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Evaluation of Diarylureas for Activity Against *Plasmodium falciparum*

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Abstract

A library of diarylurea IGFR inhibitors was screened for activity against chloroquine-sensitive (3D7) and chloroquine-resistant (K1) strains of *Plasmodium falciparum.* The 4-aminoquinaldine-derived diarylureas displayed promising antimalarial potency. Further exploration of the B ring of 4-aminoquinaldinyl ureas allowed identification of several quinaldin-4-yl ureas **4**{13, 39} and **4**{13, 58} sufficiently potent against both 3D7 and K1 strains to qualify as bone fide leads.

Keywords

Malaria; diarylurea

Introduction

Malaria affects roughly 250 million people and causes over 800,000 deaths annually, with most deaths being attributed to infection by *Plasmodium falciparum*.[1] Drug resistance has been reported against almost all available antimalarial drugs.[2–4] Therefore, it remains essential to develop and discover new effective and affordable antimalarial drugs.

Diarylureas derivatives have been reported to have antimalarial activity.[5–10] Jiang and co-workers identified a series of diphenylureas in a plasmepsin-directed screening campaign.[5]

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Supporting information available: Synthetic procedures for diarylureas. Complete LCMS characterization data of all diarylureas. Complete antimalarial data, cytotoxicity data, permeability and solubility data for all listed compounds. Kinase binding profile data. ^{1}H NMR spectra and chromatographic data of novel compounds **4**{12,1–4}, **4**{13,7}, **4**{13,12}, **4**{13,21–24}, **4**{13,27–28}, **4**{13,41–45}, **4**{13,49–50}, **4**{13,60}, **4**{19,1–4}, and **4**{19,8–9}. This material is available free of charge via the internet at http://pubs.acs.org.

Although the authors proposed the antimalarial activity of diphenylureas as inhibition of plasmepsin, the mechanism of action in *Plasmodium falciparum* remains unclear due to poor correlation between *in vitro* and *in vivo* SAR's. Baldwin and co-workers disclosed diarylureas as inhibitors of dihydroorotate dehydrogenase,[7] an enzyme in the pyrimidine biosynthetic pathway, although no correlations between enzymatic and cellular SAR were presented.[14] Domínguez and co-workers reported that phenylurenyl chalcones had activity against chloroquine resistant strains of *Plasmodium falciparum.* The phenylurenyl chalcones exerted their antimalarial activity through multiple mechanisms.[8] While there is clearly some potential for diarylureas, the mechanism or mechanisms of action remain unclear, and most of these studies revealed either poor correlation between enzyme target and cellular potencies or relatively poor potencies, or both.

We had previously examined diarylureas as inhibitors of the IGF-1R kinase for the treatment of cancer, an area that has also been explored by industrial groups.[15] The work mentioned above led us to screen our existing collection of diarylureas and with the preliminary data developed from that study, to carry out more extensive structure activity and pharmacophore determination studies to map the elements that are responsible for antimalarial activity. Here we report the results of these studies against chloroquine sensitive (3D7) and chloroquine resistant (K1) *Plasmodium falciparum* strain.

A straightforward synthesis of diarylureas has been previously reported.[15] The diarylurea library was generated in parallel by coupling of aryl amines **1**{x} (A ring) with aryl isocyanates **2**{y} (B ring), as shown in Figure 1. Phenyl isocyanates **2**{y} were either commercially available or were freshly prepared by treating commercial available aryl amines **3**{y} with 1,1′-carbonyldiimidazole. After all compounds were purified by preparative reverse phase high-pressure liquid chromatography (RP-HPLC), the identification and purity of each compound was estimated by liquid chromatography coupled to mass spectrometry (LCMS) (Table 1, Supporting Information). Novel compounds, not previously reported in the literature, were characterized by both nuclear magnetic resonance spectroscopy (NMR) and LCMS. This facile synthesis allowed us to produce a library of diarylureas **4**{x,y} with over 90 % purity.

The originally reported IGF-1R targeted library of diarylureas was tested for the antimalarial activity against chloroquine sensitive (3D7) and chloroquine resistant (K1) strains in replicate dose response experiments to generate 50% inhibitory concentrations (EC_{50}). Simultaneously, the cytotoxicity of all diarylureas was investigated against four human cell lines (BJ, HEK293, HEP G2, and Raji). In addition, the passive permeability and solubility were examined to provide a survey of structure-property relationships for the series. The results are summarized in a heat map (Figure 2).

One class of diarylureas, the 4-aminoquinaldine-derived diarylureas **4**{13, 1–9}, showed fairly strong antimalarial potency. All other classes showed weak antimalarial potency at best, although a number of compounds had EC_{50}'s in the range of 5–15 μM – similar to many of the diarylureas reported in other studies.

Diarylureas including the subclasses of diphenyl ureas **4** {1–7, 1–8} or naphthyl phenyl ureas **4** {8–9, 1–7} exhibited weak antimalarial activity. Eleven individual compounds showed modest activities with EC_{50} values of 5–15 μM against both stains. With the exception of compound **4**{4, 5} these classes were non-toxic against all four mammalian cell lines or weakly toxic with LD_{50} values between 10 and 25 μM. All compounds in these classes were generally poorly soluble in aqueous buffer at pH 7.4 but reasonably permeable.

The quinoline and the pyridine containing diarylurea series **4**{10–17, 1–9} had more compounds with modest activity and substituents on the B ring influenced the antimalarial

activity. For example, compounds **4**{13, 1–2} and **4**{13, 7–9}, carrying chloro substituents on the B ring and were more potent than **4**{13, 4–5} which lacked chloro groups. The most potent compound **4**{13, 1} (EC_{50} = 0.053 μM *vs* 3D7) gave an equivalent potency to chloroquine (EC_{50} = 0.047 ± 0.004 μM *vs.* 3D7) under the same assay conditions i*n vitro*. Although the 4-aminopyridine-derived diarylureas **4**{16, 1–8} possessed a pyridine ring structurally equivalent to that in the 4-aminoquinaldine series **4**{13, 1–9}, only one compound **4**{16, 8} among eight 4-aminopyridine-derived compounds demonstrated an EC_{50} value lower than 5 μM against 3D7 strain. Thus the activity requires both the hydrogen bonding and hydrophobic functions. In general, the 4-aminoquialdine-derived diarylureas showed only weak cytotoxicity. Most compounds **4**{10–17, 1–9} had reasonable permeability (>50 $\times 10^{-6}$ cm/s) and the pyridine-derived compounds **4**{14–17, 1–8} possessed better aqueous solubility than the quinoline-derived diarylureas **4**{10–13, 1–9}.

The set of diarylureas **4**{18–20, 1–9} containing two heteroatoms on the A ring were only weakly active. Only the 2-aminobenzimidazole-derived compounds **4**{18, 1–4} (EC_{50} = 5–15 μM) gave measurable potencies. All compounds, except **4**{18, 2} and **4**{18, 4}, were non-toxic on four mammalian cell lines. Although this set of diarylureas did not show promising antimalarial activity, the presence of benzimidazole, imidazole, and thiazole moieties enhanced both permeability and aqueous solubility of the compounds.

The most promising compounds from the original test contained the 4-aminoquinaldine system. Therefore, this ring was fixed as the A ring and variation of the B ring explored to examine the possibility of improving both the antimalarial potency and the physicochemical properties. All compounds in this series possessed good selectivity against malaria parasites and mammalian cells. The antimalarial potency, cytotoxicity, and physicochemical properties, are summarized in Figure 3. Of the substituted phenyl rings surveyed in this series, the chloro- or fluoro-substitutents gave the best antimalarial potencies with select compounds, **4**{13,1–3} and **4**{13, 7–20}, giving EC_{50}'s in the mid or high nanomolar range against 3D7 strain, equivalent to chloroquine. Some structure-activity relationships were evident. For example, compound **4**{13,7}, with a 3-Cl substituent on the B ring gave an EC_{50} of 0.60 μM, 5-fold more potent than compound **4**{13,10} with a 4-Cl substituent. Compounds **4**{13, 8–9} and **4**{13,11}, bearing two chloro- substituents on the B ring, displayed weaker antimalarial potency compared to **4**{13,7}. Introduction of a 2-OMe group to **4**{13, 7} afforded **4**{13, 1} with an improved EC_{50} value of 0.053 μM against 3D7. However, moving the methoxy group of **4**{13, 1} from the 2- to the 4-position (**4**{13, 3}) resulted in a 23-fold decrease in potency against 3D7. Compound **4** {13, 2} incorporating the 3-Cl and 4-Me groups exhibited an EC_{50} value of 0.22 μM, which was 3-fold more potent than **4**{13, 7}. Notably, compound **4**{13, 12} containing the 4-Cl and 3-CF_3 substituents, displayed an antimalarial potency of 0.47 μM. All compounds **4**{13, 13–20}, containing the fluoro substitutents on the B-ring, maintained similar levels of potency, in the range of 0.7–3.37μM. Compounds **4**{13, 26} and **4**{13, 39}, bearing 3-CF_3 and 3-NMe_2 respectively, showed EC_{50} values of 0.32 μM and 0.031 μM against 3D7, illustrating the preference for electron donating substitutents. Compounds **4**{13, 43–54}, containing hydroxyl, amino, or carbonyl-derived functional groups, showed weak growth inhibitory activities against malaria (5–15 μM) or displayed no activity at the highest assay concentration (15μM). Next in the study, we explored a series of heteroaromatic rings **4**{13, 55–60} as replacements for the phenyl ring. The most promising compound **4**{13, 58} carried the benzisothiadiazolyl ring, and displayed an EC_{50} value of 0.016 μM against 3D7, 3-fold more potent than chloroquine. Meanwhile, compound **4**{13, 58} was also active against chloroquine resistant K1 strain (EC_{50} = 0.079 μM). In general, the entire 4-aminoquinaldine-derived series **4**{13, 1–60} showed slightly more potency against chloroquine-sensitive strain 3D7 than chloroquine-resistant strain K1. With respect to structure-property relationships, there was a general correlation between potent antimalarial

activity and decreasing aqueous solubility with the compounds with EC_{50} values below 5 μM showing poor aqueous solubility (< 10 μM in 5% DMSO in aqueous buffer). However the highly active compounds had aqueous solubility roughly 100 to 1000-fold higher than their EC50's, indicating this is a liability that needs careful management, but not an inherent block to further development. Although we observed a wide range of permeability values, most compounds had reasonable permeability.

A preliminary analysis of pharmacophores in the series is illustrated with the three most active compounds **4**{13, 1}, **4**{13, 39} and **4**{13, 58}. The conformers of each compound were generated by systematic conformation search using MOE software with lowest energy conformations being used to generate pharmacophore features (Figure 4). One clear pharmacophore element is the aromatic moiety on the A ring (F2: Aro) and the aromatic nitrogen (F1: Acc) as they relate to the oxygen (F3: Acc), a distance of 4.86 Å from the centroid of the ring. Another important pharmacophore feature of **4**{13, 1} and **4**{13, 39} appears to be a hydrophobic site (F5: Hyd, in Figure 4), on the B-ring, which overlaid with 3-chloro group and 3-dimethylamino group respectively. In the case of the fused heteroaromatic ring, it may be the case that a slightly less favorable conformation also fulfills this hydrophobe by rotating the ring inwards. These pharmacophore elements can serve as a helpful tool in further designing new diarylureas.

While several antimalarial targets have been proposed for the diarylureas, including plasmepsin II,[5] dihydroorate dehydrogenase,[7] plasmodial cysteine proteases, and hemoglobin hydrolysis, the mechanism of action of diarylureas in parasites has not been understood clearly.[8] To date we have been unable to confirm any of these targets as the one acted upon by our series of compounds. We have previously investigated the use of diarylurea compounds as inhibitors of the human receptor tyrosine kinase IGF-1R, which is an important in all stage of the development of breast and other cancer. The inhibitory potencies of diarylureas against *Plasmodium falciparum* can be compared with potency against the human IGF-1R. Interestingly, while the 4-aminoquinaldine-derived diarylureas were active against IGF-1R, overall the compounds were significantly less potent (IC_{50} 9.7–36.7 μM).[15] According to these results, we tentatively suggest that the 4-aminoquinaldine-derived diarylureas may also inhibit kinases present in *Plasmodium* that contain similar catalytic domains. However, despite attempting to do so, we have been unable to validate any individual malarial kinase as the target of these compounds. Since the 4-animoquinaldine-derived diarylureas likely target kinases of *Plasmodium falciparum*, it is important to know whether those compounds inhibit any kinases of the human host besides IGF-1R. One compound **4**{13, 34}, which had some – albeit weak -- measurable mammalian cytotoxicity was submitted for comprehensive screening against 402 human kinases by Ambit Biosciences (Table 4, Supporting Information). Compound **4**{13, 34} bound to only one kinase, DDR1, out of 402 screened human kinases at concentration 10 μM (Figure 5.). Protein kinases of parasite *Plasmodium* are divergent from their human host.[16–18] In the case that the diarylureas target kinases of *Plasmodium*, it is highly possible that the diarylureas selectively inhibit kinases of *Plasmodium* over the human host since the most active antimalarial diarylureas essentially failed to bind to mammalian kinases. Further studies will be carried out to validate the targets of diarylureas, and understand their action in parasites. Additionally, further structural optimization of diarylureas will be required to overcome concerns for physicochemical properties and cross-resistance.

Herein is reported the evaluation of a library of diarylureas for antimalarial activity against two strains of *Plasmodium falciparum* (3D7 and K1). The most promising subseries of diarylureas contained a 4-aminoquinaldinyl moiety (A ring) as one of the aryl rings of the urea, a novel structural feature in the class. The further investigation on the B ring of this series revealed compounds in this series that were more active against chloroquine-sensitive

strain 3D7 than chloroqine-resistant strain K1 and with absolute potencies in 3D7 equivalent to chloroquine. The major liability observed in this class of compounds is that they tend to be quite insoluble. In order to enable clinical development, this liability will need to be addressed in future studies.

Lay Summary

A new class of antimalarial compounds was explored in order to establish relationships between the primary structure of the compounds and the activity against malaria. A small subset of compounds were highly potent but exhibited potential liabilities for further development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

IGF-1R	Insulin-like Growth Factor 1 Receptor
RP-HPLC	Reverse Phase High-Pressure Liquid Chromatography
LCMS	Liquid Chromatography coupled to Mass Spectrometry
NMR	Nuclear Magnetic Resonance spectroscopy
BJ	human fibroblast cells
HEK293	human embryonic kidney 293 cells
HEP G2	human hepatocellular liver carcinoma cells
Raji	human Burkitt's lymphoma cells
PAMPA	the Parallel Artificial Membrane Permeability Assay

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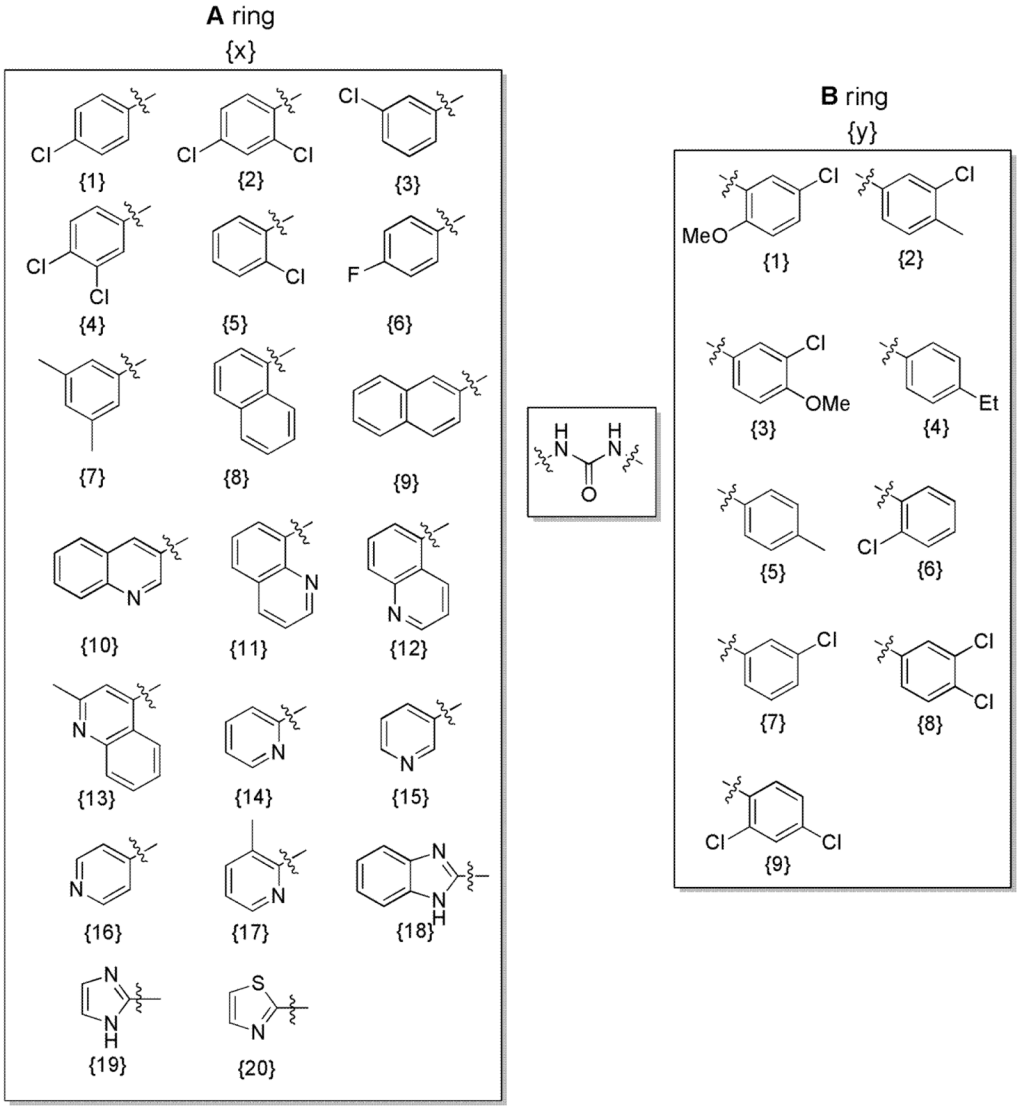


Figure 1.
Library composition of diarylurea compound chemset **4**{x,y}

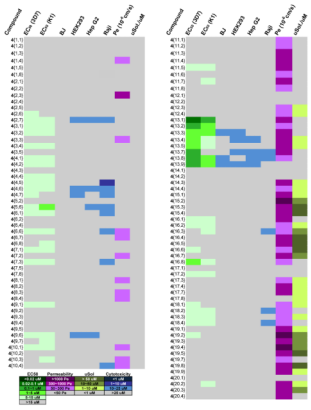


Figure 2.

Antimalarial activity, cytotoxicity, permeability and solubility of diarylurea chemset **4**{1–20, 1–9}. 1) Data represent median EC_{50} values of parasite growth inhibition against 3D7 and K1 strains of *Plasmodium falciparum*, from replicate experiments, shown in a color gradient scale with darker squares indicating a higher antimalarial potency. The positive control for the antimalarial assay was chloroquine (EC_{50} = 0.047 ± 0.004 μM against 3D7, EC_{50} = 1.23 ± 0.07 μM against K1). 2) Cytotoxicity data represent median EC_{50} values of growth inhibition against BJ, HEK-293, Hep G2 and Raji cell lines, from replicate experiments, shown in a color gradient with darker squares indicating a more potent mammalian cytotoxicity. 3) PAMPA passive permeability assay results at pH 7.4 are shown in a color gradient with darker purple squares indicating a higher permeability. 4) Aqueous solubility at pH 7.4 in isotonic buffer is shown in olive scale, as with darker squares indicating a higher solubility.

Compound	R2 (B ring)	EC_{50}/uM (3D7)	EC_{50}/ uM (K1)	BJ	HEK293 /uM	Hep G2 / uM	Raji /uM	Pe (10^{-6} cm/s)	uSol./uM
4{13,1}	3-Cl-6-OMe-phenyl	0.053	0.39	>25	>25	>25	>25	337	0.3
4{13,2}	3-Cl-4-Me-phenyl	0.22	1.57	>25	>25	>25	>25	262	0.3
4{13,3}	3-Cl-4-OMe-phenyl	1.22	3.12	23	26	>25	>25	336	0.4
4{13,4}	4-Et-phenyl	2.69	6.71	>25	16	17	>25	535	3.3
4{13,5}	4-Me-phenyl	4.38	6.90	>25	>25	>25	>25	669	1.3
4{13,7}	3-Cl-phenyl	0.60	>15	>25	21	19	18	566	0.8
4{13,8}	3,4-Cl2 phenyl	0.90	2.05	23	>25	17	10	197	0.3
4{13,9}	2,4-Cl2 phenyl	1.05	4.24	20	10	16	>25	6	0.1
4{13,10}	4-Cl-phenyl	2.76	6.80	>25	>25	>25	>25	537	1.4
4{13,11}	3,5-Cl2-phenyl	2.07	6.17	>25	>25	>25	>25	0	0.8
4{13,12}	4-Cl-3-CF3-phenyl	0.47	2.76	25	>25	>25	>25	63	1.3
4{13,13}	2-F-phenyl	1.89	0.69	>25	>25	>25	>25	817	3.8
4{13,14}	3-F-phenyl	2.43	>15	>25	>25	>25	>25	0	0.1
4{13,15}	4-F-phenyl	2.19	4.72	>25	>25	>25	>25	801	3.7
4{13,16}	2,4-F2-phenyl	0.94	4.95	20	10	17	>25	640	3.0
4{13,17}	2,5-F2-phenyl	0.69	1.64	7	12	>25	>25	541	0.5
4{13,18}	3,4-F2-phenyl	1.30	3.66	>25	>25	21	>25	478	0.5
4{13,19}	3,5-F2-phenyl	3.37	1.89	>25	>25	>25	>25	0	0.3
4{13,20}	3-F-4-OMe-phenyl	2.37	4.62	26	>25	>25	>25	50	2.7
4{13,21}	3-Me-phenyl	2.15	10.05	>25	>25	>25	>25	4	0.3
4{13,22}	2-tBu-phenyl	1.54	3.00	22	>25	>25	>25	27	0.1
4{13,23}	3-tBu-phenyl	>15	>15	>25	>25	>25	>25	555	34.7
4{13,24}	4-tBu-phenyl	2.03	3.34	20	>25	17	>25	142	6.2
4{13,25}	2-CF3-phenyl	>15	>15	>25	>25	>25	>25	945	3.8
4{13,26}	3-CF3-phenyl	0.32	>15	>25	16	21	10	0	0.1
4{13,27}	4-CF3-phenyl	1.49	2.61	>25	25	23	23	2	0.1
4{13,28}	3-CF3-4-NO2-phenyl	1.97	>15	>25	>25	>25	>25	45	0.0
4{13,29}	2-OMe-phenyl	2.05	6.90	>25	>25	>25	>25	0	0.1
4{13,30}	3-OMe-phenyl	2.01	4.74	27	>25	>25	>25	818	1.2
4{13,31}	4-OMe-phenyl	5.38	6.84	>25	>25	>25	>25	787	19.8
4{13,32}	3,4-(OMe)2-phenyl	5.67	7.04	>25	>25	>25	>25	552	4.1
4{13,33}	2-OCF3-phenyl	3.41	5.00	>25	>25	>25	>25	1107	4.6
4{13,34}	3-OCF3-phenyl	1.04	2.75	25	22	13	9	464	0.6
4{13,35}	4-OCF3-phenyl	2.63	4.85	25	14	15	10	531	0.3
4{13,36}	2-SMe-phenyl	6.10	7.31	>25	>25	>25	>25	1259	21.0
4{13,37}	4-SMe-phenyl	3.13	6.98	>25	24	16	>25	473	1.3
4{13,38}	4-SCF3-phenyl	2.86	4.33	>25	13	20	10	206	0.2
4{13,39}	3-NMe2-phenyl	0.031	0.11	>25	>25	>25	>25	0	0.0
4{13,40}	4-NMe2-phenyl	5.61	7.80	>25	>25	>25	>25	2	0.0
4{13,41}	2-NO2-phenyl	1.81	6.59	>25	>25	>25	>25	204	0.2
4{13,42}	3-NO2-phenyl	2.54	>15	>25	>25	>25	>25	42	0.0
4{13,43}	4-OH-phenyl	>15	>15	>25	>25	>25	>25	3	0.1
4{13,44}	2-NH2-phenyl	>15	>15	>25	>25	>25	>25	300	51.8
4{13,45}	4-NH2-phenyl	10.16	8.78	>25	>25	>25	>25	530	18.5
4{13,46}	4-COMe-phenyl	4.42	6.77	22	>25	19	>25	473	3.8
4{13,47}	3-COOMe-phenyl	>15	8.17	21	26	23	26	746	0.6
4{13,48}	4-COOMe-phenyl	7.18	6.16	21	15	20	17	448	1.2
4{13,49}	2-CONH2-phenyl	>15	>15	>25	>25	>25	>25	0	0.2
4{13,50}	3-CONH2-phenyl	13.13	>15	>25	>25	>25	>25	2	0.1
4{13,51}	4-CONH2-phenyl	10.73	5.48	>25	>25	>25	>25	0	0.9
4{13,52}	4-COOH-phenyl	>15	>15	>25	>25	>25	>25	0	9.3
4{13,53}	2-COOH-4-F-phenyl	>15	>15	>25	>25	>25	>25	5	44.3
4{13,54}	3-OH-4-COOH-phenyl	>15	>15	>25	>25	>25	>25	0	26.6
4{13,55}	pyridin-3-yl	3.42	6.20	>25	>25	>25	>25	63	0.0
4{13,56}	3-Me-pyridin-2-yl	>15	>15	>25	>25	>25	>25	369	1.3
4{13,57}	quinolin-3-yl	2.95	6.02	>25	>25	>25	>25	46	0.4
4{13,58}	benzisothiadiazol-4-yl	0.016	0.079	>25	>25	>25	>25	155	0.3
4{13,59}	benzodioxol-5-yl	5.08	10.10	>25	>25	>25	>25	266	1.6
4{13,60}	benzimidazol-5-yl	1.09	1.70	>25	>25	>25	>25	2	7.3

EC_{50} /uM	Permeability /Pe	uSol/uM	Cytotoxicity/uM
<0.02	>1000	> 50	<1
0.02-0.1	300~1000	10~50	1~10
0.1~1	50~300	1~10	10~25
1~5	<50	<1	>25
5-15			
>15			

Figure 3.

Antimalarial activity, cytotoxicity, permeability and solubility of the 4-aminoquinaldine-derived diarylurea compounds **4**{13, 1–60}. 1) Data represent median EC_{50} values of parasite growth inhibition against 3D7 and K1 strains of *Plasmodium falciparum*, from replicate experiments, shown in a color gradient scale with darker squares indicating a higher antimalarial potency. The positive control for the antimalarial assay was chloroquine (EC_{50} = 0.047 ± 0.004 μM against 3D7, EC_{50} = 1.23 ± 0.07 μM against K1). 2) Cytotoxicity data represent median EC_{50} values of growth inhibition against BJ, HEK 293, Hep G2 and Raji cell lines, from replicate experiments, shown in a color gradient with darker squares indicating a more potent mammalian cytotoxicity. 3) PAMPA passive permeability assay results at pH 7.4 are shown in a color gradient with darker purple squares indicating a higher permeability. 4) Aqueous solubility at pH 7.4 in isotonic buffer is shown in olive scale, as with darker squares indicating a higher solubility.

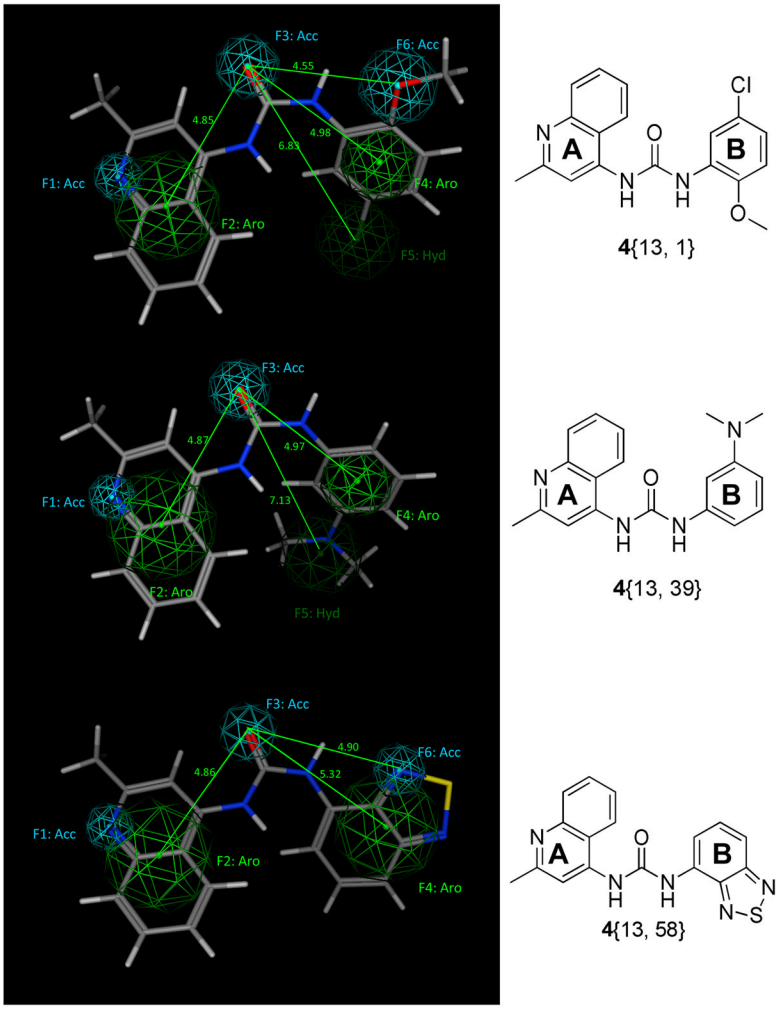


Figure 4.

Pharmacophore mapping of **4**{13, 1}, **4**{13, 39} and **4**{13, 58}. Pharmacophore features are color coded with green for aromatic feature (Aro), cyan for hydrogen-bond acceptor feature (Acc), and dark green for hydrophobic feature (Hyd).

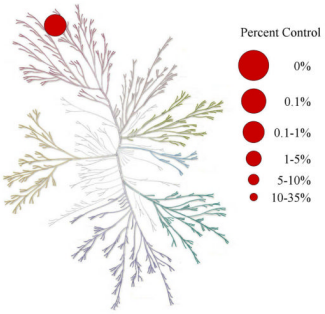
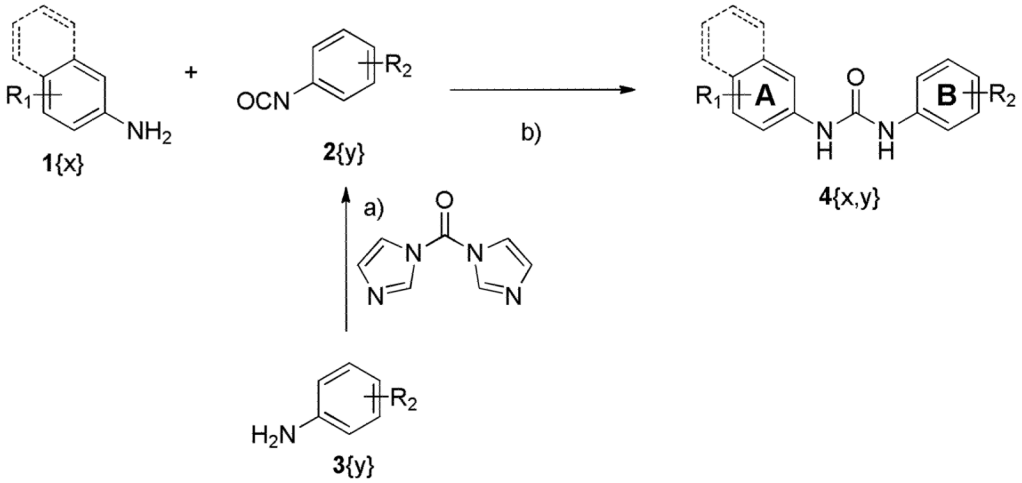


Figure 5.
Treespot™ interaction maps for **4**{13, 34} in human kinases. Kinases found to bind are marked with red circles, where larger circles indicate higher-affinity binding. Mutant and lipid kinases are not presented.



Scheme 1. General synthetic routes for diarylureas
Conditions: a) DMSO, rt, 2h; b) DMSO, rt, 5–10 min.