Synthesis and Antimalarial Evaluation of New 1,3,5-tris[(4-(Substituted-aminomethyl)phenyl)methyl]benzene Derivatives: A Novel Alternative Antiparasitic Scaffold

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Design, Synthesis of New 1,3,5-tris[(4-(Substituted-aminomethyl)phenoxy)methyl]benzene derivatives and Evaluation of their Antimalarial Activity

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In 2021, there were an estimated increase of 2 million malaria cases worldwide, since 247 million cases have been recorded compared to 245 million in 2020 according to the last WHO Malaria report[1]. Nowadays, nearly half of the world's population is at risk of malaria, in particular pregnant women and young children Effective intersectoral coordination is recommended to facilitate concerted action [2], an original strategy is to design and synthesize new original quinoline based drug candidates such as amodiaquine (AQ) that are not recognized by the protein system involved in the drug efflux. This last could serve both as natural defense mechanisms and lead to the bioavailability and disposition of drugs. Originally, such a mechanism was suggested in *Plasmodium falciparum*, where erythrocytes infected with CQ-resistant parasites accumulated specifically less drug than the sensitive ones, before its further analysis led to the identification of the Pfcrt gene among other quinoline compound resistance mechanisms. Such new series that have not been recognized by the protein system involved in the drug efflux of bisquinoline A and bisacridine B antimalarial drugs (Figure 1, Piperaquine and compounds A–B) have been described in previously published works. These original derivatives have much lower resistance indices than CQ, indicating that these bis-heterocyclic derivatives are less efficiently rejected by efflux by drug-resistant parasites.

During our research, we focused on the design and discovery of original nitrogen heterocyclic compounds that can be useful in antiprotozoal chemotherapy, and we have formerly developed and synthesized different series of phenanthrolines (series A-B), quinolines /isoquinoline and quinazoline derivatives (series C) designed as potential antiprotozoal candidates, which could bind to *Plasmodium falciparum* DNA G-quadruplexes in order to bypass the resistance mechanisms deployed by the parasites and also based on efflux. Indeed, it has previously been described that the telomeres of various protozoa could constitute interesting drug targets, and telomerase activity is noticed in gametocytes and during the transition to the erythrocytic stage of the parasite *P. falciparum*. By considering our research experience in the synthesis of new antiprotozoal heterocyclic derivatives, we have previously exposed the design and synthesis of new 1,3,5-*tris*[(4-(substituted-aminomethyl)phenyl)methyl]benzene derivatives (series D), a new alternative and potential antiparasitic scaffold against malaria [3]. We have hypothesized that substitution with three diaminobenzyl moieties in positions 1, 3, and 5 of the benzene ring could lead to more potent G4 ligands in terms of selectivity by increasing the aromatic surface. Thereby, relying on our latest antimalarial derivatives described earlier (series D), a series of novel structural analogues, the 1,3,5-*tris*[(4-(substituted-aminomethyl)phenoxy)methyl]benzene compounds **1**, were designed, prepared, and evaluated for their *in vitro* antimalarial activity against the CQ-sensitive (3D7) and the CQ-resistant (W2) strains of the malaria parasite *Plasmodium falciparum*.

These newly reported 1,3,5-*tris*[(4-(substituted-aminomethyl)phenoxy)methyl]benzene derivatives **1a-r** were synthesized starting from commercially available 1,3,5-*tris*(bromomethylbenzene (Scheme 1).

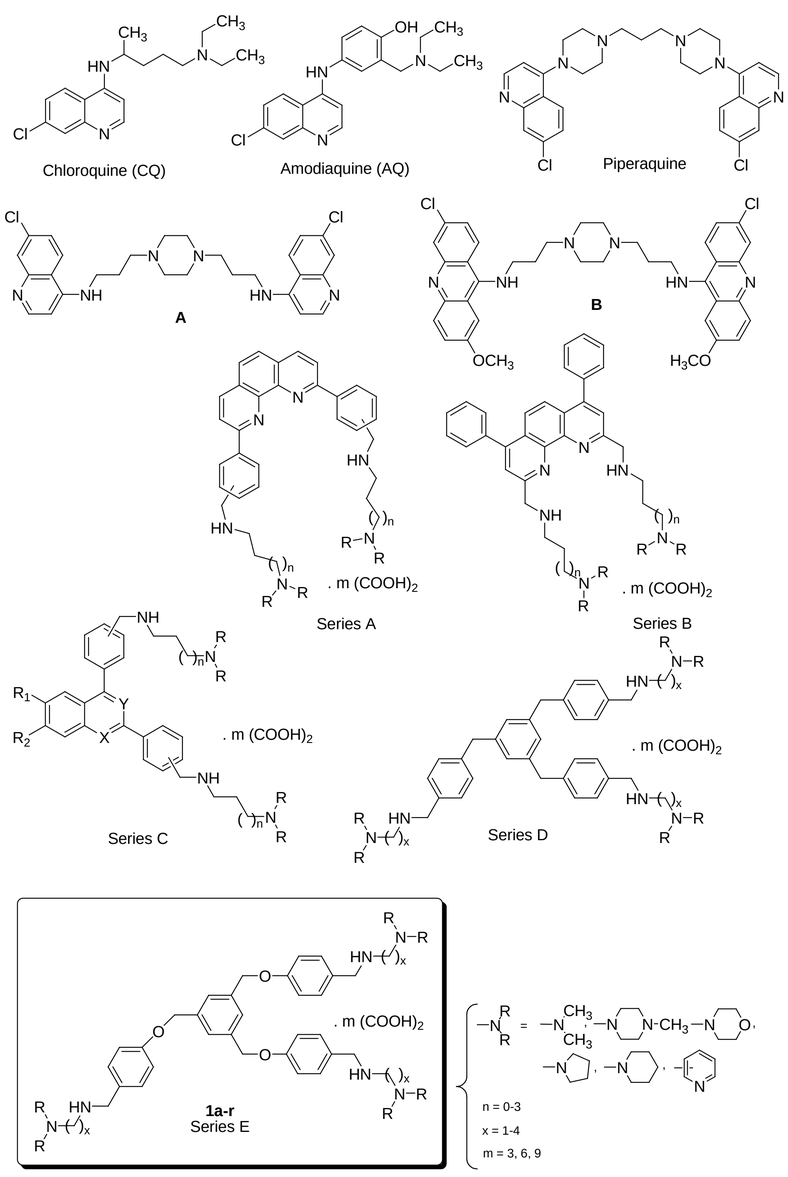
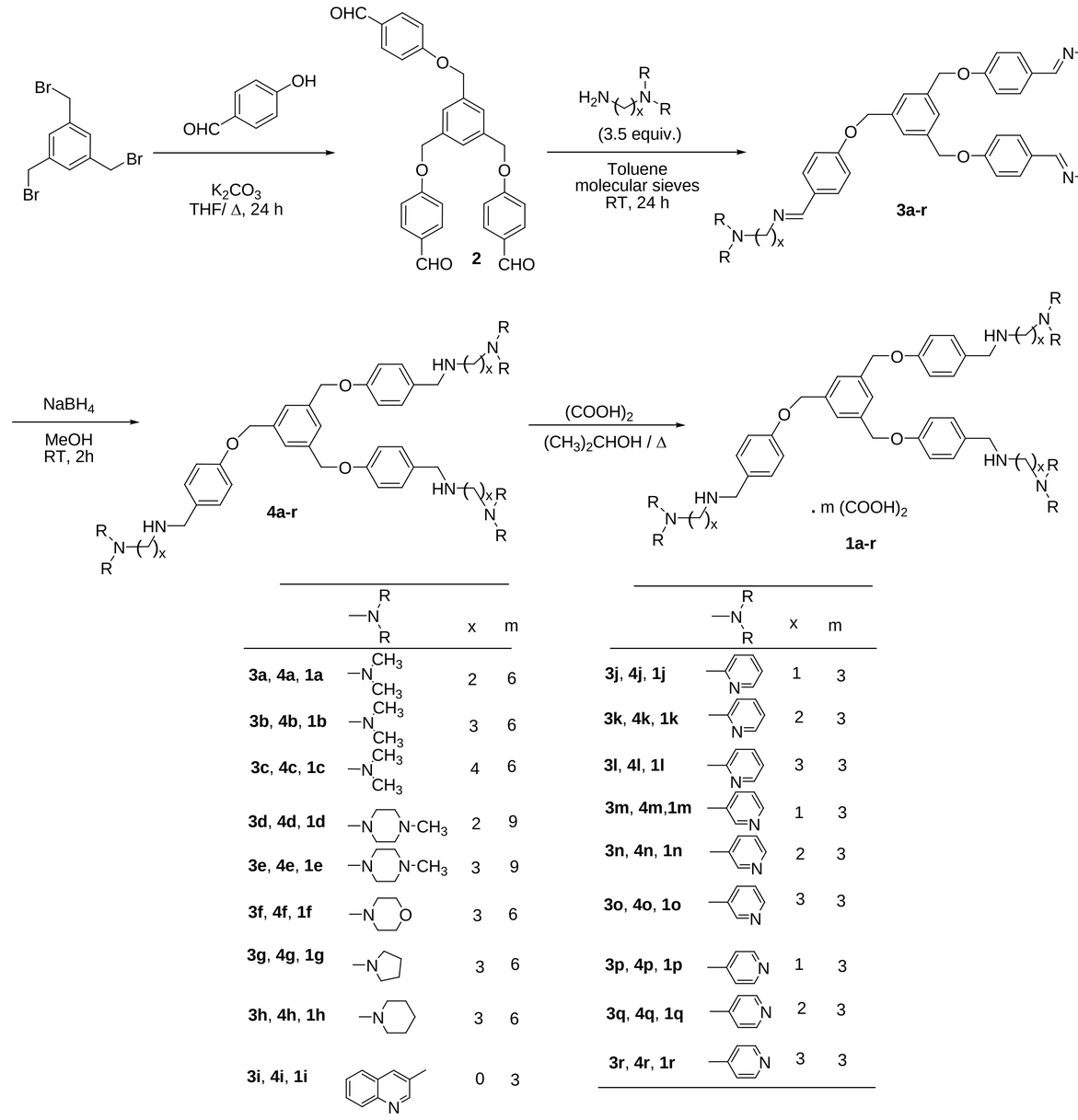
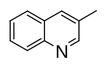


Figure 1. The structures of chloroquine (CQ), amodiaquine (AQ), piperaquine, bisquinoline **A**, bisacridine **B**, series A-D and newly synthesized 1,3,5-*tris*[(4-(substituted-aminomethyl)phenoxy)methyl]benzene derivatives **1a-r** (Series E).



		x	m
3a, **4a**, **1a**	$-N(CH_3)_2$	2	6
3b, **4b**, **1b**	$-N(CH_3)_2$	3	6
3c, **4c**, **1c**	$-N(CH_3)_2$	4	6
3d, **4d**, **1d**	$-N$⟨⟩$N-CH_3$	2	9
3e, **4e**, **1e**	$-N$⟨⟩$N-CH_3$	3	9
3f, **4f**, **1f**	$-N$⟨⟩O	3	6
3g, **4g**, **1g**	$-N$⟨⟩	3	6
3h, **4h**, **1h**	$-N$⟨⟩	3	6
3i, **4i**, **1i**		0	3

		x	m
3j, **4j**, **1j**		1	3
3k, **4k**, **1k**		2	3
3l, **4l**, **1l**		3	3
3m, **4m**,**1m**		1	3
3n, **4n**, **1n**		2	3
3o, **4o**, **1o**		3	3
3p, **4p**, **1p**		1	3
3q, **4q**, **1q**		2	3
3r, **4r**, **1r**		3	3

Scheme 1. General procedure for the preparation of target compounds **1a-r**.

The pharmacological results showed antimalarial activity with IC_{50} values in the sub and µM range. In these newly synthesized series, the 1,3,5-*tris*[(4-(substituted-aminomethyl)phenoxy)methyl]benzenes **1** bearing various pyridinyl-alkylaminomethyl side chains at position 4 of the phenoxymethyl moieties displayed better activities than their homologs substituted with alkylaminoalkylaminomethyl side chains. Against the *P. falciparum* CQ-sensitive 3D7, the 1,3,5-*tris*[(4-(substituted-aminomethyl)phenoxy)methyl]benzene **1p** bearing three pyridin-4-ylmethylaminomethyl side chains at position 4 of each of the phenoxymethyl groups was noticed to be the most active, with an IC_{50} of 0.078 µM, while compound **1m** substituted by pyridin-3-ylmethylaminomethyl side chains was found the most active ligands against the W2 strain with an IC_{50} of 0. 073 µM. All these biological results will be then studied in order to establish structure-activity relationships of these novel synthetic compounds. In order to assess their selectivity of action, the cytotoxicities of our novel synthesized antiparasitic 1,3,5-tris[(4-(substituted-aminomethyl)phenoxy)methyl]benzene compounds **1a-r** will be evaluated *in vitro* on the human cell line HepG2, which is a commonly used human-derived hepatocarcinoma cell line and expresses many hepatocyte-specific metabolic enzymes. As the telomeres of *P. falciparum* have been previously reported as potential targets of antimalarial compounds, some FRET melting assays have been carried out to test their ability to stabilize the parasitic telomeric G-quadruplexe. First results show that 1,3,5-tris[(4-(substituted-aminomethyl)phenoxy)methyl]benzenes **1a-h** bearing different dialkylaminoalkylaminomethyl side chains exhibited the more interesting stabilization profile of G-quadruplexes in FPf1T and FPf8T *P. falciparum* telomeric chromosomic sequences.

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[1] World malaria report 2022. Geneva: World Health Organization; **2022**. Licence: CC BY-NC-SA 3.0 IGO.
[2] Ending the neglect to attain the Sustainable Development Goals: a road map for neglected tropical diseases 2021–2030. Geneva: World Health Organization; **2020**. Licence: CC BY-NC-SA 3.0 IGO.
[3] *Drugs Drug Candidates* **2023**, *2*, 653-672; https://doi.org/10.3390/ddc2030033.