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Sulfonyl-phenyl-ureido benzamidines: a novel structural class of potent antimalarial agents

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Abstract—The high throughput in silico screening of a virtual library into the structure of the *P. falciparum* lactate dehydrogenase (LDH) with the 4SCan technology yielded a series of biphenyl urea compounds. These were chemically optimized to a new structural class of potent antimalarial agents. The compounds did not inhibit plasmodium LDH enough to fully explain their potency. Therefore we conclude that an unknown mode of action may be the cause of the antimalarial activity.

Malaria is a major health problem with hundreds of millions of people being infected, mostly—but not only—in tropical and subtropical countries. Drugs which are employed in the treatment of malaria are becoming increasingly ineffective due to the spread of resistant parasite strains. The development of novel drugs for the prophylactic and curative treatment of malaria is highly necessary, but has proven to be very difficult.[1]

Plasmodium falciparum lactate dehydrogenase (PfLDH) is an attractive target for antimalarial drugs.[2] We used the in silico screening method 4SCAN®[3] for the docking of our virtual library of small organic molecules into the structure of PfLDH and found a series of high scoring molecules. During the biological testing of some of these, we identified amidino biphenyl ureas, such as amicarbalide (**1**), to possess potent activity in an in vitro *P. falciparum* proliferation assay ($IC_{50} = 5$ nM), using the multidrug resistant *P. falciparum* clone Dd2. These compounds showed a high level of inhibition of parasite growth in vitro, but surprisingly they revealed low activity as inhibitor of the PfLDH enzyme (unpublished observation). Amicarbalide is reported to inhibit the growth of Babesia, a bovine parasite, which is related to *P. falciparum*,[4] but the mechanism of action remains unclear.

Because of some structural similarity with pentamidine, we hypothesized that the antiparasitic activity of amicarbalide may be a consequence of its binding ability to the minor groove of DNA. Minor groove binding compounds are known to have antiparasitic activity as is the case for pentamidine.[5]

More recently it was reported that bisarylureas with one amidine moiety show antimalarial activity due to their inhibition of malarial plasmepsin, an aspartyl protease fundamental for haemoglobin digestion inside of the parasite food vacuole.[6] To avoid a DNA binding effect, we decided to delete one amidino group and to replace it with a sulfonamide anchor, which allowed us to introduce other substituents in the second aromatic ring leading to compounds similar to the published Plasmepsin inhibitors.

The solution synthesis of 4-sulfonylamide derivatives (3 and following) was performed in a straightforward manner, allowing for the rapid production of a large number of molecules. We introduced a chemical method in which the crucial step was the urea formation using *p*-chlorosulfonyl-phenylisocyanate as the key reagent and 3- or 4-cyanophenyl aniline. When added in equimolar amounts to an aprotic solvent, these compounds reacted regiospecifically to form the urea only. The resulting chlorosulfonylphenyl cyanophenyl urea was formed almost quantitatively and was isolated as a stable compound. This intermediate was further reacted

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with primary and secondary amines and some anilines to give the respective sulfonamide. Finally, the amidine was formed by aminolysis of the cyano group following the Pinner method. The end products were obtained in high yield and purity and relatively short time (Scheme 1).

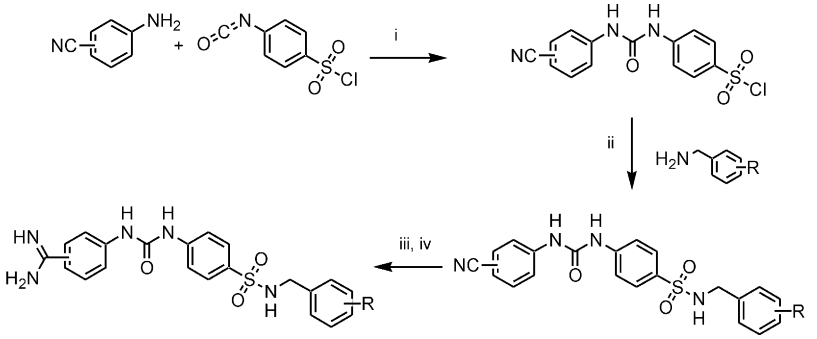
As the corresponding *m*-chlorosulfonylisocyanate was not commercially available at the time, the 3-substituted sulfonylureas were prepared according to the method outlined in Scheme 2, using the appropriate cyanoisocyanate and the 3-sulfonamides. The compounds were characterized by LC-MS and NMR and the data were consistent with the structure.[8]

The facile synthesis of the sulfonylamide derivatives allowed us to quickly screen and determine the most favourable structural properties of substituents for the sulfonylamide moiety. The compounds were evaluated in vitro against the *P. falciparum* Dd2 strain[7] (Table 1). The comparison between either polar or nonpolar aliphatic side chains and aromatic groups showed a significant increase in inhibitory activity in the case of benzylic side chain substitution (compound **8**, Table 1). The methylene spacer of the benzyl moiety played an important role for the activity, because (**10**–**12**) show low activity, whereas conformational rigidity had only a minor influence when compared to the benzyl moiety, (**9**, **8**). Also large aromatic groups were somewhat detrimental for activity (**11**–**12**).

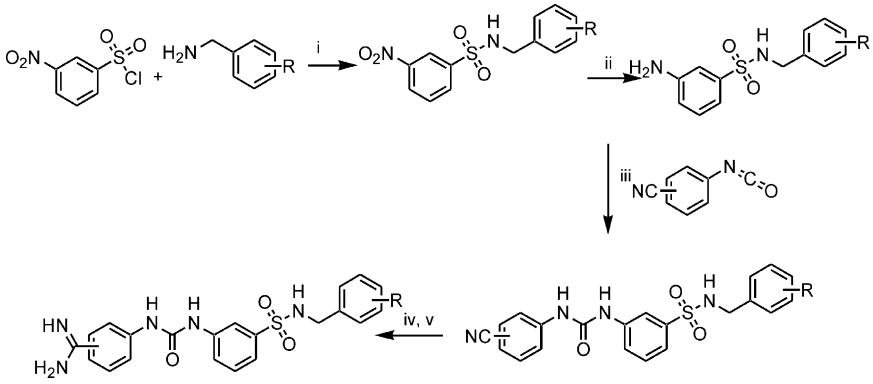
para Fluoration of the benzyl ring increased slightly the activity (compd **18**). The introduction of a further fluoro atom surprisingly decreased the activity (**19**, **20**). However, a dramatic increase in activity was achieved when the number of fluoro atoms was increased to three, with the best activity shown by 2,3,6-substitution of the fluoro atoms (**22**). It cannot be excluded that the non-linear SAR of the fluoro compounds is due to some precipitation during the assay (**16**–**25**).

A more polar substituent on the benzyl ring, a *p*-sulfonylamino group, increased activities even further (**26**).

We explored the effect of the different positions of the amidine and sufonylamide moieties on the two phenyl rings, using the unsubstituted benzyl moieties (**8**, **13**–**15**), the 2,3,6-triflourobenzyl moieties (**22**–**25**) and the *p*-sulfonamidobenzyl group (**26**–**29**). A 3,3′ substitution pattern is detrimental for activity in all three examples (**13**, **23**, **27**), with the 4,3′ substitution being more disadvantageous only for the *p*-sulfonyl-amidobenzyl compounds (**29**). While only a negligible difference in activity is observed between 3,4′, 4,3′ and 4,4′ substitution for compounds carrying the unsubstituted benzyl group, the differences are more pronounced for the other two types of substituents, showing a clear preference for 3–4′ substitution with very high activities (**22** and **26**).

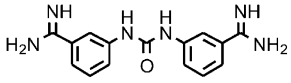
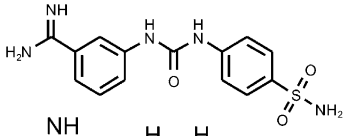
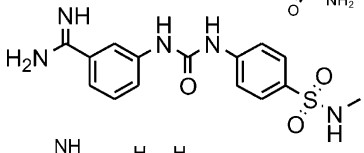
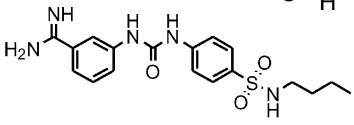
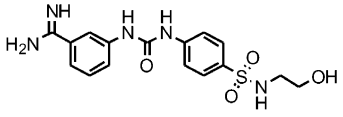
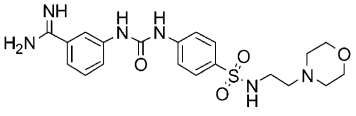
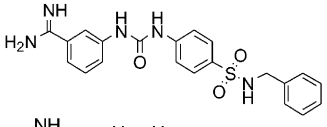
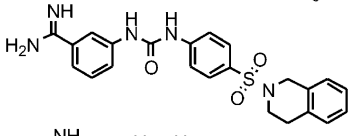
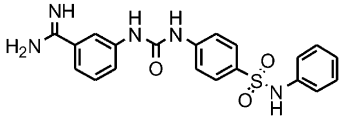
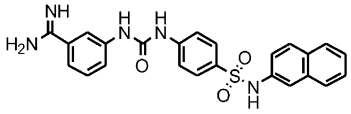
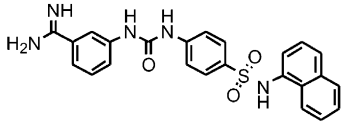
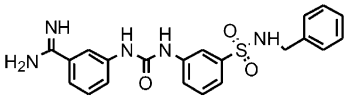
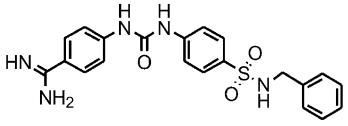
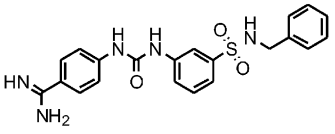
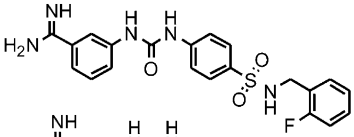
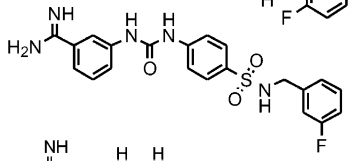
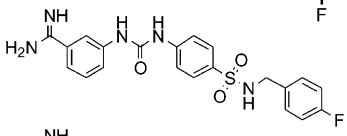
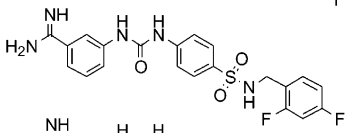
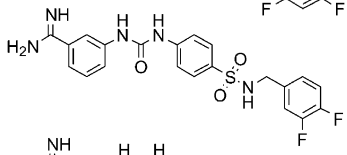
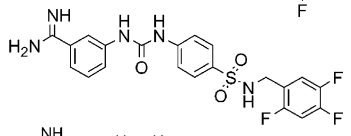
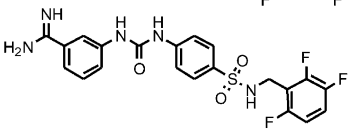
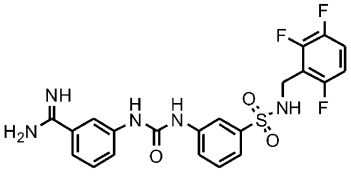
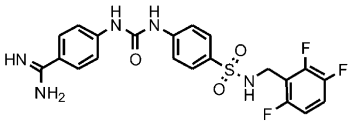
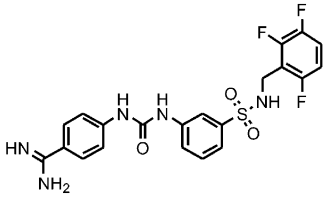
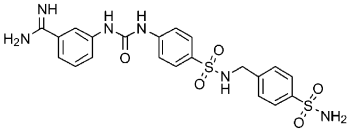


Scheme 1. Synthesis of 4-sulfonylamidobenzyl-phenylurea. Reagents and conditions: (i) AcCN, rt; (ii) R,R′-NH, DCM, TEA, rt; (iii) HCl/MeOH, rt, 2 h; (iv) NH_3/MeOH, reflux, 3 h.



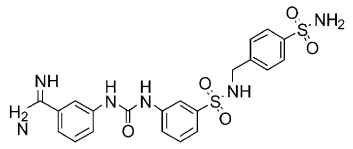
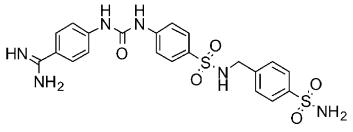
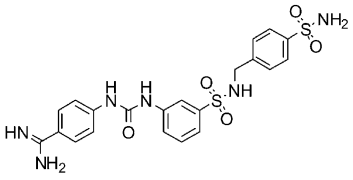
Scheme 2. Synthesis of 3-sulfonylamidobenzyl-phenylurea. Reagents and conditions: (i) DCM, TEA, rt; (ii) H_2/Pd; EtOH/EtOAc; (iii) DCM, rt; (iv) HCl/MeOH, rt, 2 h; (v) NH_3/MeOH, reflux, 3 h.

Table 1. In vitro antimalarial activity against the *P. falciparum* clone Dd2. The mean IC_{50} values of three or more determinations are shown

Compd	Activity IC_{50}, nM	Compd structure
1	5	
2	17	Chloroquine
3	>4900	
4	3000	
5	3900	
6	890	
7	>2500	
8	450	
9	610	
10	9000	
11	8200	
12	17000	
13	6200	
14	400	
15	410	
16	620	
17	500	
18	320	
19	1110	
20	1110	
21	1800	
22	17	
23	250	
24	210	
25	190	
26	7	

(*continued on next page*)

Table 1 (*continued*)

Compd	Activity IC_{50}, nM	Compd structure
27	980	
28	90	
29	> 2500	

The newly identified class of antimalarial compounds is more active in a cell based assay against *P. falciparum* than chloroquine and almost as active as amicarbalide (cmpd **1**). Since the compounds do not have a bisamidine functionality the mechamism of action is not likely to be due to DNA binding. The compounds are micromolar inhibitors of *P. falciparum* plasmepsin (unpublished observation). This activity does not explain the potent activity of these compounds against *P. falciparum*. It can be speculated that the compounds are concentrated in the food vacuole. Such an effect would amplify the activity against plasmepsin and result in the nanomolar activity in cell culture assay. However, it has to be also considered that the compounds may act against a unknown target.

These novel chemical entities with potent antimalarial activity will be an addition to the antimalarial chemotherapy. Further biological characterization of these compounds will be presented in future publications.

Acknowledgements

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7. For the determination of the antiplasmodial activity of the compounds, the multidrug resistant *Plasmodium falciparum* clone Dd2 was used. The incorporation of [8-^{3}H]hypoxanthine into the parasitic nucleic acids was measured. The plasmodia were incubated at 0.3% parasitaemia and an erythrocyte haematocrit of 2.5% in the presence of different concentrations of the compounds in a final volume of 200 μL. The medium employed was RPMI 1640 which contained 10% of heat-treated human serum and 3 mg/l of gentamycin. In the incubations, the concentrations of the compounds varied from 0.3 to 100 μM. After 48 h, each batch was treated with 50 μL of [8-^{3}H]hypoxanthine (1 mCi/mL) and incubated for a further 18 h. The cells were filtered off, washed and suspended in 20 μL of scintillation fluid. The radioactive hypoxanthine absorbed by the parasites was then quantified using a scintillation counter.The results were presented graphically and the IC_{50} values were determined using a fitting function. The value IC_{50}, the 'inhibition constant', indicates the value in nMol/l at which 50% inhibition occurs.
8. All compounds were characterized by MS and NMR (300MHz) and exhibited satisfactory properties. Examples are given for a few compounds. ^{1}H NMR (D6-DMSO).
13 δ = 3.99 (d, *J* = 6.3, 2H, CH2), 7.23–7.30 (m, 5H, Ar-H), 7.36 (d, *J* = 8.3, 1H, Ar-H), 7.40–7.43 (m, 1H, Ar-H), 7.47–7.56 (m, 2H, Ar-H), 7.58–7.61 (m, 1H, Ar-H), 7.72–7.75 (m, 1H, Ar-H), 7.98 (t, *J* = 1.8, 1H, Ar-H), 8.12 (t, *J* = 1.8, 1H, Ar-H), 8.19 (t, *J* = 6.3, 1H, N-H), 9.00 (s, 2H, N-H), 9.35 (s, 2H, N-H), 9.77 (s, 1H, N-H), 9.82 (s, 1H, N-H).
15 δ = 3.99 (d, *J* = 6.3, 2H, CH2), 7.24–7.39 (m, 5H, Ar-H), 7.42 (d, *J* = 7.9, 1H, Ar-H), 7.50 (t, *J* = 7.9, 1H, Ar-H), 7.59 (d, *J* = 7.9, 1H, Ar-H), 7.70 (d, *J* = 8.9, 2H, Ar-H), 7.81 (d, *J* = 8.9, 2H, Ar-H), 8.09 (t, *J* = 1.8, 1H, Ar-H), 8.12 (t, *J* = 1.8, 1H, Ar-H), 8.19 (t, *J* = 6.3, 1H, N-H), 8.83 (s, 2H, N-H), 9.18 (s, 2H, N-H), 9.85 (s, 1H, N-H), 10.02 (s, 1H, N-H).
24 δ = 4.01 (d, *J* = 5.3, 2H, CH2), 7.01–7.07 (m, 1H, Ar-H), 7.34–7.45 (m, 1H, Ar-H), 7.61 (d, *J* = 8.9, 2H, Ar-H), 7.68 (d, *J* = 7.5, 4H, Ar-H), 7.81 (d, *J* = 8.9, 2H, Ar-H), 8.10 (t, *J* = 5.5, 1H, N-H), 8.92 (s, 2H, N-H), 9.20 (s, 2H, N-H), 10.13 (s, 1H, N-H), 10.23 (s, 1H, N-H).