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Synthesis and antiplasmodial activity of new heteroaryl derivatives of 7-chloro-4-aminoquinoline

Manolo Casagrande [a], Anna Barteselli [a], Nicoletta Basilico [b], Silvia Parapini [c], Donatella Taramelli [c], Anna Sparatore [a,*]

[a] Dipartimento Scienze Farmaceutiche, Università degli Studi di Milano, Via Mangiagalli 25, 20133 Milano, Italy
[b] Dipartimento di Scienze Biomediche, Chirurgiche e Odontoiatriche, Università degli Studi di Milano, Via Pascal, 36, 20133 Milano, Italy
[c] Dipartimento di Scienze Farmacologiche e Biomolecolari, Università di Milano, Via Balzaretti 9, 20133 Milano, Italy

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ABSTRACT

With the aim to investigate the effect of different heterocyclic rings linked to the 4-aminoquinoline nucleus on the antimalarial activity, a set of 7-chloro-*N*-(heteroaryl)-methyl-4-aminoquinoline and 7-chloro-*N*-(heteroaryl)-4-aminoquinoline was synthesized and tested in vitro against D-10 (CQ-S) and W-2 (CQ-R) strains of *Plasmodium falciparum*. All compounds exhibited from moderate to high antiplasmodial activities. The activity was strongly influenced both by the presence of a methylenic group, as a spacer between the 4-aminoquinoline and the heterocyclic ring, and by the presence of a basic head. The most potent molecules inhibited the growth of both CQ-S and CQ-R strains of *P. falciparum* with IC_{50} < 30 nM and were not toxic against human endothelial cells. These results confirm that the presence of an heteroaryl moiety in the side chain of 7-chloro-4-aminoquinoline is useful for the design and development of new powerful antimalarial agents.

1. Introduction

Presently, the most promising and successful strategy in fighting malaria is the artemisinin-based combination therapy (ACT). Recent reports of ACT treatment failure in southeast Asia[1] and the potential emergence of artemisinin resistance[2] indicate that the search of new drugs or new combinations is still highly necessary. In order to develop new classes of antimalarial agents, we recently demonstrated that the replacement of the phenolic ring of amodiaquine and tebuquine with a pyrrole nucleus, still linked to the quinoline moiety through the usual NH (Fig.1, general stucture 1), is associated with a good activity against both chloroquine susceptible (CQ-S) and chloroquine-resistant (CQ-R) strains of *Plasmodium falciparum*.[3,4] In addition, though ferroquine continues to be a promising compound as a new chemotherapeutic agent,[5,6] it was shown by Blackie M. A. et al.[7] that analogues containing a phenyl moiety instead of ferrocene continued to exhibit high activity against both CQ-S and CQ-R strains of *P. falciparum*. Among them, phenylequine (Fig. 1), characterized by a phenyl ring linked to the 4-aminoquinoline nucleus through a methylenic group, showed good efficacy in two in vivo murine models, as well.

Starting from these evidences and with the aim to test the effect on the antimalarial activity of the presence of other heterocyclic rings linked to the 7-chloro-4-aminoquinoline nucleus, a set of twenty five 7-chloro-*N*-(heteroaryl)-4-aminoquinolines (**3a**, **3b**) and 7-chloro-*N*-(heteroaryl)-methyl-4-aminoquinolines, bearing

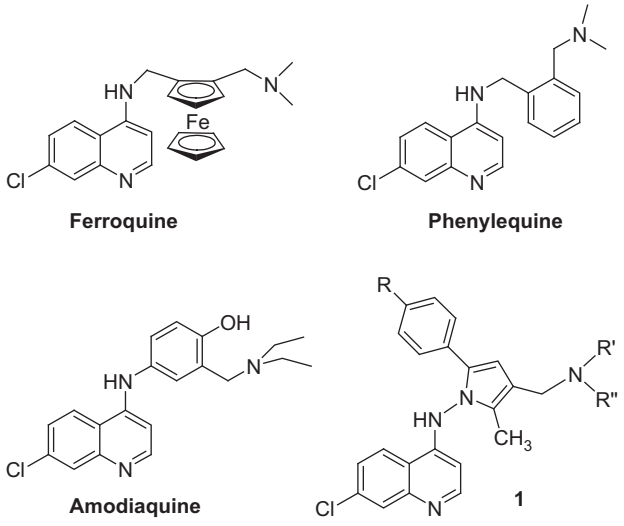

Figure 1. Structures of some 4-aminoquinoline derivatives with antimalarial activity.

* Corresponding author. Tel.: +39 02 50319365; fax: +39 02 50319359.
E-mail address: anna.sparatore@unimi.it (A. Sparatore).

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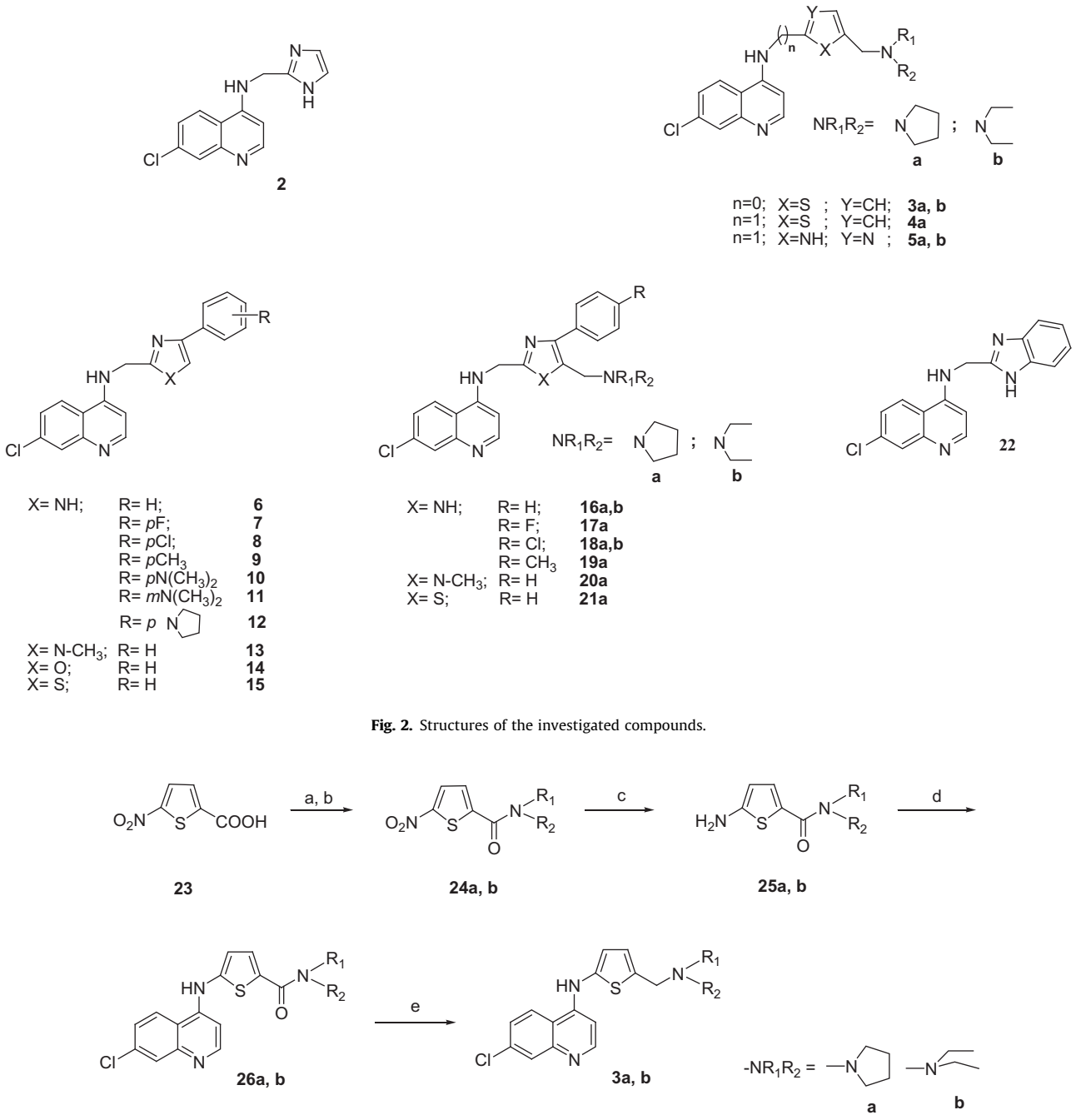


Fig. 2. Structures of the investigated compounds.

Scheme 1. Reagents and conditions: (a) $SOCl_2$, reflux; (b) anh. CH_2Cl_2, TEA, HNR_1R_2, rt (73–77%); (c) CH_3COOH, Fe, 70 °C (49–69%); (d) 4,7-dichloroquinoline, PhOH, 130 °C (83–88%); (e) $LiAlH_4$, THF, 60 °C (17–35%).

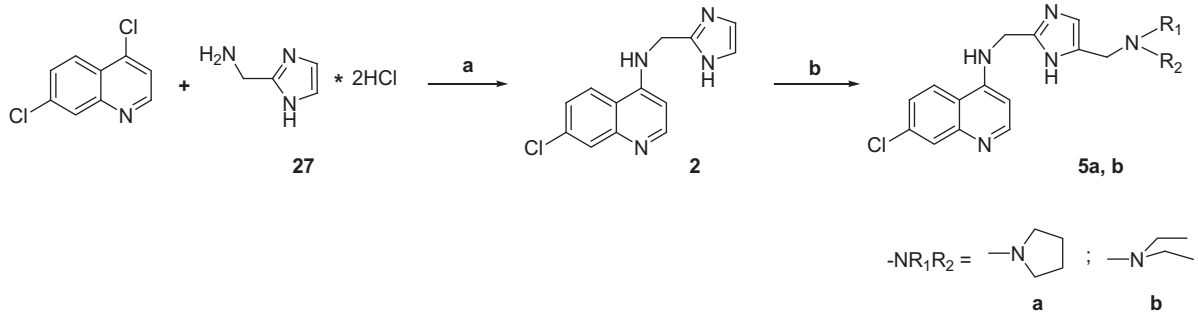
different substituents on the heterocycle ring (Fig. 2), was synthesized. Here we describe the syntheses and characterization of these compounds and the evaluation of their in vitro activity on D-10 (CQ-S) and W-2 (CQ-R) strains of *P. falciparum* and cytotoxicity against human cells. Experimental measures of pK_a and log *P* were performed to check any correlation between lipophilicity and activity.

2. Chemistry

Compounds **3a** and **3b** were obtained in four steps as indicated in Scheme 1. 5-Nitrothiophene-2-carboxylic acid (**23**) was converted into acyl chloride and reacted with the proper amine to give the corresponding amides **24a** and **24b**.[8,9] Nitrocompounds were reduced with iron and acetic acid, the amino derivatives (**25a** and **25b**) heated with 4,7-dichloroquinoline in phenol and the corresponding amides (**26a** and **26b**) reduced with $LiAlH_4$ in THF.

Starting from intermediate **2**, synthesized from (1*H*-imidazol-2yl)methanamine hydrochloride[10] (**27**) and 4,7-dichloroquinoline, a simple Mannich reaction, performed with aq. formaldehyde and the proper amine gave compounds **5a** and **5b** (Scheme 2).

According to the procedure described by Salvadori et al,[11] compounds **6–12** were obtained by reaction of the proper phenacyl bromide (**28–34**), commercially available or synthesized following methods described in literature,[12–17] and Boc-glycine cesium salt in DMF; the obtained keto ester compounds (**35–41**) were refluxed



Scheme 2. Reagents and conditions: (a) 4,7-dichloroquinoline, PhOH, DIPEA, 130 °C (81%); (b) CH_3COOH, 37% aq CH_2O, HNR_1R_2, reflux (15–21%).

with ammonium acetate in xylene, giving rise to phenyl-imidazolyl derivatives **42–48**. Boc protection was removed with trifluoroacetic acid (TFA) and the amino derivatives **49–55** reacted with 4,7-dichloroquinoline in phenol (Scheme 3).

For the preparation of compound **13**, the required *N*-methyl-imidazolyl nucleus was obtained by reacting compound **42** with methyl iodide in DMF[12] to give **56**,[18] as indicated in Scheme 4:

Mannich reactions with formaldehyde and the proper amine were used in order to obtain compounds **16a**, **16b**, **17a**, **18a**, **18b**, **19a** and **20a** (Scheme 5).

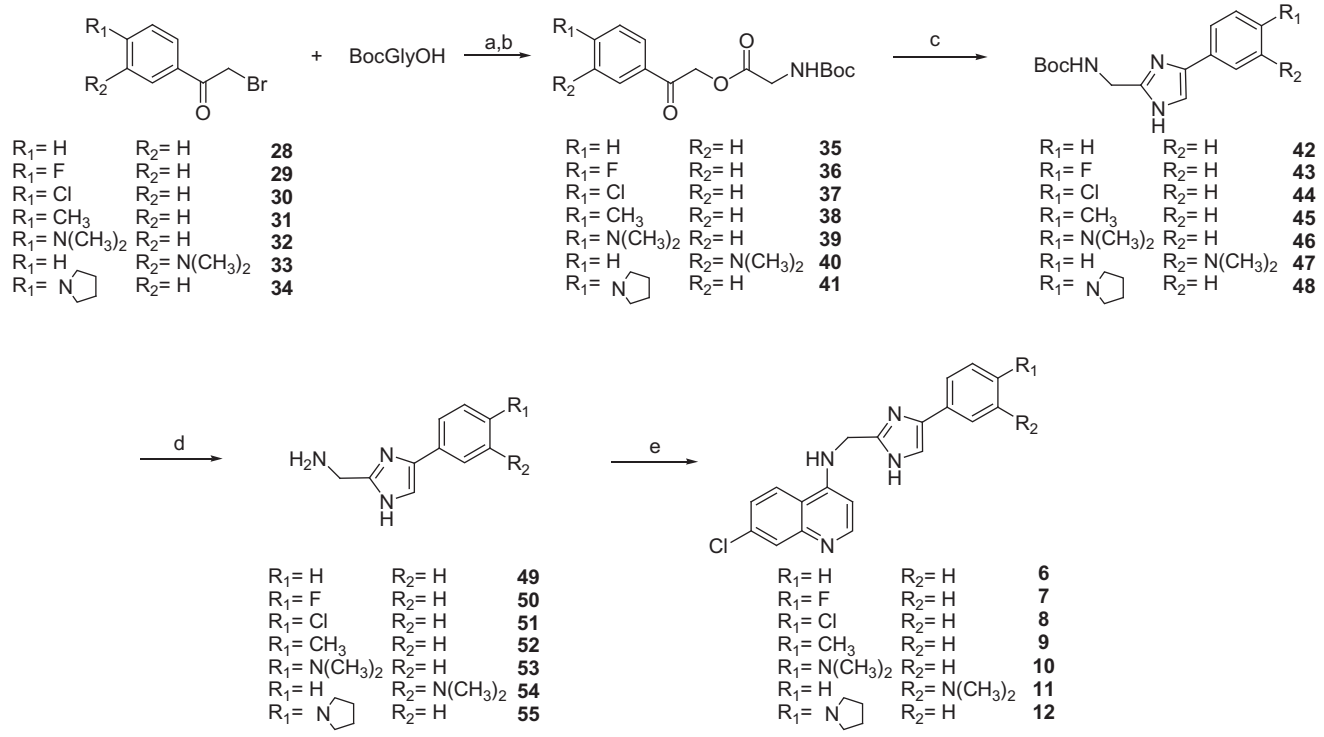
As shown in Scheme 6, compound **22** was prepared by reaction of (1*H*-benzo[d]imidazol-2-yl)methanamine[19] (**58**) and 4,7-dichloroquinoline in presence of phenol.

For the synthesis of compounds **14** and **15**, the intermediates **61** and **62** (Scheme 7) were prepared by reacting phenacyl bromide with compound **59**[20] in *N*-methylpyrrolidone (NMP) at 110 °C[21] or compound **60**[22] in a mixture of DMF/ethanol at 80 °C. Then, the obtained oxazole and thiazole derivatives were dissolved in a solution of HBr in acetic acid in order to remove Cbz group[22] and the corresponding free amino derivatives (**63**, **64**) were treated, as usual, with 4,7-dichloroquinoline to give compounds **14** and **15**.

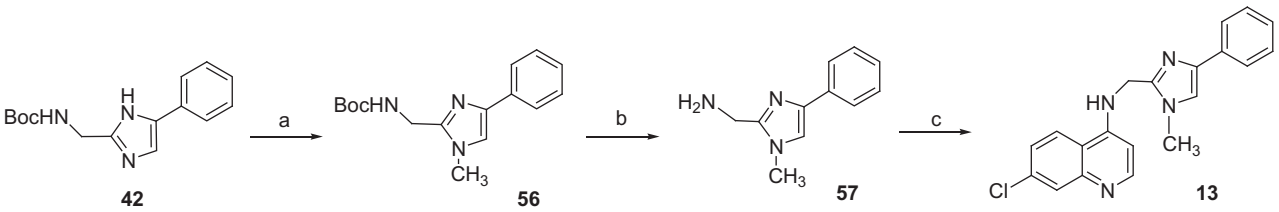
Compound **21a** could not be obtained through a Mannich reaction from compound **15**, thus an alternative synthetic pathway was set up (Scheme 8).

Likewise to compound **62**, compound **60** was condensed with ethyl 2-bromo-3-oxo-3-phenylpropanoate (**65**) and the obtained thiazolyl derivative **66** was treated with HBr in acetic acid to remove the protection on the amino moiety. Compound **67** was then heated with 4,7-dichloroquinoline in phenol and after the hydrolysis of the ester **68**, the carboxylic acid **69** was coupled with pyrrolidine in the presence of DCC and HOBt to give **70**. Finally, the amido group was reduced to the expected amino compound **21a**, by means of diphenylsilane in the presence of tris(triphenylphosphine)rhodium(I)carbonylhydride[23,24] and not with $LiAlH_4$ because its use brought to an unexpected decomposition of **70**.

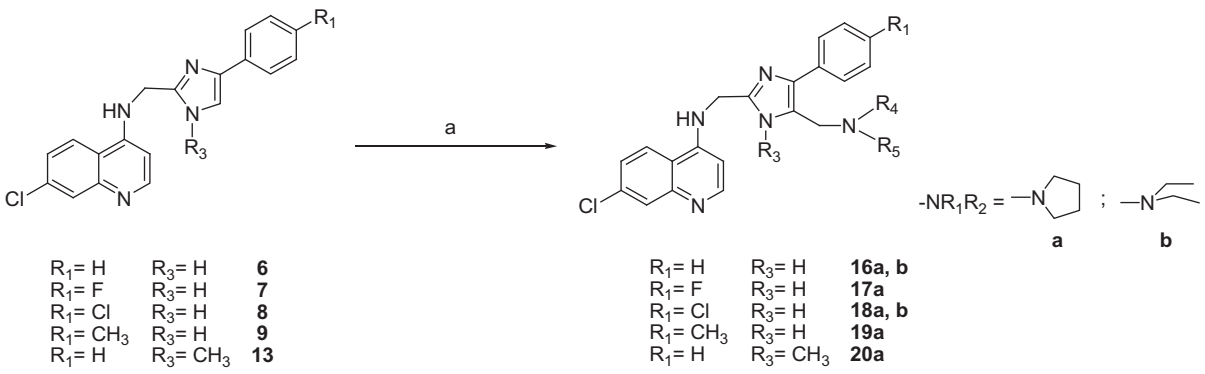
Finally, compound **4a** was synthesized starting from methyl 5-(azidomethyl)thiophene-2-caboxylate[25] (**71**) as shown in Scheme 9.



Scheme 3. Reagents and conditions: (a) Cs_2CO_3, EtOH, rt; (b) DMF, rt (47–95%); (c) CH_3COONH_4, xylene, 180 °C (46–74%); (d) CF_3COOH, rt (82–98%); (e) 4,7-dichloroquinoline, PhOH, 130 °C (24–87%).



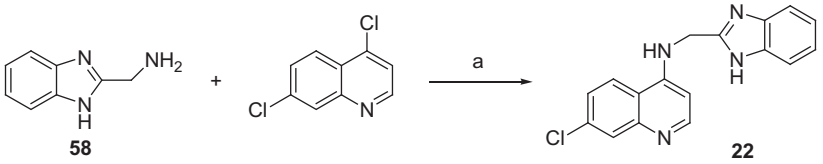
Scheme 4. Reagents and conditions: (a) CH_3I, K_2CO_3, DMF, 0 to 60 °C (57%); (b) CF_3COOH, rt (88%); (c) 4,7-dichloroquinoline, PhOH, 130 °C (74%).



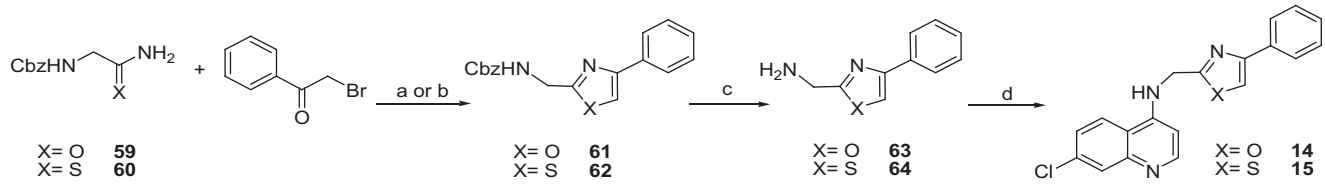
R_1= H	R_3= H	**6**	
R_1= F	R_3= H	**7**	
R_1= Cl	R_3= H	**8**	
R_1= CH_3	R_3= H	**9**	
R_1= H	R_3= CH_3	**13**	

R_1= H	R_3= H	**16a, b**
R_1= F	R_3= H	**17a**
R_1= Cl	R_3= H	**18a, b**
R_1= CH_3	R_3= H	**19a**
R_1= H	R_3= CH_3	**20a**

Scheme 5. Reagents and conditions: (a) EtOH, CH_3COOH, 37% aq CH_2O, HNR_1R_2, reflux (30–60%).



Scheme 6. Reagents and condition: (a) PhOH, 130 °C (44%).



X= O	**59**	X= O	**61**	X= O	**63**
X= S	**60**	X= S	**62**	X= S	**64**

X= O	**14**
X= S	**15**

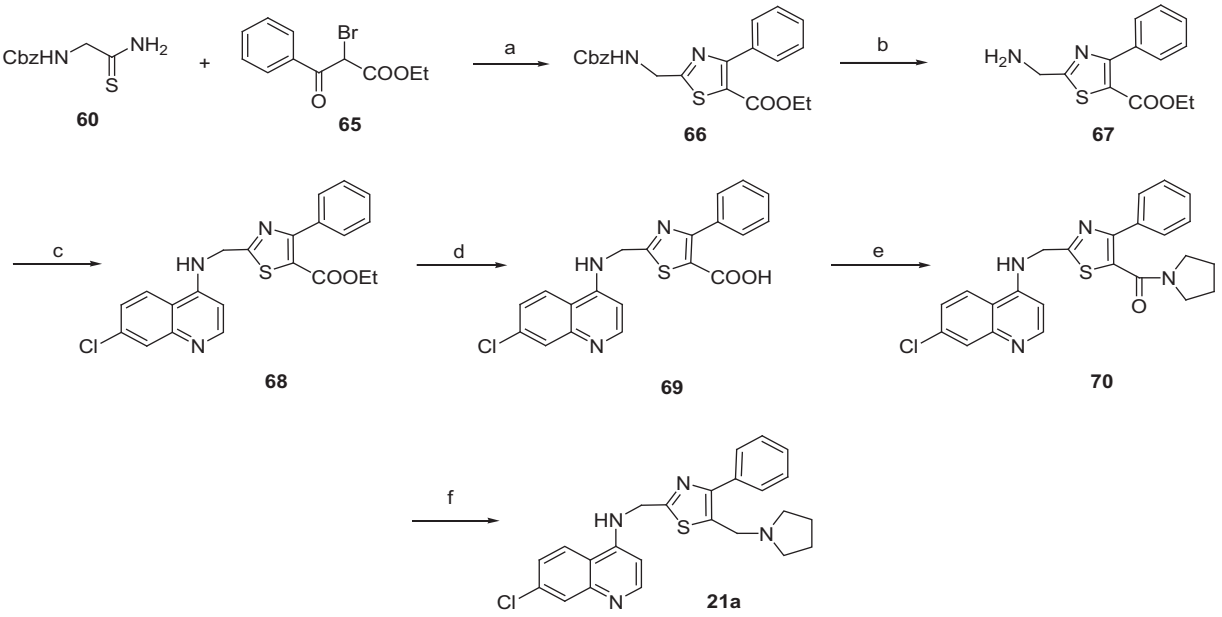
Scheme 7. Reagents and conditions: (a) NMP, 100 °C (35%); (b) DMF/EtOH, 80 °C (67%) ; (c) CH_3COOH/HBr 33%, rt (72–82%); (d) 4,7-dichloroquinoline, PhOH, 130 °C (52–68%).

The azidomethyl group was reduced using $SnCl_2$ in methanol, the corresponding amine (**72**) was heated with 4,7-dichloroquinoline in phenol and the ester (**73**) was hydrolyzed by refluxing with LiOH in ethanol/water. The carboxylic acid (**74**), was then coupled with pyrrolidine in presence of DCC and HOBt and the amide group was then reduced to the amino compound **4a** by means of $LiAlH_4$ in THF.

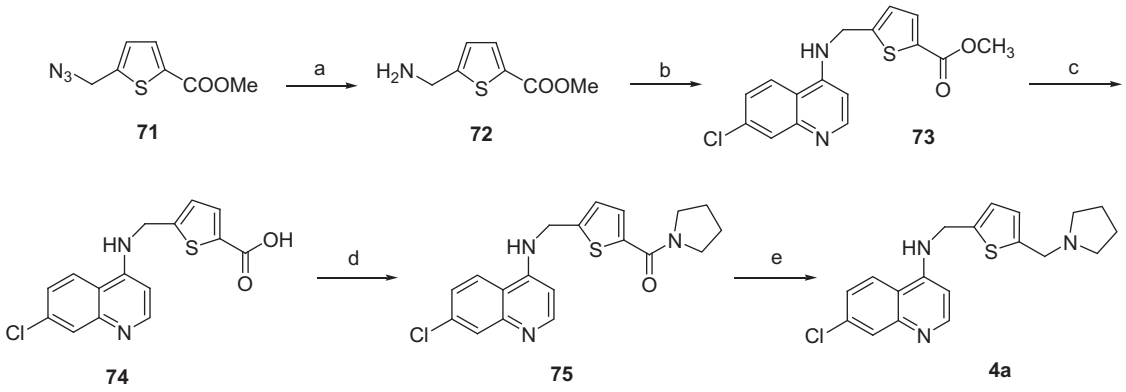
3. Results and discussion

The 25 compounds of Figure 2 were tested in vitro against D-10 (CQ-S) and W-2 (CQ-R) strains of *P. falciparum*. Their antimalarial activity was quantified as inhibition of parasite growth, measured as the activity of parasite lactate dehydrogenase (pLDH).[26] Table 1 shows the IC_{50} (nM) and the means of the ratios between the IC_{50} of CQ and that of each compound against D-10 or W-2 strains, calculated for each single experiment (since all the compounds were not tested simultaneously). The ratios between the IC_{50} of each compound against the two strains of *P. falciparum* are also indicated. The last value (resistance index, RI) is suggestive of cross-resistance between the compounds and chloroquine. Cytotoxicity on a human endothelial cell line (HMEC) was assayed using the MTT test[27] and the selectivity index (SI; IC_{50} HMEC/IC_{50} W-2) was calculated (Table 1).

To check any correlation between some physicochemical properties of the new compounds and their antiplasmodial activity, the values of pK_a, log *P* and log *D* at pH 7.4 and 5.2 were experimentally measured or calculated and are reported in Table 2. Among the compounds bearing a basic head, only those with a pyrrolidinyl moiety were considered. CQ values were in good accordance with the results published by O'Neill et al.[28] and Warhust et al.[29] All tested compounds exhibited from moderate to high activity against the CQ-S (D-10) strain, with IC_{50} ranging from 5.50 to 399 nM. CQ IC_{50} was 14.3 nM (range 11–16 nM). The majority of



Scheme 8. Reagents and conditions: (a) DMF/EtOH, 80 °C (69%); (b) CH_3COOH/HBr 33%, rt (51%); (c) 4,7-dichloroquinoline, PhOH, 130 °C (78%); (d) LiOH, EtOH/H_2O, reflux (98.5%); (e) pyrrolidine, DCC, HOBt, DMF, TEA, MW (88%); (f) Ph_2SiH_2, $(PPh_3)_3(CO)HRh$, THF, 50 °C (22%).



Scheme 9. Reagents and conditions: (a) $SnCl_2$, MeOH, rt (34%); (b) 4,7-dichloroquinoline, PhOH, 130 °C (80%); (c) LiOH, EtOH/H_2O, reflux (99%); (d) pyrrolidine, DCC, HOBt, DMF, MW (72%); (e) $LiAlH_4$, THF, 0 °C to rt (46%).

them (17/25) had an IC_{50} against D-10 lower than 100 nM, which is the empiric cut off for discriminating compounds interesting for further hit selection. Only, two compounds, **3a** and **3b** showed very low activity against D-10 strain with IC_{50} in the μM range (IC_{50} = 2656 nM and 1858 nM).

Ten of the new compounds also exhibited a strong activity against W-2, CQ-R strain, resulting from 4.8 to 16.4 times more active than CQ, with IC_{50} values as low as 20.9–60 nM (**8–12**, **4a**, **18a**, **18b**, **20a** and **21a)** compared to 317.1 nM of CQ (range 152.8–431.6 nM). Six additional compounds (**6**, **7**, **13**, **16a**, **16b** and **19a**) were from 1.7- to 4.2-fold more active than the reference drug. Compounds **2**, **3a**, **3b**, **5a**, **5b**, **14**, **15**, **17a**, **22** that had low activity or showed signs of cross resistance with CQ were not investigated further.

To get preliminary information on their mechanism of action, two representative compounds (**4a** and **10**; Table 3) were tested for their ability to inhibit β-hematin formation in vitro using the BHIA (β-hematin inhibitory activity) assay previously described.[30] The results suggest that both compounds significantly interfere with the haem detoxification process of parasites, thus acting with a mechanism similar to that of CQ. Compound **10** is 4.5-fold more active than CQ in the BHIA assay.

The substitution of the ferrocenic moiety and/or the phenyl ring, present in ferroquine and phenylequine, with different kinds of heterocyclic rings is associated with the maintenance of a good antiplasmodial activity. However, the suppression of the methylenic spacer between the 4-amino group of quinoline and the heteroaryl ring is associated with a significant reduction of the activity (compare compounds **3a** and **3b** with **4a**). This is at variance of the high activity observed in the amodiaquine-like compounds, including our 4-(*N*-pyrrolylamino)quinoline derivatives previously reported.[3,4]

In the series of N-unsubstituted imidazolyl derivatives, the presence of an aryl substituent on the imidazole ring enhanced the antiplasmodial activity (and reduced the value of RI) in both the series lacking (**6** vs **2**) or bearing an aliphatic basic head (**16a** and **16b** vs **5a** and **5b**). Indeed, arylimidazolyl derivatives, lacking the Mannich basic head (**6–12**) showed very high activity against the CQ-R strain of *P. falciparum*, with the nature (and the position) of the substituent in the phenyl ring influencing the activity in the

Table 1
In vitro antimalarial activities and cellular cytotoxicity of the compounds under study

Compd	D-10 IC_{50}[a](nM)	Ratio IC_{50} CQ/Comp[b]	W-2 IC_{50}[a] (nM)	Ratio IC_{50} CQ/Comp[b]	RI[c]	HMEC-1 IC_{50}[d](nM)	SI[e]
2	151.9 ± 46.4	0.11	383.9 ± 100.5	0.39	2.5	n.t.	—
3a	2656.6 ± 702.6	0.01	4219.9 ± 1283.6	0.04	1.6	n.t.	—
3b	1858.2 ± 418.8	0.08	2575.6 ± 270.4	0.06	1.4	n.t.	—
4a	12.5 ± 7.6	1.11	26.3 ± 13.0	16.43	2.1	24196 ± 3856	921
5a	399.0 ± 19.5	0.04	3119.8 ± 629.4	0.05	7.8	n.t.	—
5b	191.2 ± 3.8	0.07	3797.4 ± 924.8	0.04	19.9	n.t.	—
6	115.6 ± 29.9	0.14	85.1 ± 8.7	1.78	0.7	13082 ± 1314	154
7	52.1 ± 32.9	0.28	82.4 ± 20.0	5.05	1.6	9921 ± 113	120
8	25.8 ± 10.7	0.43	60.5 ± 27.5	4.77	2.3	7447 ± 840	123
9	17.2 ± 2.9	0.64	40.7 ± 4.9	7.10	2.4	7769 ± 717	191
10	13.4 ± 3.8	0.82	20.9 ± 10.9	13.80	1.6	6669 ± 291	320
11	32.0 ± 2.5	0.46	53.5 ± 13.7	7.78	1.7	8971 ± 2223	168
12	18.2 ± 5.5	0.77	35.3 ± 4.4	12.22	1.9	2426 ± 941	69
13	30.0 ± 17.1	0.46	70.3 ± 28.4	6.14	2.3	17515 ± 2236	249
14	269.5 ± 9.5	0.05	512.9 ± 42.1	0.84	1.9	n.t.	—
15	211.5 ± 56.6	0.07	395.9 ± 40.4	1.09	1.9	n.t.	—
16a	22.9 ± 9.1	0.71	91.6 ± 52.6	1.67	4.0	8661±1459	95
16b	28.0 ± 12.6	0.58	85.8 ± 23.8	1.78	3.1	9144 ± 143	107
17a	25.7 ± 6.8	0.57	197.1 ± 63.1	2.11	7.7	n.t.	—
18a	5.5 ± 1.4	2.00	27.2 ± 9.3	10.62	4.9	2056 ± 508	76
18b	8.8 ± 2.8	1.25	27.6 ± 8.0	10.47	3.1	2442 ± 308	89
19a	7.4 ± 1.5	1.49	69.3 ± 22.0	4.17	9.4	3658 ± 741	53
20a	11.8 ± 5.2	1.18	26.5 ± 5.4	16.27	2.2	28636 ± 5811	1079
21a	19.30[f]	0.81	60.1 ± 13.2	4.93	3.1	30429 ± 5436	506
22	70.6 ± 41.6	0.20	256.0 ± 54.8	1.68	3.6	2401 ± 520	9
CQ	14.3 ± 2.1	—	317.1 ± 113.1	—	22.2	> 38000	—

[a] The results are expressed as IC_{50} ± SD of at least three different experiments each performed in duplicate or triplicate.
[b] Mean of ratios between the IC_{50} of chloroquine and that of each compound against D-10 or W-2 strains of *P. falciparum* calculated for each single experiment.
[c] Ratios between the IC_{50} values of each compound against W-2(CQ-R) and D-10(CQ-S) strains of *P. falciparum.*
[d] The cytotoxic activity was assayed in vitro using the MTT assay.
[e] Selectivity Index = IC_{50} (HMEC)/IC_{50} (W-2).
[f] Mean of two experiments each performed in duplicate.

Table 2
Physicochemical characteristics of the new heteroaryl-derivatives of 7-chloro-4-aminoquinoline[a]

Compd	pK_{a1}	pK_{a2}	pK_{a3}	log*P*	log$D_{7.4}$		log$D_{5.2}$ Calc[b]
					Obs	Calc[b]	
2	7.63 ± 0.02	5.59 ± 0.06	—	2.82 ± 0.01	2.39	2.39	−0.15
3a	8.26 ± 0.13	6.40 ± 0.03	—	3.93 ± 0.01	2.98	2.98	−0.36
4a	8.56 ± 0.01	7.71 ± 0.04	—	4.42 ± 0.01	2.77	2.47	−1.45
5a	9.85 ± 0.08	8.11 ± 0.01	7.45 ± 0.13	4.78 ± 0.02	1.42	1.25	−5.03
6	7.52 ± 0.01	5.21 ± 0.10	—	4.98 ± 0.01	4.62	4.61	2.35
7	7.54 ± 0.03	5.11 ± 0.07	—	5.31	4.93	4.93	2.71
8	7.28 ± 0.13	4.54 ± 0.02	—	5.34 ± 0.04	5.10	5.09	3.17
9	7.38 ± 0.04	4.69 ± 0.06	—	4.95 ± 0.02	4.66	4.66	2.65
10	7.44 ± 0.01	5.61 ± 0.04	3.28 ± 0.09	5.07 ± 0.01	4.75	4.74	2.27
11	7.54 ± 0.03	5.67 ± 0.01	3.91 ± 0.22	5.86 ± 0.01	5.48	5.48	2.91
12	6.48 ± 0.04	5.64 ± 0.36	3.37 ± 0.09	8.87 ± 0.01	8.53	8.82	7.01
13	7.62 ± 0.10	4.89 ± 0.13	—	4.99 ± 0.02	4.57	4.56	2.40
14	6.36 ± 0.1[c]	[c]	—	4.85 ± 0.02	4.81	4.81	2.50
15	6.45 ± 0.04[c]	[c]	—	5.20 ± 0.02	5.15	5.15	2.67
16a	8.88 ± 0.16	7.86 ± 0.03	6.75 ± 0.16	6.35	4.22	4.21	−1.55
17a	8.62 ± 0.02	7.93 ± 0.01	7.14 ± 0.06	6.78 ± 0.02	4.77	4.76	−1.31
18a	9.36 ± 0.02	7.50 ± 0.05	6.13 ± 0.01	6.96 ± 0.02	4.63	4.57	−0.48
19a	9.50 ± 0.21	7.49 ± 0.02	5.76 ± 0.16	6.45 ± 0.03	4.00	3.99	−0.80
20a	8.25 ± 0.02	8.01 ± 0.02	6.45 ± 0.01	9.18 ± 0.02	7.57	7.57	2.05
21a	7.65 ± 0.02	7.63 ± 0.04	5.92 ± 0.09	6.78 ± 0.02	6.01	6.05	1.10
22	7.22 ± 0.06	3.92 ± 0.02	—	3.78 ± 0.01	3.56	3.56	1.73
CQ	10.62 ± 0.02	8.13 ± 0.01	—	4.89 ± 0.01	0.89	0.88	−3.44
	10.4[d];10.18[e]	8.1[d]; 8.38[e]	—	4.72[e]	0.96[e]	0.92[e]	−3.44[e]

[a] pK_a, log*P*, and log*D* values were calculated as described in Materials and Methods. The experiments were conducted at 25 °C. Obs, observed; Calc, calculated; log$D_{7.4}$ and log$D_{5.2}$, log*D* values at pHs 7.4 and 5.2, 'respectively'.
[b] Calculated from the following equation: $\log D = \log P - \log [1 + 10^{(pKa1 - pH)} + 10^{(pKa1 + pKa2 - 2pH)} + 10^{(pKa1 + pKa2 + pKa3 - 3pH)}]$.
[c] The pK_a values of the two basic moieties are practically superimposed.
[d] From ref.28.
[e] From ref.29.

following direction H ⩽ *p*-F < *p*-Cl < *m*-N$(CH_3)_2$ < *p*-CH_3 ⩽ pyrrolidine ⩽ *p*-N$(CH_3)_2$. Thus the highest activity of compound **10** against W-2 (CQ-R) strain might be related to the overlapping of the electronic effects and of the basicity of the amino group.

Table 3
Inhibition of β-hematin formation (BHIA method)

Compd	IC_{50} Drug: haemin molar ratio[a]
4a	1.08 ± 0.52
10	0.42 ± 0.03
CQ	1.94 ± 0.61

[a] β-hematin inhibitory activity in equivalents of test compounds relative to haemin causing 50% inhibition. Results represent the mean of three different experiments, each performed in duplicate.

The introduction of more basic Mannich-type heads (**16a**, **16b**, **17a**, **18a**, **18b**, **19a)** on the imidazole ring clearly improved the activity against the CQ-S strain, but the effects were different against the CQ-R strain and were related to the substituents on the phenyl ring (positive for Cl, negative for all the others). For all these compounds, the RI resulted greatly increased. This could be explained by the greater basicity of the aliphatic amines, which implies a more extensive protonation at pH 5.2 (which is supposed to reflect the pH of the digestive vacuole) as shown by log $D_{5.2}$ (Table 2). This means that, whereas the above derivatives of aliphatic amines and CQ itself concentrate predominantly in the aqueous regions, compounds **6–12** should concentrate in the lipid regions or at the interface between lipids and water. This may facilitate their interaction with heme molecules during the crystallization process to hemozoin, which is reported to occur in vitro at the lipid-water interface or in vivo within neutral lipid nanosphere.[31,32]

The *N*-methylation of the imidazole ring (compare **13** with **6**) only slightly affected the physicochemical characteristics of the compounds, but improved the antiplasmodial activity, particularly against the CQ-S strain, with the consequent triplication of the RI. The introduction of a pyrrolidinylmethyl group on the imidazole ring (**20a**) increased the activity against both CQ-S and CQ-R strains leaving practically unchanged the RI. Indeed this structural modification, while increasing the basicity, did not affect the log$D_{5.2}$ values (2.40 vs 2.05) that were much higher than those of the N-unsubstituted imidazole derivatives (**16a**, **17a**, **18a**, **19a** in Table 2).

The replacement of the phenylimidazole moiety in compound **6** with the planar benzimidazole (compound **22**) significantly reduced the activity against the W-2 (CQ-R) strain, as well as increased cytotoxicity against HMEC.

In compounds lacking the basic aliphatic head, imidazolyl derivatives are more active than thiazolyl or oxazolyl derivatives, as shown comparing **6** vs **14** and **15**. This indicates that the even low basicity of the heterocycle is important for the activity, contributing to the entrapment of the molecule inside the food vacuole. For the same reason, the presence of a pyrrolidinylmethyl moiety in the thiazolyl ring (**21a** vs **15**) greatly increases the antiplasmodial activity.

4. Conclusions

A set of 7-chloro-4-[*N*-(heteroaryl)methyl]aminoquinoline and 7-chloro-4-[*N*-(heteroaryl)]aminoquinoline was synthesized and tested in vitro against D-10 (CQ-S) and W-2 (CQ-R) strains of *P. falciparum*. The results confirm that the replacement of the phenyl ring of phenylequine and the ferrocenyl system of ferroquine with different heteroaromatic nuclei is still associated with antiplasmodial activity, provided that an additional basic centre, besides the quinolinic system, is present. Ten of the synthesized compounds exhibited strong activity against the CQ-R strain with $IC_{50} \leqslant 60$ nM.

Similarly to other aminoquinoline derivatives, this new class of compounds seems able to interfere with the heme detoxification process of parasites.

The most interesting compounds are the thienyl derivative **4a**, the *N*-methylimidazolyl derivative **20a** and the arylimidazolyl derivative **10**, which are associated with a very strong antiplasmodial activity, a low RI and no or very low cytotoxicity against a human endothelial cell line. For these reasons, all these compounds are worthy of further investigation in in vivo murine models of malaria.

5. Experimental

5.1. General

All commercially available solvents and reagents were used without further purification, unless otherwise stated. CC = flash column chromatography. Mps: Büchi apparatus, uncorrected. ^{1}H NMR spectra: Varian Mercury 300VX spectrometer; $CDCl_3$ or DMSO-d_6; δ in ppm, J in Hertz. High-resolution mass spectra (HRMS) on a APEX II ICR-FTMS Bruker Daltonics mass spectrometer in positive electro spray ionization (ESI). Where indicated, reactions were performed with a Biotage Iniziator Microwave Synthesis System.

5.2. N,N-Substituted-5-nitrothiophene-2-carboxamide (24a, 24b)

General method: According to the method described in literature,[8,9] 5-nitrothiophene-2-carboxylic acid (1.0 g, 5.8 mmol) was carefully added to 6 ml of $SOCl_2$ in a ice-cooled round bottom flask. The solution was refluxed and stirred under N_2 for 30 min, cooled and evaporated under reduced pressure to obtain the corresponding acyl chloride as a sticky solid. The solid was dissolved into dry CH_2Cl_2 (10 ml), cooled in ice-bath and the suitable amine (5.8 mmol) was slowly added followed by TEA (0.89 ml, 6.44 mmol). The resulting mixture was stirred under N_2 atmosphere at rt for 15 min, then CH_2Cl_2 was evaporated under reduced pressure and the residue partitioned between AcOEt and 2 N NaOH. The organic layer was washed in succession with water, 0.5 N HCl, brine, and then dried over anhydrous Na_2SO_4. The solvent was evaporated to dryness and the resulted solid residue was washed with the indicated solvent and used directly in the next step.

5.2.1. (5-Nitrothiophen-2-yl)(pyrrolidin-1-yl)methanone (24a)

Solid washed with diethyl ether. Yield: 77%. Mp 178.4–180.0 °C. ^{1}H NMR ($CDCl_3$) δ: 7.84 (d, 1H, J = 4.12 Hz); 7.41 (d, 1H, J = 4.13 Hz); 3.77–3.65 (m, 4H); 2.08–1.92 (m, 4H).

5.2.2. *N,N*-Diethyl-5-nitrothiophene-2-carboxamide (24b)

Solid washed with ethyl ether/petroleum ether (2:8). Yield: 73%. Mp 65.5–67.2 °C. ^{1}H NMR ($CDCl_3$) δ: 7.83 (d, 1H, J = 4.13 Hz); 7.19 (d, 1H, J = 4.13 Hz); 3.52 (q, 4H, J = 7.15 Hz); 1.27 (t, 6H, J = 7.15 Hz).

5.3. 5-Amino-*N,N*-(substituted)thiophene-2-carboxamide (25a, b)

General method: Iron powder (925 mg, 16.57 mmol), freshly washed with 1 N HCl and distilled water, was added to a stirred solution of the corresponding nitroderivative (**24a** or **24b**; 3.31 mmol) in 10 ml of glacial AcOH. The mixture was heated at 70 °C with vigorous stirring under N_2 atmosphere until reaction was completed. After cooling, iron was filtered and washed with ethanol. The filtered solution was evaporated to remove most of AcOH. To the residue a solution of CH_2Cl_2/MeOH (10:1) was added, followed by saturated aqueous $NaHCO_3$ until it stopped bubbling and a green suspension was formed. The solid was removed by

filtration, and the filtrate extracted three times with AcOEt. The organic phase was dried with anhydrous Na_2SO_4, evaporated and resulting crude solid was purified by CC (silica gel; different eluents and conditions as indicated for each compound).

5.3.1. (5-Aminothiophen-2-yl)(pyrrolidin-1-yl)methanone (25a)

CC (CH_2Cl_2/MeOH; 98.5:1.5); solid washed with diethyl ether. Yield: 69%. Mp 140.5–142.0 °C. ^{1}H NMR ($CDCl_3$) δ: 7.26 (d, 1H, J = 3.85 Hz); 7.13 (d, 1H, J = 3.85 Hz); 3.66 (br s, 4H); 3.43 (br s, 2H); 1.93 (br s, 4H).

5.3.2. 5-Amino-*N,N*-diethylthiophene-2-carboxamide (25b)

CC (AcOEt/cyclohexane; 60:40); solid rinsed with diethyl ether. Yield: 49%. Mp 94.9–95.6 °C. ^{1}H NMR (DMSO-d_6) δ: 6.95 (d, 1H, J = 4.13 Hz); 6.17 (s, 2H); 5.80 (d, 1H, J = 4.13 Hz); 3.40 (q, 4H, J = 6.87 Hz); 1.11 (t, 6H, J = 6.87 Hz).

5.4. 5-(7-Chloroquinolin-4-ylamino)-*N,N*-(substituted) thiophene-2-carboxamide (26a, 26b)

General method: A mixture of suitable amine (**25a**, **25b**; 1.02 mmol), 4,7-dichloroquinoline (232 mg, 1.17 mmol) and phenol (480 mg, 51 mmol) was heated for 2.5 h at 130 °C, stirring under N_2. After cooling, the mixture was diluted with CH_2Cl_2, and resulting organic layer washed three times with 2 N NaOH solution and then with brine, dried with anhydrous Na_2SO_4 and evaporated to dryness. The crude solid was purified by CC (silica gel; different eluents and conditions as indicated for each compound).

5.4.1. (5-(7-Chloroquinolin-4-ylamino)thiophen-2-yl) (pyrrolidin-1-yl)methanone (26a)

CC (AcOEt/MeOH; 99:1); solid washed with diethyl ether. Yield: 83%. Mp 226.2–228.7 °C. ^{1}H NMR (DMSO-d_6) δ: 9.73 (s, 1H); 8.55 (s, 1H); 8.40 (d, 1H, J = 9.07 Hz); 7.90 (s, 1H); 7.60 (s, 1H); 7.48 (d, 1H, J = 3.85 Hz); 7.10 (s, 1H); 6.94 (s, 1H); 3.74 (br s, 2H); 3.47 (br s, 2H); 1.92 (s, 2H); 1.84 (s, 2H).

5.4.2. 5-(7-Chloroquinolin-4-ylamino)-*N,N*-diethylthiophene-2-carboxamide (26b)

CC (CH_2Cl_2/MeOH; 98:2); solid washed with diethyl ether. Yield: 88%. Mp 161.2–162.3 °C. ^{1}H NMR (DMSO-d_6) δ: 9.66 (s, 1H); 8.54 (s, 1H); 8.39 (d, 1H, J = 9.07 Hz); 7.92 (s, 1H); 7.60 (d, 1H, J = 9.07 Hz); 7.30 (d, 1H, J = 3.58 Hz); 7.05 (s, 1H); 6.94 (s, 1H); 3.48 (q, 4H, J = 6.88 Hz); 1.17 (t, 6H, J = 6.88 Hz).

5.5. 7-Chloro-4-N-(5-((substituted)methyl)thiophen-2-yl)aminoquinoline (3a, 3b)

General method: The amido compound (**26a** or **26b**; 1.3 mmol) was suspended in 45 ml of anhydrous THF and $LiAlH_4$ (238 mg, 6.30 mmol) was carefully added; the mixture was refluxed and stirred for 3 h under N_2. After cooling in ice-bath, water and 1 N NaOH were carefully added and the suspension was filtered. After evaporation of the solvent, the residue was diluted with water and extracted with CH_2Cl_2. The joined organic layers were dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude solid was purified by CC (silica gel; different ratio of CH_2Cl_2 and MeOH as indicated for each compound).

5.5.1. 7-Chloro-4-*N*-(5-(pyrrolidin-1-ylmethyl)thiophen-2-yl)aminoquinoline (3a)

CC (CH_2Cl_2/MeOH; 98:2); solid crystallized with CH_2Cl_2/diethyl ether. Yield: 35%. Mp 179.7–181.2 °C. ^{1}H NMR (DMSO-d_6) δ: 9.25 (s, 1H); 8.46 (d, 1H, J = 5.22 Hz); 8.38 (d, 1H, J = 9.08 Hz); 7.88 (d, 1H, J = 2.20 Hz); 7.55 (dd, 1H, J = 2.20, 9.08 Hz); 6.85 (d, 1H, J = 3.30 Hz); 6.79 (d, 1H, J = 3.30 Hz); 6.71 (d, 1H, J = 5.22 Hz); 3.72 (s, 2H); 1.69 (s, 4H); the signals of four protons are missing because covered by the DMSO signal. HRMS (ESI) m/z Calcd for $C_{18}H_{19}N_3SCl$ $[M+H]^+$: 344.09827; found: 344.09870.

5.5.2. 7-Chloro-4-*N*-(5-((diethylamino)methyl)thiophen-2-yl)aminoquinoline (3b)

CC performed twice (CH_2Cl_2/MeOH; 98:2); solid rinsed with diethyl ether. Yield: 17%. Mp144.6–146.0 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 9.24 (s, 1H); 8.46 (d, 1H, J = 5.22 Hz); 8.38 (d, 1H, J = 9.08 Hz); 7.88 (s, 1H); 7.55 (d, 1H, J = 9.08 Hz); 6.85 (d, 1H, J = 3.30 Hz); 6.79 (d, 1H, J = 3.30 Hz); 6.71 (d, 1H, J = 5.22 Hz); 3.71 (s, 2H); 0.98 (t, 6H, J = 7.15 Hz); the signals of four protons are missing because covered by the DMSO signal. HRMS (ESI) m/z Calcd for $C_{18}H_{21}N_3SCl$ $[M+H]^+$: 346.11392; found: 346.11380.

5.6. 7-Chloro-4-*N*-((1*H*-imidazol-2-yl)methyl)aminoquinoline (2)

A mixture of (1H-imidazol-2-yl)methanamine dihydrochloride[10] (**27**, 440 mg, 2.59 mmol), 4,7-dichloroquinoline (615 mg, 3.10 mmol), phenol (1.2 g, 13 mmol) and DIPEA (0.9 ml, 5.18 mmol) was heated for 5 h at 130 °C, stirring under N_2. After cooling, the mixture was diluted with CH_2Cl_2 and alkalized with 2 N NaOH until a precipitate was formed. The solid was filtered off, washed in succession with CH_2Cl_2, 2 N NaOH solution, water and dried overnight in vacuo (over KOH pellets). Solid was then rinsed with diethyl ether and used directly without further purification. Yield: 81%. Mp 131.9–133.0 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 11.93 (br s, 1H); 8.33 (d, 1H, J = 5.23 Hz); 8.28 (d, 1H, J = 8.81 Hz); 7.87 (s, 1H); 7.78 (s, 1H); 7.46 (d, 1H, J = 8.81 Hz); 6.91 (s, 2H); 6.46 (d, 1H, J = 5.23 Hz); 4.49 (d, 2H, J = 4.95 Hz). HRMS (ESI) m/z Calcd for $C_{13}H_{12}N_4Cl$ $[M+H]^+$: 259.07450; found: 259.07430.

5.7. 7-Chloro-4-*N*-((5-((substituted)methyl)-1*H*-imidazol-2-yl)methyl)aminoquinoline (5a, 5b).

General method: The proper amine (1.48 mmol) and 37% aqueous formaldehyde (0.11 ml, 1.48 mmol) were added to 1 ml of glacial acetic acid in an ice-cooled flask. The solution was stirred for 2–3 min, and then compound **2** (350 mg, 1.35 mmol) was added. The mixture was heated at 110 °C and stirred under N_2 for 20 h. After cooling, AcOH was removed under reduced pressure and the obtained sticky solid was partitioned between a solution of CH_2Cl_2/MeOH (9:1) and 2 N NaOH. The organic layer was washed with water, brine, dried over anhydrous Na_2SO_4 and evaporated to dryness. The resulting crude derivative was purified by CC (silica gel; different ratio of CH_2Cl_2 and MeOH as indicated for each compound).

5.7.1. 7-Chloro-4-N-((5-(pyrrolidin-1-ylmethyl)-1H-imidazol-2-yl)methyl)aminoquinoline (5a)

CC performed twice (CH_2Cl_2/MeOH; 93:7); solid crystallized and washed with a mixture of CH_2Cl_2/diethyl ether (1:9). Yield: 15%. Mp 161.9–163.8 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 11.84 (br s, 1H); 8.33 (d, 1H, J = 5.50 Hz); 8.27 (d, 1H, J = 9.07 Hz); 7.88 (br s, 1H); 7.77 (s, 1H); 7.45 (d, 1H, J = 9.07 Hz); 6.72 (br s, 1H); 6.48 (d, 1H, J = 5.50 Hz); 4.44 (d, 2H, J = 5.50 Hz); 3.41 (s, 2H); 2.38 (s, 4H); 1.62 (s, 4H). HRMS (ESI) m/z Calcd for $C_{18}H_{21}N_5Cl$ $[M+H]^+$: 342.14800; found: 342.14823. The free base was converted to tri-hydrochloride salt (mp 252–254 °C) for biological tests.

5.7.2. 7-Chloro-4-*N*-((5-((diethylamino)methyl)-1*H*-imidazol-2-yl)methyl)aminoquinoline (5b)

CC performed twice (CH_2Cl_2/MeOH; 95:5); solid crystallized and washed with a mixture of CH_2Cl_2/diethyl ether (1:9). Yield: 21%. Mp 144.6–147 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 11.73 (br s,

1H); 8.33 (d, 1H, *J* = 5.22 Hz); 8.27 (d, 1H, *J* = 9.08 Hz); 7.81 (s, 1H); 7.77 (d, 1H, *J* = 2.20 Hz); 7.45 (dd, 1H, *J* = 9.08, 2.20 Hz); 6.76 (br s, 1H); 6.47 (d, 1H, *J* = 5.22 Hz); 4.44 (d, 2H, *J* = 5.50 Hz); 3.44 (s, 2H); 2.39 (d, 4H, *J* = 7.15 Hz); 0.93 (t, 6H, *J* = 7.15 Hz). HRMS (ESI) *m*/*z* Calcd for $C_{18}H_{23}N_5Cl$ $[M+H]^+$: 344.16365; found: 344.16388.

5.8. 2-Aryl-2-oxoethyl 2-(*tert*-butoxycarbonylamino)acetate (35–41)

General method: According to the method described in literature,[17] in a round bottom flask, cesium carbonate (3.25 g, 10 mmol) was added to a solution of BocGlyOH (3.50 g, 20 mmol) in 55 ml of EtOH. The mixture was stirred at rt until all Cs_2CO_3 disappeared (about 30 min), and then the solvent was evaporated under reduced pressure. To the resulting salt dissolved in dry DMF (60 ml) was added the suitable 2-bromo-1-aryl-1-ethanone (20 mmol). The mixture was stirred for 2 h at rt under Ar, and then concentrated to dryness under vacuum. The crude solid was suspended in 50 ml of AcOEt, CsBr filtered off, washed with AcOEt and the joined organic phases evaporated. The resulting solid was purified by CC (silica gel; different eluents and conditions as indicated for each compound) or simply rinsed with a proper solvent.

5.8.1. 2-Oxo-2-phenylethyl 2-(*tert*-butoxycarbonylamino) acetate (35)

Solid rinsed with petroleum ether. Yield: 84%. Mp 60.3–61 °C. ^{1}H NMR (DMSO-d_6) δ: 7.94 (dd, 2H, *J* = 7.94, 1.66 Hz); 7.71–7.65 (m, 1H); 7.58–7.52 (m, 1H); 7.30 (t, 1H, *J* = 4.96 Hz); 5.53 (s, 1H); 3.83 (dd, 2H, *J* = 6.06, 1.66 Hz); 1.39 (s, 9H).

5.8.2. 2-(4-Fluorophenyl)-2-oxoethyl 2-(*tert*-butoxycarbony lamino)acetate (36)

Solid rinsed with petroleum ether/diethyl ether (7:3). Yield: 85%. Mp 105.4–106.8 °C. ^{1}H NMR ($CDCl_3$) δ: 7.95–7.90 (m, 2H); 7.20–7.14 (m, 2H); 5.37 (s, 2H); 5.07 (br s, 1H); 4.11 (d, 2H, *J* = 5.77 Hz); 1.45 (s, 9H).

5.8.3. 2-(4-Chlorophenyl)-2-oxoethyl 2-(*tert*-butoxycarbonylamino)acetate (37)

Solid rinsed with petroleum ether. Yield: 95%. Mp 122.1–123.4 °C. ^{1}H NMR ($CDCl_3$) δ: 7.84 (d, 2H, *J* = 8.52 Hz); 7.48 (d, 2H, *J* = 8.52 Hz); 5.37 (s, 2H); 5.03 (br s, 1H); 4.11 (d, 2H, *J* = 5.22); 1.45 (s, 9H).

5.8.4. 2-Oxo-2-*p*-tolylethyl 2-(*tert*-butoxycarbonylamino) acetate (38)

Solid rinsed with petroleum ether/diethyl ether (8:2). Yield: 95%. Mp 87.4–89.6 °C. ^{1}H NMR ($CDCl_3$) δ: 7.79 (d, 2H, *J* = 8.25 Hz); 7.27 (d, 2H, *J* = 8.26 Hz); 5.38 (s, 2H); 5.07 (s, 1H); 4.12 (d, 2H, *J* = 5.78 Hz); 2.42 (s, 3H); 1.45 (s, 9H).

5.8.5. 2-(4-(Dimethylamino)phenyl)-2-oxoethyl 2-(*tert*-buto xycarbonylamino)acetate (39)

CC (AcOEt/cyclohexane; 45:55); solid rinsed with petroleum ether/diethyl ether (1:1). Yield: 57%. Mp 131–132.5 °C. ^{1}H NMR ($CDCl_3$) δ: 7.82 (d, 2H, *J* = 9.07 Hz); 6.65 (d, 2H, *J* = 9.07 Hz); 5.35 (s, 2H); 5.08 (s, 1H); 4.12 (d, 2H, *J* = 5.78 Hz); 3.07 (s, 6H); 1.45 (s, 9H).

5.8.6. 2-(3-(Dimethylamino)phenyl)-2-oxoethyl 2-(*tert*-butoxycarbonylamino)acetate (40)

CC (AcOEt/cyclohexane; 30:70); solid rinsed with petroleum ether/diethyl ether (1:1). Yield: 47%. Mp 83.2–84.8 °C. ^{1}H NMR ($CDCl_3$) δ: 7.35–7.17 (m, 3H); 6.96 (dd, 1H, *J* = 7.98, 1.93 Hz); 5.41 (s, 2H); 5.07 (br s, 1H); 4.12 (d, 2H, *J* = 5.70 Hz); 3.00 (s, 6H); 1.46 (s, 9H).

5.8.7. 2-Oxo-2-(4-(pyrrolidin-1-yl)phenyl)ethyl 2-(*tert*-butoxy carbonylamino)acetate (41)

CC (AcOEt/cyclohexane; 37:63); solid rinsed with petroleum ether/diethyl ether (1:1). Yield: 80%. Mp 132.7–133.8 °C. ^{1}H NMR ($CDCl_3$) δ: 7.79 (d, 2H, *J* = 8.80 Hz); 6.54 (d, 2H, *J* = 8.80 Hz); 5.35 (s, 2H); 5.06 (br s, 1H); 4.12 (d, 2H, *J* = 5.50 Hz); 3.40–3.36 (m, 4H); 2.08–2.01 (m, 4H); 1.45 (s, 9H).

5.9. *tert*-Butoxycarbonyl (4-aryl-1H-imidazol-2-yl)methylamine (42–48)

General method: A suspension of the proper 2-aryl-2-oxoethyl 2-(*tert*-butoxycarbonylamino)acetate (**35–41**; 13.3 mmol) and ammonium acetate (4.1 g, 53.2 mmol) in xylene (200 mL) was heated at 180 °C for 2.5 h in a round bottom flask, connected to a Dean-Stark trap to remove the excess of ammonium acetate and water. The mixture was then cooled to room temperature, diluted with AcOEt (100 mL) and washed with water, aq. $NaHCO_3$ (5% w/v) and brine. The organic phase was dried over anhydrous Na_2SO_4 and evaporated to dryness. The resulting solid was purified by CC (silica gel; different eluents and conditions as indicated for each compound).

5.9.1. *tert*-Butoxycarbonyl (4-phenyl-1*H*-imidazol-2-yl) methylamine (42)

CC (CH_2Cl_2/MeOH; 97:3); solid rinsed with diethyl ether/petroleum ether (1:1). Yield: 46%. Mp 164.6–165.3 °C. ^{1}H NMR (DMSO-d_6) δ: 11.82 (s, 1H); 7.70 (d, 2H, *J* = 6.60 Hz); 7.44 (s, 1H); 7.33–7.24 (m, 3H); 7.14 (t, 1H, *J* = 6.60 Hz); 4.16 (d, 2H, *J* = 4.13 Hz); 1.38 (s, 9H).

5.9.2. *tert*-Butoxycarbonyl (4-(4-fluorophenyl)-1*H*-imidazol-2-yl)methylamine (43)

(CH_2Cl_2/MeOH; 98:2); solid rinsed with diethyl ether/petroleum ether (1:1). Yield: 55%. Mp 185.5–187.8 °C (dec). ^{1}H NMR ($CDCl_3$) δ: 7.64 (dd, 2H, *J* = 5.23, 8.80 Hz); 7.17 (s, 1H); 7.06 (t, 2H, *J* = 8.80 Hz); 5.90 (br s, 1H); 4.39 (d, 2H, *J* = 6.05 Hz); 1.45 (s, 9H).

5.9.3. *tert*-Butoxycarbonyl (4-(4-chlorophenyl)-1*H*-imidazol-2-yl)methylamine (44)

CC (CH_2Cl_2/MeOH; 98.8:1.2); solid rinsed with diethyl ether/petroleum ether (1:1). Yield: 52%. Mp 182.3–183.4 °C. ^{1}H NMR (DMSO-d_6) δ: 11.90 (s, 1H); 7.74 (d, 2H, *J* = 7.70 Hz); 7.53 (s, 1H); 7.35 (d, 2H, *J* = 7.70 Hz); 7.26 (s, 1H); 4.15 (d, 2H, *J* = 5.50 Hz); 1.39 (s, 9H).

5.9.4. *tert*-Butoxycarbonyl (4-*p*-tolyl-1*H*-imidazol-2-yl) methylamine (45)

CC (CH_2Cl_2/MeOH; 98.5:1.5); solid rinsed with diethyl ether/petroleum ether (6:4). Yield: 63%. Mp 175.7–176.4 °C. ^{1}H NMR (DMSO-d_6) δ: 11.77 (s, 1H); 7.61 (d, 2H, *J* = 7.97 Hz); 7.40 (s, 1H); 7.24–7.10 (m, 3H); 4.15 (d, 2H, *J* = 5.77 Hz); 2.27 (s, 3H); 1.39 (s, 9H).

5.9.5. *tert*-Butoxycarbonyl (4-(4-(dimethylamino)phenyl)-1*H*-imidazol-2-yl)methylamine (46)

CC (CH_2Cl_2/MeOH; 97.5:2.5); solid crystallized and washed with diethyl ether. Yield: 46%. Mp 182.7–185 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 11.62 (s, 1H); 7.54 (d, 2H, *J* = 8.81 Hz); 7.22 (s, 1H); 6.74–6.67 (m, 3H); 4.13 (d, 2H, *J* = 5.78 Hz); 2.88 (s, 6H); 1.39 (s, 9H).

5.9.6. *tert*-Butoxycarbonyl (4-(3-(dimethylamino)phenyl)-1*H*-imidazol-2-yl)methylamine (47)

CC (CH_2Cl_2/MeOH; 98:2); solid crystallized with diethyl ether and rinsed with diethyl ether/petroleum ether (7:3). Yield: 74%. Mp 173.5–174.8 °C. ^{1}H NMR ($CDCl_3$) δ: 7.23–7.19 (m, 2H); 7.03 (d, 1H, J = 1.93 Hz); 6.96 (d, 1H, J = 7.43 Hz); 6.64 (dd, 1H, J = 7.43, 1.93 Hz); 5.60 (br s, 1H), 4.73-4.68 (m, 1H); 4.38–4.36 (m, 2H); 2.97 (s, 6H); 1.45 (s, 9H).

5.9.7. *tert*-Butoxycarbonyl (4-(4-(pyrrolidin-1-yl)phenyl)-1*H*-imidazol-2-yl)methylamine (48)

CC (CH_2Cl_2/MeOH; 98.5:1.5); solid crystallized with diethyl ether and rinsed with diethyl ether/petroleum ether (7:3). Yield: 55%. Mp 210.8–211.7 °C (dec.). ^{1}H NMR ($CDCl_3$) δ: 7.47 (d, 2H, J = 8.80 Hz); 7.04 (s, 1H); 6.55 (d, 2H, J = 8.80 Hz); 4.48–4.39 (m, 2H); 3.32–3.28 (m, 4H); 2.03–1.99 (m, 4H); 1.44 (s, 9H).

5.10. (4-Aryl-1*H*-imidazol-2-yl)methanamine (49–55)

General method: In a round bottom flask the appropriate boc-derivative (**42-48**; 5 mmol) was dissolved in 3 ml of TFA. The solution was stirred at rt for 30 min, then a mixture of diethyl ether/petroleum ether (1:1, 20 ml) was added. The suspension was filtered, washed with diethyl ether and dissolved in water (5 ml). Powdered K_2CO_3 (1.38 g, 10 mmol) was carefully added to the aqueous phase, the solvent was evaporated to dryness and the resulting sticky solid was filtered through a silica pad, eluting with a mixture of CH_2Cl_2/MeOH/concd NH_3 (89:10:1). The resulting solid was rinsed with different solvents as indicated for each single compound.

5.10.1. (4-Phenyl-1*H*-imidazol-2-yl)methanamine (49)

Compound appeared as a colorless gum. Yield: 98%. ^{1}H NMR (DMSO-d_6) δ: 7.79 (d, 2H, J = 8.10 Hz); 7.42 (s, 1H); 7.30 (t, 2H, J = 7.90 Hz); 7.15 (t, 1H, J = 7.50 Hz); 3.71 (s, 2H).

5.10.2. (4-(4-Fluorophenyl)-1*H*-imidazol-2-yl)methanamine (50)

Solid rinsed with diethyl ether/petroleum ether (1:1). Yield: 88%. Mp 117–118.5 °C. ^{1}H NMR (DMSO-d_6) δ: 11.79 (br s, 1H); 7.73 (s, 2H); 7.43 (s, 1H); 7.13 (t, 2H, J = 8.53 Hz); 3.70 (s, 2H); 1.84 (br s, 2H).

5.10.3. (4-(4-Chlorophenyl)-1*H*-imidazol-2-yl)methanamine (51)

Solid rinsed with diethyl ether. Yield: 85%. Mp 138.2–139.9 °C. ^{1}H NMR (DMSO-d_6) δ: 7.75 (d, 2H, J = 8.53 Hz); 7.59 (s, 1H); 7.38 (d, 2H, J = 8.53 Hz); 3.91 (s, 2H).

5.10.4. (4-*p*-Tolyl-1*H*-imidazol-2-yl)methanamine (52)

Solid rinsed with diethyl ether. Yield: 91%. Mp 81.6–84.3 °C. ^{1}H NMR (DMSO-d_6) δ: 7.59 (d, 2H, J = 7.98 Hz); 7.36 (s, 1H); 7.12 (d, 2H, J = 7.98 Hz); 3.73 (s, 2H); 3.30 (br s, 2H); 2.27 (s, 3H).

5.10.5. 4-(2-(Aminomethyl)-1*H*-imidazol-4-yl)-*N,N*-dimethyl benzenamine (53)

Solid rinsed with diethyl ether. Yield: 82%. Mp 170.8–173.3 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 7.50 (d, 2H, J = 8.810 Hz); 7.16 (s, 1H); 6.71 (d, 2H, J = 8.80 Hz); 3.71 (s, 2H); 2.87 (s, 6H).

5.10.6. 3-(2-(Aminomethyl)-1*H*-imidazol-4-yl)-*N,N*-dimethyl benzenamine (54)

Solid rinsed with diethyl ether. Yield: 91%. Mp 151–152.3 °C. ^{1}H NMR (DMSO-d_6) δ: 7.35 (br s, 1H); 7.09 (d, 2H, J = 7.98 Hz); 6.98 (d, 1H, J = 7.15 Hz); 6.53 (dd, 1H, J = 7.98, 1.65 Hz); 3.71 (s, 2H); 2.90 (s, 6H); 1.82 (br s, 2H).

5.10.7. (4-(4-(Pyrrolidin-1-yl)phenyl)-1*H*-imidazol-2-yl) methanamine (55)

Solid rinsed with diethyl ether. Yield: 86%. Mp 207.7–208.5 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 7.48 (d, 2H, J = 8.80 Hz); 7.13 (s, 1H); 6.51 (d, 2H, J = 8.80 Hz); 3.68 (s, 2H); 3.22 (s, 4H); 1.93 (s, 4H).

5.11. 7-Chloro-4-*N*-((4-aryl-1*H*-imidazol-2-yl)methyl) amminoquinoline (6–12)

General method: A mixture of proper amine (**49–55**; 1.57 mmol), 4,7-dichloroquinoline (342 mg, 1.72 mmol) and phenol (738 mg, 7.85 mmol) was heated for 3 h at 130 °C, stirring under N_2. After cooling, crude mixture was dissolved with a solution of CH_2Cl_2/MeOH (10:1) and phenol extracted with 2 N NaOH. Organic layers were washed with water and then brine, dried over anhydrous Na_2SO_4 and evaporated to yield a crude solid, which was purified by CC (silica gel; different ratio of CH_2Cl_2 and MeOH as indicated for each compound).

5.11.1. 7-Chloro-4-*N*-((4-phenyl-1*H*-imidazol-2-yl)methyl) aminoquinoline (6)

CC (CH_2Cl_2/MeOH; 95:5); solid crystallized with diethyl ether/EtOH (95:5) and rinsed with diethyl ether/CH_2Cl_2 (1:1). Yield: 82%. Mp 105.9–108.8 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 12.08 (s, 1H); 8.37 (d, 1H, J = 5.20 Hz); 8.32 (d, 1H, J = 9.08 Hz); 7.93 (s, 1H); 7.79 (d, 1H, J = 2.20 Hz); 7.73 (d, 2H, J = 5.77 Hz); 7.48 (dd, 2H, J = 8.80, 2.20 Hz); 7.34–7.29 (m, 2H); 7.17–7.12 (m, 1H); 6.50 (s, 1H, J = 5.20 Hz); 4.55 (d, 2H, J = 5.50 Hz). HRMS (ESI) m/z Calcd for $C_{19}H_{16}N_4Cl$ $[M+H]^+$: 335.10635; found: 335.10812.

5.11.2. 7-Chloro-4-N-((4-(4-fluorophenyl)-1H-imidazol-2-yl) methyl)aminoquinoline (7)

CC (CH_2Cl_2/MeOH; 95.5:4.5); solid was rinsed with diethyl ether/petroleum ether (8:2). Yield: 87%. Mp 144.3–148 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 12.09 (s, 1H); 8.37 (d, 1H, J = 5.23 Hz); 8.32 (d, 1H, J = 9.07 Hz); 7.94 (br s, 1H); 7.79 (d, 2H, J = 1.92 Hz); 7.76 (s, 1H); 7.48 (dd, 2H, J = 9.07, 1.92 Hz); 7.17-7.11 (m, 2H); 6.49 (d, 1H, J = 5.23 Hz); 4.54 (d, 2H, J = 5.50 Hz). HRMS (ESI) m/z Calcd for $C_{19}H_{15}N_4FCl$ $[M+H]^+$: 353.09638; found: 353.09612.

5.11.3. 7-Chloro-4-*N*-((4-(4-chlorophenyl)-1*H*-imidazol-2-yl) methyl)aminoquinoline (8)

CC (CH_2Cl_2/MeOH; 94:6); solid rinsed with diethyl ether/petroleum ether (1:1). Yield: 64%. Mp 158.9–161.3 °C. ^{1}H NMR (DMSO-d_6) δ: 12.14 (s, 1H); 8.37 (d, 1H, J = 5.22 Hz); 8.32 (d, 1H, J = 9.07 Hz); 7.94 (s, 1H); 7.80 (d, 1H, J = 2.20); 7.75 (d, 2H, J = 8.25 Hz); 7.57 (s, 1H); 7.48 (dd, 1H, J = 9.08, 2.20 Hz); 7.36 (d, 2H, J = 8.25 Hz); 6.49 (d, 1H, J = 5.23); 4.55 (d, 2H, J = 5.50). HRMS (ESI) m/z Calcd for $C_{19}H_{15}N_4Cl_2$ $[M+H]^+$: 369.06738; found: 369.06784.

5.11.4. 7-Chloro-4-*N*-((4-p-tolyl-1*H*-imidazol-2-yl)methyl) aminoquinoline (9)

During the work-up a suspension was formed; the solid (a first portion of compound **9**) was filtered, dried and rinsed with diethyl ether (mp 155.1–158.3 °C). Filtrate was separated, organic phases evaporated to dryness and the crude product purified by CC (CH_2Cl_2/MeOH; 93.5:6.5); solid rinsed with diethyl ether (mp 151.6–154.8 °C). Yield: 82%. ^{1}H NMR (DMSO-d_6) δ: 12.06 (s, 1H); 8.36 (d, 1H, J = 5.50 Hz); 8.32 (d, 1H, J = 9.07 Hz); 7.93 (s, 1H); 7.79 (d, 1H, J = 2.20 Hz); 7.60 (d, 2H, J = 7.15 Hz); 7.47 (dd, 1H, J = 9.70, 2.20, Hz); 7.43 (s, 1H); 7.12 (d, 2H, J = 7.97 Hz); 6.51 (d, 1H, J = 5.22 Hz); 4.53 (d, 2H, J = 5.50 Hz); 2.27 (s, 3H). HRMS (ESI) m/z Calcd for $C_{20}H_{18}N_4Cl$ $[M+H]^+$: 349.121999; found: 349.12254.

5.11.5. 7-Chloro-4-*N*-((4-(4-(dimethylamino)phenyl)-1*H*-imidazol-2-yl)methyl)aminoquinoline (10)

CC (CH_2Cl_2/MeOH; 96:4); solid rinsed with diethyl ether/CH_2Cl_2 (1:1). Yield: 63%. Mp 144–147 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 11.91 (s, 1H); 8.36 (d, 1H, J = 5.23 Hz); 8.34 (d, 1H, J = 9.07 Hz); 7.91 (s, 1H); 7.79 (d, 1H, J = 1.93 Hz); 7.52 (br s, 2H); 7.47 (dd, 1H, J = 8.80, 1.92 Hz); 7.26 (s, 1H); 6.69 (d, 2H, J = 8.80 Hz); 6.53 (d, 1H, J = 5.23 Hz); 4.51 (d, 2H, J = 5.23 Hz); 2.87 (s, 6H). HRMS (ESI) m/z Calcd for $C_{21}H_{21}N_5Cl$ $[M+H]^+$: 378.14800; found: 378.14766.

5.11.6. 7-Chloro-4-*N*-((4-(3-(dimethylamino)phenyl)-1*H*-imidazol-2-yl)methyl)aminoquinoline (11)

During the work-up, a suspension was formed and a first portion of compound **11** was collected and then joined to the product obtained at the end of the general procedure. CC (CH_2Cl_2/MeOH; 97:3); solid crystallized and rinsed with diethyl ether. Yield: 72%. Mp 150–152 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 12.06 (s, 1H); 8.37 (d, 1H, J = 4.95 Hz); 8.35 (d, 1H, J = 9.07 Hz); 7.93 (br s, 1H); 7.79 (s, 1H); 7.58–7.32 (m, 2H); 7.22–7.10 (m, 2H); 7.02 (s, 1H); 6.61–6.42 (m, 2H); 4.55 (d, 2H, J = 5.30 Hz); 2.89 (s, 6H). HRMS (ESI) m/z Calcd for $C_{21}H_{21}N_5Cl$ $[M+H]^+$: 378.14800; found: 378.14767.

5.11.7. 7-Chloro-4-*N*-((4-(4-(pyrrolidin-1-yl)phenyl)-1*H*-imidazol-2-yl)methyl)aminoquinoline (12)

During the work-up, a suspension was formed and a first portion of compound **12** was collected and then joined to the product obtained at the end of the general procedure. CC (CH_2Cl_2/MeOH; 96:4); solid was crystallized with CH_2Cl_2 and rinsed with a mixture of diethyl ether/CH_2Cl_2 (1:1). Yield: 24%. Mp 245.8–246.9 °C. ^{1}H NMR (DMSO-d_6) δ: 11.95 (br s, 1H); 8.37 (d, 1H, J = 5.22 Hz); 8.32 (d, 1H, J = 9.08 Hz); 7.91 (s, 1H); 7.79 (s, 1H); 7.52–7.46 (m, 3H); 7.19 (s, 1H); 6.50 (d, 2H, J = 9.08 Hz); 4.51 (d, 2H, J = 3.85 Hz); 3.21 (s, 4H); 1.93 (s, 4H). HRMS (ESI) m/z Calcd for $C_{23}H_{22}N_5Cl$ $[M+H]^+$: 404.16365; found: 404.16346.

5.12. *tert*-Butoxycarbonyl (1-methyl-4-phenyl-1*H*-imidazol-2-yl)methylamine (56)

Methyl iodide (0.3 ml, 4.8 mmol) was added dropwise to a well-stirred, ice-bath cooled suspension of powdered K_2CO_3 (480 mg, 3.47 mmol) and compound **42** (950 mg, 3.37 mmol) in 10 ml of dry DMF. When addition was completed, the mixture was heated at 60 °C for 24 h. After cooling, DMF was removed under reduced pressure, the sticky residue was dissolved in AcOEt, washed with water, brine, dried over anhydrous Na_2SO_4 and the organic phase evaporated to dryness. The crude solid residue was purified by CC on silica gel (AcOEt/cyclohexane; 30:70). The resulting solid was rinsed with petroleum ether/diethyl ether (8:2). Yield: 57%. Mp 146.5–148 °C. ^{1}H NMR (DMSO-d_6) δ: 7.68 (d, 2H, J = 7.15 Hz); 7.52 (s, 1H); 7.34–7.28 (m, 3H); 7.15 (t, 1H, J = 7.15 Hz); 4.21 (d, 2H, J = 5.77 Hz); 3.62 (s, 3H); 1.38 (s, 9H).

5.13. (1-Methyl-4-phenyl-1*H*-imidazol-2-yl)methanamine (57)

In a round bottom flask, compound **56** (500 mg, 1.74 mmol) was dissolved in 2 ml of TFA. The solution was stirred at rt for 45 min, then a mixture of diethyl ether/EtOH (95:5, 15 ml) was added. The obtained suspension was filtered, washed with ethyl ether and dissolved in water (5 ml). Powdered K_2CO_3 (10 mmol) was carefully added to aqueous phase, solvent evaporated to dryness and the resulted sticky solid was filtered through a silica pad, eluted with a mixture of CH_2Cl_2/MeOH/concd NH_3 (89:10:1). The resulting solid was rinsed with petroleum ether. Yield: 88%. Mp 95.5–98.5 °C. ^{1}H NMR (DMSO-d_6) δ: 7.68 (d, 2H, J = 8.53 Hz); 7.49 (s, 1H); 7.30 (t, 2H, J = 7.42 Hz); 7.14 (t, 1H, J = 7.42 Hz); 3.74 (s, 2H); 3.64 (s, 3H); 2.04 (br s, 2H).

5.14. 7-Chloro-4-*N*-((1-methyl-4-phenyl-1*H*-imidazol-2-yl)methyl)aminoquinoline (13)

In a round bottom flask a mixture of **57** (250 mg, 1.34 mmol), 4,7-dichloroquinoline (290 mg, 1.47 mmol) and phenol (650 mg, 6.75 mmol) was heated for 2 h at 130 °C, stirring under N_2. After cooling, crude mixture was dissolved with CH_2Cl_2/MeOH (10:1) and surplus of phenol extracted with 2 N NaOH. Organic layers were washed with water, brine, dried over anhydrous Na_2SO_4 and evaporated to dryness to yield a crude solid, which was purified by CC on silica gel (CH_2Cl_2/MeOH; 97:3). The resulting product was crystallized with ethyl acetate and rinsed with diethyl ether/petroleum ether (1:1). Yield: 74%. Mp 199.7–200.5 °C. ^{1}H NMR (DMSO-d_6) δ: 8.41 (d, 1H, J = 5.22 Hz); 8.31 (d, 1H, J = 9.07 Hz); 7.85 (t, 1H, J = 4.95 Hz); 7.79 (d, 1H, J = 2.20 Hz); 7.71 (d, 2H, J = 7.16 Hz); 7.58 (s, 1H); 7.47 (dd, 1H, J = 2.20, 9.07 Hz); 7.32 (t, 2H, J = 7.42 Hz); 7.16 (t, 1H, J = 7.42 Hz); 6.78 (d, 1H, J = 5.22 Hz); 4.58 (d, 2H, J = 5.00 Hz); 3.69 (s, 3H). HRMS (ESI) m/z Calcd for $C_{20}H_{18}N_4Cl$ $[M+H]^+$: 349.121999; found: 349.12216.

5.15. 7-Chloro-4-*N*-((1*H*-benzo[d]imidazol-2-yl)methyl) aminoquinoline (22)

In a round bottom flask a mixture of (1*H*-benzo[*d*]imidazol-2-yl)methanamine[19] (**58**; 720 mg, 3.8 mmol), 4,7-dichloroquinoline (1.07 g, 5.39 mmol) and phenol (2.33 g, 24.74 mmol) was heated for 3.5 h at 130 °C, stirring under N_2. After cooling, the mixture was dissolved in a solution of CH_2Cl_2/MeOH (95:5) and surplus of phenol was extracted with 2 N NaOH. The organic layers were washed with water, brine, dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude solid was purified by CC on silica gel (CH_2Cl_2/MeOH; 93:7). The resulting product was rinsed with diethyl ether. Yield: 44%. Mp 259–261 °C. ^{1}H NMR (DMSO-d_6) δ: 12.38 (s, 1H); 8.42–8.21 (m, 2H); 8.11 (br s, 1H); 7.81 (s, 1H); 7.70–7.51 (m, 2H); 7.42 (s, 1H); 7.03–7.21 (m, 2H); 6.43 (d, 1H, J = 5.50 Hz); 4.75 (d, 2H, J = 5.40 Hz). HRMS (ESI) m/z Calcd for $C_{17}H_{14}N_4Cl$ $[M+H]^+$: 309.09015; found: 309.09013.

5.16. 7-Chloro-4-N-((5-((N-substituted-amino)methyl)-4-aryl-1*H*-imidazol-2-yl)methyl)aminoquinoline and 7-chloro-4-*N*-((1-methyl-4-phenyl-5-(pyrrolidin-1-ylmethyl)-1*H*-imidazol-2-yl)methyl)aminoquinoline (16a, 16b; 17a; 18a, 18b; 19a; 20a)

General method: Aq. formaldehyde (37%, 73 μl, 0.97 mmol) was added to a stirred solution of diethylamine or pyrrolidine (0.97 mmol) in 7.5 ml of absolute ethanol; after few minutes the proper 7-chloro-4-*N*-((5-aryl-1*H*-imidazol-2-yl)methyl)aminoquinoline (**6–9**, **13**) (0.82 mmol) and 0.5 ml of AcOH were added and the suspension was heated at 95 °C for 3 h under N_2 atmosphere, during which a solution was formed. After cooling, the solvent was evaporated to dryness and the residue was dissolved in AcOEt. The organic layer was washed with 2 N NaOH, water, brine, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The resulting crude product was purified by CC (silica gel; different ratio of CH_2Cl_2 and MeOH as indicated for each compound).

5.16.1. 7-Chloro-4-*N*-((4-phenyl-5-(pyrrolidin-1-ylmethyl)-1*H*-imidazol-2-yl)methyl)aminoquinoline (16a)

CC (CH_2Cl_2/MeOH; 98:2); solid crystallized and rinsed with CH_2Cl_2. Yield: 36%. Mp 146.7–149.2 °C. ^{1}H NMR (DMSO-d_6) δ: 12.22 (s, 1/3H); 12.07 (s, 2/3H); 8.37 (d, 1H, J = 5.22 Hz); 8.32 (d, 1H, J = 9.08 Hz); 7.86–7.68 (m, 4H); 7.48–7.17 (m, 4H); 6.57 (s, 1H); 4.51 (s, 2H); 3.65 (s, 4/3H); 3.49 (s, 2/3H); 2.43 (s, 4H); 1.67

(s, 4H). HRMS (ESI) *m*/*z* Calcd for $C_{24}H_{25}N_5Cl$ $[M+H]^+$: 418.17930; found: 418.17975.

5.16.2. 7-Chloro-4-*N*-((5-((diethylamino)methyl)-4-phenyl-1*H*-imidazol-2-yl)methyl)aminoquinoline (16b)

CC (CH_2Cl_2/MeOH; 97:3); solid crystallized and rinsed with diethyl ether. Yield: 51%. Mp 145.6–147 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 12.22 (s, 1/3H); 11.95 (s, 2/3H); 8.35 (d, 1H, J = 5.22 Hz); 8.30 (d, 1H, J = 9.08 Hz); 7.87 (s, 1H); 7.78 (d, 1H, J = 2.20 Hz); 7.75–7.73 (m, 2H); 7.47 (d, 1H, J = 9.08 Hz); 7.32 (t, 2H, J = 7.70 Hz); 7.19–7.16 (m, 1H); 6.55 (d, 1H, J = 5.22 Hz); 4.52 (d, 2H, J = 5.50 Hz); 3.59 (s, 4/3H); 3.46 (s, 2/3H); 2.49–2.40 (m, 4H); 0.94-0.89 (m, 6H). HRMS (ESI) *m*/*z* Calcd for $C_{24}H_{27}N_5Cl$ $[M+H]^+$: 420.19495; found: 420.19560.

5.16.3. 7-Chloro-4-*N*-((4-(4-fluorophenyl)-5-(pyrrolidin-1-ylmethyl)-1*H*-imidazol-2-yl)methyl)aminoquinoline (17a)

CC (CH_2Cl_2/MeOH; 94.5:6.5); solid crystallized with diethyl ether/CH_2Cl_2 (95:5). Yield: 60%. Mp 144.5–148 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 12.38 (s, 1/3H); 12.10 (s, 2/3H); 8.36 (d, 1H, J = 5.22 Hz); 8.32 (d, 1H, J = 8.80 Hz); 7.87 (d, 1H, J = 8.80 Hz); 7.79 (s, 1H,); 7.71 (br s, 2H); 7.47 (d, 1H, J = 8.80 Hz); 7.17 (br s, 2H); 6.54 (d, 1H, J = 5.22 Hz); 4.51 (d, 2H, J = 5.30 Hz); 3.60 (br s, 2H); 2.44 (s, 4H); 1.66 (s, 4H). HRMS (ESI) *m*/*z* Calcd for $C_{24}H_{24}N_5ClF$ $[M+H]^+$: 436.16988; found: 436.17018.

5.16.4. 7-Chloro-4-*N*-((4-(4-chlorophenyl)-5-(pyrrolidin-1-ylmethyl)-1*H*-imidazol-2-yl)methyl)aminoquinoline (18a)

CC (CH_2Cl_2/MeOH; 96:4); solid crystallized with diethyl ether/CH_2Cl_2 (1:1). Yield: 60%. Mp 196–197 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 12.38 (s, 1/3H); 12.15 (2/3H); 8.36 (d, 1H, J = 5.22 Hz); 8.31 (d, 1H, J = 9.08 Hz); 7.87 (s, 1H); 7.79 (d, 1H, J = 2.20 Hz); 7.72 (d, 2H, J = 8.30 Hz); 7.47 (dd, 1H, J = 2.20, 9.08 Hz); 7.38 (d, 2H, J = 8.30 Hz); 6.53 (d, 1H, J = 5.22 Hz); 4.51 (d, 2H, J = 5.50 Hz); 3.63 (s, 4/3H); 3.55 (s, 2/3); 2.43 (s, 4H); 1.66 (s, 4H). HRMS (ESI) *m*/*z* calcd for $C_{24}H_{24}N_5Cl_2$ $[M+H]^+$: 452.14033; found: 452.14066.

5.16.5. 7-Chloro-4-*N*-((4-(4-chlorophenyl)-5-((diethylamino)methyl)-1*H*-imidazol-2-yl)methyl)aminoquinoline (18b)

CC (CH_2Cl_2/MeOH; 98:2); solid rinsed with diethyl ether/MeOH (95:5). Yield: 44%. Mp 165.7–167.2 °C. ^{1}H NMR (DMSO-d_6) δ: 12.18 (s, 1/3H); 12.05 (s, 2/3H); 8.22-8.09 (m, 2H); 8.01 (m, 4H); 7.46 (br s, 1H); 7.38 (br s, 2H); 6.54 (s, 1H); 4.52 (s, 2H); 3.41 (s, 2/3H); 3.58 (s, 4/3H); 2.47 (br s, 4H); 0.92 (s, 6H). HRMS (ESI) *m*/*z* Calcd for $C_{24}H_{26}N_5Cl_2$ $[M+H]^+$: 454.15598; found: 454.15660.

5.16.6. 7-Chloro-4-*N*-((5-(pyrrolidin-1-ylmethyl)-4-*p*-tolyl-1*H*-imidazol-2-yl)methyl)aminoquinoline (19a)

CC (CH_2Cl_2/MeOH; 96:4); solid crystallized with diethyl ether. Yield: 30%. Mp 128.7–133 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 12.21 (s, 1/3H); 12.03 (s, 2/3H); 8.36 (d, 1H, J = 5.22 Hz); 8.31 (d, 1H, J = 9.08 Hz); 7.86 (s, 1H); 7.78 (d, 1H, J = 2.20 Hz); 7.60–7.40 (m, 3H); 7.25–7.10 (m, 2H); 6.55 (d, 1H, J = 5.22 Hz); 4.49 (d, 2H, J = 5.50 Hz); 3.61 (s, 4/3 H); 3.55 (2/3 H); 2.42 (s, 4H); 2.27 (s, 3H); 1.65 (s, 4H). HRMS (ESI) *m*/*z* Calcd for $C_{25}H_{27}N_5Cl$ $[M+H]^+$: 432.19495; found: 432.19529.

5.16.7. 7-chloro-*N*-((1-methyl-4-phenyl-5-(pyrrolidin-1-ylmethyl)-1*H*-imidazol-2-yl)methyl)quinolin-4-amine (20a)

CC (CH_2Cl_2/MeOH; 98:2); solid crystallized with diethyl ether. Yield: 32%. Mp 203–205.3 °C. ^{1}H NMR (DMSO-d_6) δ: 8.40 (d, 1H, J = 5.50 Hz); 8.31 (d, 1H, J = 9.08 Hz); 7.85 (s, 1H); 7.78 (d, 1H, J = 2.20 Hz); 7.66 (d, 2H, J = 7.43 Hz); 7.47 (dd, 1H, J = 9.08, 2.20 Hz); 7.37 (t, 2H, J = 7.43 Hz); 7.22 (t, 1H, J = 7.43 Hz); 6.82 (d, 1H, J = 5.50 Hz); 4.58 (d, 2H, J = 4.95 Hz); 3.78–3.60 (m, 5H); 2.39 (s, 4H); 1.61 (s, 4H). HRMS (ESI) *m*/*z* Calcd for $C_{25}H_{27}N_5Cl$ $[M+H]^+$: 432.19495; found: 432.19467.

5.17. Benzyloxycarbonyl (4-phenyloxazol-2-yl)methylamine (61)

In a round bottom flask a mixture of benzyl 2-amino-2-oxoethylcarbamate[20] (**59**; 3.9 g, 18.80 mmol) and 2-bromoacetophenone (1.5 g, 7.52 mmol) in 6 ml of *N*-methyl-2-pyrrolidone (NMP) was heated for 6 h at 100 °C under N_2. After cooling, the crude mixture was diluted with AcOEt (150 ml), washed with aq. $NaHCO_3$ (5% w/v), water, brine, and dried over anhydrous Na_2SO_4. The organic phase was evaporated to dryness and the crude residue was purified by CC on silica gel (AcOEt/cyclohexane; 25:75). The resulted solid was rinsed with petroleum ether/diethyl ether (8:2). Yield: 35%. Mp 104–107.3 °C. ^{1}H NMR ($CDCl_3$) δ: 7.87 (s, 1H); 7.72 (d, 2H, J = 8.52 Hz); 7.42–7.29 (m, 8H); 5.47 (br s, 1H); 5.17 (s, 2H); 4.57 (d, 2H, J = 5.77 Hz).

5.18. Benzyloxycarbonyl (4-phenylthiazol-2-yl)methylamine (62)

Benzyl 2-amino-2-thioxoethylcarbamate[22] (**60**; 500 mg, 2.23 mmol) was added to a stirred solution of 2-bromoacetophenone (400 mg, 2 mmol) in 19 ml of DMF/EtOH (2:1) and the mixture was heated at 80 °C for 2 h. After cooling, the solvent was evaporated, the resulting sticky solid was dissolved in ethyl acetate and organic layer was washed with aq. K_2CO_3 (5% w/v), water and brine. After drying over anhydrous Na_2SO_4, the solvent was removed and the resulting solid crystallized with diethyl ether/petroleum ether (7:3). Yield: 67%. Mp 83.3–85 °C. ^{1}H NMR ($CDCl_3$) δ: 7.87 (dd, 2H, J = 1.65, 8.52 Hz); 7.45–7.32 (m, 9H); 5.64 (s, 1H); 5.18 (s, 2H); 4.76 (d, 2H, J = 6.05 Hz).

5.19. Ethyl 2-bromo-3-oxo-3-phenylpropanoate (65)

In a 500 ml round bottom flask, Br_2 (2.7 ml, 52 mmol) was carefully added dropwise to a well-stirred solution of ethyl 3-oxo-3-phenylpropanoate (10 g, 52 mmol) in 180 ml of glacial AcOH. Resulted mixture was stirred at rt for 2 h, after that solvent was evaporated under vacuum and obtained residue dissolved in AcOEt. Organic layer was then washed with saturated aq. $NaHCO_3$, water, brine, dried with anhydrous Na_2SO_4 and removed under vacuum. The crude oil was purified by CC on silica gel (cyclohexane/CH_2Cl_2; 80:20) to give a pale-yellow oil. Yield: 85%. ^{1}H NMR ($CDCl_3$) δ: 7.99 (d, 2H, J = 7.50 Hz); 7.60 (t, 1H, J = 7.51); 7.50 (t, 2H, J = 7.52 Hz); 5.66 (s, 1H); 4.28 (q, 2H, J = 7.15 Hz); 1.25 (t, 3H, J = 7.15 Hz).

5.20. Ethyl 2-((benzyloxycarbonylamino)methyl)-4-phenyl thiazole-5-carboxylate (66)

Compound **65** (5 g, 18.7 mmol) was added to a stirred solution of benzyl 2-amino-2-thioxoethylcarbamate (**60**; 4.6 g, 20.6 mmol) in 225 ml of a mixture of DMF/EtOH (2:1) and then heated at 80 °C for 2 h. After cooling, the solvent was evaporated and the resulted sticky solid dissolved in CH_2Cl_2; the organic layer was washed with aq. K_2CO_3 (5% w/v), water and brine. After drying over anhydrous Na_2SO_4, CH_2Cl_2 was removed and crude product purified by CC (CH_2Cl_2/MeOH; 98:2). The resulting solid was rinsed with diethyl ether/petroleum ether (7:3). Yield: 69%. Mp 134.8–136 °C. ^{1}H NMR ($CDCl_3$) δ: 7.73–7.70 (m, 2H); 7.44–7.35 (m, 7H); 5.59 (br s, 1H); 5.18 (s, 2H); 4.70 (d, 2H, J = 6.06 Hz); 4.27 (q, 2H, J = 7.15 Hz); 1.86 (br s, 1H); 1.28 (t, 3H, J = 7.15 Hz).

5.21. (4-Phenyloxazol-2-yl)methanamine, (4-phenylthiazol-2-yl)methanamine and ethyl 2-((benzyloxycarbonylamino) methyl)-4-phenylthiazole-5-carboxylate (63, 64, 67)

General method: In a round bottom flask compound **61** (or **62** or **66**) (3.2 mmol) was added to 1.5 ml of HBr (33% in AcOH) and after stirring the solution for 3 h at rt, diethyl ether (7 ml) was added and the salt filtered, rinsed with ether and then dissolved in water (5 ml). Aq. $NaHCO_3$ (5% w/v) was carefully added to the solution until bubbling stopped, then the mixture was extracted with AcOEt. The joined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and evaporated to dryness to yield a crude solid, which was purified by CC (silica gel; different ratio of CH_2Cl_2 and MeOH as indicated for each compound) or simply rinsed with the proper solvents.

5.21.1. (4-Phenyloxazol-2-yl)methanamine (63)

Solid rinsed with petroleum ether/diethyl ether (9:1). Yield: 82%. Mp 52–53.6 °C. ^{1}H NMR ($CDCl_3$) δ: 7.86 (s, 1H); 7.72 (d, 2H, J = 7.42 Hz); 7.40 (t, 2H, J = 7.15 Hz); 7.31 (t, 1H, J = 7.42 Hz); 4.00 (s, 2H); 1.74 (s, 2H).

5.21.2. (4-Phenylthiazol-2-yl)methanamine (64)

CC (CH_2Cl_2/MeOH; 99:1); solid crystallized with diethyl ether/petroleum ether (3:7) and rinsed with petroleum ether. Yield: 72%. Mp 46.5–48 °C. ^{1}H NMR ($CDCl_3$) δ: 7.88 (d, 2H, J = 7.43 Hz); 7.30–7.45 (m, 4H); 4.25 (s, 2H); 1.78 (s, 2H).

5.21.3. Ethyl 2-((benzyloxycarbonylamino)methyl)-4-phenyl thiazole-5-carboxylate (67)

CC (CH_2Cl_2/MeOH; 99:1); solid rinsed with petroleum ether/diethyl ether (7:3). Yield: 51%. Mp 90.2–91.6 °C. ^{1}H NMR ($CDCl_3$) δ: 7.71 (d, 2H, J = 3.85 Hz); 7.42–7.40 (m, 3H); 7.26 (q, 2H, J = 7.15 Hz); 4.55 (br s, 2H); 4.20 (s, 2H); 1.27 (t, 3H, J = 7.15 Hz).

5.22. 7-Chloro-4-*N*-((4-phenyloxazol-2-yl)methyl)aminoquinoline, 7-chloro-4-*N*-((4-phenylthiazol-2-yl)methyl)aminoquinoline and ethyl 2-((7-chloroquinolin-4-ylamino)methyl)-4-phenylthiazole-5-carboxylate (14, 15, 68)

General method: In a round bottom flask a mixture of proper amine (**63**, **64**, **67**; 3.8 mmol), 4,7-dichloroquinoline (830 mg, 4.19 mmol) and phenol (3.6 g, 38 mmol) was heated for 3 h at 130 °C, stirring under N_2. After cooling, crude mixture was dissolved in a solution of CH_2Cl_2/MeOH (95:5) and surplus of phenol extracted with 2 N NaOH. The organic layers were washed with water, brine, dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude solid was purified by CC (silica gel; different eluents and conditions as indicated for each compound).

5.22.1. 7-Chloro-4-*N*-((4-phenyloxazol-2-yl)methyl)aminoquinoline (14)

CC (CH_2Cl_2/MeOH; 98.5:1.5); solid rinsed with diethyl ether/petroleum ether (7:3). Yield: 52%. Mp 221.5–222.5 °C. ^{1}H NMR (DMSO-d_6) δ: 8.56 (s, 1H); 8.41 (d, 1H, J = 5.50 Hz); 8.28 (d, 1H, J = 8.80 Hz); 8.09 (t, 1H, J = 5.78 Hz); 7.82 (d, 1H, J = 1.92 Hz); 7.75 (dd, 2H, J = 7.15, 1.10 Hz); 7.50 (dd, 1H, J = 9.08, 2.20 Hz); 7.40 (m, 2H); 7.30 (t, 1H, J = 7.15 Hz); 6.59 (d, 1H, J = 5.50 Hz); 4.73 (d, 2H, J = 5.78 Hz). HRMS (ESI) m/z calcd for $C_{19}H_{15}N_3ClO$ $[M+H]^+$: 336.090365; found: 336.09075.

5.22.2. 7-Chloro-4-*N*-((4-phenylthiazol-2-yl)methyl)amino quinoline (15)

CC (CH_2Cl_2/MeOH; 98:2); solid rinsed with diethyl ether/petroleum ether (6:4). Yield: 68%. Mp 212-212.5 °C. ^{1}H NMR ($CDCl_3$) δ: 8.47 (d, 1H, J = 5.78 Hz); 8.09–8.04 (m, 2H); 7.92–7.87 (m, 2H); 7.48–7.33 (m, 5H); 7.13 (br s, 1H); 6.59 (d, 1H, J = 5.78 Hz); 4.96 (d, 2H, J = 3.85 Hz). HRMS (ESI) m/z calcd for $C_{19}H_{15}N_3ClS$ $[M+H]^+$: 352.06697; found: 352.06708.

5.22.3. Ethyl 2-((7-chloroquinolin-4-ylamino)methyl)-4-phenylthiazole-5-carboxylate (68)

CC (AcOEt/cyclohexane; 51:49); solid rinsed with diethyl ether/petroleum ether (6:4). Yield: 70%. Mp 190.4–192.2 °C. ^{1}H NMR ($CDCl_3$) δ: 8.49 (d, 1H, J = 5.50 Hz); 8.06–8.03 (m, 2H); 7.76–7.73 (m, 2H); 7.49–7.41 (m, 4H); 7.17 (br s, 1H); 6.55 (d, 1H, J = 5.50 Hz); 4.95 (s, 2H); 7.25 (q, 2H, J = 7.15 Hz); 1.23 (t, 3H, J = 7.15 Hz).

5.23. 2-((7-Chloroquinolin-4-ylamino)methyl)-4-phenyl thiazole-5-carboxylic acid (69)

In a round bottom flask, LiOH monohydrate (1.1 g, 26 mmol) was dissolved in 30 ml of ethanol/water (2:1) and ester **68** (1 g, 2.36 mmol) was added. The resulting mixture was heated at reflux for 1.5 h. After cooling, ethanol was evaporated and water (30 ml) was added. The resulting mixture was acidified with 1 N HCl to pH 5. The precipitate was filtered and washed with water. After drying overnight in vacuo (anhyd $CaCl_2$), solid was rinsed with diethyl ether/MeOH (8:2) to afford pure compound. Yield: 98.5%. Mp 279–281.5 °C. ^{1}H NMR (CF_3COOD) δ: 8.56 (d, 1H, J = 6.87 Hz); 8.34 (d, 1H, J = 9.07 Hz); 8.08 (d, 1H, J = 1.65 Hz); 7.88 (d, 1H, J = 9.07 Hz); 7.73–7.60 (m, 5H); 7.18 (d, 1H, J = 6.87 Hz); 5.81 (s, 2H).

5.24. (2-((7-Chloroquinolin-4-ylamino)methyl)-4-phenyl thiazol-5-yl)(pyrrolidin-1-yl)methanone (70)

In a microwave vial, acid **69** (250 mg, 0.63 mmol), TEA (88 μl, 0.63 mmol), HOBt (95 mg, 0.63 mmol) and pyrrolidine (55 μl, 0.63 mmol) were dissolved in 2 ml of well-stirred dry DMF at rt. After 5 min, DCC (130 mg, 0.63 mmol) was added and the mixture was heated with a microwave synthesizer system at 55 °C for 35 min. After cooling, the precipitate of dicyclohexylurea was filtered off and washed with DMF. The solvent was evaporated under vacuum and the resulted sticky solid was dissolved in AcOEt, washed with 2 N NaOH, water and brine. The organic solution was evaporated to dryness, the resulted crude solid was purified by CC on silica gel (CH_2Cl_2/MeOH; 98:2) and pure product was washed with diethyl ether. Yield: 80 %. Mp 200.5–201.4 °C. ^{1}H NMR ($CDCl_3$) δ: 8.44 (d, 1H, J = 5.50 Hz); 8.03–7.98 (m, 2H); 7.77–7.71 (m, 2H); 7.46–7.29 (m, 4H); 6.49 (d, 1H, J = 5.50 Hz); 4.88 (s, 2H); 3.61–3.54 (m, 2H); 2.98–2.88 (m, 2H); 1.85–1.76 (m, 2H); 1.70–1.63 (m, 2H).

5.25. 7-Chloro-4-N-((4-phenyl-5-(pyrrolidin-1-ylmethyl)thiazol-2-yl)methyl)aminoquinoline (21a)

To a stirred suspension of compound **70** (270 mg, 0.68 mmol) in 23 ml of anhydrous THF heated at 50 °C, under N_2, tris(triphenylphosphine)rhodium(I)carbonyl hydride (31.2 mg, 0.034 mmol) and diphenylsilane (2.12 ml, 11.46 mmol) were added in four portions during 2 h and the mixture was further stirred for 1 h, until the amide was completely reduced. After cooling, THF was evaporated, and the residue was partitioned between CH_2Cl_2 and a 1 N HCl solution. The acid phase was alkalized with aqueous 6 N NaOH and extracted with CH_2Cl_2, the joined organic layers were dried over anhyd Na_2SO_4 and evaporated to dryness. The crude product was purified by CC on silica gel (CH_2Cl_2/MeOH; 98:2). Pure solid was crystallized with diethyl ether/CH_2Cl_2 (9:1) and rinsed with diethyl ether. Yield: 22%. Mp 159–160.3 °C. ^{1}H NMR (DMSO-d_6) δ: 8.41 (d, 1H, J = 6.88 Hz); 8.32–8.20 (m, 2H); 7.83 (s, 1H); 7.65

(d, 2H, J = 7.15 Hz); 7.54 (d, 1H, J = 9.08 Hz); 7.50-7.30 (m, 3H); 6.55 (d, 1H, J = 6.88 Hz); 4.83 (d, 2H, J = 5.77); 3.81 (s, 2H); 2.43 (s, 4H); 1.62 (s, 4H). HRMS (ESI) m/z calcd for $C_{24}H_{24}N_4ClS$ $[M+H]^+$: 435.14047; found: 435.14023.

5.26. Methyl 5-(aminomethyl)thiophene-2-carboxylate (72)

To a stirred solution of methyl 5-(azidomethyl)thiophene-2-carboxylate[25] (**71**; 3 g, 15.2 mmol) in 100 ml of dry MeOH under N_2 atmosphere, anhydrous $SnCl_2$ (8.65 g, 45.6 mmol) was added. After stirring for 5 h at rt, the mixture was concentrated, diluted with CH_2Cl_2, cooled at 0 °C and extracted with cold 1 N HCl. The precipitate was filtered off and the acid phase was cooled, alkalized with 2 N NaOH and extracted with CH_2Cl_2. The joined organic layers were dried over anhydrous Na_2SO_4 and evaporated to dryness. The solid residue was rinsed with petroleum ether/diethyl ether (9:1) to give pure **72**. Yield: 34%. Mp 49–51 °C. ^{1}H NMR ($CDCl_3$) δ: 7.66 (d, 1H, J = 3.85 Hz); 6.91 (d, 1H, J = 3.85 Hz); 4.08 (s, 2H); 3.87 (s, 3H); 1.62 (s, 2H).

5.27. Methyl 5-((7-chloroquinolin-4-ylamino)methyl) thiophene-2-carboxylate (73)

In a round bottom flask a mixture of compound **72** (860 mg, 5.02 mmol), 4,7-dichloroquinoline (1.09 g, 5.52 mmol) and phenol (4.72 g, 50.2 mmol) was heated for 3.5 h at 130 °C, stirring under N_2. After cooling, crude mixture was dissolved with a solution of CH_2Cl_2/MeOH (95:5) and surplus of phenol extracted with 2 N NaOH. Organic layers were washed with water, brine, dried with anhydrous Na_2SO_4 and evaporated to give a crude solid, which was purified by CC on silica gel (CH_2Cl_2/MeOH; 97:3) and washed with diethyl ether to give pure compound. Yield: 80%. Mp 189.5–190.7 °C. ^{1}H NMR (DMSO-d_6) δ: 8.37 (d, 1H, J = 5.22 Hz); 8.25 (d, 1H, J = 9.08 Hz); 8.15 (t, 1H, J = 6.33 Hz); 7.81 (d, 1H, J = 2.20 Hz); 7.67 (d, 1H, J = 3.85 Hz); 7.50 (dd, 1H, J = 9.08, 2.20 Hz); 7.19 (d, 1H, J = 3.85 Hz); 6.51 (d, 1H, J = 5.22 Hz); 4.77 (d, 2H, J = 6.33 Hz); 3.74 (s, 3H).

5.28. 5-((7-Chloroquinolin-4-ylamino)methyl)thiophene-2-carboxylic acid (74)

In a round bottom flask, LiOH monohydrate (698 mg, 16.6 mmol) was dissolved in 15 ml of ethanol/water (2:1) and ester **73** (500 mg, 1.5 mmol) was added. The resulting mixture was heated at reflux for 1.5 h. After cooling, ethanol was evaporated and water was added. The resulting suspension was acidified with 1 N HCl to pH 4. The suspension was filtered and washed with water. After drying overnight in vacuo (anhydrous $CaCl_2$), the solid was washed with diethyl ether to afford pure compound **74**. Yield: 99%. Mp 269.7–272 °C (dec). ^{1}H NMR (DMSO-d_6) δ: 9.18 (s, 1H); 8.46–8.41 (m, 2H); 7.90 (s, 1H); 7.63–7.58 (m, 2H); 7.19 (s, 1H); 6.68 (d, 1H, J = 5.78 Hz); 4.86 (s, 2H).

5.29. (5-((7-Chloroquinolin-4-ylamino)methyl)thiophen-2-yl)(pyrrolidin-1-yl)methanone (75)

In a microwave vial, compound **74** (467 mg, 1.47 mmol), HOBt (224.3 mg, 1.47 mmol) and pyrrolidine (0.15 ml, 1.75 mmol) were dissolved in 5 ml of dry DMF at room temperature. After 5 min., DCC (256.5 mg, 1.47 mmol) was added and the mixture was heated with a microwave synthesizer system at 55 °C for 1 h. After cooling, the precipitate of dicyclohexylurea was filtered and washed with DMF. The solvent was removed under vacuum and the obtained sticky solid was dissolved in CH_2Cl_2, washed with aq. $NaHCO_3$ (5% w/v), water and brine. The organic phase was evaporated to dryness and the resulted crude solid purified by CC on silica gel (CH_2Cl_2/MeOH; 97:3) and pure product rinsed with diethyl ether. Yield: 72%. Mp 210.3–212.5 °C ^{1}H NMR (DMSO-d_6) δ: 8.37 (d, 1H, J = 5.22 Hz); 8.26 (d, 1H, J = 9.08 Hz); 8.11 (t, 1H, J = 5.77 Hz); 7.80 (d, 1H, J = 2.20 Hz); 7.49 (dd, 1H, J = 9.08, 2.20 Hz); 7.43 (d, 1H, J = 3.85 Hz); 7.11 (d, 1H, J = 3.85 Hz); 6.52 (d, 1H, J = 5.22 Hz); 4.72 (d, 2H, J = 5.77 Hz); 3.66 (br s, 2H); 3.41 (br s, 2H); 1.87 (d, 2H, J = 5.22 Hz); 1.79 (d, 2H, J = 5.22 Hz).

5.30. 7-Chloro-4-*N*-((5-(pyrrolidin-1-ylmethyl)thiophen-2-yl)methyl)aminoquinoline (4a)

The amido compound **75** (392 mg, 1.05 mmol) was added carefully to a cooled ice-bath suspension of $LiAlH_4$ (200 mg, 5.27 mmol) in 45 ml of anhydrous THF and the mixture was stirred for 3 h at rt, under N_2. After cooling in ice-bath, water and 2 N NaOH were carefully added and the formed suspension filtered off; THF was evaporated and the aqueous layer diluted with water and extracted with CH_2Cl_2. The organic phase was washed with water, dried over anhydrous Na_2SO_4, and evaporated to dryness. Crude solid was purified by CC on silica gel (CH_2Cl_2/MeOH; 98:2) and the resulting solid was washed with diethyl ether. Yield: 46%. Mp 160.8–162 °C. ^{1}H NMR (DMSO-d_6) δ: 8.36 (d, 1H, J = 5.22 Hz); 8.25 (d, 1H, J = 9.08 Hz); 7.90–8.10 (m, 1H); 7.79 (d, 1H, J = 1.93 Hz); 7.47 (dd, 1H, J = 9.08, 1.93 Hz); 6.90 (d, 1H, J = 3.30 Hz); 6.74 (d, 1H, J = 3.30 Hz); 6.55 (d, 1H, J = 5.22 Hz); 4.65 (d, 2H, J = 5.23 Hz); 3.63 (s, 2H); 2.39 (s, 4H); 1.63 (s, 4H). HRMS (ESI) m/z Calcd for $C_{19}H_{21}N_3ClS$ $[M+H]^+$: 358.11392; found: 358.11362.

5.31. Parasite cultures and drug susceptibility assay

Plasmodium falciparum cultures were carried out according to Trager and Jensen with slight modifications.[33] The CQ-susceptible, strain D10 and the CQ-resistant, strain W2 were maintained at 5% hematocrit (human type A-positive red blood cells) in RPMI 1640 (EuroClone, Celbio) medium with the addition of 1% AlbuMax (Invitrogen, Milan, Italy), 0.01% hypoxanthine, 20 mM Hepes, and 2 mM glutamine. All the cultures were maintained at 37 °C in a standard gas mixture consisting of 1% O_2, 5% CO_2, and 94% N_2. Compounds were dissolved in either water or DMSO and then diluted with medium to achieve the required concentrations (final DMSO concentration <1%, which is non-toxic to the parasite). Drugs were placed in 96-well flat-bottomed microplates (COSTAR) and serial dilutions made. Asynchronous cultures with parasitaemia of 1–1.5% and 1% final hematocrit were aliquoted into the plates and incubated for 72 h at 37 °C. Parasite growth was determined spectrophotometrically (OD650) by measuring the activity of the parasite lactate dehydrogenase (pLDH), according to a modified version of the method of Makler in control and drug-treated cultures.[26] The antimalarial activity is expressed as 50% inhibitory concentrations (IC_{50}); each IC_{50} value is the mean and standard deviation of at least three separate experiments performed in duplicate.[34]

5.32. Cytotoxicity assay

The long-term human microvascular endothelial cell line (HMEC-1) immortalized by SV 40 large *T antigen*[35] was maintained in MCDB 131 medium (Invitrogen, Milan, Italy) supplemented with 10% fetal calf serum (HyClone, Celbio, Milan, Italy), 10 ng/ml of epidermal growth factor (Chemicon), 1 μg/ml of hydrocortisone, 2 mM glutamine, 100 U/ml of penicillin, 100 μg/ml of streptomycin, and 20 mM Hepes buffer (EuroClone). Unless stated otherwise, all reagents were from Sigma Italia, Milan, Italy. For the cytotoxicity assays, cells were treated with serial dilutions of test compounds for 72 h and cell proliferation evaluated using the

MTT assay already described.[27] The results are expressed as IC_{50}, which is the dose of compound necessary to inhibit cell growth by 50%. All the tests were performed in duplicate at least three times.

5.33. BHIA Assay

The inhibition of β-hematin formation by compounds **4a** and **10** was carried out using a β-hematin inhibitory activity (BHIA) assay. The full details of the assay have been published[30] and the assay was performed exactly as described in the indicated reference.

5.34. Determination of physicochemical properties

The pK_a values of the studied compounds were determined potentiometrically at 25 °C using a PCA 101 instrument from Sirius Analytical Instruments (East Sussex, United Kingdom). Compounds were dissolved in 0.15 M KCl and the resulting solutions acidified to pH 1.8 with 0.5 M HCl. Titrations were done in triplicate with 0.5 M KOH. Partition coefficients of the compounds in *n*-octanol–0.15 M KCl were determined potentiometrically using the same instrument with three different volume ratios of organic and aqueous phases (0.2, 0.4 and 0.6). The pK_a, $\log P_{Octanol}$, and $\log D$ (distribution coefficient, defined as the ratio of the sum of concentrations for all solute species in the *n*-octanol phase to the same of the aqueous phase at selected pH) values were calculated from the titration curves using the Refinement Pro software program, v. 1.0 (Sirius Analytical Instruments). Alternatively, $\log D$ values were calculated using the following equation: $\log D = \log P - \log[1 + 10^{(pKa1 - pH) +} 10^{(pKa1 + pKa2 - 2pH) +} 10^{(pKa1 + pKa2 + pKa3 - 3pH)}]$.[36]

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References and notes

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