascent hydrogene cyanide was neutralized by passing a solution of $KMnO_4$.

^{1}H NMR (400 MHz, DMSO) δ 12.69 (s, 1H, COO**H**), 8.01 (d, J = 8.5 Hz, 2H, Ar–**H**), 7.98–7.88 (m, 6H, Ar–**H**), 7.36 (s, 1H, furan–**H**), 3.76 (d, J = 9.2 Hz, 2H, furan–C**H**$_2$). ^{13}C NMR (101 MHz, DMSO) δ 171.43, 150.70, 148.34, 133.83, 133.28, 132.95, 132.84, 125.79, 124.29, 120.64, 118.76, 118.71, 114.66, 109.93, 109.89, 31.82. HR-MS: m/z: 329.0918 [calculated for $C_{20}H_{13}N_2O_3^+$: 329.0921].

*5.1.14. General method for the synthesis of amides **13a-y***

A mixture of **12** (0.3 mmol) and PyBOP (0.45 mmol) in THF (20 mL) was stirred for 10 min. Afterwards, the corresponding amine (0.34 mmol) and DIPEA (0.76 mmol) were given to the mixture and stirred overnight at RT. The crude mixture was evaporated, the product was dissolved in EtOAc, washed with Na_2CO_3 solution, and dried over Na_2SO_4. After evaporation, the product was purified by column chromatography (eluent: $CHCl_3$/MeOH).

*5.1.14.1. 2-[2,5-Bis(4-cyanophenyl)furan-3-yl]-N-methylacetamide **(13a)**.* Light yellow solid (75% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85–7.77 (m, 4H, Ar–**H**), 7.75–7.69 (m, 4H, Ar–**H**), 6.93 (s, 1H, furan–**H**), 5.79 (s, 1H, CON**H**), 3.66 (s, 2H, furan–C**H**$_2$), 2.84 (d, J = 4.7 Hz, 3H, C**H**$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.68, 152.15, 149.59, 134.03, 133.58, 132.90, 132.88, 126.14, 124.43, 119.89, 118.76, 118.70, 113.03, 111.56, 111.51, 34.38, 26.84. ESI-MS: 340.27 [M–H]$^-$.

*5.1.14.2. 2-[2,5-Bis(4-cyanophenyl)furan-3-yl]-N,N-dimethylacetamide **(13b)**.* Light yellow solid (71% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, J = 8.4 Hz, 4H, Ar–**H**), 7.73 (d, J = 8.5 Hz, 2H, Ar–**H**), 7.68 (d, J = 8.4 Hz, 2H, Ar–**H**), 6.91 (s, 1H, furan–**H**), 3.76 (s, 2H, furan–C**H**$_2$), 3.06 (s, 3H, C**H**$_3$), 3.01 (s, 3H, C**H**$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.27, 151.68, 149.06, 134.35, 133.72, 132.63, 132.59, 126.17, 124.18, 120.26, 118.71, 118.63, 112.79, 111.09, 110.97, 37.65, 35.84, 31.29. ESI-MS: m/z: 354.32 [M–H]$^-$.

*5.1.14.3. N-(Cyclohexylmethyl)-2-[2,5-bis(4-cyanophenyl)furan-3-yl] acetamide **(13c)**.* Off-white solid (50% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85–7.78 (m, 4H, Ar–**H**), 7.76–7.68 (m, 4H, Ar–**H**), 6.92 (s, 1H, furan–**H**), 5.67 (s, 1H, CON**H**), 3.66 (s, 2H, furan–C**H**$_2$), 3.13–3.10 (m, 2H, CONHC**H**$_2$), 1.82–0.75 (m, 11H, cyclohexane–**H**). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.62, 152.19, 149.52, 134.03, 133.58, 132.92, 132.87, 126.09, 124.46, 119.99, 118.76, 113.08, 113.07, 111.57, 111.55, 46.24, 38.03, 34.72, 30.92, 26.42, 25.86. HR-MS: m/z: 422,1860 [calculated for $C_{27}H_{24}N_3O_2^-$: 422.1863].

*5.1.14.4. 2-[2,5-Bis(4-cyanophenyl)furan-3-yl]-N-(2-phenylethyl) acetamide **(13d)**.* Light yellow solid (66% yield). ^{1}H NMR (400 MHz, DMSO) δ 8.24 (t, J = 5.5 Hz, 1H, CON**H**), 8.03–7.96 (m, 4H, Ar–**H**), 7.95–7.88 (m, 4H, Ar–**H**), 7.29–7.14 (m, 6H, phenyl–**H**, furan–**H**), 3.57 (s, 2H, furan–C**H**$_2$), 3.39–3.27 (m, 2H, C**H**$_2$CH$_2$Ph, H_2O), 2.73 (t, J = 7.2 Hz, 2H, CH$_2$C**H**$_2$Ph). ^{13}C NMR (101 MHz, DMSO) δ 168.66, 150.61, 148.37, 139.31, 133.91, 133.33, 132.96, 132.72, 128.60, 128.24, 126.03, 125.92, 124.25, 121.71, 118.77, 118.76, 114.29, 109.87, 109.80, 40.31, 34.97, 32.95. ESI-MS: m/z: 430.15 [M–H]$^-$.

*5.1.14.5. 2-[2,5-Bis(4-cyanophenyl)furan-3-yl]-N-(3-phenylpropyl) acetamide **(13e)**.* Light yellow solid (74% yield). ^{1}H NMR (400 MHz, DMSO) δ 8.20 (t, J = 5.5 Hz, 1H, CON**H**), 8.04–7.97 (m, 4H, Ar–**H**), 7.96–7.88 (m, 4H, Ar–**H**), 7.31 (s, 1H, furan–**H**), 7.29–7.22 (m, 2H, phenyl–**H**), 7.20–7.12 (m, 3H, phenyl–**H**), 3.61 (s, 2H, furan–C**H**$_2$), 3.13–3.08 (m, 2H, C**H**$_2$CH$_2$CH$_2$Ph), 2.56 (t, J = 7.5 Hz, 2H, CH$_2$CH$_2$C**H**$_2$Ph), 1.75–1.67 (m, 2H, CH$_2$C**H**$_2$CH$_2$Ph). ^{13}C NMR (101 MHz, DMSO) δ 168.65, 150.64, 148.38, 141.59, 133.95, 133.34, 132.95, 132.73, 128.22, 128.21, 125.90, 125.70, 124.25, 121.85, 118.77, 118.74, 114.37, 109.86, 109.80, 38.39, 32.99, 32.45, 30.77. ESI-MS: m/z: 444.23 [M–H]$^-$.

*5.1.14.6. N-(2,2-Diphenylethyl)-2-[2,5-bis(4-cyanophenyl)furan-3-yl] acetamide **(13f)**.* Off-white solid (63% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78–7.62 (m, 8H, Ar-**H**), 7.25–7.18 (m, 5H, phenyl-**H**), 7.18–7.10 (m, 5H, phenyl-**H**), 6.61 (s, 1H, furan-**H**), 5.57 (s, 1H, CON**H**), 4.15 (t, J = 8.1 Hz, 1H, C**H**), 3.95–3.88 (m, 2H, C**H**$_2$), 3.54 (s, 2H, furan-C**H**$_2$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.91, 152.12, 149.51, 141.41, 133.81, 133.50, 132.87, 132.84, 128.90, 128.05, 127.10, 126.06, 124.44, 119.49, 118.76, 118.68, 112.68, 111.57, 111.54, 50.52, 44.00, 34.51. ESI-MS: m/z: 506.13 [M–H]$^-$.

*5.1.14.7. N-(3,3-Diphenylpropyl)-2-[2,5-bis(4-cyanophenyl)furan-3-yl]acetamide **(13g)**.* Off-white solid (64% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82–7.76 (m, 4H, Ar-**H**), 7.75–7.68 (m, 4H, Ar-**H**), 7.28–7.20 (m, 5H, phenyl-**H**), 7.18–7.11 (m, 5H, phenyl-**H**), 6.84 (s, 1H, furan-**H**), 5.54 (t, J = 5.3 Hz, 1H, CON**H**), 3.85 (t, J = 7.8 Hz, 1H, C**H**), 3.56 (s, 2H, furan-C**H**$_2$), 3.30–3.25 (m, 2H, CONHC**H**$_2$),

2.27–2.21 (m, 2H, C**H**$_2$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.90, 152.15, 149.49, 144.06, 133.98, 133.54, 132.93, 132.89, 128.79, 127.78, 126.68, 126.04, 124.43, 119.83, 118.75, 118.65, 112.99, 111.58, 111.57, 49.41, 39.10, 35.23, 34.55. ESI-MS: *m/z*: 520.16 [M−H]$^-$.

5.1.14.8. N-(2-(Methylthio)ethyl)-2-[2,5-bis(4-cyanophenyl)furan-3-yl]acetamide ***(13h)***. Off-white solid (52% yield). ^{1}H NMR (400 MHz, DMSO) δ 8.31 (t, J = 5.4 Hz, 1H, CON**H**), 8.00 (d, J = 8.4 Hz, 4H, Ar-**H**), 7.96–7.87 (m, 4H, Ar-**H**), 7.31 (s, 1H, furan-**H**), 3.61 (s, 2H, furan-C**H**$_2$), 3.30–3.25 (m, 2H, C**H**$_2$CH$_2$S), 2.58–2.52 (m, 2H, CH$_2$C**H**$_2$S), 2.06 (s, 3H, C**H**$_3$). ^{13}C NMR (101 MHz, DMSO) δ 168.78, 150.63, 148.40, 133.91, 133.34, 132.96, 132.74, 125.92, 124.25, 121.64, 118.77, 118.76, 114.41, 109.86, 109.81, 38.13, 32.94, 32.69, 14.50. ESI-MS: *m/z*: 400.08 [M−H]$^-$.

5.1.14.9. N-(3-(Methylthio)propyl)-2-[2,5-bis(4-cyanophenyl)furan-3-yl]acetamide ***(13i)***. Light yellow solid (61% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, J = 8.4 Hz, 4H, Ar-**H**), 7.77–7.68 (m, 4H, Ar-**H**), 6.92 (s, 1H, furan-**H**), 3.66 (s, 2H, furan-C**H**$_2$), 3.40 (dd, J = 12.1, 6.3 Hz, 2H, C**H**$_2$CH$_2$CH$_2$S), 2.46 (t, J = 6.9 Hz, 2H, CH$_2$CH$_2$C**H**$_2$S), 2.01 (s, 3H, C**H**$_3$), 1.78 (p, J = 6.8 Hz, 2H, CH$_2$C**H**$_2$CH$_2$S). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.94, 152.00, 149.36, 133.85, 133.38, 132.74, 132.71, 125.91, 124.26, 119.64, 118.58, 118.49, 112.87, 111.41, 111.37, 39.26, 34.41, 31.90, 28.11, 15.46. ESI-MS: *m/z*: 414.10 [M−H]$^-$.

5.1.14.10. N-(3-Hydroxypropyl)-2-(2,5-bis(4-cyanophenyl)furan-3-yl)acetamide ***(13j)***. White solid (76% yield). ^{1}H NMR (400 MHz, DMSO) δ 8.14 (t, J = 5.4 Hz, 1H, CON**H**), 8.04–7.97 (m, 4H, Ar–**H**), 7.96–7.88 (m, 4H, Ar–**H**), 7.30 (s, 1H, furan–**H**), 3.59 (s, 2H, furan–C**H**$_2$), 3.41 (t, J = 6.3 Hz, 2H, C**H**$_2$OH), 3.17–3.12 (m, 2H, C**H**$_2$, CONHC**H**$_2$), 1.60–1.54 (m, 2H, C**H**$_2$). ^{13}C NMR (101 MHz, DMSO) δ 168.68, 150.63, 148.39, 133.95, 133.36, 132.96, 132.73, 125.91, 124.27, 121.84, 118.77, 114.36, 109.86, 109.80, 58.35, 36.06, 32.93, 32.27. ESI-MS: *m/z*: 384.16 [M−H]$^-$.

5.1.14.11. N-[2-(Dimethylamino)ethyl]-2-[2,5-bis(4-cyanophenyl) furan-3-yl]acetamide ***(13k)***. Light yellow solid (61% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89–7.82 (m, 2H, Ar–**H**), 7.82–7.77 (m, 2H, Ar–**H**), 7.75–7.71 (m, 2H, Ar–**H**), 7.71–7.66 (m, 2H, Ar–**H**), 6.99 (s, 1H, furan–**H**), 6.56 (s, 1H, CON**H**), 3.64 (s, 2H, furan–C**H**$_2$), 3.40–3.36 (m, 2H, CONHC**H**$_2$), 2.46 (t, J = 5.7 Hz, 2H, C**H**$_2$N(CH$_3$)$_2$), 2.24 (s, 6H, CH$_2$N(C**H**$_3$)$_2$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.27, 151.91, 149.50, 134.26, 133.77, 132.84, 132.76, 126.27, 124.37, 120.19, 118.83, 118.79, 113.15, 111.33, 111.27, 57.81, 45.04, 36.92, 34.30. ESI-MS: *m/z*: 399.23 [M+H]$^+$.

5.1.14.12. N-[2-(Diethylamino)ethyl]-2-[2,5-bis(4-cyanophenyl) furan-3-yl]acetamide ***(13l)***. Light yellow solid (70% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, J = 8.5 Hz, 2H, Ar–**H**), 7.79 (d, J = 8.4 Hz, 2H, Ar–**H**), 7.72 (d, J = 8.5 Hz, 2H, Ar–**H**), 7.68 (d, J = 8.4 Hz, 2H, Ar–**H**), 7.01–6.89 (m, 2H, furan–**H**, CON**H**), 3.64 (s, 2H, furan–C$_2$), 3.41–3.37 (m, 2H, CONHC**H**$_2$), 2.71–2.69 (m, 2H, C**H**$_2$N(CH$_2$CH$_3$)$_2$), 2.63–2.58 (m, 4H, CH$_2$N(C**H**$_2$CH$_3$)$_2$)), 0.97 (t, J = 7.1 Hz, 6H, C**H**$_2$N(CH$_2$C**H**$_3$)$_2$)). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.21, 151.97, 149.49, 134.20, 133.72, 132.84, 132.78, 126.17, 124.36, 120.17, 118.84, 118.79, 113.27, 111.32, 111.28, 51.31, 47.07, 36.50, 34.24, 11.09. ESI-MS: *m/z*: 427.21 [M+H]$^+$.

5.1.14.13. N-(2-Piperidin-1-ylethyl)-2-[2,5-bis(4-cyanophenyl)furan-3-yl]acetamide ***(13m)***. Light yellow solid (49% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90–7.85 (m, 2H, Ar–**H**), 7.82–7.77 (m, 2H, Ar–**H**), 7.75–7.70 (m, 2H, Ar–**H**), 7.68 (d, J = 8.4 Hz, 2H, Ar–**H**), 7.05 (s, 1H, furan–**H**), 3.67 (s, 2H, furan–C**H**$_2$), 3.45–3.40 (m, 2H, CONHC**H**$_2$CH$_2$), 2.60–2.58 (m, 2H, CONHCH$_2$C**H**$_2$), 2.55–2.41 (m, 4H, piperidine–**H**), 1.61–1.52 (m, 4H, piperidine–**H**), 1.45–1.35 (m, 2H, piperidine–**H**). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.46, 151.85, 149.48, 134.21, 133.75, 132.81, 132.76, 126.18, 124.32, 120.36, 118.82, 118.79, 113.43, 111.27, 111.23, 57.04, 54.20, 35.56, 34.31, 24.97, 23.58. ESI-MS: *m/z*: 439.18 [M+H]$^+$.

5.1.14.14. N-[2-(Morpholin-4-yl)ethyl]-2-[2,5-bis(4-cyanophenyl) furan-3-yl]acetamide ***(13n)***. Off-white solid (47% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 8.3 Hz, 2H, Ar-**H**), 7.82 (d, J = 8.4 Hz, 2H, Ar-**H**), 7.74 (d, J = 8.5 Hz, 2H, Ar-**H**), 7.68 (d, J = 8.4 Hz, 2H, Ar-**H**), 7.26 (m, 1H, furan-**H**, $CHCl_3$), 4.40–3.82 (m, 6H), 3.81–3.64 (m, 4H, furan-C**H**$_2$), 3.59–3.32 (m, 2H), 3.01–2.74 (m, 2H), 1.72–1.52 (m, 2H). ESI-MS: *m/z*: 441.13 [M+H]$^+$.

5.1.14.15. N-[2-(4-Methylpiperazin-1-yl)ethyl]-2-[2,5-bis(4-cyanophenyl)furan-3-yl]acetamide ***(13o)***. The compound was used for the next step without purification. ESI-MS: *m/z*: 454.19 [M+H]$^+$.

5.1.14.16. N-[3-(Dimethylamino)propyl]-2-[2,5-bis(4-cyanophenyl) furan-3-yl]acetamide ***(13p)***. Light yellow solid (63% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (s, 1H, CON**H**), 7.86 (d, J = 8.4 Hz, 2H, Ar–**H**), 7.82 (d, J = 8.4 Hz, 2H, Ar-**H**), 7.76–7.72 (m, 2H, Ar-**H**), 7.70 (d, J = 8.4 Hz, 2H, Ar–**H**), 7.01 (s, 1H, furan–**H**), 3.64 (s, 2H, furan–C**H**$_2$), 3.42–3.38 (m, 2H, CONHC**H**$_2$), 2.47 (t, J = 5.9 Hz, 2H, C**H**$_2$N(CH$_3$)$_2$), 2.13 (s, 6H, CH$_2$N(C**H**$_3$)$_2$), 1.75–1.66 (m, 2H, CH$_2$C**H**$_2$CH$_2$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.51, 151.86, 149.57, 134.14, 133.66, 132.95, 132.85, 126.03, 124.32, 120.37, 118.78, 118.75, 113.56, 111.41, 111.38, 58.40, 44.69, 39.73, 34.63, 24.65. ESI-MS: *m/z*: 413.13 [M+H]$^+$.

5.1.14.17. N-[4-(Dimethylamino)butyl]-2-[2,5-bis(4-cyanophenyl) furan-3-yl]acetamide ***(13q)***. Light yellow solid (82% yield). ^{1}H NMR (400 MHz, DMSO) δ 8.25 (t, J = 5.7 Hz, 1H, CON**H**), 8.05–7.98 (m, 4H, Ar–**H**), 7.97–7.89 (m, 4H, Ar–**H**), 7.32 (s, 1H, furan–**H**), 3.61 (s, 2H, furan–C**H**$_2$), 3.13–3.10 (m, 2H, C**H**$_2$), 3.05–2.97 (m, 2H, C**H**$_2$), 2.71 (s, 6H, C**H**$_3$), 1.65–1.54 (m, 2H, C**H**$_2$), 1.49–1.39 (m, 2H, C**H**$_2$). ^{13}C NMR (101 MHz, DMSO) δ 168.72, 150.67, 148.43, 133.94, 133.32, 132.98, 132.75, 125.93, 124.27, 121.74, 121.73, 118.76, 114.30, 109.92, 109.85, 56.24, 42.07, 37.93, 32.97, 26.03, 21.16. ESI-MS: *m/z*: 427.18 [M+H]$^+$.

5.1.14.18. 1-{[2,5-Bis(4-cyanophenyl)furan-3-yl]acetyl}piperidine ***(13r)***. Off-white solid (71% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82–7.76 (m, 4H, Ar–**H**), 7.73 (d, J = 8.5 Hz, 2H, Ar–**H**), 7.68 (d, J = 8.5 Hz, 2H, Ar–**H**), 6.91 (s, 1H, furan–**H**), 3.77 (s, 2H, furan–C**H**$_2$), 3.52 (s, 4H, piperidine–**H**), 1.71–1.62 (m, 2H, piperidine–**H**), 1.61–1.49 (m, 4H, piperidine–**H**). ESI-MS: *m/z*: 394.24 [M−H]$^-$.

5.1.14.19. 4-{[2,5-Bis(4-cyanophenyl)furan-3-yl]acetyl}morpholine ***(13s)***. Light yellow solid (45% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79–7.74 (m, 4H, Ar–**H**), 7.74–7.70 (m, 2H, Ar–**H**), 7.69–7.64 (m, 2H, Ar–**H**), 6.88 (s, 1H, furan–**H**), 3.75 (s, 2H, furan–C**H**$_2$), 3.72–3.59 (m, 6H, morpholine–**H**), 3.46 (s, 2H, morpholine–**H**). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.13, 152.01, 149.16, 134.35, 133.74, 132.84, 132.81, 126.34, 124.38, 119.99, 118.82, 118.70, 112.65, 111.44, 111.31, 66.99, 66.67, 46.58, 42.56, 31.23. ESI-MS: *m/z*: 396.21 [M−H]$^-$.

5.1.14.20. 1-{[2,5-Bis(4-cyanophenyl)furan-3-yl]acetyl}-4-methylpiperazine ***(13t)***. Light yellow solid (53% yield). ^{1}H NMR (400 MHz, DMSO) δ 8.05–7.99 (m, 2H, Ar–**H**), 7.96–7.87 (m, 6H, Ar–**H**), 7.27 (s, 1H, furan–**H**), 3.88 (s, 2H, furan–C**H**$_2$), 3.60–3.53 (m, 2H, piperazine–**H**), 3.53–3.46 (m, 2H, piperazine–**H**), 2.35–2.32 (m, 2H, piperazine–**H**), 2.31–2.25 (m, 2H, piperazine–**H**), 2.20 (s, 3H, C**H**$_3$). ^{13}C NMR (101 MHz, DMSO) δ 167.39, 150.59, 148.40, 133.98, 133.36, 132.94, 132.75, 125.83, 124.26, 121.74, 118.78, 118.75, 114.60, 109.84, 109.74, 54.80, 54.32, 45.61, 45.22, 41.37, 30.48. ESI-

MS: *m/z*: 411.08 $[M+H]^+$.

*5.1.14.21. 2-(4-{[2,5-Bis(4-cyanophenyl)furan-3-yl]acetyl}piperazine-1-yl)ethanol **(13u)**.* Light yellow solid (66% yield). ^{1}H NMR (400 MHz, DMSO) δ 8.04–7.99 (m, 2H, Ar–**H**), 7.96–7.88 (m, 6H, Ar–**H**), 7.27 (s, 1H, furan–**H**), 4.43 (s, 1H, O**H**), 3.88 (s, 2H, furan–C**H**$_2$), 3.63–3.44 (m, 6H, C**H**$_2$OH, piperazine–**H**), 3.31–3.28 (m, 2H, NC**H**$_2$, H_2O), 2.43 (s, 4H, piperazine–**H**). ESI-MS: *m/z*: 441.11 $[M+H]^+$.

*5.1.14.22. 2-(4-{[2,5-Bis(4-cyanophenyl)furan-3-yl]acetyl}piperazine-1-yl)pyrimidine **(13v)**.* Light yellow solid (73% yield). ^{1}H NMR (400 MHz, DMSO) δ 8.40 (d, *J* = 4.8 Hz, 2H, **H**3, **H**5), 8.03 (d, *J* = 8.3 Hz, 2H, Ar-**H**), 8.00–7.87 (m, 6H, Ar-**H**), 7.30 (s, 1H, furan-**H**), 6.67 (t, *J* = 4.8 Hz, 1H, **H**4), 3.96 (s, 2H, furan-C**H**$_2$), 3.82 (s, 2H, piperazine-**H**), 3.77 (s, 2H, piperazine-**H**), 3.69 (s, 2H, piperazine-**H**), 3.60 (s, 2H, piperazine-**H**). ESI-MS: *m/z*: 475.10 $[M+H]^+$.

*5.1.14.23. 1-{[2,5-Bis(4-cyanophenyl)furan-3-yl]acetyl}-4-benzoylpiperazine **(13w)**.* Light yellow solid (54% yield). ^{1}H NMR (400 MHz, DMSO) δ 8.02–8.00 (m, 2H, Ar–**H**), 7.98–7.87 (m, 6H, Ar–**H**), 7.48–7.42 (m, 5H, benzoyl–**H**), 7.28 (s, 1H, furan–**H**), 3.92 (s, 2H, piperazine–**H**), 3.73–3.51 (m, 6H, piperazine–**H**), 3.31 (s, 2H, furan–C**H**$_2$). ^{13}C NMR (101 MHz, DMSO) δ 169.21, 167.72, 150.63, 148.42, 135.64, 133.93, 133.33, 132.92, 132.75, 129.64, 128.42, 126.98, 125.82, 124.27, 121.50, 118.78, 118.75, 114.50, 109.85, 109.73, 79.14, 30.55. ESI-MS: *m/z*: 523.11 $[M+Na]^+$.

*5.1.14.24. 1-{[2,5-Bis(4-cyanophenyl)furan-3-yl]acetyl}-4-(diphenylmethyl)piperazine **(13x)**.* White solid (64% yield). ^{1}H NMR (400 MHz, DMSO) δ 8.00 (d, *J* = 8.4 Hz, 2H, Ar–**H**), 7.96–7.85 (m, 6H, Ar–**H**), 7.44 (d, *J* = 7.3 Hz, 4H, phenyl–**H**), 7.31 (t, *J* = 7.6 Hz, 4H, phenyl–**H**), 7.25 (s, 1H, furan–**H**), 7.20 (t, *J* = 7.3 Hz, 2H, phenyl–**H**), 4.38 (s, 1H, C**H**), 3.84 (s, 2H, furan–C**H**$_2$), 3.61 (s, 2H, piperazine–**H**), 3.53 (s, 2H, piperazine–**H**), 2.39–2.24 (m, 4H, piperazine–**H**). ^{13}C NMR (101 MHz, DMSO) δ 167.31, 150.59, 148.38, 142.30, 133.92, 133.33, 132.92, 132.69, 128.53, 127.62, 126.95, 125.79, 124.25, 121.65, 118.78, 118.73, 114.54, 109.83, 109.70, 74.51, 51.73, 51.17, 45.43, 41.54, 30.42. ESI-MS: *m/z*: 561.32 $[M-H]^-$.

*5.1.14.25. 1-{[2,5-Bis(4-cyanophenyl)furan-3-yl]acetyl}-4-[bis(4-fluorphenyl)methyl]piperazine **(13y)**.* Off-white solid (51% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80–7.64 (m, 8H, Ar-**H**), 7.39–7.27 (m, 4H, phenyl-**H**), 7.04–6.92 (m, 4H, phenyl-**H**), 6.86 (s, 1H, furan-**H**), 4.25 (s, 2H, C**H**), 3.73 (s, 2H, furan-C**H**$_2$), 3.68 (s, 2H, piperazine-**H**), 3.49 (s, 2H, piperazine-**H**), 2.43–2.28 (m, 4H, piperazine-**H**). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.83, 163.27, 160.85, 151.86, 149.05, 137.42, 134.32, 133.74, 132.79, 132.73, 129.36, 129.28, 126.24, 124.32, 120.25, 118.82, 118.71, 115.85, 115.62, 112.72, 111.26, 111.19, 74.24, 51.94, 51.47, 46.34, 42.32, 31.33. ESI-MS: *m/z*: 597,24 $[M-H]^-$.

*5.1.15. General Pinner-method for amidination to get **14a-y***

HCl-gas was led into abs. ethanol (10–15 mL) under cooling for 30 min. Afterwards, the bisbenzonitrile derivative (0.1–0.2 mmol) was added and stirred for one week under argon. After conversion the crude mixture was evaporated. The residue was dissolved in dry ethanolic ammonia (10–15 mL) and stirred for another week. After evaporation the free base can converted into the formate salt by adding a mixture of 10 mL ethanol/formic acid (9:1). After precipitation in diethyl ether (20–30 mL) the salt was separated by centrifugation. Purification was achieved by preparative HPLC (eluent: MeOH/ammonium formate solution) to get **14a-y** as formate salts.

*5.1.15.1. 2-[2,5-Bis(4-carbamimidoylphenyl)furan-3-yl]-N-methylacetamide formate **(14a)**.* Yellow solid (41% yield). ^{1}H NMR (400 MHz, DMSO) δ 8.48 (s, 2.5H, formate), 8.22–8.15 (m, 1H, CON**H**), 8.04 (d, 4H, Ar–**H**), 7.95–7.89 (m, 4H, Ar–**H**), 7.30 (s, 1H, furan-**H**), 3.61 (s, 2H, furan-C**H**$_2$), 2.64 (d, *J* = 4.6 Hz, 3H, C**H**$_3$). ^{13}C NMR (101 MHz, DMSO) δ 169.37, 168.01, 165.50, 150.81, 148.56, 134.34, 133.79, 128.56, 128.31, 127.66, 127.54, 125.51, 123.83, 121.21, 113.87, 32.94, 25.81. HR-MS: *m/z*: 376.1766 [calculated for $C_{21}H_{22}N_5O_2^+$: 376.1768].

*5.1.15.2. 2-[2,5-Bis(4-carbamimidoylphenyl)furan-3-yl]-N,N-dimethylacetamide formate **(14b)**.* Yellow solid (32% yield). ^{1}H NMR (500 MHz, DMSO) δ 8.47 (s, 2.5H, formate), 8.05 (d, *J* = 8.5 Hz, 2H, Ar–**H**), 7.97 (d, *J* = 8.7 Hz, 2H, Ar–**H**), 7.95–7.87 (m, 4H, Ar–**H**), 7.26 (s, 1H, furan–**H**), 3.88 (s, 2H, furan–C**H**$_2$), 3.11 (s, 3H, C**H**$_3$), 2.90 (s, 3H, C**H**$_3$). ^{13}C NMR (126 MHz, DMSO) δ 168.90, 167.77, 165.48, 150.70, 148.63, 134.41, 133.80, 128.51, 128.29, 127.64, 127.49, 125.42, 123.79, 121.26, 114.12, 37.15, 35.21, 30.73. HR-MS: *m/z*: 390.1925 [calculated for $C_{22}H_{24}N_5O_2^+$: 390.1925].

*5.1.15.3. N-(Cyclohexylmethyl)-2-[2,5-bis(4-carbamimidoylphenyl)furan-3-yl]acetamide formate **(14c)**.* Yellow solid (17% yield). ^{1}H NMR (400 MHz, DMSO) δ 8.47 (s, 2.5H, formate), 8.18 (t, *J* = 5.6 Hz, 1H, CON**H**), 8.05–8.03 (m, 4H, Ar–**H**), 7.93–7.89 (m, 4H, Ar–**H**), 7.29 (s, 1H, furan–**H**), 3.63 (s, 2H, furan–C**H**$_2$), 2.97–2.93 (m, 2H, CONHC**H**$_2$), 1.72–0.76 (m, 11H, cyclohexane–**H**). ^{13}C NMR (101 MHz, DMSO) δ 168.80, 165.40, 165.33, 150.75, 148.48, 148.21, 134.32, 133.74, 128.54, 128.21, 127.76, 127.58, 125.49, 123.78, 121.40, 113.87, 45.08, 39.73, 37.43, 30.40, 26.00, 25.38. HR-MS: *m/z*: 458.2548 [calculated for $C_{27}H_{32}N_5O_2^+$: 458.2551].

*5.1.15.4. 2-[2,5-Bis(4-carbamimidoylphenyl)furan-3-yl]-N-(2-phenylethyl)acetamide formate **(14d)**.* Yellow solid (21% yield).^{1}H NMR (500 MHz, DMSO) δ 8.42 (s, 2.5H, formate), 8.31 (t, *J* = 5.6 Hz, 1H, CON**H**), 8.05–8.02 (m, 4H, Ar–**H**), 7.94–7.90 (m, 4H, Ar–**H**), 7.30–7.16 (m, 6H, furan–**H**, phenyl–**H**), 3.60 (s, 2H, furan–C**H**$_2$), 3.37–3.33 (m, 2H, C**H**$_2$CH$_2$Ph), 2.75 (t, *J* = 7.3 Hz, 2H, CH$_2$C**H**$_2$Ph). ^{13}C NMR (126 MHz, DMSO) δ 168.82, 166.94, 165.45, 165.42, 150.76, 148.52, 139.34, 134.33, 133.78, 128.62, 128.57, 128.28, 128.27, 127.60, 127.47, 126.06, 125.52, 123.80, 121.20, 113.77, 40.37, 35.04, 32.99. HR-MS: *m/z*: 466.2234 [calculated for $C_{28}H_{28}N_5O_2^+$: 466.2238].

*5.1.15.5. 2-[2,5-Bis(4-carbamimidoylphenyl)furan-3-yl]-N-(3-phenylpropyl)acetamide formate **(14e)**.* Yellow solid (76% yield). ^{1}H NMR (500 MHz, DMSO) δ 8.45 (s, 2.5H, formate), 8.30 (t, *J* = 5.6 Hz, 1H, CON**H**), 8.07–8.03 (m, 4H, Ar–**H**), 7.94–7.90 (m, 4H, Ar–**H**), 7.31 (s, 1H, furan–**H**), 7.28–7.23 (m, 2H, phenyl–**H**), 7.20–7.13 (m, 3H, phenyl–**H**), 3.63 (s, 2H, furan–C**H**$_2$), 3.14–3.10 (m, 2H, C**H**$_2$CH$_2$CH$_2$Ph), 2.58 (t, 2H, CH$_2$CH$_2$C**H**$_2$Ph), 1.76–1.70 (m, 2H, CH$_2$C**H**$_2$CH$_2$Ph). ^{13}C NMR (126 MHz, DMSO) δ 168.83, 167.42, 165.48, 165.42, 150.79, 148.52, 141.61, 134.35, 133.78, 128.54, 128.26, 128.24, 127.65, 127.48, 125.72, 125.51, 123.81, 121.34, 113.83, 38.42, 33.02, 32.49, 30.82. HR-MS: *m/z*: 480.2391 [calculated for $C_{29}H_{30}N_5O_2^+$: 480.2394].

*5.1.15.6. N-(2,2-Diphenylethyl)-2-[2,5-bis(4-carbamimidoylphenyl)furan-3-yl]acetamide formate **(14f)**.* Yellow solid (38% yield). ^{1}H NMR (500 MHz, DMSO) δ 8.45 (s, 2.5H, formate), 8.31 (t, *J* = 5.6 Hz, 1H, CON**H**), 8.00–7.94 (m, 4H, Ar-**H**), 7.94–7.90 (m, 2H, Ar-**H**), 7.90–7.86 (m, 2H, Ar-**H**), 7.31–7.25 (m, 8H, phenyl-**H**), 7.20–7.15 (m, 2H, phenyl-**H**), 7.01 (s, 1H, furan-**H**), 4.25 (t, *J* = 7.9 Hz, 1H, C**H**), 3.81–3.75 (m, 2H, C**H**$_2$), 3.51 (s, 2H, furan-C**H**$_2$). ^{13}C NMR (126 MHz, DMSO) δ 168.98, 167.17, 165.48, 150.69, 148.39, 142.72, 134.21, 133.68, 128.52, 128.38, 128.23, 127.86, 127.85, 127.69, 127.54, 126.33, 125.49, 123.77, 121.13, 113.37, 50.03, 43.31, 32.94. HR-MS: *m/z*:

271.6311 [calculated for $C_{34}H_{33}N_5O_2^{2+}$: 271,6312].

5.1.15.7. N-(3,3-Diphenylpropyl)-2-[2,5-bis(4-carbamimidoylphenyl) furan-3-yl]acetamide formate ***(14g)***. Yellow solid (79% yield). ^{1}H NMR (500 MHz, DMSO) δ 8.47 (s, 2.5H, formate), 8.28 (t, J = 5.4 Hz, 1H, CONH), 8.07–8.01 (m, 4H, Ar-H), 7.95–7.88 (m, 4H, Ar-H), 7.31 (s, 1H, furan-H), 7.29–7.23 (m, 8H, phenyl-H), 7.18–7.12 (m, 2H, phenyl-H), 3.98 (t, J = 7.8 Hz, 1H, CH), 3.61 (s, 2H, furan-CH_2), 3.04–2.97 (m, 2H, CONHCH_2), 2.22–2.15 (m, 2H, CH_2). ^{13}C NMR (126 MHz, DMSO) δ 168.80, 167.69, 165.39, 165.32, 150.78, 148.52, 144.60, 134.29, 133.72, 128.51, 128.38, 128.23, 127.74, 127.57, 127.53, 126.08, 125.50, 123.79, 121.26, 113.79, 47.89, 37.63, 34.41, 33.00. HR-MS: m/z: 556.2703 [calculated for $C_{35}H_{34}N_5O_2^+$: 556.2707].

5.1.15.8. N-(2-(Methylthio)ethyl)-2-[2,5-bis(4-carbamimidoylphenyl) furan-3-yl]acetamide formate ***(14h)***. Yellow solid (50% yield). ^{1}H NMR (500 MHz, DMSO) δ 8.46 (s, 2.5H, formate), 8.39 (t, J = 5.7 Hz, 1H, CONH), 8.06–8.01 (m, 4H, Ar-H), 7.96–7.89 (m, 4H, Ar-H), 7.31 (s, 1H, furan-H), 3.63 (s, 3H, furan-CH_2), 3.32–3.29 (m, 2H, CH_2CH$_2$S), 2.59–2.54 (m, 2H, CH$_2$CH_2S), 2.07 (s, 3H, CH_3). ^{13}C NMR (126 MHz, DMSO) δ 168.94, 167.53, 165.46, 165.43, 150.77, 148.54, 134.29, 133.76, 128.54, 128.27, 127.68, 127.55, 125.51, 123.79, 121.09, 113.87, 38.15, 32.98, 32.72, 14.52. HR-MS: m/z: 436.1799 [calculated for $C_{35}H_{34}N_5O_2^+$: 436.1802].

5.1.15.9. N-(3-(Methylthio)propyl)-2-[2,5-bis(4-carbamimidoylphenyl)furan-3-yl]acetamide formate ***(14i)***. Yellow solid (78% yield). ^{1}H NMR (500 MHz, DMSO) δ 8.47 (s, 2H, formate), 8.27 (t, J = 5.6 Hz, 1H, CONH), 8.07–8.01 (m, 4H, Ar-H), 7.95–7.88 (m, 4H, Ar-H), 7.30 (s, 1H, furan-H), 3.62 (s, 2H, furan-CH_2), 3.18 (dd, J = 12.5, 6.7 Hz, 2H, CH_2CH$_2$CH$_2$S), 2.46 (t, J = 7.1 Hz, 2H, CH$_2$CH$_2$CH_2S), 2.02 (s, 3H, CH$_3$), 1.69 (p, J = 6.9 Hz, 2H. CH$_2$CH_2CH$_2$S). ^{13}C NMR (126 MHz, DMSO) δ 168.88, 167.68, 165.45, 165.40, 150.79, 148.52, 134.32, 133.76, 128.53, 128.25, 127.70, 127.55, 125.49, 123.80, 121.26, 113.83, 37.82, 33.00, 30.63, 28.50, 14.61. HR-MS: m/z: 450.1957 [calculated for $C_{24}H_{28}N_5O_2S^+$: 450.1958].

5.1.15.10. N-(3-Hydroxypropyl)-2-(2,5-bis(4-carbamimidoylphenyl) furan-3-yl)acetamide formate ***(14j)***. Yellow solid (33% yield). ^{1}H NMR (500 MHz, DMSO) δ 8.46 (s, 2.5H, formate), 8.24 (t, J = 5.5 Hz, 1H, CON), 8.05–8.03 (4H, Ar–H), 7.94–7.90 (4H, Ar–H), 7.30 (s, 1H, furan–H), 3.61 (s, 2H, furan–CH_2), 3.42 (t, J = 6.3 Hz, 2H, CH_2OH), 3.18–3.14 (m, 2H, CONHCH_2), 1.61–1.56 (m, 2H, CH_2). ^{13}C NMR (126 MHz, DMSO) δ 168.82, 167.55, 165.46, 165.43, 150.76, 148.51, 134.31, 133.76, 128.52, 128.25, 127.68, 127.54, 125.49, 123.80, 121.30, 113.84, 58.35, 36.05, 32.97, 32.30. HR-MS: m/z: 420.2028 [calculated for $C_{23}H_{26}N_5O_3^+$: 420,2030].

5.1.15.11. N-[2-(Dimethylamino)ethyl]-2-[2,5-bis(4-carbamimidoylphenyl)furan-3-yl]acetamide formate ***(14k)***. Yellow solid (53% yield). ^{1}H NMR (500 MHz, DMSO) δ 8.35 (m, 4.5H, formate, CONH), 8.05–8.03 (m, 4H, Ar–H), 7.96–7.93 (m, 4H, Ar–H), 7.33 (s, 1H, furan–H), 3.64 (s, 1H, furan–CH_2), 3.26–3.22 (m, 2H, CONHCH_2), 2.43 (t, J = 6.6 Hz, 2H, CH_2N(CH$_3$)$_2$), 2.23 (s, 6H, CH$_2$N(CH_3)$_2$). ^{13}C NMR (126 MHz, DMSO) δ 168.94, 165.32, 165.30, 150.76, 148.53, 134.45, 133.91, 128.73, 128.45, 127.26, 127.12, 125.51, 123.79, 121.37, 113.99, 57.73, 44.71, 36.55, 32.97. HR-MS: m/z: 433.2345 [calculated for $C_{24}H_{29}N_6O_2^+$: 433.2347].

5.1.15.12. N-[2-(Diethylamino)ethyl]-2-[2,5-bis(4-carbamimidoylphenyl)furan-3-yl]acetamide formate ***(14l)***. Yellow solid (50% yield). ^{1}H NMR (500 MHz, DMSO) δ 8.35 (s, 3H, formate), 8.27 (s, 1H, CONH), 8.08–8.02 (m, 4H, Ar–H), 8.00–7.90 (m, 4H, Ar–H), 7.34 (s, 1H, furan–H), 3.63 (s, 4H, furan–CH_2), 3.21–3.18 (m, 2H, CONHCH_2), 2.59–2.44 (m, 4H, CH_2N(CH_2CH$_3$, DMSO), 0.95 (t, J = 7.1 Hz, 6H, CH$_2$N(CH$_2$CH_3)$_2$. ^{13}C NMR (126 MHz, DMSO) δ 168.85, 165.70, 165.18, 165.15, 150.77, 148.54, 134.48, 133.93, 128.78, 128.50, 127.10, 126.96, 125.49, 123.78, 121.35, 114.03, 46.56, 11.35. HR-MS: m/z: 461.2659 [calculated for $C_{26}H_{33}N_6O_2^+$: 461,2660].

5.1.15.13. N-(2-Piperidin-1-ylethyl)-2-[2,5-bis(4-carbamimidoylphenyl)furan-3-yl]acetamid formate ***(14m)***. Yellow solid (44% yield). ^{1}H NMR (500 MHz, DMSO) δ 8.48 (t, 1H, J = 5.6 Hz, CONH), 8.25 (s, 2H, formate), 8.09–8.03 (m, 4H, Ar–H), 8.02–7.96 (m, 4H, Ar–H), 7.37 (s, 1H, furan–H), 3.66 (s, 6H, furan–CH_2), 3.34–3.30 (m, 2H, CONHCH_2CH$_2$), 2.69–2.65 (m, 6H, CONHCH$_2$CH_2, piperidine–H), 1.59–1.55 (m, 4H, piperidine–H), 1.43–1.36 (m, 2H, piperidine–H). ^{13}C NMR (126 MHz, DMSO) δ 168.97, 165.00, 164.35, 150.77, 148.56, 134.60, 134.06, 128.95, 128.69, 126.64, 126.50, 125.48, 123.77, 121.39, 114.16, 56.62, 53.27, 35.43, 33.03, 24.29, 23.00. HR-MS: m/z: 473.2657 [calculated for $C_{27}H_{33}N_6O_2^+$: 473.2656].

5.1.15.14. N-[2-(Morpholin-4-yl)ethyl]-2-[2,5-bis(4-carbamimidoylphenyl)furan-3-yl]acetamide formate ***(14n)***. Yellow solid (81% yield). ^{1}H NMR (500 MHz, DMSO) δ 8.32 (s, 2H, formate), 8.27 (t, J = 5.6 Hz, 1H, CONH), 8.08–8.02 (m, 4H, Ar-H), 8.00–7.93 (m, 4H, Ar-H), 7.34 (s, 1H, furan-H), 3.64 (s, 2H, furan-CH_2), 3.55–3.49 (m, 4H, morpholine-H), 3.24–3.20 (m, 2H, CONHCH_2CH$_2$), 2.39–2.32 (m, 6H, CONHCH$_2$CH_2, morpholine-H). ^{13}C NMR (126 MHz, DMSO) δ 168.83, 165.24, 165.12, 165.09, 150.77, 148.53, 134.53, 133.98, 128.85, 128.57, 126.92, 126.77, 125.49, 123.78, 121.46, 114.03, 66.13, 57.28, 53.21, 36.07, 33.03. HR-MS: m/z: 475.2450 [calculated for $C_{26}H_{31}N_6O_3^+$: 475.2452].

5.1.15.15. N-[2-(4-Methylpiperazin-1-yl)ethyl]-2-[2,5-bis(4-carbamimidoylphenyl)furan-3-yl]-acetamide formate ***(14o)***. Yellow solid (43% yield). ^{1}H NMR (500 MHz, DMSO) δ 8.38 (s, 3.5H, formate), 8.28 (s, 1H, CONH), 8.07–8.01 (m, 4H, Ar–H), 8.00–7.93 (m, 4H, Ar–H), 7.33 (s, 1H, furan–H), 3.63 (s, 2H, furan–CH_2), 3.22–3.18 (m, 2H, CONHCH_2CH$_2$), 2.47–2.25 (m, 10H, CONHCH$_2$CH_2, piperazine–H), 2.18–2.11 (m, 3H, CH$_3$). ^{13}C NMR (126 MHz, DMSO) δ 168.85, 165.95, 165.23, 150.79, 148.54, 134.48, 133.94, 128.79, 128.53, 127.10, 126.95, 125.49, 123.80, 121.42, 114.01, 56.67, 54.33, 52.18, 45.26, 36.35, 33.05. HR-MS: m/z: 488.2767 [calculated for $C_{27}H_{34}N_7O_2^+$: 488.2769].

5.1.15.16. N-[3-(Dimethylamino)propyl]-2-[2,5-bis(4-carbamimidoylphenyl)furan-3-yl] acetamide formate ***(14p)***. Yellow solid (42% yield). ^{1}H NMR (500 MHz, DMSO) δ 8.47 (t, J = 5.5 Hz, 1H, CONH), 8.34 (s, 2.5H, formate), 8.08–8.03 (m, 4H, Ar–H), 8.02–7.94 (m, 4H, Ar–H), 7.36 (s, 1H, furan–H), 3.64 (s, 2H, furan–CH_2), 3.15–3.10 (m, 2H, CONHCH_2), 2.53–2.45 (m, 2H, CH_2N(CH$_3$)$_2$, DMSO), 2.28 (s, 6H, CH$_2$N(CH_3)$_2$), 1.69–1.61 (m, 2H, CH$_2$). ^{13}C NMR (126 MHz, DMSO) δ 169.13, 164.96, 164.92, 150.79, 148.52, 134.65, 134.10, 128.99, 128.80, 126.47, 126.28, 125.40, 123.82, 121.48, 114.30, 54.33, 41.86, 36.02, 32.98, 24.03. HR-MS: m/z: 447.2500 [calculated for $C_{25}H_{31}N_6O_2^+$: 447.2503].

5.1.15.17. N-[4-(Dimethylamino)butyl]-2-[2,5-bis(4-carbamimidoylphenyl)furan-3-yl]acetamide formate ***(14q)***. Yellow solid (46% yield). ^{1}H NMR (500 MHz, DMSO) δ 8.42 (t, J = 5.4 Hz, 1H, CONH), 8.34 (s, 2H, formate), 8.08–8.04 (m, 4H, Ar–H), 8.00–7.95 (m, 4H, Ar–H), 7.36 (s, 1H, furan–H), 3.64 (s, 2H, furan–CH_2), 3.13–3.09 (m, 2H, CONHCH_2), 2.53–2.47 (m, 2H, CH_2N(CH$_3$)$_2$, DMSO), 2.31 (s, 6H, CH$_2$N(CH_3)$_2$), 1.53–1.41 (m, 4H, CH_2CH_2). ^{13}C NMR (126 MHz, DMSO) δ 168.77, 165.58, 165.09, 165.07, 150.75, 148.52, 134.56, 134.00, 128.85, 128.59, 126.83, 126.68, 125.46, 123.78, 121.56, 114.09, 57.58, 43.74, 38.39, 32.99,

26.55, 23.08. HR-MS: *m*/*z*: 461.2660 [calculated for $C_{26}H_{33}N_6O_2^+$: 461.2660].

*5.1.15.18. 1-{[2,5-Bis(4-carbamimidoylphenyl)furan-3-yl]acetyl} piperidin formate **(14r)**.* Yellow solid (58% yield). ^{1}H NMR (500 MHz, DMSO) δ 8.45 (s, 2H, formate), 8.06 (d, J = 8.5 Hz, 2H, Ar–**H**), 7.97 (d, J = 8.6 Hz, 2H, Ar–**H**), 7.95–7.88 (m, 4H, Ar–**H**), 7.27 (s, 1H, furan–**H**), 3.88 (s, 2H, furan–C**H**$_2$), 3.55–3.50 (m, 2H, piperidine–**H**), 3.50–3.45 (m, 2H, piperidine–**H**), 1.65–1.57 (m, 2H, piperidine–**H**), 1.55–1.50 (m, 2H, piperidine–**H**), 1.49–1.43 (m, 2H, piperidine–**H**). ^{13}C NMR (126 MHz, DMSO) δ 167.41, 167.13, 165.46, 165.41, 150.73, 148.48, 134.37, 133.78, 128.52, 128.29, 127.62, 127.42, 125.38, 123.80, 121.39, 114.07, 46.36, 42.37, 30.73, 26.11, 25.37, 23.98. HR-MS: *m*/*z*: 430.2234 [calculated for $C_{25}H_{28}N_5O_2^+$: 430.2238].

*5.1.15.19. 4-{[2,5-Bis(4-carbamimidoylphenyl)furan-3-yl]acetyl}morpholine formate **(14s)**.* Yellow solid (35% yield). ^{1}H NMR (500 MHz, DMSO) δ 8.43 (s, 2.5H, formate), 8.07 (d, J = 8.6 Hz, 2H, Ar–**H**), 7.98 (d, J = 8.7 Hz, 2H, Ar–**H**), 7.93 (d, J = 8.8 Hz, 2H, Ar–**H**), 7.91 (d, J = 8.6 Hz, 2H, Ar–**H**), 7.28 (s, 1H, furan–**H**), 3.92 (s, 2H, furan–C**H**$_2$), 3.65–3.57 (m, 6H, morpholine–**H**), 3.53–3.48 (m, 2H, morpholine–**H**). ^{13}C NMR (126 MHz, DMSO) δ 167.79, 167.24, 165.44, 165.39, 150.76, 148.55, 134.36, 133.78, 128.52, 128.30, 127.60, 127.41, 125.41, 123.82, 121.05, 114.05, 66.14, 45.84, 41.88, 30.39. HR-MS: *m*/*z*: 432.2029 [calculated for $C_{24}H_{26}N_5O_3^+$: 432.2030].

*5.1.15.20. 1-{[2,5-Bis(4-carbamimidoylphenyl)furan-3-yl]acetyl}-4-methylpiperazine formate **(14t)**.* Yellow solid (61% yield). ^{1}H NMR (500 MHz, DMSO) δ 8.23 (s, 2H, formate), 8.08 (d, J = 8.6 Hz, 2H, Ar–**H**), 8.01–7.94 (m, 6H, Ar–**H**), 7.30 (s, 1H, furan–**H**), 3.91 (s, 2H, furan–C**H**$_2$), 3.63–3.58 (m, 2H, piperazine–**H**), 3.55–3.49 (m, 2H, piperazine–**H**), 2.41–2.36 (m, 2H, piperazine–**H**), 2.35–2.30 (m, 2H, piperazine–**H**), 2.23 (s, 3H, C**H**$_3$). ^{13}C NMR (126 MHz, DMSO) δ 167.49, 165.00, 164.97, 164.16, 150.74, 148.56, 134.65, 134.06, 128.91, 128.68, 126.68, 126.48, 125.36, 123.80, 121.39, 114.36, 54.73, 54.25, 45.49, 45.12, 41.27, 30.58. HR-MS: *m*/*z*: 445.2345 [calculated for $C_{25}H_{29}N_6O_2^+$: 445.2347].

*5.1.15.21. 2-(4-{[2,5-Bis(4-carbamimidoylphenyl)furan-3-yl]acetyl} piperazin-1-yl)ethanol formate **(14u)**.* Yellow solid (82% yield). ^{1}H NMR (500 MHz, DMSO) δ 8.38 (s, 3.5H, formate), 8.07 (d, J = 8.5 Hz, 2H, Ar–**H**), 7.97 (d, J = 8.7 Hz, 2H, Ar–**H**), 7.95–7.88 (m, 4H, Ar–**H**), 7.27 (s, 1H, furan–**H**), 3.90 (s, 2H, furan–C**H**$_2$), 3.59–3.57 (m, 2H, piperazine–**H**), 3.53–3.49 (m, 4H, C**H**$_2$OH, piperazine–**H**), 2.48–2.44 (m, 2H, piperazine–**H**), 2.44–2.38 (m, 4H, NC**H**$_2$, piperazine–**H**). ^{13}C NMR (126 MHz, DMSO) δ 167.43, 166.23, 165.41, 165.37, 150.73, 148.53, 134.40, 133.82, 128.57, 128.34, 127.51, 127.31, 125.39, 123.81, 121.22, 114.13, 60.05, 58.51, 53.44, 52.92, 45.38, 41.52, 30.56. HR-MS: *m*/*z*: 475.2450 [calculated for $C_{26}H_{31}N_6O_3^+$: 475.2452].

*5.1.15.22. 2-(4-{[2,5-Bis(4-carbamimidoylphenyl)furan-3-yl]acetyl} piperazin-1-yl)pyrimidine formate **(14v)**.* Yellow solid (86% yield). ^{1}H NMR (500 MHz, DMSO) δ 8.44 (s, 2.5H, formate), 8.40 (d, J = 4.8 Hz, 2H, **H**4, **H**6), 8.07 (d, J = 8.6 Hz, 2H, Ar-**H**), 8.00 (d, J = 8.7 Hz, 2H, Ar-**H**), 7.94 (d, J = 8.7 Hz, 2H, Ar-**H**), 7.90 (d, J = 8.7 Hz, 2H, Ar-**H**), 7.30 (s, 1H, furan-**H**), 6.68 (t, J = 4.8 Hz, 1H, **H**5), 3.98 (s, 2H, furan-C**H**$_2$), 3.85–3.81 (m, 2H, piperazine-**H**), 3.80–3.75 (m, 2H, piperazine-**H**), 3.74–3.68 (m, 2H, piperazine-**H**), 3.64–3.59 (m, 2H, piperazine-**H**). ^{13}C NMR (126 MHz, DMSO) δ 167.83, 167.29, 165.45, 165.42, 161.09, 157.98, 150.78, 148.59, 134.36, 133.78, 128.50, 128.31, 127.63, 127.44, 125.42, 123.82, 121.08, 114.07, 110.48, 45.04, 43.48, 43.14, 41.22, 30.68. HR-MS: *m*/*z*: 509.2405 [calculated for $C_{28}H_{29}N_8O_2^+$: 509.2408].

*5.1.15.23. 1-{[2,5-Bis(4-carbamimidoylphenyl)furan-3-yl]acetyl}-4-benzoylpiperazine formate **(14w)**.* Yellow solid (91% yield). ^{1}H NMR (500 MHz, DMSO) δ 8.44 (s, 2.5H, formate), 8.07 (d, J = 8.5 Hz, 2H, Ar–**H**), 8.00–7.97 (m, 2H, Ar–**H**), 7.94–7.90 (m, 4H, Ar–**H**), 7.50–7.41 (m, 5H, benzoyl–**H**), 7.28 (s, 1H, furan–**H**), 3.95 (s, 2H, furan–C**H**$_2$), 3.80–3.30 (m, 8H, piperazine–**H**). ^{13}C NMR (126 MHz, DMSO) δ 169.22, 167.85, 167.22, 165.44, 150.79, 148.59, 135.62, 134.32, 133.77, 129.67, 128.51, 128.44, 128.31, 127.62, 127.45, 126.99, 125.42, 123.83, 120.92, 113.99, 40.11–39.02 (m, piperazine-**C**, DMSO), 30.61. HR-MS: *m*/*z*: 535.2451 [calculated for $C_{31}H_{31}N_6O_3^+$: 535.2452].

*5.1.15.24. 1-{[2,5-Bis(4-carbamimidoylphenyl)furan-3-yl]acetyl}-4-(diphenylmethyl)-piperazine formate **(14x)**.* Yellow solid (87% yield). ^{1}H NMR (500 MHz, DMSO) δ 8.45 (s, 2.5H, formate), 8.05 (d, J = 8.6 Hz, 2H, Ar–**H**), 7.97 (d, J = 8.7 Hz, 2H, Ar–**H**), 7.91 (d, J = 8.6 Hz, 4H, Ar–**H**), 7.47–7.43 (m, 4H, phenyl–**H**), 7.31 (t, J = 7.7 Hz, 4H, phenyl–**H**), 7.26 (s, 1H, furan–**H**), 7.23–7.18 (m, 2H, phenyl–**H**), 4.36 (s, 1H, C**H**), 3.86 (s, 2H, furan–C**H**$_2$), 3.66–3.62 (m, 2H, piperazine–**H**), 3.56–3.52 (m, 2H, piperazine–**H**), 2.37–2.33 (m, 2H, piperazine–**H**), 2.33–2.29 (m, 2H, piperazine–**H**). ^{13}C NMR (126 MHz, DMSO) δ 167.45, 167.27, 165.44, 165.43, 150.75, 148.56, 142.37, 134.30, 133.76, 128.55, 128.50, 128.27, 127.63, 127.60, 127.48, 126.96, 125.39, 123.81, 121.03, 113.99, 74.62, 51.78, 51.23, 45.43, 41.54, 30.50. HR-MS: *m*/*z*: 597.2970 [calculated for $C_{37}H_{37}N_6O_2^+$: 597.2973].

*5.1.15.25. 1-{[2,5-Bis(4-carbamimidoylphenyl)furan-3-yl]acetyl}-4-[bis(4-fluorphenyl)-methyl]-piperazine formate **(14y)**.* Yellow solid (36% yield). ^{1}H NMR (500 MHz, DMSO) δ 8.44 (s, 2.5H, formate), 8.05 (d, J = 8.5 Hz, 2H, Ar-**H**), 7.97 (d, J = 8.6 Hz, 2H, Ar-**H**), 7.91 (d, J = 8.5 Hz, 4H, Ar-**H**), 7.48–7.43 (m, 4H, phenyl-**H**), 7.26 (s, 1H, furan-**H**), 7.18–7.12 (m, 4H, phenyl-**H**), 4.45 (s, 1H, C**H**), 3.87 (s, 2H, furan-C**H**$_2$), 3.66–3.60 (m, 2H, piperazine-**H**), 3.56–3.51 (m, 2H, piperazine-**H**), 2.35–2.31 (m, 2H, piperazine-**H**), 2.31–2.26 (m, 2H, piperazine-**H**). ^{13}C NMR (126 MHz, DMSO) δ 167.93, 167.60, 165.87, 165.86, 162.53, 160.60, 149.02, 138.75. 138.72, 134.77, 134.23, 129.93, 129.86, 128.98, 128.75, 128.09, 127.94, 125.87, 124.28, 121.49, 115.92, 115.75, 114.46, 73.02, 68.17, 52.06, 51.49, 41.99, 30.97. HR-MS: *m*/*z*: 633.2786 [calculated for $C_{37}H_{35}F_2N_6O_2^+$: 633.2784].

*5.1.15.26. Ethyl-2-[2,5-bis(4-carbamimidoylphenyl)furan-3-yl]acetate formate **(15)**.* Yellow solid (53% yield). ^{1}H NMR (500 MHz, DMSO) δ 8.47 (s, 2.5H, formate), 8.06 (d, J = 8.5 Hz, 2H, Ar–**H**), 7.90–7.98 (m, 6H, Ar–**H**), 7.36 (s, 1H, furan–**H**), 4.14 (q, J = 7.1 Hz, 2H, C**H**$_2$CH$_3$), 3.89 (s, 2H, furan–C**H**$_2$), 1.20 (t, J = 7.1 Hz, 3H, CH$_2$C**H**$_3$). ^{13}C NMR (126 MHz, DMSO) δ 170.08, 167.83, 165.45, 165.42, 150.97, 148.62, 134.06, 133.63, 128.54, 128.39, 127.79, 127.76, 125.47, 123.88, 119.42, 113.89, 60.74, 31.64, 14.07. HR-MS: *m*/*z*: 391.1764 [calculated for $C_{22}H_{23}N_4O_3^+$: 391.1765].

*5.1.16. Synthesis of the 3-chloro-furamidines **20a** and **b***

*5.1.16.1. 2,5-Bis(4-bromophenyl)-3-chlorofuran **(16)** [36].* PCl_5 (5 g) and **1** (2.5 g) were heated in a flask without solvent for 3 h at 40 °C. The crude mixture was dissolved in water and $CHCl_3$. The organic layer was separated, dried over Na_2SO_4 and evaporated.

White solid (>95% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89–7.81 (m, 2H, Ar-**H**), 7.61–7.51 (m, 6H, Ar-**H**), 6.73 (s, 1H, furan-**H**). ^{13}C NMR (101 MHz, $CDCl_3$) δ 151.34, 146.18, 132.21, 131.97, 128.59, 128.32, 126.54, 125.47, 122.42, 122.10, 114.24, 109.88.

*5.1.16.2. 2,5-Bis(4-bromophenyl)-3-(bromomethyl)-4-chlorofuran **(17)** [20].* A suspension of **16** (0.02 mol), paraformaldehyde (0.1 mol) and 30% HBr in AcOH (80 g) was stirred for 5 days at RT. The solid was filtered off, washed with water and recrystallized in

acetone to give **17**. The crude product was used without further purification for next steps.

*5.1.16.3. 2,5-Bis(4-bromophenyl)-3-chloro-4-(methoxymethyl)furan (**18a**).* Synthesis and purification was achieved according to 5.5. by consumption of **17** with sodium methoxide in MeOH.

White solid (12% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88–7.83 (m, 2H, Ar-**H**), 7.68–7.64 (m, 2H, Ar-**H**), 7.61–7.55 (m, 4H, Ar-**H**), 4.45 (s, 2H, C**H**$_2$), 3.48 (s, 3H, C**H**$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 150.64, 145.49, 132.20, 131.99, 128.75, 128.22, 127.82, 126.65, 123.03, 122.23, 119.23, 115.50, 63.27, 58.10.

*5.1.16.4. 2,5-Bis(4-bromophenyl)-3-chloro-4-(propoxymethyl)furan (**18b**).* Synthesis and purification was achieved according to 5.5. by consumption of **17** with sodium *n*-propoxide in nPrOH.

White solid (12% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87–7.82 (m, 2H, Ar-**H**), 7.71–7.66 (m, 2H, Ar-**H**), 7.61–7.54 (m, 4H, Ar-**H**), 4.48 (s, 2H, C**H**$_2$), 3.55 (t, J = 6.6 Hz, 2H. OC**H**$_2$CH$_2$), 1.74–1.63 (m, 2H, OCH$_2$C**H**$_2$), 0.97 (t, J = 7.4 Hz, 3H, C**H**$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 150.50, 145.41, 132.13, 131.95, 128.81, 128.26, 127.79, 126.62, 122.91, 122.16, 119.56, 115.52, 72.25, 61.57, 23.09, 10.90.

*5.1.16.5. 4,4'-[3-Chloro-4-(methoxymethyl)furan-2,5-diyl]bis(benzonitrile) (**19a**).* Synthesis and purification was achieved according to 5.6. by consumption of **18a**.

White solid (76% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14–8.10 (m, 2H, Ar-**H**), 7.96–7.91 (m, 2H, Ar-**H**), 7.79–7.72 (m, 4H, Ar-**H**), 4.51 (s, 2H, furan-C**H**$_2$), 3.50 (s, 3H, C**H**$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 150.58, 145.45, 133.34, 132.85, 132.83, 132.69, 126.72, 125.45, 121.75, 118.71, 118.63, 118.16, 112.34, 111.70, 62.95, 58.32. HR-MS: *m/z*: 349.0740 [calculated for $C_{22}H_{25}N_4O_3^+$: 349.07383].

*5.1.16.6. 4,4'-[3-Chloro-4-(propoxymethyl)furan-2,5-diyl]bis(benzonitril) (**19b**).* Synthesis and purification was achieved according to 5.6. by consumption of **18b**.

White solid (47% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14–8.09 (m, 2H, Ar-**H**), 7.99–7.93 (m, 2H, Ar-**H**), 7.79–7.71 (m, 4H, Ar-**H**), 4.54 (s, 2H, furan-C**H**$_2$), 3.58 (t, J = 6.6 Hz, 2H, OC**H**$_2$CH$_2$CH$_3$), 1.75–1.62 (m, 2H, OCH$_2$C**H**$_2$CH$_3$), 0.97 (t, J = 7.4 Hz, 3H, OCH$_2$CH$_2$C**H**$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 150.55, 145.49, 133.51, 132.97, 132.89, 132.77, 126.79, 125.53, 122.21, 118.82, 118.75, 118.27, 112.33, 111.73, 72.61, 61.38, 23.16, 10.96. HR-MS: *m/z*: 377.1050 [calculated for $C_{22}H_{18}ClN_2O_2^+$: 377.1051].

*5.1.16.7. 4,4'-[3-Chloro-4-(methoxymethyl)furan-2,5-diyl]bis(benzenecarboximidamide) formate (**20a**).* Synthesis and purification was achieved according to 5.13. by consumption of **19a**.

Yellow solid (68% yield). ^{1}H NMR (400 MHz, DMSO) δ 8.43 (s, 2H, formate), 8.24 (d, J = 8.7 Hz, 2H, Ar-**H**), 8.07 (d, J = 8.7 Hz, 2H, Ar-**H**), 8.03–7.98 (m, 4H, Ar-**H**), 4.53 (s, 2H, furan-C**H**$_2$), 3.40 (s, 3H, C**H**$_3$). ^{13}C NMR (126 MHz, DMSO) δ 167.19, 165.29, 165.25, 149.92, 144.98, 132.96, 132.30, 128.67, 128.25, 126.10, 124.99, 120.98, 116.75, 62.34, 57.65. HR-MS: *m/z*: 383.1264 [calculated for $C_{20}H_{20}ClN_4O_2^+$: 383.1269].

*5.1.16.8. 4,4'-[3-Chloro-4-(propoxymethyl)furan-2,5-diyl]bis(benzenecarboxyimidamide) formate (**20b**).* Synthesis and purification was achieved according to 5.13. by consumption of **19b**.

Yellow solid (63% yield). ^{1}H NMR (400 MHz, DMSO) δ 8.46 (s, 2.5H, formate), 8.23 (d, J = 8.6 Hz, 2H, Ar-**H**), 8.08 (d, J = 8.6 Hz, 2H, Ar-**H**), 8.03–7.95 (m, 4H, Ar-**H**), 4.56 (s, 2H, furan-C**H**$_2$), 3.53 (t, J = 6.5 Hz, 2H, OC**H**$_2$CH$_2$CH$_3$), 1.65–1.53 (m, 2H, OCH$_2$C**H**$_2$CH$_3$), 0.90 (t, J = 7.4 Hz, 3H, OCH$_2$CH$_2$C**H**$_3$). ^{13}C NMR (101 MHz, DMSO) δ 167.71, 165.44, 165.42, 149.89, 144.97, 132.90, 132.20, 128.98, 128.63, 128.50, 126.08, 125.00, 121.22, 116.65, 71.47, 60.63, 22.36, 10.62. HR-MS: *m/z*: 411.1582 [calculated for $C_{22}H_{24}ClN_4O_2^+$: 411.1582].

*5.1.17. 1,3-Bis(4-cyanophenyl)urea (**21**) [43]*

DIPEA (3.6 mmol) was given to a suspension of 4-cyanophenylisocyanate (1.6 mmol) and 4-aminobenzonitrile (2.4 mmol) in DCM (20 mL) at RT, and the reaction mixture was stirred for 12 h. The formed solid was filtered off and recrystallized with DCM to get **21** as a white solid (87% yield).

^{1}H NMR (500 MHz, DMSO) δ 9.37 (s, 2H, N**H**), 7.78–7.72 (m, 4H, Ar-**H**), 7.68–7.61 (m, 4H, Ar **H**). ^{13}C NMR (126 MHz, DMSO) δ 151.74, 143.64, 133.28, 119.14, 118.34, 103.81. ESI-MS: *m/z*: 261.03 [M–H]$^-$.

*5.1.18. General method for the synthesis of the guanidine derivatives **22a-d***

A suspension of Ph_3PBr_2 (0.38 mmol) in $CHCl_3$ (10 mL) was cooled at 0 °C. Afterwards, DIPEA (0.76 mmol) was given to the mixture and stirred for 15 min at 0 °C. After addition of **21** (0.38 mmol), the mixture was stirred for 1 h and subsequently the corresponding amine was added and the reaction mixture was heated under reflux for another 30 min. After cooling to RT, the crude mixture was evaporated, the product was dissolved in $CHCl_3$, washed with saturated Na_2CO_3 solution, dried over Na_2SO_4 and the solvent was evaporated. The product was purified by column chromatography (eluent: $CHCl_3$/MeOH) to get the guanidine derivatives **22a-d** as colourless oils.

*5.1.18.1. 1,3-Bis(4-cyanophenyl)-2-(2-phenylethyl)guanidine (**22a**).* Colorless oil (53% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, J = 8.6 Hz, 4H, Ar-**H**), 7.38–7.27 (m, 3H, phenyl-**H**), 7.24–7.18 (m, 2H, phenyl-**H**), 6.96 (d, J = 8.6 Hz, 4H, Ar-**H**), 3.60 (t, J = 6.6 Hz, 2H, C**H**$_2$CH$_2$Ph), 2.91 (t, J = 6.5 Hz, 2H, CH$_2$C**H**$_2$Ph). ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.14, 147.46, 138.53, 133.53, 129.08, 128.98, 127.10, 121.72, 119.25, 105.44, 43.68, 35.73. ESI-MS: *m/z*: 366.13 [M+H]$^+$.

*5.1.18.2. 2,3-Bis(4-cyanophenyl)-1-[2-(dimethylamino)ethyl]guanidine (**22b**).* Off-white solid (63% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53–7.47 (m, 4H, Ar-**H**), 7.24–7.14 (m, 4H, Ar-**H**), 3.33–3.26 (m, 2H, C**H**$_2$CH$_2$N(CH$_3$)$_2$), 2.65–2.59 (m, 2H, CH$_2$C**H**$_2$N(CH$_3$)$_2$), 2.48 (s, 6H, CH$_2$CH$_2$N(C**H**$_3$)$_2$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 149.47, 133.50, 121.31, 119.60, 104.77, 62.04, 45.30, 41.35. ESI-MS: *m/z*: 333.08 [M+H]$^+$.

*5.1.18.3. 2,3-Bis(4-cyanophenyl)-1-[2-(diethylamino)ethyl]guanidine (**22c**).* White solid (48% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52–7.47 (m, 4H, Ar–**H**), 7.20 (d, J = 7.2 Hz, 4H, Ar–**H**), 3.34–3.25 (m, 2H, C**H**$_2$CH$_2$N(Et)$_2$), 2.75 (q, J = 7.2 Hz, 4H, N(C**H**$_2$CH$_3$)$_2$), 2.70–2.62 (m, 2H, CH$_2$C**H**$_2$N(Et)$_2$), 1.14 (t, J = 7.2 Hz, 6H, N(CH$_2$C**H**$_3$)$_2$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 149.58, 133.42, 132.21, 132.11, 128.72, 128.60, 120.56, 104.50, 55.56, 48.34, 42.54, 11.16. ESI-MS: *m/z*: 361.07 [M+H]$^+$.

*5.1.18.4. 2,3-Bis(4-cyanophenyl)-1-(2-piperidin-1-ylethyl)guanidine (**22d**).* White solid (41% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53–7.46 (m, 4H, Ar-**H**), 7.22 (d, J = 7.9 Hz, 4H, Ar-**H**), 3.34–3.26 (m, 2H, C**H**$_2$CH$_2$-piperidine), 2.69–2.50 (m, 6H, CH$_2$C**H**$_2$-piperidine, piperidine-**H**), 1.76–1.61 (m, 4H, piperidine-**H**), 1.62–1.49 (m, 2H, piperidine-**H**). ^{13}C NMR (101 MHz, $CDCl_3$) δ 149.44, 133.38, 121.80, 119.60, 104.71, 60.94, 55.21, 40.76, 25.52, 23.73. ESI-MS: *m/z*: 373.10 [M+H]$^+$.

*5.1.19. General method for the synthesis of the derivatives **23a-d***

Conversion of the nitrile derivatives **21** and **22a-d** into the amidines was achieved with Pinner-method according to 5.13. to get **23a-d** and **24** as formate salts.

*5.1.19.1. 2,3-Bis(4-carbamimidoylphenyl)-1-(2-phenylethyl)guanidine formate **(23a)**.* White solid (27% yield). ^{1}H NMR (500 MHz, DMSO) δ 8.33 (s, 2.5H, formate), 7.68–7.63 (m, 4H, Ar-**H**), 7.32–7.27 (m, 2H, phenyl-**H**), 7.24–7.18 (m, 3H, phenyl-**H**), 7.04–6.98 (m, 4H, Ar-**H**), 3.40 (t, J = 7.3 Hz, 2H, C**H**$_2$CH$_2$Ph), 2.84 (t, J = 7.3 Hz, 2H, C**H**$_2$CH$_2$Ph). ^{13}C NMR (126 MHz, DMSO) δ 165.59, 164.74, 148.50, 139.36, 128.77, 128.72, 128.32, 126.12, 119.49, 118.44, 43.38, 35.01. HR-MS: m/z: 400.2239 [calculated for $C_{23}H_{26}N_7^+$: 400.2244].

*5.1.19.2. 2,3-Bis(4-carbamimidoylphenyl)-1-[2-(dimethylamino) ethyl]guanidine formate **(23b)**.* White solid (80% yield). ^{1}H NMR (500 MHz, DMSO) δ 8.15 (s, 1H, formate), 7.74–7.70 (m, 4H, Ar-**H**), 7.09 (d, J = 8.4 Hz, 4H, Ar-**H**), 3.58 (t, J = 5.9 Hz, 2H, C**H**$_2$CH$_2$N(CH$_3$)$_2$), 3.18 (t, J = 5.9 Hz, 2H, CH$_2$C**H**$_2$N(CH$_3$)$_2$), 2.74 (s, 6H, CH$_2$CH$_2$N(C**H**$_3$)$_2$). ^{13}C NMR (126 MHz, DMSO) δ 164.53, 162.99, 148.83, 129.00, 118.64, 56.35, 42.75, 42.63. HR-MS: m/z: 367.2353 [calculated for $C_{19}H_{27}N_8^+$: 367.2353].

*5.1.19.3. 2,3-Bis(4-carbamimidoylphenyl)-1-[2-(diethylamino)ethyl] guanidine formate **(23c)**.* White solid (62% yield). ^{1}H NMR (500 MHz, DMSO) δ 8.17 (s, 1,5H, formate), 7.74–7.70 (m, 4H, Ar–**H**), 7.06 (d, J = 8.6 Hz, 4H, Ar–**H**), 3.59 (t, J = 6.1 Hz, 2H, C**H**$_2$CH$_2$N(Et)$_2$), 3.20 (t, J = 6.1 Hz, 2H, CH$_2$C**H**$_2$N(Et)$_2$), 3.11 (q, J = 7.2 Hz, 4H, N(C**H**$_2$CH$_3$)$_2$), 1.22 (t, J = 7.2 Hz, 6H, N(CH$_2$C**H**$_3$)$_2$). ^{13}C NMR (126 MHz, DMSO) δ 164.56, 163.13, 149.06, 129.04, 119.75, 118.66, 50.40, 46.79, 37.25, 8.87. HR-MS: m/z: 395.2661 [calculated for $C_{21}H_{31}N_8^+$: 395.2666].

*5.1.19.4. 2,3-Bis(4-carbamimidoylphenyl)-1-(2-piperidin-1-ylethyl) guanidine formate **(23d)**.* White solid (88% yield). ^{1}H NMR (500 MHz, DMSO) δ 8.16 (s, 1,5H, formate), 7.75–7.70 (m, 4H, Ar-**H**), 7.09 (d, J = 8.5 Hz, 4H, Ar-**H**), 3.65 (t, J = 5.8 Hz, 2H, C**H**$_2$CH$_2$-piperidine), 3.18 (t, J = 6.0 Hz, 2H, CH$_2$C**H**$_2$-piperidine), 3.15 (s, 4H, piperidine-**H**), 1.82–1.74 (m, 4H, piperidine-**H**), 1.52 (s, 2H, piperidine-**H**). ^{13}C NMR (126 MHz, DMSO) δ 164.57, 163.02, 149.03, 129.04, 119.85, 118.85, 55.62, 52.39, 36.98, 22.58, 21.48. HR-MS: m/z: 407.2660 [calculated for $C_{22}H_{31}N_8^+$: 407.2666].

*5.1.19.5. 1,3-Bis(4-carbamimidoylphenyl)urea formate **(24)** [45].* White solid (84% yield). ^{1}H NMR (500 MHz, DMSO) δ 8.41 (s, 0.5H, formate), 7.85–7.81 (m, 4H, Ar-**H**), 7.71–7.67 (m, 4H, Ar-**H**). ^{13}C NMR (126 MHz, DMSO) δ 164.79, 152.26, 144.83, 129.33, 120.32, 117.24. HR-MS: m/z: 297.1457 [calculated for $C_{15}H_{17}N_6O^+$: 297.1458].

*5.1.20. 4,4'-(3,4-Dimethylfuran-2,5-diyl)bis[N-hydroxybenzenecarboximidamide] formate **(25)***

To a suspension of hydroxylamine hydrochloride (20 mmol) in DMSO (15 mL) cooled to 0 °C using an ice bath *t*-BuOK (20 mmol) was added slowly while stirring over 1 h. 1 mmol of **8** was added and the mixture was stirred overnight at RT. The mixture was poured into 100 mL water and the precipitated solid was filtered off. The crude product was purified by column chromatography (eluent: CHCl$_3$/MeOH) to give **25**.

Yellow solid (78% yield). ^{1}H NMR (500 MHz, DMSO) δ 9.69 (s, 2H, O**H**), 8.14 (s, 1H, formate), 7.80–7.76 (m, 4H, Ar–**H**), 7.73–7.69 (m, 4H, Ar–**H**), 5.84 (s, 4H, N**H**$_2$), 2.24 (s, 6H, C**H**$_3$). ^{13}C NMR (126 MHz, DMSO) δ 163.07, 150.41, 146.49, 131.66, 131.22, 125.73, 124.66, 119.98, 9.63. HR-MS: m/z: 365.1605 [calculated for $C_{20}H_{21}N_4O_3^+$: 365.1608].

*5.1.21. 4-{5-[4-(N-Methoxycarbaimidoyl)phenyl]-3,4-dimethylfuran-2-yl}-N-hydroxybenzenecarboximidamide **(26)***

To a cooled suspension of **25** (0.28 mmol) in dioxane (1 mL) 2 M NaOH (5.5 mL) was added slowly while stirring. Afterwards a solution of dimethylsulfate (0.7 mmol) in dioxane (0.5 mL) was added and the mixture was allowed to stire overnight at RT. The reaction mixture was extracted 3 times with ethylacetate. The combined organic layers were washed with water and saturated NaCl solution, dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography (eluent: CHCl$_3$/MeOH) to give **26**.

Yellow solid (17% yield). ^{1}H NMR (500 MHz, DMSO) δ 7.79–7.74 (m, 4H, Ar–**H**), 7.74–7.69 (m, 4H, Ar–**H**), 6.09 (s, 4H, N**H**$_2$), 3.76 (s, 6H, OC**H**$_3$), 2.24 (s, 6H, C**H**$_3$). ^{13}C NMR (126 MHz, DMSO) δ 150.65, 146.48, 131.59, 130.84, 126.12, 124.69, 120.22, 60.60, 9.64. HR-MS: m/z: 393.1921 [calculated for $C_{22}H_{25}N_4O_3^+$: 393.1921].

*5.1.22. General method for the synthesis of the N-alkylated bisamidines **27a, b** and **c***

Conversion of the nitrile derivative **8** into the *N*-alkylated amidines was achieved with Pinner-method according to 5.13. to get **27a, b** and **c** as formate salts. In the second step, the corresponding amine (2 mL) solved in ethanol (10 mL) was used instead of ethanolic ammonia.

*5.1.22.1. 4,4'-(3,4-Dimethylfuran-2,5-diyl)bis(N-ethylbenzenecarboximidamide) formate **(27a)**.* Yellow solid (47% yield). ^{1}H NMR (500 MHz, DMSO) δ 8.46 (s, 2,5H, formate), 7.95–7.81 (m, 8H, Ar-**H**), 3.46 (d, J = 5.6 Hz, 4H, NHC**H**$_2$CH$_3$), 2.25 (s, 6H, C**H**$_3$), 1.23 (t, J = 7.0 Hz, 6H, NHCH$_2$C**H**$_3$). ^{13}C NMR (126 MHz, DMSO) δ 165.66, 161.69, 146.39, 134.32, 128.72, 127.82, 124.94, 122.16, 37.62, 13.16, 9.69. HR-MS: m/z: 389.2334 [calculated for $C_{24}H_{29}N_4O^+$: 389.2336].

*5.1.22.2. 4,4'-(3,4-Dimethylfuran-2,5-diyl)bis[N-(1-methylethyl)benzenecarboximidamide] formate **(27b)** [47].* Yellow solid (73% yield). ^{1}H NMR (500 MHz, DMSO) δ 8.42 (s, 3.5H, formate), 7.94–7.88 (m, 4H, Ar–**H**), 7.89–7.81 (m, 4H, Ar–**H**), 4.08 (s, 2H, C**H**(CH$_3$)$_2$), 2.28 (s, 6H, C**H**$_3$), 1.27 (d, J = 5.6 Hz, 12H, CH(C**H**$_3$)$_2$). ^{13}C NMR (126 MHz, DMSO) δ 165.34, 159.46, 157.69, 146.45, 134.04, 128.67, 124.95, 121.95, 44.83, 21.57, 9.68. HR-MS: m/z: 417.2645 [calculated for $C_{26}H_{33}N_4O^+$: 417.2649].

*5.1.22.3. 4,4'-(3,4-Dimethylfuran-2,5-diyl)bis[N-(2-methylpropyl) benzenecarboximidamide] formate **(27c)**.* Yellow solid (38% yield). ^{1}H NMR (500 MHz, DMSO) δ 8.43 (s, 3H, formate), 7.90 (s, 8H, Ar–**H**), 3.26 (d, J = 5.8 Hz, 4H, C**H**$_2$), 2.27 (s, 6H, C**H**$_3$), 2.07–1.95 (m, 2H, C**H**(CH$_3$)$_2$), 0.96 (d, J = 6.0 Hz, 12H, CH(C**H**$_3$)$_2$). ^{13}C NMR (126 MHz, DMSO) δ 165.45, 162.11, 146.41, 134.35, 128.75, 128.01, 125.00, 122.15, 49.73, 27.09, 19.94, 9.68. HR-MS: m/z: 445.2952 [calculated for $C_{28}H_{37}N_4O^+$: 445.2962].

*5.1.22.4. 4,4'-(3,4-Dimethylfuran-2,5-diyl)bis(benzenecarboximidamide) hydrochloride **(27d)**.* Resynthesis of NSC305836 **(27d)** was realized according to the reported procedure [20].

Light yellow solid (76% yield). ^{1}H NMR (400 MHz, DMSO) δ 9.65 (s, 4H, N**H**$_2$), 9.46 (s, 4H, N**H**$_2$), 8.04 (m, 4H, Ar–**H**), 7.97 (m, 4H, Ar–**H**), 2.30 (s, 6H, C**H**$_3$). ^{13}C NMR (101 MHz, DMSO) δ 165.02, 146.49, 135.21, 128.86, 125.84, 125.17, 122.84, 9.77. HR-MS: m/z: 333.1709 [calculated for $C_{20}H_{21}N_4O^+$: 333.1710].

5.2. Biological evaluation

5.2.1. In-vitro activity against P. falciparum

For *P. falciparum* 3D7, growth inhibition assays were performed using SYBR Green I [53] as previously described in Fréville et al. [54]. Briefly, *in vitro* cultured *P. falciparum* 3D7 infected erythrocytes (0.5% parasitemia, 1% hematocrit) were seeded into 96-well tissue culture plates containing vehicle control (DMSO, <0.5%), positive control (chloroquine, Sigma Aldrich) or test compound and incubated under standard *P. falciparum* culture conditions for 48 h.

Cultures were stained for 30 min in the dark with SYBR Green I 1X (Invitrogen) diluted in 20 mM Tris pH 8.8, 138 mM NaCl, and fixed with 1% paraformaldehyde. Fixed parasitized red blood cells (RBC) were stored at 4 °C in the dark until flow cytometry analysis. Parasite growth was assessed by flow cytometry on a BD FACS Canto II (BD Biosciences). Cell pairs were excluded from the analysis using a forward scatter (FSC)-width versus FSC-area dot plot. Infected and uninfected erythrocytes were gated on the basis of their FSC and side scatter (SSC) signals. Fluorescence analysis (Green fluorescence FITC) was performed using BD FACSDiva software (version 6.1.3, BD Biosciences) on a total of 200,000 acquired events. Fluorescence was observed as described by Izumiyama et al. [55] on a two-parameter dot plot (FTIC-FSC). Fluorescence of non-infected RBC was adjusted to plot between 10^0 and 10^2. Results are expressed as the percentage of growth inhibition. Each independent experiment was carried out in duplicate and performed at least two times.

P. falciparum Dd2 growth inhibition assays were carried out using ^{3}H-hypoxanthine incorporation similar to the method previously described [56]. Briefly, *in vitro* cultured *P. falciparum* line Dd2 infected erythrocytes (1.0% parasitemia and 1.0% hematocrit) were seeded in triplicate wells into 96 well tissue culture plates containing vehicle control (DMSO, <0.5%), positive control (chloroquine, Sigma Aldrich) or test compound and incubated under standard *P. falciparum* culture conditions with 0.5 μCi [^{3}H]-hypoxanthine for 48 h. Cells were then harvested onto 1450 MicroBeta filter-mats (Filter Mat A; Perkin Elmer) and [^{3}H]-hypoxanthine incorporation determined using a 1450 Trilux MicroBeta liquid scintillation counter. Growth was compared to matched DMSO controls (<0.5%). Each independent experiment was carried out in triplicate and performed at least three times. 50% inhibitory concentrations IC_{50}(s) were determined *via* log linear interpolation [57].

5.2.2. Toxicity on human HEK293 cells

HEK293 cells (DSMZ Braunschweig, ACC305) were incubated at 37 °C in a humidified incubator with 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FCS and 5 mM glutamine. Cells were seeded at 1.5×10^3 cells per well in a 96-well cell culture plate (TPP, Switzerland). The compounds were added immediately to the medium at 50 μM to determine the percentage viability. For hit compounds IC_{50} values were determined in dose response assays. After 24 h, AlamarBlue® reagent (Invitrogen, CA) was added according to the manufacturer instructions and incubated again for 21 h before samples were analyzed. Detection of viable cells, which convert resazurin (dye of AlmarBlue® reagent) into the high fluorescent resorufin, was performed by using a FLUOstarOPTIMA microplate reader (BMG Labtec) with the following filter set: Ex 560 nm/Em 590 nm. All measurements were performed in triplicates over three independent experiments and data are means with SD ≤ 12%.

5.3. In silico prediction of pharmacokinetic and tox data

For the *in silico* prediction the PreADMET web application was used [58]. The PreADMET approach is based on different classes of molecular descriptors that are considered for generating quantitative structure property relationship or binary classification models. The following properties were calculated: blood brain barrier penetration (BBB) [59], Caco2 cell permeability (Caco2 Papp nm/s) [60], human intestinal absorption (% HIA) [51], plasma protein binding, buffer solubility (classification model), log P (consensus logP), and total polar surface area (TPSA Å^2). Classification models were used to predict the inhibition of several.cytochromes, hERG and para-glycoprotein (p-gp). Druglikeness was predicted using Lipinski's rule of five [61], MDDR [62] and World Drug Index rule (WDI) [63].

To predict the human toxicity the PROTOX approach developed by Preissner et al. [52] which is available as web service (http://tox.charite.de/tox/) was used. The prediction method is based on the analysis of the similarity of compounds with known median lethal doses (LD_{50}) and incorporates the identification of toxic fragments.

Author contribution

B. Sauer: organic synthesis, preparation of manuscript.

T. Skinner-Adams: anti-plasmodial activities Dd2, data analysis, preparation of manuscript.

A. Bouchut: anti-plasmodial activities 3D7, data analysis, preparation of manuscript.

C. Pierrot: anti-plasmodial activities 3D7, data analysis, preparation of manuscript.

M.J. Chua: anti-plasmodial activities Dd2, data analysis.

F. Erdmann: cytotoxicity (HEK293), preparation of manuscript.

D. Robaa: data analysis, research planning, preparation of manuscript.

M. Schmidt: analytics, supervision, research planning, preparation of manuscript.

J. Khalife: research planning, anti-plasmodial activities 3D7, preparation of manuscript.

K. Andrews: research planning, anti-plasmodial data analysis, preparation of manuscript.

W. Sippl: supervision, research planning, preparation of manuscript.

Acknowledgment

This work and the authors of this manuscript received funding from the European Union's Seventh Framework Program for research, technological development, and demonstration (under grant agreements nos. 241865 (SEtTReND) and 602080 (A-Para-DDisE)) and the Australian National Health and Medical Research Council (APP1093378 and APP1074016 to KTA). We thank Simone Kniesa for technical assistance and the Australian Red Cross Blood Service for the provision of human blood and sera for *P. falciparum* culture. We also thank the BioImaging Center Lille Facility for access to the BD FACSCanto II cytometer.

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2016.12.041. These data include MOL files and InChiKeys of the most important compounds described in this article.

Abbreviations

aq	aqueous
$CDCl_3$	deuterated chloroform
$CHCl_3$	chloroform
corresp.	corresponding
DCC	dicyclohexylcarbodiimide
DIPEA	diisopropylethylamine
dDMSO	deuterated dimethyl sulfoxide
DMSO	dimethylsulfoxide
EtOAc	ethyl acetate
EtOH	ethanol
Et_3N	triethylamine
eq	equivalent
DB75	furamidine

HEK	human embryonic kidney
LIHMDS	Lithium hexamethyldisilazide
MeOH	methanol
n.d.	not determined
DB 289	pafuramidine
NSC305836	dimethylfuramidine
Pfcrt	plasmodium falciparum chloroquine resistance transporter
PyBOP	benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
sol	solution
t-BuOK	potassium *tert*-butoxide
TEA	triethylamine
TFA	trifluoracetic acid
THF	tetrahydrofuran
TLC	thin-layer chromatography

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