

Article

Synthesis of New 1,3-bis[(4-(Substituted-Aminomethyl)Phenyl)methyl]benzene and 1,3-bis[(4-(Substituted-Aminomethyl)Phenoxy)methyl]benzene Derivatives, Designed as Novel Potential G-Quadruplex Antimalarial Ligands

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Abstract

Background: Based on our previously reported series of novel 1,3,5-*tris*[(4-(substituted-aminomethyl)phenyl)methyl]benzene and 1,3,5-*tris*[(4-(substituted-aminomethyl)phenoxy)methyl]benzene derivatives, we have now designed, synthesized, and tested a new series of novel restricted and simplified structural analogues of these compounds against *Plasmodium falciparum* in vitro; i.e., the 1,3-*bis*[(4-(substituted-aminomethyl)phenyl)methyl]benzene and 1,3-*bis*[(4-(substituted-aminomethyl)phenoxy)methyl]benzene compounds. **Methods & Results:** The pharmacological results revealed significant antimalarial activity, with IC₅₀ values in the submicromolar to micromolar range. Additionally, the in vitro cytotoxicity of these new nitrogen-containing polyphenyl- or -phoxymethylbenzene compounds was evaluated on human HepG2 cells. The compound **1f**, the 1,3-*bis*[(4-(3-(morpholin-1-yl)propyl)aminomethyl)phenoxy)methyl]benzene derivative, emerged as one of the most potent and promising antimalarial candidates, demonstrating a cytotoxicity / antiprotozoal activity ratio of 594 against the chloroquine-sensitive *Plasmodium falciparum* 3D7 strain. Additionally, the 1,3-*bis*[(substituted aminomethyl)phenyl)methyl]benzene compound **1j** and the 1,3-*bis*[(substituted aminomethyl)phenoxy)methyl]benzenes **2p** and **2q** also showed strong antimalarial potential, with selectivity indexes (SI) of over 303, 280, and 217, respectively, against the 3D7 strain, which has mefloquine-reduced sensitivity. Furthermore,

the 1,3-bis[(4-(pyridin-2-ylethylaminomethyl)phenyl)methyl]benzene **2k** was identified as the most noteworthy antimalarial compound, exhibiting a selectivity index (SI) that was superior to 178 against the chloroquine-resistant *Plasmodium falciparum* W2 strain. It has previously been suggested that the telomeres of *P. falciparum* may serve as potential targets for these polyaromatic compounds; thus, we assessed the ability of our novel derivatives to stabilize parasitic telomeric G-quadruplexes using a FRET melting assay. **Conclusions:** However, regarding the stabilization of the protozoan G-quadruplex, it was noted that the few substituted derivatives, which showed interesting stabilization profiles, were not necessarily the most effective antimalarial compounds against both *Plasmodium* strains. Moreover, these new compounds did not show promising stabilizing effects on the different G4 sequences. Therefore, no correlation arises between their antimalarial activity and the selectivity of their binding to G-quadruplexes.

Keywords: antimalarial activity; 1,3-bis[(4-(substituted-aminomethyl)phenoxy)methyl]benzene; 1,3-bis[(4-(substituted-aminomethyl)phenyl)methyl]benzene; G-quadruplexes; synthesis

1. Introduction

Since the beginning of the millennium, an estimated 2.2 billion malaria cases and 12.7 million deaths linked to this vector-borne disease have been avoided worldwide according to the latest world malaria report [1]. Nevertheless, malaria still represents a real global health challenge, with nearly 600,000 deaths in 2023 [1]. Rising threats such as insecticide and drug resistance are worrying, especially as they are associated with climate change and the population displacement inherent in the current situation in the wake of the COVID-19 pandemic. In this context, managing the resistance of mosquito vectors to pyrethrinoids [2] and controlling the spread of the *Anopheles stephensi* species in Africa [3] are among the major challenges to be resolved in the fight against malaria. In particular, the spread of antimalarial drug resistance in Africa is alarming, notably the presence of partial resistance to artemisinin in a growing number of countries. Considering the importance of artemisinin-based combination therapies (ACTs) as part of the recommended curative treatment [4], the WHO has launched a “strategy to respond to antimalarial drug resistance in Africa” in 2022 [5]. Among the pillars of this strategy, there is the stimulation of research and innovation to leverage existing tools and the development of original tools against antimalarial drug resistance.

A potentially innovative approach would be to develop original compounds against *Plasmodium*, which would involve the design and synthesis of quinoline-based derivatives that are not recognizable by resistance mechanisms such as plasmodial efflux pumps. From this perspective, previous work has presented original synthons, such as bisquinoline A and bisacridine B (Figure 1, compounds A–B and Piperaquine), which display antiplasmodial activity without being recognized by the protein system involved in drug efflux [6–13]. These newly developed compounds exhibit significantly lower resistance indexes than chloroquine (CQ), which indicates that these bis-heterocyclic derivatives are less rejected by the efflux pumps of drug-resistant parasites. Similarly, tafenoquine, an 8-aminoquinoline compound developed to prevent all types of malaria [10,12,14], has recently been described as an efflux pump inhibitor (Figure 1) [15].

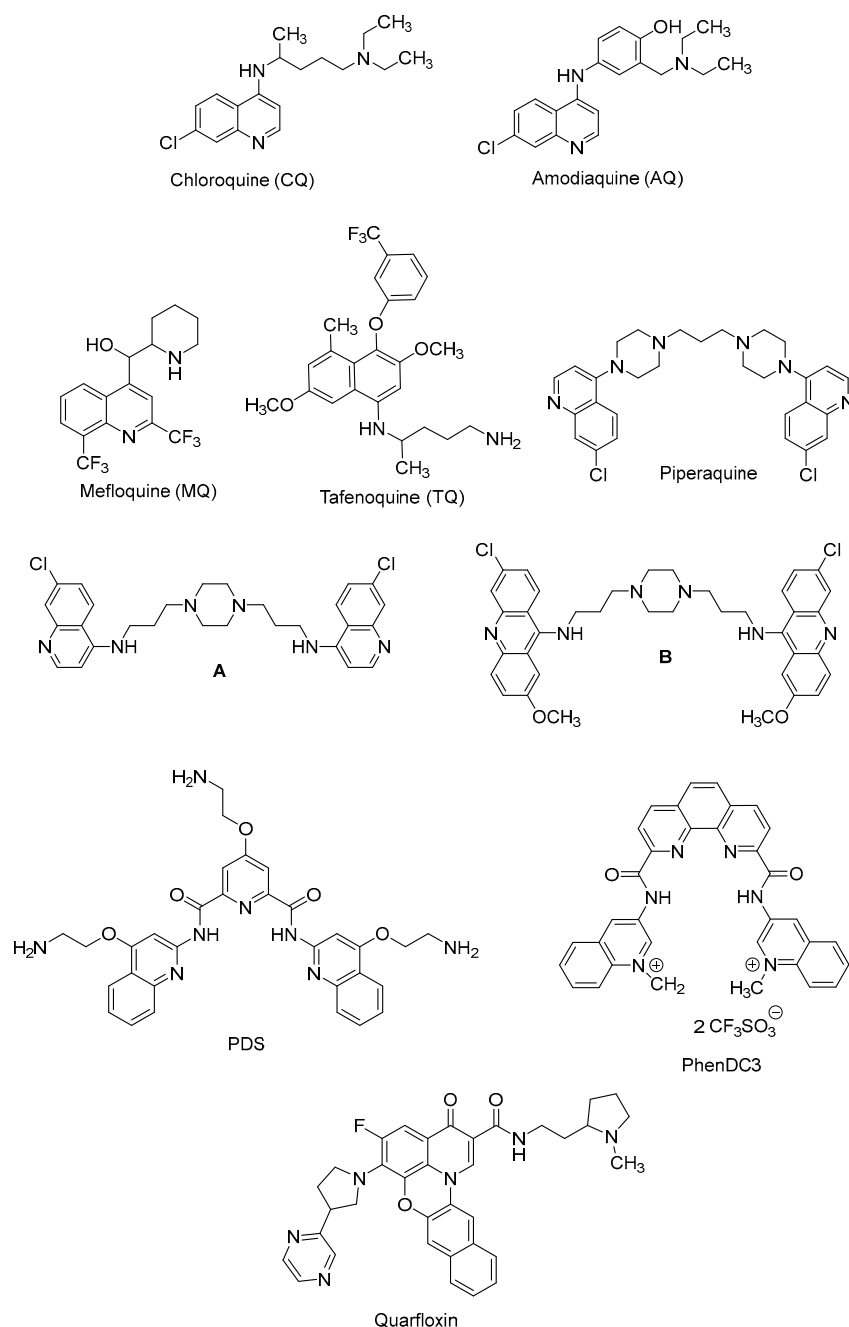


Figure 1. The structures of chloroquine (CQ), amodiaquine (AQ), mefloquine (MQ), tafenoquine (TQ), piperaquine, bisquinoline A, bisacridine B, and G-quadruplex ligands PDS, PhenDC3, and quarfloxin.

In our research endeavors, we concentrated on the exploration of new nitrogen heterocyclic derivatives with potentialities in antiprotozoal chemotherapy [16–26]. Previously, we have synthesized various series, including 2,9-*bis*[(substituted-aminomethyl)phenyl]phenanthrolines (series A–B), 2,4-*bis*[(substituted-aminomethyl)phenyl]quinoline, 1,3-*bis*[(substituted-aminomethyl)phenyl]isoquinoline, and 2,4-*bis*[(substituted-aminomethyl)phenyl]quinazoline derivatives (series C) [16,19–22]. More recently, we have developed two novel series of 1,3,5-*tris*[(4-(substituted-aminomethyl)phenyl)methyl]benzene and 1,3,5-*tris*[(4-(substituted-aminomethyl)phenoxy)methyl]benzene derivatives (series D and E), which could be considered as novel alternative antiparasitic scaffolds (Figure 2). These new series, series D and E, were developed as potential candidates for antiprotozoal agents, and were specifically designed to interact with *Plasmodium falciparum* DNA G-quadruplexes via π -stacking interactions [25,26].

PhenDC3, PDS, and quarfloxin (Figure 1). The latter compound, the phase 2 anticancer drug quarfloxin, was potent against the *P. falciparum* 3D7 strain, with an IC_{50} of 0.114 μ M. Its selection window on *P. falciparum* is at least 40 times greater than that on human cells (0.114 μ M against 3D7 versus 4.44 μ M on MCF10A human mammary epithelial cells) [29,33].

Importantly, the distinction between parasitic and human (5'GGGTTA3') G-quadruplexes opens the door to the development of antiprotozoal ligands that specifically target G-quadruplexes found in this parasitic species.

Given our research expertise in the preparation of new antimalarial derivatives, we present here the design and synthesis of original 1,3-bis[(4-(substituted-aminomethyl)phenyl)methyl]benzenes **1a–r** (series F) and 1,3-bis[(4-(substituted-aminomethyl)phenoxy)methyl]benzenes **2a–r** (series G), which could be considered as novel, restricted, and simplified structural analogues of our previously described 1,3,5-tris[(4-(substituted-aminomethyl)phenyl)methyl]benzenes and 1,3,5-tris[(4-(substitutedaminomethyl)phenyl)methyl]benzenes (series D and E) (Figure 2).

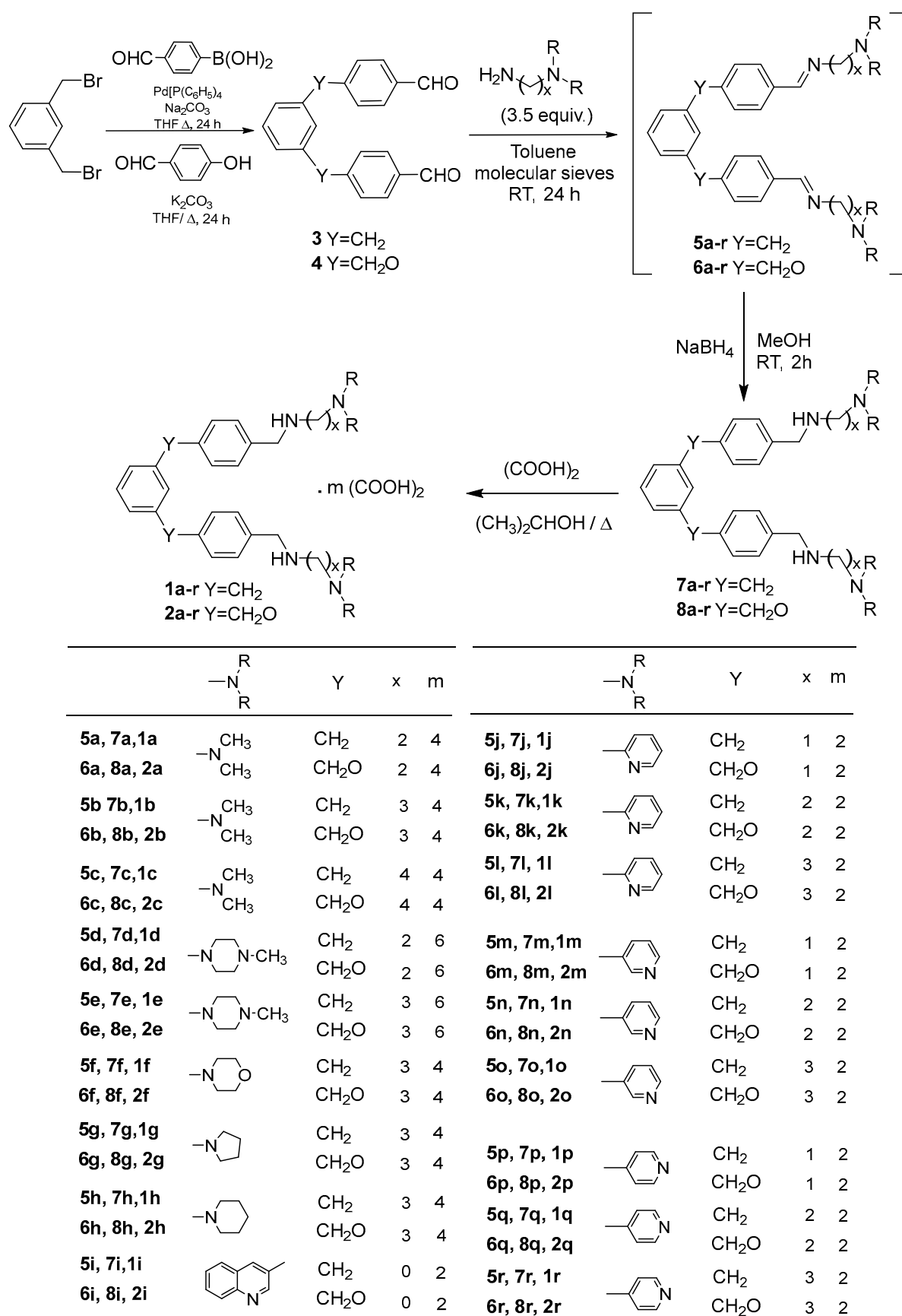
We anticipate that these novel, simplified, and extended aromatic structures will facilitate π – π stacking interactions with various G-quartets, which is the classical strategy and mechanism to stabilize G-quadruplexes.

We examine the in vitro antimalarial activities of these new derivatives against both the chloroquine-sensitive (3D7) and chloroquine-resistant (W2) strains of the malaria parasite *Plasmodium falciparum*. The in vitro cytotoxicity of our 1,3-bis[(4-(substituted-aminomethyl)-phenyl or -phenoxy)methyl]benzene derivatives (**1–2**) was assessed in human HepG2 cells. For each compound, we determine the index of selectivity and the ratio of cytotoxicity to antiprotozoal activity. Additionally, we investigate the potential of these novel polyaromatic compounds to stabilize specific telomeric DNA G-quadruplex structures in parasites. To evaluate this, we used a FRET melting assay to assess the stabilization of G-quadruplexes found in *Plasmodium falciparum* telomeric DNA.

2. Results & Discussion

2.1. Chemistry

These novel reported 1,3-bis[(4-(substituted-aminomethyl)phenyl)methyl]benzenes **1a–r** and 1,3-bis[(4-(substituted-aminomethyl)phenoxy)methyl]benzenes **2a–r** have both been synthesized starting from the commercially available 1,3-bis(bromomethyl)benzene (Scheme 1). The first intermediate 1,3-bis[(4-formylphenyl)methyl]benzene (**3**) was synthesized by a double-Suzuki–Miyaura cross-coupling reaction of 1,3-bis(bromomethyl)benzene with an excess of 4-formylphenylboronic acid in the presence of $Pd(PPh_3)_4$ as a catalyst and sodium carbonate, which was used as the base [37]. The synthesis of the second intermediate involved the preparation of 1,3-bis[(4-formylphenoxy)methyl]benzene **4** through a coupling reaction between 1,3-bis(bromomethyl)benzene and an excess of 4-hydroxybenzaldehyde in the presence of potassium carbonate in THF [38]. The structure of intermediate compound **4** was established by X-ray crystallography (Figure 3) [39].



Scheme 1. General procedure for the preparation of novel compounds 1–2a–r.

Then, the reaction of various primary substituted alkylaminoalkylamines with dialdehydes **3** or **4** resulted in the formation of 1,3-*bis*[(4-(substituted-iminomethyl)phenyl)methyl]benzenes **5a–r** and 1,3-*bis*[(4-(substituted-iminomethyl)phenoxy)methyl]benzenes **6a–r**, which were then both reduced into 1,3,5-tris[(4-(substituted-aminomethyl)phenyl)methyl]benzenes **7a–r** and 1,3-*bis*[(4-(substituted-aminomethyl)phenoxy)methyl]benzenes **8a–r**, respectively, using sodium borohydride in methanol [18,21–26]. These new polyamino derivatives, **7–8a–r**, were then converted into ammonium oxalate salts **1–2a–r** through a reaction with oxalic acid in reflux isopropanol. These novel derivatives **7–8a–r**, Figures S1(a–c) to S36(a–c) have been converted into their oxalate salts to acquire water solubility parameters for pharmacological testing. These oxalates were observed to be less hygroscopic than the hydrochloride ones and were also found soluble in water. Table 1 provides their physical properties.

Table 1. Physical properties of ammonium oxalate salts **1–2a–r**.

Compound		Salt ^a	mp (°C) ^b	% Yield ^c
1a	Beige crystals	4 (COOH) ₂	233–235	15.8
2a	Beige crystals	4 (COOH) ₂	247–249	42.5
1b	White crystals	4 (COOH) ₂	180–182	30
2b	Beige crystals	4 (COOH) ₂	245–247	68
1c	Beige crystals	4 (COOH) ₂	181–183	32
2c	White crystals	4 (COOH) ₂	185–187	67
1d	Beige crystals	6 (COOH) ₂	239–241	59
2d	Beige crystals	6 (COOH) ₂	237–239	78
1e	White crystals	6 (COOH) ₂	203–205	21
2e	White crystals	6 (COOH) ₂	243–245	31
1f	White crystals	4 (COOH) ₂	169–171	38
2f	White crystals	4 (COOH) ₂	211–213	52
1g	White crystals	4 (COOH) ₂	212–214	31
2g	White crystals	4 (COOH) ₂	218–220	48
1h	White crystals	4 (COOH) ₂	187–189	38
2h	White crystals	4 (COOH) ₂	214–216	63
1i	Yellow crystals	2 (COOH) ₂	138–140	30
2i	Yellow crystals	2 (COOH) ₂	133–135	49
1j	Yellow crystals	2 (COOH) ₂	213–215	44
2j	Yellow crystals	2 (COOH) ₂	209–211	61
1k	Yellow crystals	2 (COOH) ₂	191–193	42
2k	Yellow crystals	2 (COOH) ₂	181–183	79
1l	Yellow crystals	2 (COOH) ₂	171–173	52
2l	Yellow crystals	2 (COOH) ₂	167–169	75
1m	White crystals	2 (COOH) ₂	247–249	24
2m	Beige crystals	2 (COOH) ₂	243–245	58
1n	White crystals	2 (COOH) ₂	201–203	27.5
2n	Yellow crystals	2 (COOH) ₂	202–204	54
1o	Yellow crystals	2 (COOH) ₂	161–63	48
2o	Beige crystals	2 (COOH) ₂	165–167	62
1p	Green crystals	2 (COOH) ₂	153–155	32
2p	Green crystals	2 (COOH) ₂	207–209	50
1q	White crystals	2 (COOH) ₂	151–153	28
2q	Beige crystals	2 (COOH) ₂	143–145	87
1r	White crystals	2 (COOH) ₂	128–130	52
2r	Beige crystals	2 (COOH) ₂	141–143	80

^a The stoichiometry and composition of the salts were determined using elemental analyses, and obtained values were within ±0.4% of the theoretical values. ^b Crystallization solvent: 2-PrOH–H₂O. ^c The total yields included the conversions into the ammonium oxalates starting from commercially available 1,3-*bis*(bromomethyl)benzene.

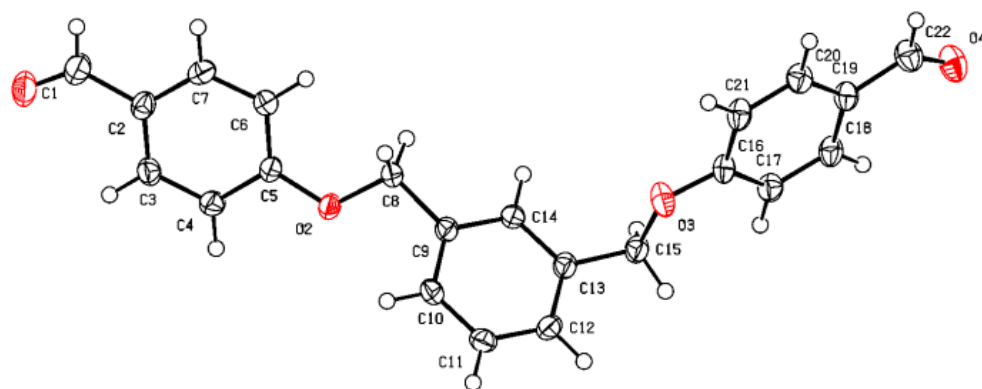


Figure 3. The ORTEP (Oak Ridge Thermal Ellipsoid Plot) drawing of 1,3-bis[(4-formylphenoxy)methyl]benzene **4** with atom labeling and thermal ellipsoids at 30% probability level. Hydrogen atoms are represented as small spheres of arbitrary radii.

2.2. Biological Evaluation

2.2.1. In Vitro Antimalarial Activity

All these novel 1,3-bis[(4-(substituted-aminomethyl)phenyl)methyl]benzenes, **1a–r**, and 1,3-bis[(4-(substituted-aminomethyl)phenoxy)methyl]benzenes, **2a–r**, were tested for their in vitro antimalarial activity by incubation with *P. falciparum* chloroquine (CQ)-resistant strain W2 (IC_{50} CQ = 0.40 μ M, IC_{50} mefloquine (MQ) = 0.016 μ M) and the strain 3D7, which is CQ-sensitive and has decreased sensitivity to MQ (IC_{50} CQ = 0.11 μ M, MQ = 0.06 μ M). The IC_{50} values of these new active 1,3-bis[(4-(substituted-aminomethyl)phenyl)methyl]benzenes, **1a–r**, and 1,3-bis[(4-(substituted-aminomethyl)phenoxy)methyl]benzenes, **2a–r**, were observed to be between 0.11 μ M and greater than 40 μ M against the CQ-resistant strain W2 and between 0.038 μ M and greater than 40 μ M against the 3D7 *P. falciparum* strains (Table 2).

In these novel series, we can observe that the 1,3-bis[(4-(substituted-aminomethyl)phenyl)methyl]benzene **1r**, bearing two pyridin-4-ylpropylaminomethyl side chains, was found to be the most active compound against the CQ-resistant W2 strain, with an IC_{50} of 0.11 μ M. 1,3-Bis[(4-(substituted-aminomethyl)phenyl)methyl]benzene derivative **1a–h**, bearing alkylaminoalkylaminomethyl side chains, were noticed to display better activities than their analogues, 1,3-bis[(4-(substituted-aminomethyl)phenoxy)methyl]benzenes **2a–h**, except for compounds **2e–d**, which were slightly more active than **1e–d**. While compound **1i** was found to be completely inactive, with an IC_{50} greater than 40 μ M, its homologue **2i** exhibits moderate activity, with an IC_{50} of 5.37 μ M.

From a general point of view, it is possible to say that the derivatives **1j–r** and **2j–r**, bearing two pyridinylalkylaminomethyl side chains, generally show a more interesting antimalarial activity than their homologue compounds **1a–h** and **2a–h**, which are substituted by the alkylaminoalkylaminomethyl groups; however, it remains difficult to establish truly successful structure–activity relationships.

Against the 3D7 CQ-sensitive strain, in the first subseries, the 1,3-bis[(4-(pyridin-4-ylpropylaminomethyl)phenyl)methyl]benzene **1r** was found to be the most active derivative, with an IC_{50} of 0.07 μ M, while the best antimalarial activities of the second subseries were observed for the two 1,3-bis[(4-(pyridinylethylaminomethyl)phenoxy)methyl]benzenes **2n** and **2q**, which had IC_{50} of 0.038 and 0.095 μ M against this 3D7 *P. falciparum* strain, respectively. These first results seem to indicate that a pyridinylethylaminomethyl)phenoxy)methyl substitution of the benzene ring (derivatives **2n** and **2q**) is more interesting than a methyl or propyl chain between the pyridine nucleus and the amino function in the side chains, i.e., compounds **2m**, **2o**, **2p**, and **2r**.

Table 2. In vitro sensitivity of *P. falciparum* strains to compounds **1–2a–r** and cytotoxicity of these derivatives in HepG2 cells.

Compound	<i>P. falciparum</i> Strains IC ₅₀ Values (μM) ^a		Cytotoxicity to HepG2 Cells CC ₅₀ Values (μM) ^b	Resistance Index	
	W2	3D7		W2 ^e	3D7 ^f
CQ ^c	0.40 ± 0.04	0.11 ± 0.01	30	3.64	0.28
MQ ^c	0.016 ± 0.002	0.06 ± 0.003	n.d. ^d	0.27	3.75
Quarflorin	n.d. ^d	0.114 ± 0.05 [29]	n.d. ^d	n.d. ^d	n.d. ^d
PDS	n.d. ^d	5.20 ± 0.90 [28]	n.d. ^d	n.d. ^d	n.d. ^d
1a	8.38 ± 1.06	n.d. ^d	0.87 ± 0.12	n.d. ^d	n.d. ^d
1b	1.98 ± 0.53	0.14 ± 0.07	3.17 ± 0.16	14.14	0.07
1c	1.61 ± 0.42	2.22 ± 0.42	18.45 ± 1.10	0.73	1.34
1d	2.12 ± 0.14	0.30 ± 0.05	0.28 ± 0.08	7.07	0.14
1e	>40	1.21 ± 0.43	5.23 ± 0.56	>33.0	0.03>
1f	1.21 ± 0.13	0.12 ± 0.06	71.38 ± 1.23	10.8	0.10
1g	1.81 ± 0.34	2.37 ± 0.40	2.60 ± 0.23	0.76	1.31
1h	0.72 ± 0.21	0.37 ± 0.09	20.89 ± 0.87	1.95	0.51
1i	>40	>40	>100	n.d.	n.d.
1j	2.83 ± 0.78	0.33 ± 0.06	>100	8.58	0.12
1k	1.47 ± 0.36	0.25 ± 0.10	7.54 ± 0.57	5.88	0.17
1l	0.66 ± 0.11	0.31 ± 0.06	0.05 ± 0.03	2.13	0.47
1m	1.22 ± 0.21	1.24 ± 0.15	>100	0.98	1.02
1n	4.91 ± 0.72	0.33 ± 0.07	18.17 ± 0.74	14.88	0.07
1o	7.35 ± 0.85	0.12 ± 0.06	22.65 ± 0.68	61.25	0.02
1p	1.75 ± 0.54	1.78 ± 0.41	18.91 ± 0.97	0.98	1.02
1q	0.36 ± 0.08	0.20 ± 0.05	15.60 ± 0.82	1.80	0.53
1r	0.11 ± 0.08	0.07 ± 0.02	2.46 ± 0.34	1.57	0.64
2a	>20	n.d. ^d	2.74 ± 0.46	n.d.	n.d.
2b	4.10 ± 0.81	3.86 ± 0.89	0.40 ± 0.10	1.06	0.94
2c	8.70 ± 1.03	2.40 ± 0.65	24.60 ± 0.74	3.63	0.28
2d	1.82 ± 0.72	0.70 ± 0.08	4.74 ± 0.23	2.60	0.38
2e	7.22 ± 0.92	n.d. ^d	20.92 ± 1.10	n.d.	n.d.
2f	2.00 ± 0.27	0.33 ± 0.08	0.05 ± 0.02	6.06	0.17
2g	11.22 ± 1.07	4.48 ± 0.39	2.60 ± 0.32	2.50	0.40
2h	1.35 ± 0.12	1.03 ± 0.07	4.51 ± 0.77	1.31	0.76
2i	5.37 ± 0.89	0.92 ± 0.25	>100	5.84	0.17
2j	1.28 ± 0.42	3.79 ± 0.60	56.16 ± 1.24	0.34	2.96
2k	0.56 ± 0.48	n.d. ^d	>100	n.d.	n.d.
2l	2.10 ± 0.11	3.76 ± 0.52	1.76 ± 0.24	0.56	1.79
2m	0.31 ± 0.04	0.48 ± 0.11	14.63 ± 0.65	0.64	1.55
2n	0.17 ± 0.06	0.038 ± 0.04	0.11 ± 0.05	4.47	0.22
2o	2.76 ± 0.24	>40	1.04 ± 0.15	0.07>	>14.49
2p	>40	0.32 ± 0.07	89.86 ± 1.31	>125	0.01>
2q	2.12 ± 0.09	0.095 ± 0.06	20.62 ± 1.12	22.31	0.04
2r	0.35 ± 0.08	0.52 ± 0.12	2.76 ± 0.35	0.67	1.48

^a Values were measured against CQ-resistant and mefloquine-sensitive strain W2 and the CQ-sensitive and decreased-MQ-sensitivity strain 3D7. The IC₅₀ (μM) values correspond to the means ± standard deviations from three independent experiments. ^b CC₅₀ values were measured against HepG2 cells. The CC₅₀ (μM) values correspond to the means ± standard deviations from three independent experiments. ^c CQ and MQ were used as antiparasitic compounds of reference. ^d n.d.: not determined. ^e IC₅₀ (W2)/IC₅₀ (3D7). ^f IC₅₀ (3D7)/IC₅₀ (W2).

The situation seems very complex in terms of SAR, but it is also possible to see that the aryl substitution of compound **1** (probably by its planarity) contributes to the activity and that the extension of the alkyl side chain from one to three carbon atoms further decreases the activity, with a propyl group being presented as optimal for series **1**. However, if we consider the pyridine compounds **1m,n,o**, a chain with a single carbon is presented as optimal. The trend is totally reversed if we consider the derivatives **1j,k,l**, which show an improvement in their antimalarial activity when the alkyl chain is increased. The position of the nitrogen atom also seems to play a role here, which could further contribute to an interaction. From this point of view, it seems that the positions of this pyridine nitrogen in meta and para present more interesting antimalarial activities against both strains than the compounds bearing the pyridine ring in ortho.

The resistance index values (Table 2), which represent the ratio of the IC_{50} of the resistant strain to that of the sensitive strain, were calculated based on the activities of a CQ-resistant strain (W2) and a strain with reduced sensitivity to MQ (3D7). For CQ, the W2/3D7 ratio is approximately ~3.64. Since W2 is resistant to CQ and 3D7 is sensitive to it, this ratio (~3.64) reflects the level of CQ resistance. Any ligand with a W2/3D7 ratio higher than 3.64 exhibits greater resistance to W2 than CQ, which suggests a potential shared mechanism of action. From the data in Table 2, it appears that several derivatives, **1b** (W2/3D7 ~ 14.14), **1d** (~7.07), **1f** (~10.8), **1j** (~8.58), **1n** (~14.88), **1o** (~61.25), **2f** (~6.06), **2i** (~5.84), **2n** (~4.47), and **2q** (~22.31), could all share the CQ mechanism of action. Likewise, for MQ, the 3D7/W2 ratio is around 3.75. Given that 3D7 is less susceptible to MQ, a derivative showing a 3D7/W2 ratio above 3.75 might act through a similar mechanism to that of MQ. Among the tested compounds, only compound **2o** exhibits this property (ratio 3D7/W2 > 14.49). In the vast majority, it is possible to observe that these new compounds, **1–2**, are more active against the 3D7 CQ-sensitive strain than against the CQ-resistant W2 strain. Additional studies are required to investigate their potential mode of action.

2.2.2. Cytotoxicity and Selectivity Index

In order to assess their selectivity of action, the cytotoxicities of our novel antiprotozoal 1,3-bis[(4-(substituted-aminomethyl)phenyl)methyl]benzenes, **1a–r**, and 1,3-bis[(4-(substituted-aminomethyl)phenoxy)methyl]benzenes, **2a–r**, were evaluated in vitro on the human cell line HepG2, a commonly used human-derived hepatocarcinoma cell line that expresses many hepatocyte-specific metabolic enzymes. This test not only highlights the potential cytotoxicity of a test compound, but can also detect the formation of toxic metabolites, although it does not allow their identification. This makes the HepG2 cell line particularly effective for assessing the in vitro cytotoxicity of new compounds. [40,41]. The 50% cytotoxic concentration (CC_{50}) values were determined and the selectivity index (SI), which represent the ratio between cytotoxic and antiparasitic activities ($SI = CC_{50}/IC_{50}$), were calculated (Table 3).

Our newly synthesized 1,3-bis[(4-(substituted-aminomethyl)phenyl)methyl]benzenes **1a–r** and 1,3-bis[(4-(substituted-aminomethyl)phenoxy)methyl]benzenes **2a–r** exhibited varying degrees of cytotoxicity against HepG2 cells, with CC_{50} values ranging from 0.05 μ M to over 100 μ M.

Concerning the *P. falciparum* strain W2, the calculated SIs were observed to be between 0.10 and a value superior to 178.6. For the CQ-sensitive strain 3D7, the SIs were observed to be from 0.026 to 594.8. Based on the selectivity index (SI) data, the 1,3-bis[(4-(3-(morpholin-1-yl)propyl)aminomethyl)phenoxy)methyl]benzene derivative **1f** was identified as the most promising antimalarial candidate, showing an SI of 594.8 against the *Plasmodium falciparum* 3D7 strain. Additionally, the 1,3-bis[(substituted aminomethyl)phenyl)methyl]benzene derivative **1j**, along with the 1,3-bis[(substituted aminomethyl)phenoxy)methyl]benzenes **2p** and **2q**, demonstrated strong antimalarial activity against the mefloquine-reduced sensitivity *Plasmodium falciparum* 3D7 strain, with selectivity index (SI) that exceeded 303 and were equal to 280 and 217, respectively. Moreover, the compound **2k**, a 1,3-bis[(4-(pyridin-2-ylethylaminomethyl)phenyl)methyl]benzene derivative, was identified as the most notable antimalarial candidate against the chloroquine-resistant *P. falciparum* W2 strain, displaying a selectivity index greater than 178.

Table 3. Selectivity Index of compounds **1–2a–r**.

Compound	Selectivity Index (SI) ^a	
	HepG2/W2	HepG2/3D7
CQ	75	272
1a	0.10	n.d. ^b
1b	1.60	22.64
1c	11.46	8.31
1d	0.13	0.93
1e	0.13>	4.32
1f	59.0	594.83
1g	1.44	1.10
1h	29.01	56.46
1i	n.d. ^b	n.d. ^b
1j	>35.33	>303.03
1k	5.13	30.16
1l	0.08	0.16
1m	>82.0	>80.6
1n	3.70	55.06
1o	3.08	188.8
1p	10.81	10.62
1q	43.33	78.0
1r	22.36	35.14
2a	n.d. ^b	n.d. ^b
2b	0.10	0.10
2c	2.83	10.25
2d	2.60	6.77
2e	2.90	n.d. ^b
2f	0.025	0.15
2g	0.23	0.58
2h	3.34	4.38
2i	>18.62	>108.7
2j	43.87	14.82
2k	>178.6	n.d. ^b
2l	0.84	0.47
2m	47.2	30.48
2n	0.65	2.89
2o	0.38	0.026>
2p	2.24>	280.81
2q	9.73	217.1
2r	7.89	5.31

^a SI was defined as the ratio between the CC₅₀ value on the HepG2 cells and the IC₅₀ value against the *P. falciparum* W2 or 3D7 strains. ^b n.d.: not determined.

The interesting selectivity index values suggest that the novel 1,3-bis[(4-(substituted-aminomethyl)phenyl)methyl]benzenes **1f** and **1j** and the 1,3-bis[(4-(substituted-aminomethyl)phenoxy)methyl]benzene derivatives **2k** and **2p–q** probably merit further investigation for their potential as antimalarial drug candidates.

2.3. FRET Melting Experiments

In *Plasmodium falciparum*, G-quadruplexes appear to be involved in the regulation of virulence genes and genome stability and could represent potential therapeutic targets, but their exact functional role remains largely to be characterized. However, given the potential of *P. falciparum* telomeres as promising targets for polyaromatic derivatives [22,29,32–36], we further investigated the ability of our biologically active compounds **1–2** to potentially stabilize *P. falciparum* telomeric chromosomal G-quadruplexes. This study was conducted

using a FRET melting assay. The goal was to evaluate how effectively the new polyaromatic derivatives **1–2** could stabilize G-quadruplex structures formed by oligonucleotides with sequences from both *P. falciparum* and human telomeres. Two fluorescently labeled *P. falciparum* telomeric chromosomal sequences (FPf1T and FPf8T) and one human telomeric sequence (F21T) were used in this assay.

To assess the G4 selectivity of our novel 1,3-bis[(4-(substituted-aminomethyl)phenyl)methyl]benzenes **1a–r** and 1,3-bis[(4-(substituted-aminomethyl)phenoxy)methyl]benzenes **2a–r** over duplex DNA, we performed a FRET melting assay using a duplex control sequence, FdxT. We also evaluated the reference G4 ligand PhenDC3 (inactive against the *P. falciparum* W2 and 3D7 strains, but active against the F32, FcB1, and K1 strains [29]), as well as the antimalarial reference drugs CQ and MQ. To obtain a meaningful comparison of selectivities, we calculated the difference (ΔT_m) in melting temperature (T_m) of the G-quadruplexes formed by FPf1T, FPf8T, F21T, or FdxT in the presence or absence of each selected derivative. The ΔT_m values are summarized in Table 4, which shows that the selected ligands from series **1–2** exhibited ΔT_m values ranging from 0.24 to 26.43 °C at a 2 μ M derivative concentration.

Table 4. FRET melting values for compounds **1–2a–r** (2 μ M) with FPf1T, FPf8T, F21T, and FdxT (0.2 μ M) in K⁺ conditions.

Compound	ΔT_m (°C) ^a	ΔT_m (°C) ^a	ΔT_m (°C) ^a	ΔT_m (°C) ^a
	FPf1T	FPf8T	F21T	FdxT
PhenDC3	21.92 ± 3.30	26.48 ± 1.66	22.28 ± 0.52	0.83 ± 0.04
CQ	1.90 ± 0.10	2.40 ± 1.20	2.40 ± 1.10	n.d. ^b
MQ	3.10 ± 0.50	6.60 ± 2.30	2.60 ± 0.50	n.d. ^b
1a	3.59 ± 0.42	5.22 ± 0.8	3.69 ± 0.44	0.53 ± 0.16
1b	11.38 ± 0.32	11.50 ± 0.73	10.40 ± 0.63	0.27 ± 0.12
1c	10.02 ± 0.23	10.55 ± 1.31	9.42 ± 0.39	0.26 ± 0.13
1d	0.24 ± 0.47	0.58 ± 0.43	0.26 ± 0.38	−0.04 ± 0.03
1e	8.34 ± 0.31	9.39 ± 0.13	8.58 ± 0.42	0.34 ± 0.11
1f	2.04 ± 0.25	3.27 ± 0.39	2.72 ± 0.07	0.19 ± 0.14
1g	10.82 ± 1.32	10.88 ± 0.32	10.32 ± 0.73	0.26 ± 0.12
1h	11.89 ± 0.31	13.01 ± 0.01	11.77 ± 0.52	0.19 ± 0.12
1i	−0.13 ± 0.01	−0.53 ± 0.33	−0.34 ± 0.11	0.16 ± 0.17
1j	5.83 ± 2.92	4.91 ± 1.26	3.27 ± 0.06	0.11 ± 0.08
1k	4.08 ± 0.65	7.10 ± 1.24	4.34 ± 1.24	0.17 ± 0.03
1l	4.97 ± 0.31	16.80 ± 1.01	13.82 ± 0.63	0.23 ± 0.27
1m	0.47 ± 0.03	0.53 ± 0.43	0.48 ± 0.25	0.31 ± 0.15
1n	3.55 ± 0.54	4.13 ± 0.77	3.74 ± 0.63	0.36 ± 0.13
1o	4.15 ± 0.42	4.50 ± 0.15	3.91 ± 0.19	−0.01 ± 0.11
1p	0.67 ± 0.20	0.64 ± 0.39	0.37 ± 0.26	0.04 ± 0.21
1q	2.72 ± 0.53	3.16 ± 0.16	2.07 ± 0.11	0.13 ± 0.05
1r	5.78 ± 0.72	6.37 ± 0.53	5.51 ± 0.44	0.18 ± 0.01
2a	4.09 ± 0.31	5.10 ± 0.13	4.32 ± 0.25	0.00 ± 0.17
2b	11.59 ± 0.22	10.91 ± 2.53	10.08 ± 0.52	0.39 ± 0.01
2c	23.35 ± 0.29	26.43 ± 0.14	21.23 ± 0.29	3.77 ± 0.23
2d	7.77 ± 0.42	9.57 ± 0.33	7.65 ± 0.43	0.09 ± 0.17
2e	9.28 ± 0.14	10.95 ± 0.034	9.22 ± 0.21	0.29 ± 0.07
2f	3.09 ± 0.22	3.57 ± 0.23	3.57 ± 0.54	0.19 ± 0.13
2g	10.31 ± 0.33	10.80 ± 1.14	9.99 ± 0.42	0.27 ± 0.23
2h	11.05 ± 0.62	12.63 ± 0.83	10.54 ± 0.43	0.20 ± 0.01
2i	−0.43 ± 0.09	−0.39 ± 0.08	−0.32 ± 0.32	0.14 ± 0.04
2j	3.78 ± 0.42	3.55 ± 0.58	4.06 ± 0.07	0.17 ± 0.03
2k	4.25 ± 0.30	5.41 ± 0.97	4.26 ± 0.25	0.06 ± 0.06
2l	5.88 ± 0.71	7.58 ± 0.80	4.58 ± 0.77	0.01 ± 0.04
2m	1.66 ± 0.42	2.49 ± 0.22	1.51 ± 0.15	0.08 ± 0.02
2n	4.30 ± 0.18	5.06 ± 0.52	4.39 ± 0.37	0.25 ± 0.06
2o	5.67 ± 0.43	6.10 ± 0.38	6.57 ± 0.28	0.10 ± 0.11
2p	0.67 ± 0.20	0.64 ± 0.39	0.37 ± 0.26	0.04 ± 0.21
2q	3.66 ± 0.42	5.15 ± 0.08	3.71 ± 0.67	0.09 ± 0.13
2r	7.02 ± 2.12	8.56 ± 0.83	7.84 ± 0.11	0.33 ± 0.12

^a ΔT_m of FPf1T, FPf8T, F21T, and FdxT (0.2 μ M) were recorded in 10 mM lithium cacodylate (pH 7.2), 10 mM KCl, and 90 mM LiCl. PhenDC3 was tested at 0.5 μ M, whereas CQ and MQ were tested at 1 μ M. Error margins correspond to SD of two replicates. ^b n.d.: not determined.

Only compound **2c** was found to be the best ligand of both series, as it stabilized the three G-quadruplex sequences (the two parasitic FPf1T and FPf8T, and the human F21T) with ΔT_m values ranging from 21.23 to 26.43 °C (Table 4).

On the other hand, all other ligands, whether they belong to the series F (1,3-bis[(4-(substituted-aminomethyl)phenyl)methyl]benzenes) or series G (1,3-bis[(4-(substituted-aminomethyl)phenoxy)methyl]benzenes), stabilize the FPf1T, FPf8T, and F21T G-quadruplexes with moderate or even relatively weak ΔT_m values, i.e., ranging from 0.24 to 11.89 °C, from 0.53 to 12.63 °C, and from 0.26 to 11.77 °C, respectively, on the FPf1T, FPf8T, and F21T sequences.

Moreover, in terms of structure–activity relationships, we noticed that only the polyaromatic compounds **1–2b**, **1c**, **1–2g**, and **1–2h**, respectively, substituted with 4-(3-dimethylaminopropyl)aminomethyl)phenyl(oxy)methyl, 4-(3-dimethylaminobutyl)aminomethyl)phenylmethyl, 4-(3-(pyrrolidin-1-yl)propyl)aminomethyl)phen(oxy)methyl, or 4-(3-(piperidin-1-yl)propyl)aminomethyl)phen(oxy)methyl side chains, moderately stabilize the FPf1T, FPf8T, and F21T G-quadruplexes. Thus, the ΔT_m values ranged from 10.02 to 13.01 °C at a 2 μ M ligand concentration. In addition, derivatives **1–2e**, bearing two 4-(3-(4-methylpiperazin-1-yl)propyl)aminomethyl)phen(oxy)methyl substituents, also showed moderate stabilization of the three FPf1T, FPf8T, and F21T G-quadruplexes (ΔT_m values found to be between 8.34 and 10.95 °C). No difference is observed in the stabilization of the *Plasmodium* telomeric sequences FPf1T and FPf8T G-quadruplexes and the human F21T G-quadruplex by our novel derivatives. Unfortunately, the 1,3-bis[(4-(pyridin-4-ylpropylaminomethyl)phenyl)methyl]benzene **1r** and 1,3-bis[(4-(pyridin-3-ylethylaminomethyl)phenoxy)methyl]benzene **2n**, which were found to be the most active derivatives against W2 and 3D7 *P. falciparum* strains, exhibited low stabilization of the two FPf1T and FPf8T sequences. Moreover, all of the 1,3-bis[(4-(substituted-aminomethyl)phenoxy)methyl]benzene compounds that were substituted by two pyridinylalkylaminomethyl chains (compounds **1–2j–r**) or which bore two quinolinylaminomethyl chains (derivatives **1–2i**) exhibited much lower stabilization profiles on the FPf1T and FPf8T *Plasmodium* telomeric sequences and also on the human one, F21T.

The radar plots presented in Figure 4 show that the compounds **1–2a–r** may be able to more or less moderately stabilize some of the G4-forming sequences.

No real binding to duplex DNA sequence was noted for all of our compounds when using FRET assays.

Interestingly, although some derivatives showed notable stabilization of protozoal G-quadruplex structures, these were not the most potent antimalarial agents. As a result, no clear correlation could be established between G-quadruplex stabilization and antimalarial activity within this compound series. It therefore appears unlikely that the antimalarial effects of 1,3-bis[(4-(substituted-aminomethyl)phenyl)methyl]benzenes **1** and 1,3-bis[(4-(substituted-aminomethyl)phenoxy)methyl]benzenes **2** are mediated through specific G-quadruplex binding mechanisms.

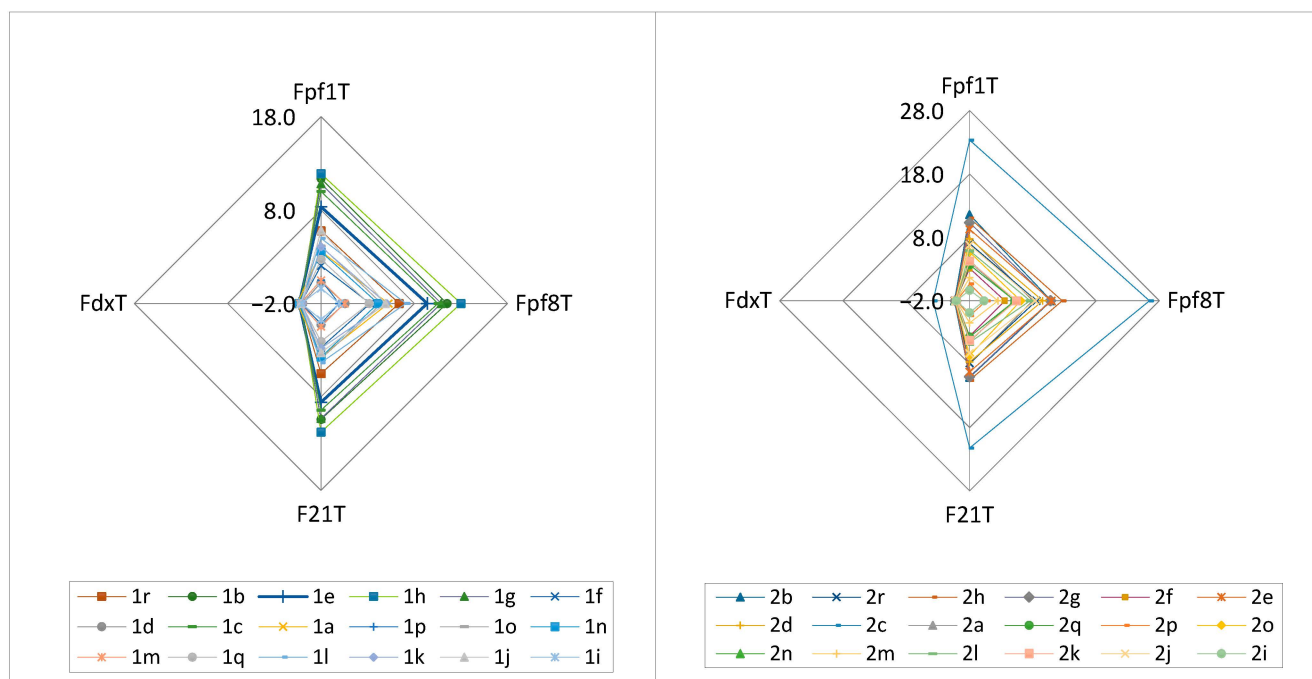


Figure 4. Stabilization specificity profile of **1-2a-r** (2 μ M) toward various G4 oligonucleotides. The difference in T_m in presence and absence of **1-2a-r**, ΔT_m , in $^{\circ}\text{C}$, is plotted for each sequence. Three quadruplexes and one duplex (FdxT) were tested.

3. Materials and Methods

3.1. Chemistry

3.1.1. General

Commercially available reagents were used without additional purification. Melting points were determined with an SM-LUX-POL Leitz hot-stage microscope (Leitz GMBH, Midland, ON, USA) and are uncorrected.

IR spectra were recorded on a NICOLET 380FT-IR spectrophotometer (Bruker BioSpin, Wissembourg, France). NMR spectra were recorded with tetramethylsilane as an internal standard using a BRUKER AVANCE 300 spectrometer (Bruker BioSpin, Wissembourg, France). Splitting patterns have been reported as follows: s = singlet; bs = broad singlet; d = doublet; t = triplet; q = quartet; dd = double doublet; ddd = double double doublet; qt = quintuplet; and m = multiplet.

Analytical TLCs were carried out on 0.25 precoated silica gel plates (POLYGRAM SIL G/UV254) (Merck KGaA, Darmstadt, Germany) and compounds were visualized after UV light irradiation. Silica gel 60 (70–230 mesh) was used for column chromatography. Mass spectra were recorded on an ESI LTQ Orbitrap Velos mass spectrometer (ThermoFisher, Bremen, Germany). Ionization was performed using an Electrospray ion source operating in positive ion mode with a capillary voltage of 3.80 kV and a capillary temperature of 250 $^{\circ}\text{C}$. The scan type analyzed was full scan, and all MS recordings were in the m/z range between 150 to 2000 m/z . No fragmentation was carried out and the resolution used for the analysis was 60,000.

3.1.2. Synthesis of 1,3-bis[(4-formylphenyl)methyl]benzene 3

To a suspension of α,α' -dibromo-*m*-xylene (2 mmol), 4-formylphenylboronic acid (5 mmol), and $\text{Pd}(\text{PPh}_3)_4$ (0.225 mmol) in THF (40 mL) under nitrogen were added 4 mL of a 2 M aqueous solution of Na_2CO_3 . The reaction mixture was refluxed for 24 h. The suspension was then evaporated to dryness and extracted with AcOEt (2×30 mL). The organic layer was filtered and washed with water (2×40 mL). The organic layer was

dried over sodium sulfate, filtered, and evaporated under reduced pressure. The crude residue was purified by column chromatography on silica gel using EtOAc/petroleum ether (*v/v*: 30/70) as eluent to give the pure product **2**. Colourless oil (59%): ^1H NMR (CDCl_3) δ ppm: 9.97 (s, 2H, CHO), 7.80 (d, 4H, $J = 8.10$ Hz, H-3_{phen} and H-5_{phen}), 7.29 (d, 4H, $J = 8.10$ Hz, H-2_{phen} and H-6_{phen}), 7.25 (t, 1H, $J = 7.2$ Hz, H_{benz}), 7.02 (s, 1H, H_{benz}), 7.05 (d, 2H, $J = 6.1$ Hz, H_{benz}), 3.92 (s, 4H, CH₂); ^{13}C NMR (CDCl_3) δ ppm: 193.3 (CO), 149.6 (C-1_{phen}), 141.6 (C-q_{benz}), 136.1 (C-4_{phen}), 131.4 (C-3_{phen} and C-5_{phen}), 130.9 (C-2_{phen} and C-6_{phen}), 130.43 (CH_{benz}), 129.4 (CH_{benz}), 128.6 (CH_{benz}), 43.4 (CH₂).

3.1.3. General Procedure for the Synthesis of 1,3-bis[(4-(substituted-iminomethyl)phenyl)methyl]benzenes **5a–r**

The 1,3-bis[(4-formylphenyl)methyl]benzene **3** (62 mg, 0.2 mmol) was dissolved in 6 mL of toluene. Activated molecular sieves 4 Å (800 mg) were then introduced in the reaction mixture, after which dialkylamine (0.5 mmol) was added. The reaction mixture was stirred in a stoppered flask for 24 h. The suspension that was obtained was filtered and washed with dichloromethane, and the solvent was removed under reduced pressure to afford the di-imine **5**. The crude products were then used without further purification.

1,3-bis[(4-(2-dimethylaminoethyl)iminomethyl)phenyl)methyl]benzene (**5a**)

Yellow oil (99%); ^1H NMR (CDCl_3) δ ppm: 8.28 (s, 2H, CH=N), 7.65 (d, 4H, $J = 8.10$ Hz, H-3_{phen} and H-5_{phen}), 7.22 (d, 4H, $J = 8.10$ Hz, H-2_{phen} and H-6_{phen}), 7.18 (m, 1H, H_{benz}), 7.03 (s, 2H, H_{benz}), 7.02 (s, 1H, H_{benz}), 3.96 (s, 4H, CH₂), 3.75 (t, 4H, $J = 6.90$ Hz, NCH₂), 2.67 (t, 4H, $J = 6.90$ Hz, NCH₂), 2.33 (s, 12H, N(CH₃)₂).

1,3-bis[(4-(3-dimethylaminopropyl)iminomethyl)phenyl)methyl]benzene (**5b**)

Yellow oil (98%); ^1H NMR (CDCl_3) δ ppm: 8.30 (s, 2H, CH=N), 7.70 (d, 4H, $J = 8.10$ Hz, H-3_{phen} and H-5_{phen}), 7.30 (d, 4H, $J = 8.10$ Hz, H-2_{phen} and H-6_{phen}), 7.18 (m, 1H, H_{benz}), 7.21 (s, 2H, H_{benz}), 7.20 (s, 1H, H_{benz}), 4.00 (s, 4H, CH₂), 3.68 (t, 4H, $J = 6.90$ Hz, NCH₂), 2.40 (t, 6H, $J = 6.90$ Hz, NCH₂), 2.18 (s, 12H, N(CH₃)₂), 1.92 (qt, 4H, $J = 8.40$ Hz, CH₂).

1,3-bis[(4-(4-dimethylaminobutyl)iminomethyl)phenyl)methyl]benzene (**5c**)

Yellow oil (98%); ^1H NMR (CDCl_3) δ ppm: 8.24 (s, 2H, CH=N), 7.64 (d, 4H, $J = 8.1$ Hz, H-3_{phen} and H-5_{phen}), 7.19 (d, 4H, $J = 8.10$ Hz, H-2_{phen} and H-6_{phen}), 7.18 (m, 1H, H_{benz}), 7.02 (s, 2H, H_{benz}), 7.01 (s, 1H, H_{benz}), 3.96 (s, 4H, CH₂), 3.62 (t, 4H, $J = 6.90$ Hz, NCH₂), 2.33 (t, 4H, $J = 6.90$ Hz, NCH₂), 2.24 (s, 12H, N(CH₃)₂), 1.72–1.69 (m, 4H, CH₂), 1.58–1.55 (m, 4H, CH₂).

1,3-bis[(4-(2-(4-methylpiperazin-1-yl)ethyl)iminomethyl)phenyl)methyl]benzene (**5d**)

Yellow oil (98%); ^1H NMR (CDCl_3) δ ppm: 8.25 (s, 2H, CH=N), 7.62 (d, 4H, $J = 8.10$ Hz, H-3_{phen} and H-5_{phen}), 7.20 (d, 4H, $J = 8.10$ Hz, H-2_{phen} and H-6_{phen}), 7.19 (m, 1H, H_{benz}), 6.99 (s, 2H, H_{benz}), 6.98 (s, 1H, H_{benz}), 3.94 (s, 4H, CH₂), 3.74 (t, 4H, $J = 6.0$ Hz, NCH₂), 2.72 (t, 4H, $J = 8.40$ Hz, NCH₂), 2.46 (s, 16H, NCH₂_{2pip}), 2.28 (s, 6H, NCH₃).

1,3-bis[(4-(3-(4-methylpiperazin-1-yl)propyl)iminomethyl)phenyl)methyl]benzene (**5e**)

Yellow oil (98%); ^1H NMR (CDCl_3) δ ppm: 8.25 (s, 2H, CH=N), 7.64 (d, 4H, $J = 8.10$ Hz, H-3_{phen} and H-5_{phen}), 7.27 (d, 4H, $J = 8.10$ Hz, H-2_{phen} and H-6_{phen}), 7.28 (m, 1H, H_{benz}), 7.03 (s, 2H, H_{benz}), 7.01 (s, 1H, H_{benz}), 3.96 (s, 4H, CH₂), 3.63 (t, 4H, $J = 6.00$ Hz, NCH₂), 2.48 (t, 4H, $J = 6.00$ Hz, NCH₂), 2.33 (s, 16H, NCH₂_{2pip}), 2.28 (s, 6H, NCH₃), 1.90–1.88 (m, 4H, CH₂).

1,3-bis[(4-(3-(morpholin-1-yl)propyl)iminomethyl)phenyl)methyl]benzene (**5f**)

Yellow oil (99%); ^1H NMR (CDCl_3) δ ppm: 8.24 (s, 2H, CH=N), 7.62 (d, 4H, $J = 8.10$ Hz, H-3_{phen} and H-5_{phen}), 7.19 (d, 4H, $J = 8.10$ Hz, H-2_{phen} and H-6_{phen}), 7.20 (s, 1H, H_{benz}), 7.01 (s, 2H, H_{benz}), 6.98 (s, 1H, H_{benz}), 3.94 (s, 4H, CH₂), 3.69 (t, 8H, $J = 4.20$ Hz, OCH₂), 3.58 (t, 4H, $J = 6.9$ Hz, NCH₂), 2.39–2.28 (m, 10H, NCH₂ and NCH₂_{2morph}), 1.83 (qt, 4H, $J = 6.90$ Hz, CH₂).

1,3-bis[(4-(3-(pyrrolidin-1-yl)propyl)iminomethyl)phenyl)methyl]benzene (**5g**)

Yellow oil (98%); ^1H NMR (CDCl_3) δ ppm: 8.24 (s, 2H, $\text{CH}=\text{N}$), 7.62 (d, 4H, $J = 8.1$ Hz, H-3_{phen} , and H-5_{phen}), 7.20 (d, 4H, $J = 8.10$ Hz, H-2_{phen} , and H-6_{phen}), 7.19 (s, 1H, H_{benz}), 7.01 (s, 2H, H_{benz}), 6.98 (s, 1H, H_{benz}), 3.95 (s, 4H, CH_2), 3.64 (t, 4H, $J = 6.60$ Hz, NCH_2), 2.54–2.47 (m, 12H, NCH_2 and $\text{NCH}_2_{\text{pyrrol}}$), 1.94–1.92 (m, 4H, $J = 6.90$ Hz, CH_2), 1.79–1.76 (m, 8H, $\text{CH}_2_{\text{pyrrol}}$).

1,3-bis[(4-(3-(piperidin-1-yl)propyl)iminomethyl)phenyl)methyl]benzene (**5h**)

Yellow oil (99%); ^1H NMR (CDCl_3) δ ppm: 8.23 (s, 2H, $\text{CH}=\text{N}$), 7.62 (d, 4H, $J = 8.10$ Hz, H-3_{phen} , and H-5_{phen}), 7.19 (s, 1H, H_{benz}), 7.18 (d, 4H, $J = 8.10$ Hz, H-2_{phen} , and H-6_{phen}), 7.01 (s, 2H, H_{benz}), 6.98 (s, 1H, H_{benz}), 3.94 (s, 4H, CH_2), 3.60 (t, 4H, $J = 6.90$ Hz, NCH_2), 2.42–2.31 (m, 12H, NCH_2 and $\text{NCH}_2_{\text{pip}}$), 1.62–1.54 (m, 16H, CH_2 and CH_2_{pip}).

1,3-bis[(4-((quinolin-3-yl)iminomethyl)phenyl)methyl]benzene (**5i**)

Pale-yellow oil (98%); ^1H NMR (CDCl_3) δ ppm: 8.92 (d, 2H, $J = 2.70$ Hz, $\text{H-2}_{\text{quinol}}$), 8.59 (s, 2H, $\text{CH}=\text{N}$), 8.15–8.12 (m, 2H, $\text{H-8}_{\text{quinol}}$), 8.02–7.99 (m, 2H, $\text{H-5}_{\text{quinol}}$), 7.97 (d, 2H, $J = 2.70$ Hz, $\text{H-4}_{\text{quinol}}$), 7.89 (d, 4H, $J = 7.80$ Hz, H-3_{phen} , and H-5_{phen}), 7.50–7.39 (m, 4H, $\text{H-6}_{\text{quinol}}$, and $\text{H-7}_{\text{quinol}}$), 7.23 (d, 4H, $J = 7.80$ Hz, H-2_{phen} , and H-6_{phen}), 7.19 (s, 1H, H_{benz}), 7.01 (s, 2H, H_{benz}), 6.98 (s, 1H, H_{benz}), 4.05 (s, 4H, CH_2).

1,3-bis[(4-(pyridin-2-yl)methyliminomethyl)phenyl)methyl]benzene (**5j**)

Pale-yellow oil (93%); ^1H NMR (CDCl_3) δ ppm: 8.58 (d, 2H, $J = 6.90$ Hz, $\text{H-6}_{\text{pyrid}}$), 8.45 (s, 2H, $\text{CH}=\text{N}$), 7.75–7.67 (m, 6H, H-3_{phen} , H-5_{phen} and $\text{H-4}_{\text{pyrid}}$), 7.42 (d, 2H, $J = 6.90$ Hz, $\text{H-5}_{\text{pyrid}}$), 7.25–7.17 (m, 7H, H-2_{phen} , H-6_{phen} , $\text{H-3}_{\text{pyrid}}$ and H_{benz}), 7.04 (s, 2H, H_{benz}), 7.01 (s, 1H, H_{benz}), 4.95 (s, 4H, NCH_2), 3.98 (s, 4H, CH_2).

1,3-bis[(4-(pyridin-2-ylethyliminomethyl)phenyl)methyl]benzene (**5k**)

Yellow oil (97%); ^1H NMR (CDCl_3) δ ppm: 8.55 (d, 2H, $J = 5.90$ Hz, $\text{H-6}_{\text{pyrid}}$), 8.17 (s, 2H, $\text{CH}=\text{N}$), 7.61–7.54 (m, 6H, H-3_{phen} , H-5_{phen} , and $\text{H-4}_{\text{pyrid}}$), 7.20–7.09 (m, 9H, $J = 8.10$ Hz, H-2_{phen} , H-6_{phen} , $\text{H-3}_{\text{pyrid}}$, H_{benz} , and $\text{H-5}_{\text{pyrid}}$), 7.01 (s, 2H, H_{benz}), 7.00 (s, 1H, H_{benz}), 4.00 (t, 4H, $J = 7.20$ Hz, NCH_2), 3.94 (s, 4H, CH_2), 3.18 (t, 4H, $J = 7.20$ Hz, $\text{CH}_2_{\text{Pyrid}}$).

1,3-bis[(4-(pyridin-2-ylpropyliminomethyl)phenyl)methyl]benzene (**5l**)

Yellow oil (97%); ^1H NMR (CDCl_3) δ ppm: 8.54 (d, 2H, $J = 5.80$ Hz, $\text{H-6}_{\text{pyrid}}$), 8.25 (s, 2H, $\text{CH}=\text{N}$), 7.61–7.54 (m, 6H, H-3_{phen} , H-5_{phen} , and $\text{H-4}_{\text{pyrid}}$), 7.23–7.17 (m, 8H, $J = 8.10$ Hz, H-2_{phen} , H-6_{phen} , $\text{H-3}_{\text{pyrid}}$, and $\text{H-5}_{\text{pyrid}}$), 7.10 (m, 1H, H_{benz}), 7.03 (s, 2H, H_{benz}), 7.01 (s, 1H, H_{benz}), 3.96 (s, 4H, CH_2), 3.67 (t, 4H, $J = 6.60$ Hz, NCH_2), 2.88 (t, 4H, $J = 6.60$ Hz, $\text{CH}_2_{\text{Pyrid}}$), 2.16 (qt, 4H, $J = 6.60$ Hz, CH_2).

1,3-bis[(4-(pyridin-3-yl)methyliminomethyl)phenyl)methyl]benzene (**5m**)

Pale-yellow oil (98%); ^1H NMR (CDCl_3) δ ppm: 8.61 (d, 2H, $J = 2.20$ Hz, $\text{H-2}_{\text{pyrid}}$), 8.54 (dd, 2H, $J = 4.80$ and 1.60 Hz, $\text{H-6}_{\text{pyrid}}$), 8.40 (s, 2H, $\text{CH}=\text{N}$), 7.72–7.68 (m, 6H, H-3_{phen} , H-5_{phen} and $\text{H-4}_{\text{pyrid}}$), 7.28–7.22 (m, 7H, H-2_{phen} , H-6_{phen} , H_{benz} , and $\text{H-5}_{\text{pyrid}}$), 7.04 (s, 2H, H_{benz}), 7.03 (s, 1H, H_{benz}), 4.80 (s, 4H, NCH_2), 3.98 (s, 4H, CH_2).

1,3-bis[(4-(pyridin-3-ylethyliminomethyl)phenyl)methyl]benzene (**5n**)

Yellow oil (99%); ^1H NMR (CDCl_3) δ ppm: 8.52–8.44 (m, 4H, $\text{H-2}_{\text{pyrid}}$ and $\text{H-6}_{\text{pyrid}}$), 8.13 (s, 2H, $\text{CH}=\text{N}$), 7.63 (d, 4H, $J = 7.80$ Hz, H-3_{phen} , and H-5_{phen}), 7.53–7.54 (d, 2H, $J = 7.80$ Hz, $\text{H-4}_{\text{pyrid}}$), 7.27–7.20 (m, 7H, H-2_{phen} , H-6_{phen} , H_{benz} , and $\text{H-5}_{\text{pyrid}}$), 7.05 (s, 2H, H_{benz}), 7.02 (s, 1H, H_{benz}), 3.97 (s, 4H, CH_2), 3.85 (t, 2H, $J = 7.20$ Hz, NCH_2), 3.02 (t, 4H, $J = 7.20$ Hz, $\text{CH}_2_{\text{Pyrid}}$).

1,3-bis[(4-(pyridin-3-ylpropyliminomethyl)phenyl)methyl]benzene (**5o**)

Yellow oil (91%); ^1H NMR (CDCl_3) δ ppm: 8.52 (d, 2H, $J = 2.20$ Hz, $\text{H-2}_{\text{pyrid}}$), 8.49 (dd, 2H, $J = 4.80$ and 1.60 Hz, $\text{H-6}_{\text{pyrid}}$), 8.28 (s, 2H, $\text{CH}=\text{N}$), 7.70 (d, 4H, $J = 8.10$ Hz, H-3_{phen} , and H-5_{phen}), 7.56–7.53 (m, 2H, $\text{H-4}_{\text{pyrid}}$), 7.32–7.18 (m, 7H, $\text{H-5}_{\text{pyrid}}$, H_{benz} , H-2_{phen} , and H-6_{phen}), 7.04 (s, 2H, H_{benz}), 7.03 (s, 1H, H_{benz}), 4.01 (s, 4H, CH_2), 3.65 (t, 4H, $J = 6.60$ Hz, NCH_2), 2.74 (t, 4H, $J = 6.60$ Hz, $\text{CH}_2_{\text{Pyrid}}$), 2.08 (qt, 4H, $J = 6.60$ Hz, CH_2).

1,3-bis[(4-(pyridin-4-ylmethyliminomethyl)phenyl)methyl]benzene (5p)

Pale-yellow oil (99%); ^1H NMR (CDCl_3) δ ppm: 8.54 (d, 4H, $J = 5.40$ Hz, $\text{H-2}_{\text{pyrid}}$, and $\text{H-6}_{\text{pyrid}}$), 8.37 (s, 2H, CH=N), 7.72 (d, 4H, $J = 8.10$ Hz, H-3_{phen} , and H-5_{phen}), 7.28–7.23 (m, 9H, H-2_{phen} , H-6_{phen} , H_{benz} , $\text{H-3}_{\text{pyrid}}$, and $\text{H-5}_{\text{pyrid}}$), 7.04 (s, 2H, H_{benz}), 7.03 (s, 1H, H_{benz}), 4.78 (s, 4H, NCH_2), 3.98 (s, 4H, CH_2).

1,3-bis[(4-(pyridin-4-ylethyliminomethyl)phenyl)methyl]benzene (5q)

Yellow oil (97%); ^1H NMR (CDCl_3) δ ppm: 8.50–8.48 (m, 4H, $\text{H-2}_{\text{pyrid}}$, and $\text{H-6}_{\text{pyrid}}$), 8.12 (s, 2H, CH=N), 7.62 (d, 4H, $J = 8.20$ Hz, H-3_{phen} , and H-5_{phen}), 7.20 (d, 4H, $J = 8.20$ Hz, H-2_{phen} , and H-6_{phen}), 7.16–7.14 (m, 5H, $\text{H-3}_{\text{pyrid}}$, and $\text{H-5}_{\text{pyrid}}$, H_{benz}), 7.03 (s, 2H, H_{benz}), 7.02 (s, 1H, H_{benz}), 3.96 (s, 4H, CH_2), 3.85 (t, 4H, $J = 7.20$ Hz, NCH_2), 3.00 (t, 4H, $J = 7.20$ Hz, CH_2Pyrid).

1,3-bis[(4-(pyridin-4-ylpropyliminomethyl)phenyl)methyl]benzene (5r)

Yellow oil (98%); ^1H NMR (CDCl_3) δ ppm: 8.50 (d, 4H, $J = 5.40$ Hz, $\text{H-2}_{\text{pyrid}}$, and $\text{H-6}_{\text{pyrid}}$), 8.25 (s, 2H, CH=N), 7.68 (d, 4H, $J = 7.80$ Hz, H-3_{phen} , and H-5_{phen}), 7.28–7.24 (m, 4H, $\text{H-3}_{\text{pyrid}}$, and $\text{H-5}_{\text{pyrid}}$), 7.18 (s, 1H, H_{benz}), 7.15 (d, 4H, $J = 8.20$ Hz, H-2_{phen} , and H-6_{phen}), 7.06 (s, 2H, H_{benz}), 7.05 (s, 1H, H_{benz}), 3.99 (s, 4H, CH_2), 3.64 (t, 4H, $J = 7.20$ Hz, NCH_2), 2.72 (t, 4H, $J = 7.20$ Hz, CH_2Pyrid), 2.07 (qt, 6H, $J = 7.20$ Hz, CH_2).

3.1.4. General Procedure for the Synthesis of 1,3-bis[(4-(Substituted-Aminomethyl)Phenyl)Methyl]Benzenes 7a–r

To a solution of compounds 5a–r (0.4 mmol) in methanol (10 mL) was added, portion-wise at 0°C , sodium borohydride (2.4 mmol, 6 eq.). The reaction mixture was then stirred at room temperature for 2 h. Then, it was evaporated to dryness under reduced pressure. After cooling, the residue was triturated in water and extracted with dichloromethane (40 mL). The organic layer was separated, dried over sodium sulfate and activated charcoal, and evaporated to dryness. The residue was then purified by column chromatography on silica gel using dichloromethane/methanol (90/10: v/v) as eluent to give the pure products 7a–r.

1,3-bis[(4-(2-dimethylaminoethyl)aminomethyl)phenyl)methyl]benzene (7a)

Yellow oil (44%); ^1H NMR (CDCl_3) δ ppm: 7.38 (m, 1H, H_{benz}), 7.23 (d, 4H, $J = 8.10$ Hz, H-3_{phen} , and H-5_{phen}), 7.11 (d, 4H, $J = 8.10$ Hz, H-2_{phen} , and H-6_{phen}), 7.01 (s, 2H, H_{benz}), 6.98 (s, 1H, H_{benz}), 3.92 (s, 4H, CH_2), 3.78 (s, 4H, NCH_2), 2.70 (t, 4H, $J = 6.90$ Hz, NCH_2), 2.43 (t, 4H, $J = 6.90$ Hz, NCH_2), 2.21 (s, 12H, $\text{N}(\text{CH}_3)_2$); ^{13}C NMR (CDCl_3) δ ppm: 141.29 ($\text{C-4}_{\text{phenyl}}$), 141.17 (C_{qbenz}), 137.81 ($\text{C-1}_{\text{phenyl}}$), 130.24 (C-3_{phen} and C-5_{phen}), 129.65 (C-2_{phen} and C-6_{phen}), 128.81 (CH_{benz}), 126.66 (CH_{benz}), 58.90 (NCH_2), 53.66 (NCH_2), 46.47 (NCH_2), 45.44 ($\text{N}(\text{CH}_3)_2$), 41.53 (CH_2). ESI-MS m/z $[\text{M}+\text{H}]^+$ calculated for $\text{C}_{30}\text{H}_{43}\text{N}_4$: 459.3488, found: 459.3474.

1,3-bis[(4-(3-dimethylaminopropyl)aminomethyl)phenyl)methyl]benzene (7b)

Yellow oil (51%); ^1H NMR (CDCl_3) δ ppm: 7.25 (m, 1H, H_{benz}), 7.20 (d, 4H, $J = 8.10$ Hz, H-3_{phen} , and H-5_{phen}), 7.12 (d, 4H, $J = 8.10$ Hz, H-2_{phen} , and H-6_{phen}), 6.98 (m, 3H, H_{benz}), 3.95 (s, 4H, CH_2), 3.76 (s, 4H, NCH_2), 2.68 (t, 4H, $J = 7.10$ Hz, NCH_2), 2.33 (t, 4H, $J = 7.10$ Hz, NCH_2), 2.22 (s, 12H, $\text{N}(\text{CH}_3)_2$), 1.69 (qt, 4H, $J = 7.10$ Hz, CH_2); ^{13}C NMR (CDCl_3) δ ppm: 142.60 ($\text{C-4}_{\text{phenyl}}$), 141.13 (C_{qbenz}), 139.41 ($\text{C-1}_{\text{phenyl}}$), 130.94 (CH_{benz}), 130.29 (C-3_{phen} and C-5_{phen}), 129.91 (CH_{benz}), 129.61 (C-2_{phen} and C-6_{phen}), 128.84 (CH_{benz}), 59.40 (NCH_2), 55.10 (NCH_2), 49.22 (NCH_2), 46.90 ($\text{N}(\text{CH}_3)_2$), 42.91 (CH_2), 29.32 (CH_2). ESI-MS m/z $[\text{M} + 2\text{H}]^+$ calculated for $\text{C}_{32}\text{H}_{47}\text{N}_4$: 487.3722, found: 487.3784.

1,3-bis[(4-(4-dimethylaminobutyl)aminomethyl)phenyl)methyl]benzene (7c)

Yellow oil (85%); ^1H NMR (CDCl_3) δ ppm: 7.22 (d, 4H, $J = 7.90$ Hz, H-3_{phen} , and H-5_{phen}), 7.17 (m, 1H, H_{benz}), 7.13 (d, 4H, $J = 7.90$ Hz, H-2_{phen} , and H-6_{phen}), 7.00 (m, 3H, H_{benz}), 3.91 (s, 4H, CH_2), 3.75 (s, 4H, NCH_2), 2.65 (t, 4H, $J = 6.90$ Hz, NCH_2), 2.24 (t, 4H, $J = 6.90$ Hz, NCH_2), 2.20 (s, 12H, $\text{N}(\text{CH}_3)_2$), 1.54–1.51 (m, 8H, CH_2); ^{13}C NMR

(CDCl₃) δ ppm: 142.61 (C-4_{phenyl}), 141.30 (Cq_{benz}), 138.97 (C-1_{phenyl}), 130.35 (C-3_{phen} and C-5_{phen}), 130.95 (CH_{benz}), 129.92 (C-2_{phen} and C-6_{phen}), 128.41 (CH_{benz}), 128.06 (CH_{benz}), 60.88 (NCH₂), 54.87 (NCH₂), 50.49 (NCH₂), 46.87 (N(CH₃)₂), 42.91 (CH₂), 29.14 (CH₂), 26.77 (CH₂). ESI-MS m/z [M + H]⁺ calculated for C₃₄H₅₁N₄: 515.4035, found: 515.5112.

1,3-bis[(4-(2-(4-methylpiperazin-1-yl)ethyl)aminomethyl)phenyl)methyl]benzene (**7d**)

Yellow oil (61%); ¹H NMR (CDCl₃) δ ppm: 7.21 (d, 4H, J = 8.10 Hz, H-3_{phen} and H-5_{phen}), 7.14 (m, 1H, H_{benz}), 7.13 (d, 4H, J = 8.10 Hz, H-2_{phen} and H-6_{phen}), 7.03 (m, 3H, H_{benz}), 3.90 (s, 4H, CH₂), 3.64 (s, 4H, NCH₂), 2.46 (t, 4H, J = 6.90 Hz, NCH₂), 2.50 (t, 4H, J = 6.90 Hz, NCH₂), 2.48–2.35 (m, 16H, NCH₂₂piperazine), 2.27 (s, 6H, NCH₃); ¹³C NMR (CDCl₃) δ ppm: 142.65 (C-4_{phenyl}), 141.17 (Cq_{benz}), 139.39 (C-1_{phenyl}), 130.92 (CH_{benz}), 130.29 (C-3_{phen} and C-5_{phen}), 129.58 (C-2_{phen} and C-6_{phen}), 128.38 (CH_{benz}), 128.03 (CH_{benz}), 58.98 (NCH₂), 56.30 (NCH₂₂piperazine), 54.97 (NCH₂), 54.44 (NCH₂₂piperazine), 47.37 (NCH₃), 46.90 (NCH₂), 42.90 (CH₂). ESI-MS m/z [M + H]⁺ calculated for C₃₆H₅₃N₆: 569.4253, found: 569.43.

1,3-bis[(4-(3-(4-methylpiperazin-1-yl)propyl)aminomethyl)phenyl)methyl]benzene (**7e**)

Yellow oil (75%); ¹H NMR (CDCl₃) δ ppm: 7.20 (d, 4H, J = 8.10 Hz, H-3_{phen} and H-5_{phen}), 7.16 (m, 3H, H_{benz}), 7.12 (d, 4H, J = 8.10 Hz, H-2_{phen} and H-6_{phen}), 6.99 (m, 3H, H_{benz}), 3.91 (s, 4H, CH₂), 3.72 (s, 4H, NCH₂), 2.65 (t, 4H, J = 7.10 Hz, NCH₂), 2.37 (t, 4H, J = 7.10 Hz, NCH₂), 2.41–2.34 (m, 16H, NCH₂₂piperazine), 2.25 (s, 6H, NCH₃), 1.72 (qt, 4H, J = 7.10 Hz, CH₂); ¹³C NMR (CDCl₃) δ ppm: 142.59 (C-4_{phenyl}), 141.10 (Cq_{benz}), 139.35 (C-1_{phenyl}), 130.90 (CH_{benz}), 130.25 (C-3_{phen} and C-5_{phen}), 130.26 (CH_{benz}), 129.55 (C-2_{phen} and C-6_{phen}), 128.07 (CH_{benz}), 59.10 (NCH₂), 56.45 (NCH₂₂piperazine), 55.05 (NCH₂), 54.55 (NCH₂₂piperazine), 49.46 (NCH₂), 47.39 (NCH₃), 42.88 (CH₂), 28.20 (CH₂). ESI-MS m/z [M + H]⁺ calculated for C₃₈H₅₇N₆: 597.456, found: 597.4630.

1,3-bis[(4-(3-(morpholin-1-yl)propyl)aminomethyl)phenyl)methyl]benzene (**7f**)

Yellow oil (90%); ¹H NMR (CDCl₃) δ ppm: 7.40 (m, 1H, H_{benz}), 7.27 (d, 4H, J = 8.10 Hz, H-3_{phen} and H-5_{phen}), 7.11 (d, 4H, J = 8.10 Hz, H-2_{phen} and H-6_{phen}), 6.99 (m, 3H, H_{benz}), 3.93 (s, 4H, CH₂), 3.71 (s, 4H, NCH₂), 3.69 (t, 8H, J = 4.65 Hz, OCH₂), 2.69 (t, 4H, J = 6.90 Hz, NCH₂), 2.44–2.37 (m, 12H, NCH₂₂morpholine, NCH₂), 1.71 (qt, 4H, J = 6.90 Hz, CH₂); ¹³C NMR (CDCl₃) δ ppm: 142.64 (C-4_{phenyl}), 141.23 (Cq_{benz}), 139.30 (C-1_{phenyl}), 130.94 (CH_{benz}), 130.32 (C-3_{phen} and C-5_{phen}), 130.01 (CH_{benz}), 129.94 (C-2_{phen} and C-6_{phen}), 128.82 (CH_{benz}), 68.35 (OCH₂), 58.76 (NCH₂), 55.16 (NCH₂₂morpholine), 55.04 (NCH₂), 49.35 (NCH₂), 42.82 (CH₂), 27.94 (CH₂). ESI-MS m/z [M + H]⁺ calculated for C₃₆H₅₁N₄O₂: 571.3934, found: 571.40.

1,3-bis[(4-(3-(pyrrolidin-1-yl)propyl)aminomethyl)phenyl)methyl]benzene (**7g**)

Yellow oil (84%); ¹H NMR (CDCl₃) δ ppm: 7.24 (m, 1H, H_{benz}), 7.20 (d, 4H, J = 8.10 Hz, H-3_{phen} and H-5_{phen}), 7.17 (d, 4H, J = 8.10 Hz, H-2_{phen} and H-6_{phen}), 7.05 (m, 3H, H_{benz}), 3.92 (s, 4H, CH₂), 3.76 (s, 4H, NCH₂), 2.70 (t, 4H, J = 6.90 Hz, NCH₂), 2.53–2.48 (m, 12H, NCH₂ and NCH₂₂pyrrolidine), 1.79–1.72 (m, 12H, CH₂ and CH₂₂pyrrolidine); ¹³C NMR (CDCl₃) δ ppm: 141.29 (C-4_{phenyl}), 139.74 (Cq_{benz}), 138.11 (C-1_{phenyl}), 129.58 (CH_{benz}), 128.92 (C-3_{phen} and C-5_{phen}), 128.55 (CH_{benz}), 128.21 (C-2_{phen} and C-6_{phen}), 126.67 (CH_{benz}), 54.77 (NCH₂), 54.25 (NCH₂₂pyrrolidine), 53.67 (NCH₂), 48.07 (NCH₂), 41.55 (CH₂), 29.19 (CH₂), 23.44 (CH₂₂pyrrolidine). ESI-MS m/z [M + H]⁺ calculated for C₃₆H₅₁N₄: 539.035, found: 539.41.

1,3-bis[(4-(3-(piperidin-1-yl)propyl)aminomethyl)phenyl)methyl]benzene (**7h**)

Yellow oil (95%); ¹H NMR (CDCl₃) δ ppm: 7.24 (m, 1H, H_{benz}), 7.20 (d, 4H, J = 8.10 Hz, H-3_{phen} and H-5_{phen}), 7.17 (d, 4H, J = 8.10 Hz, H-2_{phen} and H-6_{phen}), 7.01–6.98 (m, 3H, H_{benz}), 3.92 (s, 4H, CH₂), 3.75 (s, 4H, NCH₂), 2.69 (t, 4H, J = 6.90 Hz, NCH₂), 2.39–2.32 (m, 12H, NCH₂ and NCH₂₂piperidine), 1.71 (qt, 4H, J = 6.90 Hz, CH₂), 1.61–1.50 (m, 8H, CH₂₂piperidine), 1.44–1.40 (m, 4H, CH₂₂piperidine); ¹³C NMR (CDCl₃) δ ppm: 142.67 (C-4_{phenyl}), 141.11 (Cq_{benz}), 139.45 (C-1_{phenyl}), 130.95 (CH_{benz}), 130.30 (C-3_{phen} and C-5_{phen}), 129.93 (CH_{benz}), 129.61 (C-2_{phen} and C-6_{phen}), 128.05 (CH_{benz}), 59.15

(NCH₂), 56.04 (NCH₂ piperidine), 55.06 (NCH₂), 49.63 (NCH₂), 42.92 (CH₂), 28.34 (CH₂), 27.35 (CH₂piperidine), 25.84 (CH₂piperidine). ESI-MS *m/z* [M + H]⁺ calculated for C₃₈H₅₅N₄: 567.4348, found: 567.4424.

1,3-bis[(4-((quinolin-3-yl)aminomethyl)phenyl)methyl]benzene (7i)

Pale-yellow oil (87%); ¹H NMR (CDCl₃) δ ppm: 8.47 (d, 2H, *J* = 2.70 Hz, H-2_{quinol}), 7.98 (dd, 2H, *J* = 6.90 and 3.60 Hz, H-8_{quinol}), 7.57 (dd, 2H, *J* = 6.90 and 3.60 Hz, H-5_{quinol}), 7.46–7.39 (m, 4H, H-6_{quinol} and H-7_{quinol}), 7.29 (d, 4H, *J* = 7.80 Hz, H-3_{phen} and H-5_{phen}), 7.24 (m, 1H, H_{benz}), 7.15 (d, 4H, *J* = 7.80 Hz, H-2_{phen} and H-6_{phen}), 7.13 (d, 2H, *J* = 2.70 Hz, H-4_{quinol}), 6.98 (m, 3H, H_{benz}), 4.34 (d, 4H, *J* = 5.1 Hz, NCH₂); 3.44 (s, 4H, CH₂); ¹³C NMR (CDCl₃) δ ppm: 144.70 (C-2_{quinol}), 144.50 (C-8a_{quinol}), 143.4 (C-4_{phenyl} and C-3_{quinol}), 142.93 (Cq_{benz}), 137.43 (C-1_{phenyl}), 137.3 (C-4a_{quinol}), 130.61 (C-3_{phen} and C-5_{phen}), 130.35 (C-4_{quinol}), 129.02 (C-2_{phen} and C-6_{phen}), 129.00 (C-8_{quinol}), 128.33 (C-5_{quinol}), 127.37 (C-6_{quinol}), 127.30 (C-7_{quinol}), 116.32, (CH_{benz}), 111.75 (CH_{benz}), 48.96 (NCH₂), 42.94 (CH₂). ESI-MS *m/z* [M + H]⁺ calculated for C₄₀H₃₅N₄: 571.2783, found: 571.2860.

1,3-bis[(4-(pyridin-2-ylmethylaminomethyl)phenyl)methyl]benzene (7j)

Yellow oil (80%); ¹H NMR (CDCl₃) δ ppm: 8.57 (d, 2H, *J* = 6.90 Hz, H-6_{pyrid}), 7.65–7.60 (m, 2H, H-4_{pyrid}), 7.33–7.30 (m, 6H, H-3_{phen}, H-5_{phen}, and H-3_{pyrid}), 7.21 (s, 1H, H_{benz}), 7.20–7.13 (m, 6H, H-6_{phen}, H-2_{phen}, and H-5_{pyrid}), 7.07–7.00 (m, 3H, H_{benz}), 3.94 (m, 8H, NCH₂), 3.82 (s, 4H, CH₂). ¹³C NMR (CDCl₃) δ ppm: 161.18 (C-2_{pyrid}), 150.70 (C-6_{pyrid}), 142.69 (C-4_{phenyl}), 141.25 (Cq_{benz}), 139.24 (C-1_{phenyl}), 137.82 (C-4_{pyrid}), 130.98 (CH_{benz}), 130.34 (C-3_{phen} and C-5_{phen}), 129.95 (C-2_{phen} and C-6_{phen}), 128.46 (CH_{benz}), 128.08 (CH_{benz}), 123.75 (C-3_{pyrid}), 123.32 (C-5_{pyrid}), 55.92 (NCH₂), 54.61 (NCH₂), 42.94 (CH₂). ESI-MS *m/z* [M + H]⁺ calculated for C₃₄H₃₅N₄: 499.2783, found: 499.2860.

1,3-bis[(4-(pyridin-2-ylethylaminomethyl)phenyl)methyl]benzene (7k)

Yellow oil (95%); ¹H NMR (CDCl₃) δ ppm: 8.54–8.51 (m, 2H, H-6_{pyrid}), 7.60–7.52 (m, 2H, H-4_{pyrid}), 7.39–7.36 (m, 1H, H_{benz}), 7.20–7.11 (m, 6H, H-3_{phen}, H-5_{phen}, and H-3_{pyrid}), 7.09–7.05 (m, 6H, H-6_{phen}, H-2_{phen}, and H-5_{pyrid}), 7.02–6.98 (m, 3H, H_{benz}), 3.92 (s, 4H, NCH₂), 3.79 (s, 4H, CH₂), 3.05–3.01 (m, 8H, NCH₂, and CH₂Pyrid); ¹³C NMR (CDCl₃) δ ppm: 161.61 (C-2_{pyrid}), 150.69 (C-6_{pyrid}), 142.67 (C-4_{phenyl}), 141.17 (Cq_{benz}), 139.30 (C-1_{phenyl}), 137.78 (C-4_{pyrid}), 130.96 (CH_{benz}), 129.94 (C-3_{phen} and C-5_{phen}), 129.65 (C-2_{phen} and C-6_{phen}), 128.40 (CH_{benz}), 128.06 (CH_{benz}), 124.71 (C-3_{pyrid}), 122.66 (C-5_{pyrid}), 54.93 (NCH₂), 50.22 (NCH₂), 42.92 (CH₂), 39.74 (CH₂). ESI-MS *m/z* [M + H]⁺ calculated for C₃₆H₃₉N₄: 527.3096, found: 527.3166.

1,3-bis[(4-(pyridin-2-ylpropylaminomethyl)phenyl)methyl]benzene (7l)

Yellow oil (90%); ¹H NMR (CDCl₃) δ ppm: 8.51–8.49 (m, 2H, H-6_{pyrid}), 7.58–7.53 (m, 2H, H-4_{pyrid}), 7.24 (d, 4H, *J* = 8.10 Hz, H-3_{phen} and H-5_{phen}), 7.24–7.22 (m, 1H, H_{benz}), 7.23–7.07 (m, 8H, H-6_{phen}, H-2_{phen}, H-5_{pyrid}, and H-3_{pyrid}), 7.01–6.99 (m, 3H, H_{benz}), 3.92 (s, 4H, NCH₂), 3.74 (s, 4H, CH₂), 2.83 (t, 4H, *J* = 6.90 Hz, NCH₂), 2.68 (t, 4H, *J* = 6.90 Hz, CH₂Pyrid), 1.95 (qt, 4H *J* = 6.90 Hz, CH₂); ¹³C NMR (CDCl₃) δ ppm: 163.17 (C-2_{pyrid}), 150.72 (C-6_{pyrid}), 142.69 (C-4_{phenyl}), 141.16 (Cq_{benz}), 139.44 (C-1_{phenyl}), 137.86 (C-4_{pyrid}), 130.96 (CH_{benz}), 130.31 (C-3_{phen} and C-5_{phen}), 128.76 (C-2_{phen} and C-6_{phen}), 128.73 (CH_{benz}), 128.03 (CH_{benz}), 124.19 (C-3_{pyrid}), 122.44 (C-5_{pyrid}), 54.98 (NCH₂), 50.12 (NCH₂), 42.92 (CH₂), 37.34 (CH₂), 31.41 (CH₂). ESI-MS *m/z* [M + H]⁺ calculated for C₃₈H₄₃N₄: 555.3409, found: 555.3476.

1,3-bis[(4-(pyridin-3-ylmethylaminomethyl)phenyl)methyl]benzene (7m)

Pale-yellow oil (80%); ¹H NMR (CDCl₃) δ ppm: 8.58–8.51 (m, 4H, H-6_{pyrid}, and H-3_{pyrid}), 7.72 (d, 2H, *J* = 7.80 Hz, H-4_{pyrid}), 7.28–7.26 (m, 6H, H-5_{pyrid}, H-3_{phen}, and H-5_{phen}), 7.21 (m, 1H, H_{benz}), 7.15 (d, 4H, *J* = 8.10 Hz, H-6_{phen}, and H-2_{phen}), 7.07–7.01 (m, 3H, H_{benz}), 3.92 (s, 4H, NCH₂), 3.82 (s, 4H, NCH₂), 3.78 (s, 4H, CH₂); ¹³C NMR (CDCl₃) δ ppm: 151.15 (C-2_{pyrid}), 149.89 (C-6_{pyrid}), 142.63 (C-4_{phenyl}), 141.43 (Cq_{benz}), 138.99 (C-1_{phenyl}), 137.25 (C-

4_{pyrid}), 137.24 (C-3_{pyrid}), 130.98 (CH_{benz}), 130.40 (C-3_{phen} and C-5_{phen}), 129.66 (C-2_{phen} and C-6_{phen}), 128.52 (CH_{benz}), 128.11 (CH_{benz}), 124.81 (C-5_{pyrid}), 54.28 (NCH₂), 51.82 (NCH₂), 42.93 (CH₂). ESI-MS m/z [M + H]⁺ calculated for C₃₄H₃₅N₄: 499.2783, found: 499.2855.

1,3-bis[(4-(pyridin-3-ylethylaminomethyl)phenyl)methyl]benzene (**7n**)

Yellow oil (66%); ¹H NMR (CDCl₃) δ ppm: 8.48–8.46 (m, 4H, H-6_{pyrid} and H-3_{pyrid}), 7.53 (d, 2H, J = 7.80 Hz, H-4_{pyrid}), 7.23–7.18 (m, 6H, H-5_{pyrid}, H-3_{phen} and H-5_{phen}), 7.20–7.12 (m, 1H, H_{benz}), 7.12 (d, 6H, J = 7.80 Hz, H-6_{phen} and H-2_{phen}), 7.03–7.00 (m, 3H, H_{benz}), 3.93 (s, 4H, NCH₂), 3.78 (s, 4H, NCH₂), 2.91–2.82 (m, 8H, CH₂ and CH₂Pyrid); ¹³C NMR (CDCl₃) δ ppm: 151.53 (C-2_{pyrid}), 149.06 (C-6_{pyrid}), 142.64 (C-4_{phenyl}), 141.32 (C_{qbenz}), 139.16 (C-1_{phenyl}), 137.58 (C-4_{pyrid}), 136.84 (C-3_{pyrid}), 130.96 (CH_{benz}), 130.36 (C-3_{phen} and C-5_{phen}), 129.97 (C-2_{phen} and C-6_{phen}), 129.19 (CH_{benz}), 129.09 (CH_{benz}), 124.77 (C-5_{pyrid}), 54.94 (NCH₂), 51.53 (NCH₂), 42.92 (CH₂), 34.97 (CH₂). ESI-MS m/z [M + H]⁺ calculated for C₃₆H₃₉N₄: 527.3096, Found: 527.3169.

1,3-bis[(4-(pyridin-3-ylpropylaminomethyl)phenyl)methyl]benzene (**7o**).

Yellow oil (90%); ¹H NMR (CDCl₃) δ ppm: 8.46–8.41 (m, 4H, H-6_{pyrid} and H-3_{pyrid}), 7.8 (d, 2H, J = 7.80 Hz, H-4_{pyrid}), 7.21 (d, 4H, J = 8.10 Hz, H-3_{phen} and H-5_{phen}), 7.23–7.21 (m, 2H, H-5_{pyrid}), 7.20–7.12 (m, 1H, H_{benz}), 7.09 (d, 4H, J = 8.10 Hz, H-6_{phen} and H-2_{phen}), 7.01–6.99 (m, 3H, H_{benz}), 3.92 (s, 4H, NCH₂), 3.74 (s, 4H, CH₂), 2.68–2.63 (m, 8H, NCH₂ and CH₂Pyrid), 1.82 (qt, 4H, J = 7.20 Hz, CH₂); ¹³C NMR (CDCl₃) δ ppm: 151.24 (C-2_{pyrid}), 148.68 (C-6_{pyrid}), 142.65 (C-4_{phenyl}), 141.26 (C_{qbenz}), 139.28 (C-1_{phenyl}), 138.76 (C-3_{pyrid}), 137.23 (C-4_{pyrid}), 130.96 (CH_{benz}), 130.33 (C-3_{phen} and C-5_{phen}), 129.97 (CH_{benz}), 129.65 (C-2_{phen} and C-6_{phen}), 128.08 (CH_{benz}), 124.71 (C-5_{pyrid}), 55.08 (NCH₂), 49.96 (NCH₂), 42.91 (CH₂), 32.76 (CH₂), 32.00 (CH₂). ESI-MS m/z [M + H]⁺ calculated for C₃₈H₄₃N₄: 555.3409, found: 555.3480.

1,3-bis[(4-(pyridin-4-ylmethylaminomethyl)phenyl)methyl]benzene (**7p**).

Yellow oil (60%); ¹H NMR (CDCl₃) δ ppm: 8.54 (d, 4H, J = 6.00 Hz, H-2_{pyrid} and H-6_{pyrid}), 7.72 (d, 4H, J = 6.00 Hz, H-3_{pyrid} and H-5_{pyrid}), 7.28–7.23 (m, 8H, H-3_{phen}, H-5_{phen}, H-6_{phen} and H-2_{phen}), 7.21 (m, 1H, H_{benz}), 7.04 (m, 3H, H_{benz}), 4.77 (s, 4H, NCH₂), 3.98 (s, 4H, NCH₂), 2.34 (s, 4H, CH₂); ¹³C NMR (CDCl₃) δ ppm: 151.16 (C-2_{pyrid} and C-6_{pyrid}), 150.6 (C-4_{pyrid}), 142.61 (C-4_{phenyl}), 141.47 (C_{qbenz}), 138.89 (C-1_{phenyl}), 130.96 (CH_{benz}), 130.39 (C-3_{phen} and C-5_{phen}), 130.00 (CH_{benz}), 129.65 (C-2_{phen} and C-6_{phen}), 128.11 (CH_{benz}), 124.39 (C-3_{pyrid} and C-5_{pyrid}), 54.29 (NCH₂), 53.21 (NCH₂), 42.96 (CH₂). ESI-MS m/z [M + H]⁺ calculated for C₃₄H₃₅N₄: 499.2783, found: 499.2853.

1,3-bis[(4-(pyridin-4-ylethylaminomethyl)phenyl)methyl]benzene (**7q**).

Yellow oil (89%); ¹H NMR (CDCl₃) δ ppm: 8.47–8.45 (m, 6H, H-2_{pyrid} and H-6_{pyrid}), 7.56 (d, 4H, J = 6.00 Hz, H-3_{pyrid} and H-5_{pyrid}), 7.28–7.23 (m, 9H, H-3_{phen}, H-5_{phen}, H_{benz} H-6_{phen} and H-2_{phen}), 7.02–7.00 (m, 3H, H_{benz}), 3.93 (s, 4H, NCH₂), 3.77 (s, 4H, CH₂), 2.92 (t, 4H, J = 6.90 Hz, NCH₂), 2.80 (t, 4H, J = 6.90 Hz, CH₂); ¹³C NMR (CDCl₃) δ ppm: 151.06 (C-2_{pyrid} and C-6_{pyrid}), 150.62 (C-4_{pyrid}), 142.65 (C-4_{phenyl}), 141.38 (C_{qbenz}), 139.01 (C-1_{phenyl}), 130.95 (CH_{benz}), 130.37 (C-3_{phen} and C-5_{phen}), 129.62 (C-2_{phen} and C-6_{phen}), 128.74 (CH_{benz}), 128.48 (CH_{benz}), 125.59 (C-3_{pyrid} and C-5_{pyrid}), 54.85 (NCH₂), 50.65 (NCH₂), 42.92 (CH₂), 37.08 (CH₂). ESI-MS m/z [M + H]⁺ calculated for C₃₆H₃₉N₄: 527.3096, found: 527.3164.

1,3-bis[(4-(pyridin-4-ylpropylaminomethyl)phenyl)methyl]benzene (**7r**).

Yellow oil (99%); ¹H NMR (CDCl₃) δ ppm: 8.45 (d, 4H, J = 6.00 Hz, H-2_{pyrid} and H-6_{pyrid}), 7.23 (d, 6H, J = 8.10 Hz, H-3_{phen} and H-5_{phen}), 7.14 (d, 4H, J = 8.10 Hz, H-6_{phen} and H-2_{phen}), 7.19 (m, 1H, H_{benz}), 7.08–7.03 (m, 7H, H-3_{pyrid}, H-5_{pyrid} and H_{benz}), 3.91 (s, 4H, NCH₂), 3.72 (s, 4H, CH₂), 2.63 (t, 8H, J = 7.20 Hz, NCH₂ and CH₂Pyrid), 1.81 (qt, 4H, J = 7.20 Hz, CH₂); ¹³C NMR (CDCl₃) δ ppm: 152.57 (C-4_{pyrid}), 150.97 (C-2_{pyrid} and C-6_{pyrid}), 142.63 (C-4_{phenyl}), 141.27 (C_{qbenz}), 139.29 (C-1_{phenyl}), 130.96 (CH_{benz}), 130.33 (C-3_{phen} and C-5_{phen}), 129.98 (CH_{benz}), 129.65 (C-2_{phen} and C-6_{phen}), 128.08 (CH_{benz}), 125.30 (C-3_{pyrid}

and C-5_{pyrid}), 55.99 (NCH₂), 49.87 (NCH₂), 42.92 (CH₂), 34.22 (CH₂), 31.88 (CH₂). ESI-MS m/z [M + H]⁺ calculated for C₃₈H₄₃N₄: 555.3409, found: 555.3489.

3.1.5. Synthesis of the 1,3-bis[(4-Formylphenoxy)methyl]benzene 4

To a suspension of 1,3-bis(bromomethyl)benzene (2.5 mmol) and 4-hydroxybenzaldehyde (7.5 mmol) in THF (25 mL) were added 17.5 mmol of powder K₂CO₃. The reaction mixture was refluxed for 24 h. The suspension was then filtered, evaporated to dryness, and extracted with DCM (2 × 30 mL). The organic layer was filtered and washed with a solution of NaOH 2.5M (2 × 20 mL). The organic layer was dried over sodium sulfate, filtered, and evaporated under reduced pressure to give the pure product **2**. Beige crystals (90%); Mp = 90 °C [38]. ¹H NMR (CDCl₃) δ ppm: 9.89 (s, 2H, CHO), 7.84 (d, 4H, J = 8.10 Hz, H-3_{phen} and H-5_{phen}), 7.52 (s, 3H, H_{benz}), 7.42 (s, 1H, H_{benz}), 7.43 (s, 2H, H_{benz}), 7.07 (d, 4H, J = 8.10 Hz, H-2_{phen} and H-6_{phen}), 5.16 (s, 4H, CH₂); ¹³C NMR (CDCl₃) δ ppm: 192.1(CO), 164.97(C-4_{phen}), 138.03 (Cq_{benz}), 133.37 (C-3_{phen} and C-5_{phen}), 132.3 (C-1_{phen}), 130.54 (CH_{benz}), 128.76 (CH_{benz}), 127.82 (CH_{benz}), 116.54 (C-2_{phen} and C-6_{phen}), 71.35 (CH₂). Colorless single crystal **4** was obtained by slow evaporation from a methanol/chloroform solution (*v/v*: 20/80): monoclinic, space group P21/n, a = 16.0383(7) Å, b = 5.9521(3) Å, c = 18.1048(9) Å, α = 90°, β = 90.005(2)°, γ = 90°, V = 1728.31(14) Å³, Z = 4, δ(calcd) = 1.331 Mg·m⁻³, FW = 346.36 for C₂₂H₁₈O₄, F(000) = 728.0. Full crystallographic results were deposited at the Cambridge Crystallographic Data Centre (CCDC-2427200), UK [39].

3.1.6. General Procedure for the Synthesis of 1,3-bis[(4-(Substituted-iminomethyl)phenoxy)methyl]benzenes **6a-r**

The 1,3-bis[(4-formylphenoxy)methyl]benzene **4** (69 mg, 0.2 mmol) was dissolved in 6 mL of toluene. Activated molecular sieves of 4 Å (800 mg) were introduced, followed by dialkylamine (0.5 mmol). The reaction mixture was stirred for 24 h. The obtained suspension was filtered and washed with dichloromethane and the solvent was removed under reduced pressure to afford the di-imine **6**. The crude products were then used without further purification.

1,3-bis[(4-(2-dimethylaminoethyl)iminomethyl)phenoxy)methyl]benzene (**6a**)

Yellow oil (99%); ¹H NMR (CDCl₃) δ ppm: 8.23 (s, 2H, CH = N), 7.65 (d, 6H, J = 8.10 Hz, H-3_{phen} and H-5_{phen}), 7.49 (s, 1H, H_{benz}), 7.39 (s, 2H, H_{benz}), 7.28 (s, 1H, H_{benz}), 6.97 (d, 6H, J = 8.10 Hz, H-2_{phen} and H-6_{phen}), 5.09 (s, 4H, CH₂), 3.72 (t, 4H, J = 6.90 Hz, NCH₂), 2.62 (t, 4H, J = 6.90 Hz, NCH₂), 2.30 (s, 12H, N(CH₃)₂).

1,3-bis[(4-(3-dimethylaminopropyl)iminomethyl)phenoxy)methyl]benzene (**6b**)

Yellow oil (98%); ¹H NMR (CDCl₃) δ ppm: 8.35 (s, 2H, CH=N), 7.86 (d, 4H, J = 8.10 Hz, H-3_{phen} and H-5_{phen}), 7.62 (s, 1H, H_{benz}), 7.53 (s, 2H, H_{benz}), 7.44 (s, 1H, H_{benz}), 7.13 (d, 4H, J = 8.10 Hz, H-2_{phen} and H-6_{phen}), 5.17 (s, 4H, CH₂), 3.79 (t, 4H, J = 6.90 Hz, NCH₂), 2.54 (t, 4H, J = 6.90 Hz, NCH₂), 2.41 (s, 12H, N(CH₃)₂), 2.06 (qt, 4H, J = 8.40 Hz, CH₂).

1,3-bis[(4-(4-dimethylaminobutyl)iminomethyl)phenoxy)methyl]benzene (**6c**)

Yellow oil (90%); ¹H NMR (CDCl₃) δ ppm: 8.20 (s, 2H, CH=N), 7.67 (d, 4H, J = 8.1 Hz, H-3_{phen} and H-5_{phen}), 7.50 (s, 1H, H_{benz}), 7.40 (s, 2H, H_{benz}), 7.23 (s, 1H, H_{benz}), 6.98 (d, 4H, J = 8.10 Hz, H-2_{phen} and H-6_{phen}), 5.10 (s, 4H, CH₂), 3.60 (t, 4H, J = 6.90 Hz, NCH₂), 2.30 (t, 4H, J = 6.90 Hz, NCH₂), 2.22 (s, 12H, N(CH₃)₂), 1.71 (qt, 4H, J = 8.40 Hz, CH₂), 1.55 (qt, 4H, J = 8.40 Hz, CH₂).

1,3-bis[(4-(2-(4-methylpiperazin-1-yl)ethyl)iminomethyl)phenoxy)methyl]benzene (**6d**)

Yellow oil (99%); ¹H NMR (CDCl₃) δ ppm: 8.20 (s, 2H, CH=N), 7.64 (d, 4H, J = 8.1 Hz, H-3_{phen} and H-5_{phen}), 7.47 (s, 1H, H_{benz}), 7.37 (s, 2H, H_{benz}), 7.23 (s, 1H, H_{benz}), 6.98 (d, 4H, J = 8.10 Hz, H-2_{phen} and H-6_{phen}), 5.06 (s, 4H, CH₂), 3.72 (t, 4H, J = 6.0 Hz, NCH₂), 2.8 (t, 4H, J = 8.40 Hz, NCH₂), 2.23 (s, 16H, NCH₂_{2pip}), 2.26 (s, 6H, NCH₃).

1,3-bis[(4-(3-(4-methylpiperazin-1-yl)propyl)iminomethyl)phenoxy)methyl]benzene (**6e**)

Yellow oil (99%); ^1H NMR (CDCl_3) δ ppm: 8.20 (s, 2H, $\text{CH}=\text{N}$), 7.65 (d, 4H, $J = 8.1$ Hz, H-3_{phen} , and H-5_{phen}), 7.50 (s, 1H, H_{benz}), 7.39 (s, 2H, H_{benz}), 7.28 (s, 1H, H_{benz}), 6.98 (d, 4H, $J = 8.10$ Hz, H-2_{phen} , and H-6_{phen}), 5.10 (s, 4H, CH_2), 3.60 (t, 6H, $J = 6.00$ Hz, NCH_2), 2.47–2.34 (m, 20H, NCH_2 , $\text{NCH}_2_{\text{pip}}$), 2.28 (s, 6H, NCH_3), 1.91–1.86 (qt, 4H, $J = 8.40$ Hz, CH_2).

1,3-bis[(4-(3-(morpholin-1-yl)propyl)iminomethyl)phenoxy)methyl]benzene (**6f**)

Yellow oil (99%); ^1H NMR (CDCl_3) δ ppm: 8.20 (s, 2H, $\text{CH}=\text{N}$), 7.65 (d, 4H, $J = 8.1$ Hz, H-3_{phen} , and H-5_{phen}), 7.50 (s, 1H, H_{benz}), 7.39 (s, 2H, H_{benz}), 7.17 (s, 1H, H_{benz}), 6.98 (d, 4H, $J = 8.10$ Hz, H-2_{phen} , and H-6_{phen}), 5.09 (s, 4H, CH_2), 3.72 (t, 8H, $J = 4.20$ Hz, OCH_2), 3.63 (t, 4H, $J = 6.9$ Hz, NCH_2), 2.44–2.34 (m, 12H, NCH_2 , and $\text{NCH}_2_{\text{morph}}$), 1.86 (qt, 6H, $J = 6.90$ Hz, CH_2).

1,3,5-bis[(4-(3-(pyrrolidin-1-yl)propyl)iminomethyl)phenoxy)methyl]benzene (**6g**)

Yellow oil (98%); ^1H NMR (CDCl_3) δ ppm: 8.20 (s, 2H, $\text{CH}=\text{N}$), 7.65 (d, 4H, $J = 8.1$ Hz, H-3_{phen} , and H-5_{phen}), 7.49 (s, 1H, H_{benz}), 7.39 (s, 2H, H_{benz}), 7.17 (s, 1H, H_{benz}), 6.99 (d, 4H, $J = 8.10$ Hz, H-2_{phen} , and H-6_{phen}), 5.09 (s, 4H, CH_2), 3.62 (t, 4H, $J = 6.60$ Hz, NCH_2), 2.54–2.48 (m, 12H, NCH_2 , and $\text{NCH}_2_{\text{pyrrol}}$), 1.93 (t, 4H, $J = 6.60$ Hz, CH_2), 1.79–1.74 (m, 8H, $\text{CH}_2_{\text{pyrrol}}$).

1,3-bis[(4-(3-(piperidin-1-yl)propyl)iminomethyl)phenoxy)methyl]benzene (**6h**)

Yellow oil (99%); ^1H NMR (CDCl_3) δ ppm: 8.20 (s, 2H, $\text{CH}=\text{N}$), 7.65 (d, 4H, $J = 8.1$ Hz, H-3_{phen} , and H-5_{phen}), 7.50 (s, 1H, H_{benz}), 7.39 (s, 2H, H_{benz}), 7.17 (s, 1H, H_{benz}), 6.99 (d, 4H, $J = 8.10$ Hz, H-2_{phen} , and H-6_{phen}), 5.09 (s, 4H, CH_2), 3.60 (t, 4H, $J = 6.90$ Hz, NCH_2), 2.40–2.35 (m, 12H, NCH_2 , and $\text{NCH}_2_{\text{pip}}$), 1.88 (t, 4H, $J = 6.90$ Hz, CH_2), 1.62–1.54 (m, 8H, CH_2_{pip}), 1.45–1.42 (m, 4H, CH_2_{pip}).

1,3-bis[(4-((quinolin-3-yl)iminomethyl)phenoxy)methyl]benzene (**6i**)

Pale-yellow oil (98%); ^1H NMR (CDCl_3) δ ppm: 8.91 (d, 2H, $J = 2.70$ Hz, $\text{H-2}_{\text{quinol}}$), 8.57–8.53 (m, 2H, $\text{H-8}_{\text{quinol}}$), 8.15 (s, 2H, $\text{CH}=\text{N}$), 7.99–7.92 (m, 4H, $\text{H-5}_{\text{quinol}}$, $\text{H-4}_{\text{quinol}}$), 7.81 (d, 4H, $J = 7.80$ Hz, H-3_{phen} , and H-5_{phen}), 7.69–7.24 (m, 8H, $\text{H-6}_{\text{quinol}}$, and $\text{H-7}_{\text{quinol}}$, H_{benz}), 7.12 (d, 4H, $J = 7.80$ Hz, H-2_{phen} and H-6_{phen}), 5.19 (s, 4H, CH_2).

1,3-bis[(4-(pyridin-2-ylmethyliminomethyl)phenoxy)methyl]benzene (**6j**)

Pale-yellow oil (93%); ^1H NMR (CDCl_3) δ ppm: 8.53 (d, 2H, $J = 6.90$ Hz, $\text{H-6}_{\text{pyrid}}$), 8.40 (s, 2H, $\text{CH}=\text{N}$), 7.76 (d, 4H, $J = 8.1$ Hz, H-3_{phen} , and H-5_{phen}), 7.69–7.66 (m, 2H, $\text{H-4}_{\text{pyrid}}$), 7.44–7.41 (m, 3H, $J = 6.10$ Hz, $\text{H-3}_{\text{pyrid}}$), 7.19–7.17 (m, 5H, H_{benz} , $\text{H-5}_{\text{pyrid}}$), 7.03 (d, 4H, $J = 8.1$ Hz, H-2_{phen} , and H-6_{phen}), 5.10 (s, 4H, OCH_2), 4.93 (s, 4H, NCH_2).

1,3-bis[(4-(pyridin-2-ylethyliminomethyl)phenoxy)methyl]benzene (**6k**)

Yellow oil (97%); ^1H NMR (CDCl_3) δ ppm: 8.55 (d, 2H, $J = 5.90$ Hz, $\text{H-6}_{\text{pyrid}}$), 8.14 (s, 2H, $\text{CH}=\text{N}$), 7.63 (d, 4H, $J = 8.10$ Hz, H-3_{phen} , and H-5_{phen}), 7.71–7.50 (m, 3H, $\text{H-4}_{\text{pyrid}}$, H_{benz}), 7.39 (s, 3H, H_{benz}), 7.22–7.11 (m, 4H, $\text{H-3}_{\text{pyrid}}$, and $\text{H-5}_{\text{pyrid}}$), 6.99 (t, 4H, $J = 8.10$ Hz, H-2_{phen} , H-6_{phen}), 5.09 (s, 4H, $J = 7.20$ Hz, OCH_2), 4.01 (s, 4H, NCH_2), 3.18 (m, 4H, $\text{CH}_2_{\text{Pyrid}}$).

1,3-bis[(4-(pyridin-2-ylpropyliminomethyl)phenoxy)methyl]benzene (**6l**)

Yellow oil (97%); ^1H NMR (CDCl_3) δ ppm: 8.53 (d, 2H, $J = 5.80$ Hz, $\text{H-6}_{\text{pyrid}}$), 8.21 (s, 2H, $\text{CH}=\text{N}$), 7.68 (d, 4H, $J = 8.10$ Hz, H-3_{phen} , and H-5_{phen}), 7.58–7.57 (m, 2H, $\text{H-4}_{\text{pyrid}}$), 7.22 (s, 1H, H_{benz}), 7.41 (s, 3H, H_{benz}), 7.12–7.03 (m, 2H, $\text{H-3}_{\text{pyrid}}$), 7.10–7.09 (m, 2H, $\text{H-5}_{\text{pyrid}}$), 6.99 (t, 4H, $J = 8.10$ Hz, H-2_{phen} , H-6_{phen}), 5.11 (s, 4H, OCH_2), 3.65 (t, 4H, $J = 6.60$ Hz, NCH_2), 2.89 (t, 4H, $J = 6.60$ Hz, $\text{CH}_2_{\text{Pyrid}}$), 2.15 (qt, 4H, $J = 6.60$ Hz, CH_2).

1,3-bis[(4-(pyridin-3-ylmethyliminomethyl)phenoxy)methyl]benzene (**6m**)

Pale-yellow oil (98%); ^1H NMR (CDCl_3) δ ppm: 8.61–8.50 (m, 4H, $\text{H-2}_{\text{pyrid}}$, and $\text{H-6}_{\text{pyrid}}$), 8.34 (s, 2H, $\text{CH}=\text{N}$), 7.73 (d, 4H, $J = 8.10$ Hz, H-3_{phen} , and H-5_{phen}), 7.67–7.64 (m, 2H, $\text{H-4}_{\text{pyrid}}$), 7.50 (s, 1H, H_{benz}), 7.40 (s, 3H, H_{benz}), 7.18 (m, 2H, $\text{H-5}_{\text{pyrid}}$), 7.02 (t, 4H, $J = 8.10$ Hz, H-2_{phen} , H-6_{phen}), 5.09 (s, 4H, OCH_2), 4.76 (s, 4H, NCH_2).

1,3-bis[(4-(pyridin-3-ylethyliminomethyl)phenoxy)methyl]benzene (**6n**)

Pale Yellow oil (97%); ^1H NMR (CDCl_3) δ ppm: 8.51–8.44 (m, 4H, H-2_{pyrid}, and H-6_{pyrid}), 8.09 (s, 2H, CH=N), 7.65 (d, 4H, J = 7.80 Hz, H-3_{phen}, and H-5_{phen}), 7.56–7.52 (m, 3H, H-4_{pyrid}, H_{benz}), 7.51 (s, 3H, H_{benz}), 7.26–7.21 (m, 2H, H-5_{pyrid}), 7.00 (t, 4H, J = 8.10 Hz, H-2_{phen}, H-6_{phen}), 5.10 (s, 4H, OCH₂), 3.79 (t, 4H, J = 7.20 Hz, NCH₂), 3.00 (t, 4H, J = 7.20 Hz, CH₂Pyrid).

1,3-bis[(4-(pyridin-3-yl)propyliminomethyl)phenoxy)methyl]benzene (**6o**)

Yellow oil (91%); ^1H NMR (CDCl_3) δ ppm: 8.47–8.43 (m, 4H, H-2_{pyrid}, and H-6_{pyrid}), 8.20 (s, 2H, CH=N), 7.65 (d, 4H, J = 7.80 Hz, H-3_{phen}, and H-5_{phen}), 7.54–7.50 (m, 3H, H-4_{pyrid}, H_{benz}), 7.51 (s, 3H, H_{benz}), 7.26–7.21 (m, 2H, H-5_{pyrid}), 7.00 (t, 4H, J = 8.10 Hz, H-2_{phen}, H-6_{phen}), 5.10 (s, 4H, OCH₂), 3.60 (t, 4H, J = 7.20 Hz, NCH₂), 2.71 (t, 4H, J = 7.20 Hz, CH₂Pyrid), 2.04 (qt, 6H, J = 6.60 Hz, CH₂).

1,3-bis[(4-(pyridin-4-yl)methyliminomethyl)phenoxy)methyl]benzene (**6p**)

Pale-yellow oil (98%); ^1H NMR (CDCl_3) δ ppm: 8.53 (d, 4H, J = 5.40 Hz, H-2_{pyrid}, and H-6_{pyrid}), 8.33 (s, 2H, CH=N), 7.74 (d, 4H, J = 8.10 Hz, H-3_{phen}, and H-5_{phen}), 7.52 (s, 1H, H_{benz}), 7.41 (s, 3H, H_{benz}), 7.28–7.11 (m, 4H, H-4_{pyrid}, and H-5_{pyrid}), 7.00 (d, 4H, J = 8.10 Hz, H-2_{phen} and H-6_{phen}), 5.12 (s, 4H, OCH₂), 4.74 (s, 4H, NCH₂).

1,3-bis[(4-(pyridin-4-ylethyliminomethyl)phenoxy)methyl]benzene (**6q**)

Yellow oil (97%); ^1H NMR (CDCl_3) δ ppm: 8.50 (d, 4H, J = 5.40 Hz, H-2_{pyrid}, and H-6_{pyrid}), 8.07 (s, 2H, CH=N), 7.63 (d, 4H, J = 8.10 Hz, H-3_{phen}, and H-5_{phen}), 7.50 (s, 1H, H_{benz}), 7.40 (s, 3H, H_{benz}), 7.18–7.14 (m, 4H, H-4_{pyrid}, and H-5_{pyrid}), 7.00 (d, 4H, J = 8.10 Hz, H-2_{phen}, and H-6_{phen}), 5.09 (s, 4H, OCH₂), 3.82 (s, 4H, NCH₂), 2.99 (t, 6H, J = 7.20 Hz, CH₂Pyrid).

1,3-bis[(4-(pyridin-4-yl)propyliminomethyl)phenoxy)methyl]benzene (**6r**)

Yellow oil (98%); ^1H NMR (CDCl_3) δ ppm: 8.48 (d, 4H, J = 5.40 Hz, H-2_{pyrid}, and H-6_{pyrid}), 8.17 (s, 2H, CH=N), 7.67 (d, 4H, J = 8.10 Hz, H-3_{phen}, and H-5_{phen}), 7.50 (s, 1H, H_{benz}), 7.40 (s, 3H, H_{benz}), 7.18–7.14 (m, 4H, H-4_{pyrid}, and H-5_{pyrid}), 7.00 (d, 4H, J = 8.10 Hz, H-2_{phen}, and H-6_{phen}), 5.09 (s, 4H, OCH₂), 3.59 (s, 4H, NCH₂), 2.69 (t, 6H, J = 7.20 Hz, CH₂Pyrid), 2.03 (qt, 4H, J = 7.20 Hz, CH₂).

3.1.7. General Procedure for the Synthesis of 1,3-bis[(4-(Substituted-aminomethyl)phenoxy)methyl]benzenes **8a–r**

Compounds **6a–r** (0.4 mmol) were dissolved in methanol (10 mL), and sodium borohydride (2.4 mmol, 6 eq.) was added gradually at 0 °C. The resulting mixture was stirred at room temperature for 2 h. Subsequently, the solvent was removed under reduced pressure, and the residue obtained was cooled, triturated in water, and then extracted with dichloromethane (40 mL). After separating, the organic layer was dried with sodium sulfate, filtered, and evaporated to dryness, yielding the products **8a–r**.

1,3-bis[(4-(2-dimethylaminoethyl)aminomethyl)phenoxy)methyl]benzene (**8a**)

Yellow oil (90%); ^1H NMR (CDCl_3) δ ppm: 7.49 (s, 1H, H_{benz}), 7.38 (s, 3H, H_{benz}), 7.22 (d, 4H, J = 8.10 Hz, H-3_{phen}, and H-5_{phen}), 6.92 (d, 4H, J = 8.10 Hz, H-2_{phen}, and H-6_{phen}), 5.04 (s, 4H, OCH₂), 3.74 (t, 4H, J = 6.90 Hz, NCH₂), 2.68 (t, 4H, J = 6.90 Hz, NCH₂), 2.41 (t, 4H, J = 6.90 Hz, NCH₂), 2.18 (s, 12H, N(CH₃)₂); ^{13}C NMR (CDCl_3) δ ppm: 159.14 (C-4_{phenyl}), 138.88 (C_{qbenz}), 134.07 (C-1_{phenyl}), 130.80 (C-3_{phen} and C-5_{phen}), 130.20 (CH_{benz}), 128.36 (CH_{benz}), 127.79 (CH_{benz}), 116.13 (C-2_{phen} and C-6_{phen}), 71.26 (OCH₂), 60.31 (NCH₂), 54.80 (NCH₂), 46.84 (NCH₂), 46.87 (N(CH₃)₂), ESI-MS m/z [$\text{M} + \text{H}$]⁺ calculated for C₃₀H₄₃N₄O₂: 490.3308, found: 491.3386.

1,3-bis[(4-(3-dimethylaminopropyl)aminomethyl)phenoxy)methyl]benzene (**8b**)

Yellow oil (81%); ^1H NMR (CDCl_3) δ ppm: 7.42 (s, 1H, H_{benz}), 7.36 (s, 3H, H_{benz}), 7.22 (d, 4H, J = 8.10 Hz, H-3_{phen}, and H-5_{phen}), 6.92 (d, 4H, J = 8.10 Hz, H-2_{phen}, and H-6_{phen}), 5.02 (s, 4H, OCH₂), 3.70 (t, 4H, J = 6.90 Hz, NCH₂), 2.68 (t, 4H, J = 6.90 Hz, NCH₂), 2.29 (t, 4H, J = 6.90 Hz, NCH₂), 2.19 (s, 12H, N(CH₃)₂); 1.66 (qt, 4H, J = 7.10 Hz, CH₂); ^{13}C NMR

(CDCl₃) δ ppm: 159.12(C-4_{phenyl}), 138.88 (Cq_{benz}), 134.24 (C-1_{phenyl}), 130.67 (C-3_{phen} and C-5_{phen}), 130.67 (CH_{benz}), 128.31 (CH_{benz}), 127.74 (CH_{benz}), 116.26 (C-2_{phen} and C-6_{phen}), 71.22 (OCH₂), 59.38 (NCH₂), 54.75 (NCH₂), 49.11 (NCH₂), 46.86 (N(CH₃)₂), 29.28 (CH₂). ESI-MS m/z [M + H]⁺ calculated for C₃₂H₄₇N₄: 519.3621, found: 519.3703.

1,3-bis[(4-(4-dimethylaminobutyl)aminomethyl)phenoxy)methyl]benzene (8c)

Yellow oil (91%); ¹H NMR (CDCl₃) δ ppm: 7.50 (s, 1H, H_{benz}), 7.38 (s, 3H, H_{benz}), 7.22 (d, 4H, J = 8.10 Hz, H-3_{phen}, and H-5_{phen}), 6.92 (d, 4H, J = 8.10 Hz, H-2_{phen}, and H-6_{phen}), 5.05 (s, 4H, OCH₂), 3.71 (t, 4H, J = 6.90 Hz, NCH₂), 2.63 (t, 4H, J = 6.90 Hz, NCH₂), 2.25 (t, 4H, J = 6.90 Hz, NCH₂), 2.19 (s, 12H, N(CH₃)₂); ¹³C NMR (CDCl₃) δ ppm: 159.16 (C-4_{phenyl}), 138.88 (Cq_{benz}), 134.19 (C-1_{phenyl}), 130.74 (C-3_{phen} and C-5_{phen}), 130.21 (CH_{benz}), 128.37 (CH_{benz}), 127.8 (CH_{benz}), 116.13 (C-2_{phen} and C-6_{phen}), 71.23 (OCH₂), 61.01 (NCH₂), 54.72 (NCH₂), 50.55 (NCH₂), 46.78 (N(CH₃)₂), 29.27 (CH₂), 26.87 (CH₂). ESI-MS m/z [M + H]⁺ calculated for C₃₄H₅₁N₄: 547.3934, found: 547.40.

1,3-bis[(4-(2-(4-methylpiperazin-1-yl)ethyl)aminomethyl)phenoxy)methyl]benzene (8d)

Yellow oil (99%); ¹H NMR (CDCl₃) δ ppm: 7.48 (s, 1H, H_{benz}), 7.36 (s, 3H, H_{benz}), 7.20 (d, 4H, J = 8.10 Hz, H-3_{phen}, and H-5_{phen}), 6.92 (d, 4H, J = 8.10 Hz, H-2_{phen}, and H-6_{phen}), 5.03 (s, 4H, OCH₂), 3.71 (s, 4H, NCH₂), 2.66 (t, 4H, J = 6.90 Hz, NCH₂), 2.48–2.40 (m, 20H, NCH₂_{piperazine}, NCH₂), 2.25 (s, 6H, NCH₃); ¹³C NMR (CDCl₃) δ ppm: 159.11 (C-4_{phenyl}), 138.87 (Cq_{benz}), 134.29 (C-1_{phenyl}), 130.69 (C-3_{phen} and C-5_{phen}), 116.09 (C-2_{phen} and C-6_{phen}), 71.22 (OCH₂), 59.06 (NCH₂), 56.49 (NCH₂_{piperazine}), 54.49 (NCH₂_{piperazine}), 47.40 (NCH₃), 46.88 (CH₂). ESI-MS m/z [M + H]⁺ calculated for C₃₆H₅₃N₆ O₂: 601.42, found: 601.42.

1,3bis[(4-(3-(4-methylpiperazin-1-yl) propyl)aminomethyl)phenoxy)methyl]benzene (8e)

Yellow oil (63%); ¹H NMR (CDCl₃) δ ppm: 7.48 (s, 1H, H_{benz}), 7.36 (s, 3H, H_{benz}), 7.24 (d, 4H, J = 8.10 Hz, H-3_{phen}, and H-5_{phen}), 6.93 (d, 4H, J = 8.10 Hz, H-2_{phen}, and H-6_{phen}), 5.06 (s, 4H, OCH₂), 3.70 (s, 4H, NCH₂), 2.66 (t, 4H, J = 7.10 Hz, NCH₂), 2.37 (t, 6H, J = 7.10 Hz, NCH₂), 2.47–2.36 (m, 20H, NCH₂, and NCH₂_{piperazine}), 2.26 (s, 6H, NCH₃), 1.67 (qt, 4H, J = 7.10 Hz, CH₂); ¹³C NMR (CDCl₃) δ ppm: 159.15 (C-4_{phenyl}), 138.81 (Cq_{benz}), 134.21 (C-1_{phenyl}), 130.71 (C-3_{phen} and C-5_{phen}), 130.23 (CH_{benz}), 128.40 (CH_{benz}), 127.82 (CH_{benz}), 116.12 (C-2_{phen} and C-6_{phen}), 71.25 (OCH₂), 58.32 (NCH₂), 56.47 (NCH₂_{piperazine}), 54.76 (NCH₂), 54.57 (NCH₂_{piperazine}), 49.39 (NCH₂), 47.40 (NCH₃), 28.27 (CH₂). ESI-MS m/z [M + H]⁺ calculated for C₃₈H₅₇N₆O₂: 629.4465, found: 629.45.

1,3-bis[(4-(3-(morpholin-1-yl)propyl)aminomethyl)phenoxy)methyl]benzene (8f)

Yellow oil (83%); ¹H NMR (CDCl₃) δ ppm: 7.50 (s, 1H, H_{benz}), 7.38 (s, 3H, H_{benz}), 7.22 (d, 4H, J = 8.10 Hz, H-3_{phen} and H-5_{phen}), 6.91 (d, 4H, J = 8.10 Hz, H-2_{phen}, and H-6_{phen}), 5.06 (s, 4H, OCH₂), 3.71–3.67 (m, 12H, NCH₂, and OCH₂), 2.66 (t, 4H, J = 6.90 Hz, NCH₂), 2.43–2.36 (m, 12H, NCH₂, and NCH₂_{morpholine}), 1.69 (qt, 4H, J = 6.90 Hz, CH₂); ¹³C NMR (CDCl₃) δ ppm: 159.13 (C-4_{phenyl}), 138.88 (Cq_{benz}), 134.27 (C-1_{phenyl}), 130.65 (C-3_{phen} and C-5_{phen}), 130.21 (CH_{benz}), 128.37 (CH_{benz}), 127.80 (CH_{benz}), 116.12 (C-2_{phen} and C-6_{phen}), 71.23 (OCH₂), 68.35 (OCH₂_{morpholine}), 58.76 (NCH₂), 55.16 (NCH₂_{morpholine}), 54.80 (NCH₂), 49.27 (NCH₂), 28.02 (CH₂). ESI-MS m/z [M + H]⁺ calculated for C₃₆H₅₁N₄O₄: 603.3832, found: 603.39.

1,3-bis[(4-(3-(pyrrolidin-1-yl)propyl)aminomethyl)phenoxy)methyl]benzene (8g)

Yellow oil (70%); ¹H NMR (CDCl₃) δ ppm: 7.49 (s, 1H, H_{benz}), 7.38 (s, 3H, H_{benz}), 7.22 (d, 4H, J = 8.10 Hz, H-3_{phen}, and H-5_{phen}), 6.93 (d, 4H, J = 8.10 Hz, H-2_{phen}, and H-6_{phen}), 5.05 (s, 4H, OCH₂), 3.72 (s, 4H, NCH₂), 2.7 (t, 4H, J = 6.90 Hz, NCH₂), 2.51–2.46 (m, 12H, NCH₂, and NCH₂_{pyrrolidine}), 1.78–1.70 (m, 12H, CH₂, and CH₂_{pyrrolidine}); ¹³C NMR (CDCl₃) δ ppm: 159.10 (C-4_{phenyl}), 138.88 (Cq_{benz}), 134.37 (C-1_{phenyl}), 130.64 (C-3_{phen} and C-5_{phen}), 130.19 (CH_{benz}), 128.35 (CH_{benz}), 127.79 (CH_{benz}), 116.09 (C-2_{phen} and C-6_{phen}), 71.23 (OCH₂), 56.15 (NCH₂), 55.64 (NCH₂_{pyrrolidine}), 54.76 (NCH₂), 49.37 (NCH₂), 30.67

(CH₂), 24.79 (CH₂_{pyrrolidine}). ESI-MS m/z [M + H]⁺ calculated for C₃₆H₅₁N₄O₂: 571.3934, found: 571.40.

1,3-bis[(4-(3-(piperidin-1) propyl)aminomethyl)phenoxy)methyl]benzene (**8h**)

Yellow oil (98%); ¹H NMR (CDCl₃) δ ppm: 7.50 (s, 1H, H_{benz}), 7.38 (s, 3H, H_{benz}), 7.22 (d, 4H, J = 8.10 Hz, H-3_{phen}, and H-5_{phen}), 6.93 (d, 4H, J = 8.10 Hz, H-2_{phen}, and H-6_{phen}), 5.05 (s, 4H, OCH₂), 3.71 (s, 4H, NCH₂), 2.64 (t, 6H, J = 6.90 Hz, NCH₂), 2.36–2.31 (m, 12H, NCH₂, and NCH₂_{piperidine}), 1.70 (qt, 6H, J = 6.90 Hz, CH₂), 1.58–1.54 (m, 8H, CH₂_{piperidine}), 1.43–1.41 (m, 4H, CH₂_{piperidine}); ¹³C NMR (CDCl₃) δ ppm: 159.10 (C-4_{phenyl}), 138.88 (C_{qbenz}), 134.37 (C-1_{phenyl}), 130.66 (C-3_{phen} and C-5_{phen}), 130.20 (CH_{benz}), 128.37 (CH_{benz}), 127.81 (CH_{benz}), 116.09 (C-2_{phen} and C-6_{phen}), 71.23 (OCH₂), 56.17 (NCH₂), 56.06 (NCH₂_{piperidine}), 54.78 (NCH₂), 49.56 (NCH₂), 28.41 (CH₂), 27.37 (CH₂_{piperidine}), 25.85 (CH₂_{piperidine}). ESI-MS m/z [M + H]⁺ calculated for C₃₈H₅₅N₄O₂: 599.4247, found: 599.4321.

1,3-bis[(4-((quinolin-3-yl)aminomethyl)phenoxy)methyl]benzene (**8i**)

Pale-yellow oil (85%); ¹H NMR (CDCl₃) δ ppm: 8.47 (d, 2H, J = 2.70 Hz, H-2_{quinol}), 7.98 (dd, 2H, J = 6.90 and 3.60 Hz, H-8_{quinol}), 7.57 (dd, 2H, J = 6.90 and 3.60 Hz, H-5_{quinol}), 7.50 (m, 1H, H_{benz}), 7.46–7.39 (m, 7H, H_{benz}, H-6_{quinol}, and H-7_{quinol}), 7.35 (d, 4H, J = 7.80 Hz, H-3_{phen}, and H-5_{phen}), 7.00 (d, 4H, J = 7.80 Hz, H-2_{phen}, and H-6_{phen}), 6.96 (d, 2H, J = 2.70 Hz, H-4_{quinol}), 5.08 (s, 4H, OCH₂), 4.34 (d, 4H, J = 5.1 Hz, NCH₂). ¹³C NMR (CDCl₃) δ ppm: 159.59 (C-4_{phenyl}), 144.54 (C-2_{quinol}), 143.48 (C_{qbenz}), 142.83 (C-4a_{quinol}), 138.76 (C-8a_{quinol}), 131.97 (C-1_{phenyl}), 130.86 (C-3_{quinol}), 130.30 (C-3_{phen} and C-5_{phen}), 128.51 (CH_{benz}), 128.34 (CH_{benz}), 127.87 (CH_{benz}), 127.41 (C-4_{quinol}), 126.99 (C-6_{quinol}), 126.38 (C-5_{quinol}), 116.54 (C-2_{phen} and C-6_{phen}), 111.77 (C-7_{quinol}), 71.28 (OCH₂), 48.77 (NCH₂). ESI-MS m/z [M + H]⁺ calculated for C₄₀H₃₅N₄O₂: 603.2782, found: 603.2756.

1,3-bis[(4-(pyridin-2-ylmethylaminomethyl) phenoxy)methyl]benzene (**8j**)

Yellow oil (75%); ¹H NMR (CDCl₃) δ ppm: 8.57–8.55 (m, 2H, H-6_{pyrid}), 7.67–7.60 (m, 2H, H-4_{pyrid}), 7.52 (s, 1H, H_{benz}), 7.39 (s, 3H, H_{benz}), 7.33–7.25 (m, 6H, H-3_{phen}, H-5_{phen}, and H-3_{pyrid}), 7.18–7.15 (m, 2H, H-5_{pyrid}), 6.98–6.95 (d, 4H, J = 7.80 Hz, H-6_{phen}, and H-2_{phen}), 5.07 (s, 4H, OCH₂), 3.92 (s, 4H, NCH₂), 3.79 (s, 4H, NCH₂); ¹³C NMR (CDCl₃) δ ppm: 161.19 (C-4_{phenyl}), 159.21 (C-2_{pyrid}), 150.69 (C-6_{pyrid}), 138.91 (C_{qbenz}), 137.81 (C-4_{pyrid}), 134.06 (C-1_{phenyl}), 130.88 (C-3_{phen} and C-5_{phen}), 130.24 (CH_{benz}), 128.39 (CH_{benz}), 127.82 (CH_{benz}), 123.75 (C-5_{pyrid}), 123.32 (C-3_{pyrid}), 116.15 (C-2_{phen} and C-6_{phen}), 71.25 (OCH₂), 55.83 (NCH₂), 54.29 (NCH₂). ESI-MS m/z [M + H]⁺ calculated for C₃₄H₃₅N₄O₂: 531.2682, found: 531.2760.

1,3-bis[(4-(pyridin-2-ylethylaminomethyl)phenoxy)methyl]benzene (**8k**)

Orange oil (89%); ¹H NMR (CDCl₃) δ ppm: 8.51–8.49 (m, 2H, H-6_{pyrid}), 7.59–7.53 (m, 2H, H-4_{pyrid}), 7.49 (s, 1H, H_{benz}), 7.37 (s, 3H, H_{benz}), 7.20–7.18 (m, 6H, H-3_{phen}, H-5_{phen}, and H-3_{pyrid}), 7.18–7.15 (m, 2H, H-5_{pyrid}), 6.96–6.90 (d, 4H, J = 7.80 Hz, H-6_{phen}, and H-2_{phen}), 5.03 (s, 4H, OCH₂), 3.74 (s, 4H, NCH₂), 3.01 (m, 4H, CH₂); ¹³C NMR (CDCl₃) δ ppm: 161.19 (C-4_{phenyl}), 159.21 (C-2_{pyrid}), 150.65 (C-6_{pyrid}), 138.91 (C_{qbenz}), 137.81 (C-4_{pyrid}), 134.10 (C-1_{phenyl}), 130.76 (C-3_{phen} and C-5_{phen}), 130.22 (CH_{benz}), 128.37 (CH_{benz}), 127.80 (CH_{benz}), 124.71 (C-5_{pyrid}), 122.67 (C-3_{pyrid}), 116.14 (C-2_{phen} and C-6_{phen}), 71.22 (OCH₂), 54.57 (NCH₂), 50.11 (NCH₂), 39.68 (CH₂). ESI-MS m/z [M + H]⁺ calculated for C₃₆H₃₉N₄O₂: 559.2995, found: 559.3061.

1,3-bis[(4-(pyridin-2-ylpropylaminomethyl)phenoxy)methyl]benzene (**8l**)

Yellow oil (85%); ¹H NMR (CDCl₃) δ ppm: 8.50–8.48 (m, 2H, H-6_{pyrid}), 7.59–7.53 (m, 2H, H-4_{pyrid}), 7.50 (s, 1H, H_{benz}), 7.38 (s, 3H, H_{benz}), 7.24 (d, 4H, J = 8.10 Hz, H-3_{phen}, and H-5_{phen}), 7.21–7.06 (m, 4H, H-5_{pyrid}, and H-3_{pyrid}), 6.96–6.91 (d, 4H, J = 7.80 Hz, H-6_{phen}, and H-2_{phen}), 5.05 (s, 4H, OCH₂), 3.71 (s, 4H, NCH₂), 3.71 (s, 4H, NCH₂), 2.81 (t, 4H, J = 6.90 Hz, NCH₂), 2.66 (t, 4H, J = 6.90 Hz, CH₂_{Pyrid}), 1.94 (qt, J = 6.90 Hz, CH₂); ¹³C NMR (CDCl₃) δ ppm: 161.19 (C-4_{phenyl}), 159.12 (C-2_{pyrid}), 150.65 (C-6_{pyrid}), 138.87 (C_{qbenz}),

137.80 (C-4_{pyrid}), 134.21 (C-1_{phenyl}), 130.75 (C-3_{phen} and C-5_{phen}), 130.23 (CH_{benz}), 128.40 (CH_{benz}), 127.80 (CH_{benz}), 124.18 (C-5_{pyrid}), 122.44 (C-3_{pyrid}), 116.09 (C-2_{phen} and C-6_{phen}), 71.22 (OCH₂), 54.64 (NCH₂), 50.01 (NCH₂), 37.33 (CH₂), 31.38 (CH₂). ESI-MS m/z [M + H]⁺ calculated for C₅₄H₆₂N₆: 841.47, found: 841.48.

1,3-bis[(4-(pyridin-3-ylmethylaminomethyl)phenoxy)methyl]benzene (8m)

Pale-yellow oil (94%); ¹H NMR (CDCl₃) δ ppm: 8.57–8.48 (m, 4H, H-6_{pyrid}, and H-2_{pyrid}), 7.70 (d, 2H, J = 7.80 Hz, H-4_{pyrid}), 7.52 (s, 1H, H_{benz}), 7.40 (s, 3H, H_{benz}), 7.25–7.23 (m, 6H, H-5_{pyrid}, H-3_{phen}, and H-5_{phen}), 6.93 (d, 4H, J = 8.10 Hz, H-6_{phen}, and H-2_{phen}), 5.05 (s, 4H, OCH₂), 3.79 (s, 4H, NCH₂), 3.74 (s, 4H, CH₂); ¹³C NMR (CDCl₃) δ ppm: 159.28 (C-4_{phenyl}), 151.12 (C-2_{pyrid}), 149.83 (C-6_{pyrid}), 138.86 (Cq_{benz}), 137.25 (C-3_{pyrid}), 133.80 (C-1_{phenyl}), 130.76 (C-3_{phen} and C-5_{phen}), 130.25 (CH_{benz}), 128.42 (CH_{benz}), 127.83 (CH_{benz}), 124.79 (C-5_{pyrid}), 116.21 (C-2_{phen} and C-6_{phen}), 71.25 (OCH₂), 53.96 (NCH₂), 51.71 (CH₂). ESI-MS m/z [M + H]⁺ calculated for C₃₄H₃₅N₄O₂: 531.2682, found: 531.2753.

1,3-bis[(4-(pyridin-3-ylethylaminomethyl)phenoxy)methyl]benzene (8n)

Yellow oil (62%); ¹H NMR (CDCl₃) δ ppm: 8.48–8.44 (m, 4H, H-6_{pyrid}, and H-2_{pyrid}), 7.70 (d, 2H, J = 7.80 Hz, H-4_{pyrid}), 7.52 (s, 1H, H_{benz}), 7.40 (s, 3H, H_{benz}), 7.23–7.19 (m, 6H, H-5_{pyrid}, H-3_{phen}, and H-5_{phen}), 6.91 (d, 4H, J = 7.80 Hz, H-6_{phen} and H-2_{phen}), 5.06 (s, 4H, OCH₂), 3.75 (s, 4H, NCH₂), 2.90–2.81 (m, 8H, NCH₂, and CH₂Pyrid); ¹³C NMR (CDCl₃) δ ppm: 159.22 (C-4_{phenyl}), 151.57 (C-2_{pyrid}), 149.04 (C-6_{pyrid}), 138.86 (C-3_{pyrid}), 137.57 (C-4_{pyrid}), 136.87 (Cq_{benz}), 133.97 (C-1_{phenyl}), 130.68 (C-3_{phen} and C-5_{phen}), 130.24 (CH_{benz}), 128.41 (CH_{benz}), 127.83 (CH_{benz}), 124.76 (C-5_{pyrid}), 116.18 (C-2_{phen} and C-6_{phen}), 71.25 (OCH₂), 54.61 (NCH₂), 51.42 (NCH₂), 34.95 (CH₂). ESI-MS m/z [M + H]⁺ calculated for C₃₆H₃₉N₄O₂: 559.2995, found: 559.3065.

1,3-bis[(4-(pyridin-3-ylpropylaminomethyl)phenoxy)methyl]benzene (8o)

Yellow oil (90%); ¹H NMR (CDCl₃) δ ppm: 8.43–8.39 (m, 4H, H-6_{pyrid}, and H-2_{pyrid}), 7.48 (d, 2H, J = 7.80 Hz, H-4_{pyrid}), 7.31 (s, 3H, H_{benz}), 7.23–7.14 (m, 7H, H-5_{pyrid}, H_{benz}, H-3_{phen}, and H-5_{phen}), 7.12 (d, 4H, J = 7.80 Hz, H-6_{phen}, and H-2_{phen}), 5.04 (s, 4H, OCH₂), 3.69 (s, 4H, NCH₂), 2.66–2.61 (m, 8H, NCH₂, and CH₂Pyrid), 1.80 (qt, 4H, J = 7.20 Hz, CH₂); ¹³C NMR (CDCl₃) δ ppm: 159.17 (C-4_{phenyl}), 151.24 (C-2_{pyrid}), 148.67 (C-6_{pyrid}), 138.84 (Cq_{benz}), 138.85 (C-1_{phenyl}), 137.20 (C-4_{pyrid}), 134.23 (C-3_{pyrid}), 130.71 (C-3_{phen} and C-5_{phen}), 130.21 (CH_{benz}), 128.38 (C-5_{pyrid}), 127.80 (CH_{benz}), 124.70 (CH_{benz}), 116.14 (C-2_{phen} and C-6_{phen}), 71.23 (OCH₂), 54.75 (NCH₂), 49.87 (NCH₂), 32.75 (CH₂), 32.04 (CH₂). ESI-MS m/z [M + H]⁺ calculated for C₃₈H₄₃N₄O₂: 587.3308, found: 587.3375.

1,3-bis[(4-(pyridin-4-ylmethylaminomethyl)phenoxy)methyl]benzene (8p)

Yellow oil (65%); ¹H NMR (CDCl₃) δ ppm: 8.54 (d, 4H, J = 6.00 Hz, H-2_{pyrid}, and H-6_{pyrid}), 7.51 (s, 1H, H_{benz}), 7.40 (s, 3H, H_{benz}), 7.29–7.28 (m, 8H, H-3_{pyrid}, H-5_{pyrid}, H-3_{phen}, and H-5_{phen}), 6.96 (d, 4H, J = 8.10 Hz, H-6_{phen}, and H-2_{phen}), 5.06 (s, 4H, OCH₂), 3.79 (s, 6H, NCH₂), 3.73 (s, 4H, NCH₂); ¹³C NMR (CDCl₃) δ ppm: 159.31 (C-4_{phenyl}), 151.13 (C-2_{pyrid} and C-6_{pyrid}), 150.2 (C-4_{pyrid}), 138.84 (Cq_{benz}), 133.69 (C-1_{phenyl}), 130.76 (C-3_{phen} and C-5_{phen}), 130.27 (CH_{benz}), 128.44 (CH_{benz}), 127.84 (CH_{benz}), 124.43 (C-3_{pyrid} and C-5_{pyrid}), 116.21 (C-2_{phen} and C-6_{phen}), 71.25 (OCH₂), 53.96 (NCH₂), 53.09 (NCH₂). ESI-MS m/z [M + H]⁺ calculated for C₃₄H₃₅N₄O₂: 531.2682, found: 531.2751.

1,3-bis[(4-(pyridin-4-ylethylaminomethyl)phenoxy)methyl]benzene (8q)

Yellow oil (96%); ¹H NMR (CDCl₃) δ ppm: 8.49–8.47 (m, 4H, H-2_{pyrid}, and H-6_{pyrid}), 7.50 (s, 1H, H_{benz}), 7.38 (s, 3H, H_{benz}), 7.18 (d, 4H, J = 8.10 Hz, H-3_{phen}, and H-5_{phen}), 7.12–7.10 (m, 8H, H-3_{pyrid}, H-5_{pyrid}), 6.91 (d, 4H, J = 8.10 Hz, H-6_{phen}, and H-2_{phen}), 5.04 (s, 4H, OCH₂), 3.73 (s, 4H, NCH₂), 2.89 (t, 4H, J = 6.90 Hz, CH₂), 2.78 (t, 4H, J = 6.90 Hz, CH₂); ¹³C NMR (CDCl₃) δ ppm: 159.22 (C-4_{phenyl}), 151.13 (C-2_{pyrid} and C-6_{pyrid}), 150.58 (C-4_{pyrid}), 138.84 (Cq_{benz}), 133.84 (C-1_{phenyl}), 130.68 (C-3_{phen} and C-5_{phen}), 130.23 (CH_{benz}), 128.42 (CH_{benz}), 127.81 (CH_{benz}), 125.55 (C-3_{pyrid} and C-5_{pyrid}), 116.16 (C-2_{phen} and C-6_{phen}), 71.23

(OCH₂), 54.57 (NCH₂), 50.60 (NCH₂). 37.12 (CH₂). ESI-MS m/z [M + H]⁺ calculated for C₃₆H₃₉N₄O₂: 559.2995, found: 559.3061.

1,3-bis[(4-(pyridin-4-yl)propylaminomethyl)phenoxy]methyl]benzene (**8r**)

Yellow oil (99%); ¹H NMR (CDCl₃) δ ppm: 8.44–8.41 (m, 4H, H-2_{pyrid}, and H-6_{pyrid}), 7.50 (s, 1H, H_{benz}), 7.36 (s, 3H, H_{benz}), 7.18 (d, 4H, J = 8.10 Hz, H-3_{phen}, and H-5_{phen}), 7.07–7.04 (m, 8H, H-3_{pyrid}, H-5_{pyrid}) 6.91 (d, 4H, J = 8.10 Hz, H-6_{phen}, and H-2_{phen}), 5.03 (s, 4H, OCH₂), 3.68 (s, 4H, NCH₂), 2.65–2.58 (m, 8H, NCH₂, and CH₂), 1.78 (t, 4H, J = 6.90 Hz, CH₂); ¹³C NMR (CDCl₃) δ ppm: 159.19 (C-4_{phenyl}), 151.58 (C-4_{pyrid}), 150.93 (C-2_{pyrid} and C-6_{pyrid}), 138.84 (C_{qbenz}), 134.16 (C-1_{phenyl}), 130.71 (C-3_{phen} and C-5_{phen}), 130.20 (CH_{benz}), 128.42 (CH_{benz}), 127.78 (CH_{benz}), 125.27 (C-3_{pyrid} and C-5_{pyrid}), 116.16 (C-2_{phen} and C-6_{phen}), 71.23 (OCH₂), 54.68 (NCH₂), 49.79 (NCH₂). 34.22 (CH₂), 31.87 (CH₂). ESI-MS m/z [M + H]⁺ calculated for C₃₈H₄₃N₄O₂: 587.3308, found: 587.3387.

3.1.8. General Procedure for the Synthesis of 1,3-bis[(4-(Substituted-aminomethyl)phenyl)methyl]benzene ammonium oxalates salts **1a-r** and 1,3-bis[(4-(substituted-aminomethyl)phenoxy)methyl]benzene ammonium oxalates salts **2a-r**

To a solution of compounds **7–8a–r** (0.3 mmol) in isopropanol (11 mL) was added oxalic acid (2.4 mmol, 8 eq.). The reaction mixture was heated under reflux for 30 min. The precipitate was filtered, washed with isopropanol then with diethyl ether, and dried under reduced pressure to give ammonium oxalate salts **1–2**, which were crystallized using a mixture of 2-PrOH–H₂O solvents.

3.2. Biological Evaluation

3.2.1. In Vitro Antiplasmodial Activity

Derivatives **1–2** were dissolved in DMSO and then diluted in sterile water in order to obtain a range of concentration from 40 nM to 40 mM for the first screening against culture-adapted *Plasmodium falciparum* reference strains 3D7 and W2. The former strain is susceptible to CQ but displays a decreased susceptibility to MQ; the latter is considered resistant to CQ. These two strains were obtained from the collection of the National Museum of Natural History (Paris, France). The parasites were cultivated in RPMI medium with human erythrocytes that was (Sigma-Aldrich, Lyon, France) supplemented with 0.5% Albumax I (Life Technologies Corporation, Paisley, UK), hypoxanthine (Sigma-Aldrich), and gentamicin (Sigma-Aldrich) and were incubated at 37 °C in a candle jar, as described previously [42]. The *P. falciparum* drug susceptibility test was carried out in 96-well flat-bottom sterile plates in a final volume of 250 µL. After 48 h incubation period with the drugs, quantities of DNA in treated and control cultures of parasites in human erythrocytes were quantified using the SYBR Green I (Sigma-Aldrich) fluorescence-based method [43,44]. Briefly, after incubation, plates were frozen at –20 °C until use. Plates were then thawed for 2 h at room temperature, and 100 µL of each homogenized culture was transferred to a well of a 96-well flat-bottom sterile black plate (Sigma-Aldrich, Lyon, France) that contained 100 µL of the SYBR Green I lysis buffer (2xSYBR Green, 20 mM Tris base pH 7.5, 5 mM EDTA, 0.008% w/v saponin, 0.08% w/v Triton X-100). Negative controls treated with solvent (typically DMSO or H₂O) and positive controls (CQ and MQ) were added to each set of experiments. Plates were incubated for 1 h at room temperature and then read on a fluorescence plate reader (Tecan Trading AG, Männedorf, Switzerland) using excitation and emission wavelengths of 485 and 535 nm, respectively. The concentrations at which the screening drug or antimalarial can inhibit 50% of parasitic growth (IC₅₀) were calculated from a sigmoid inhibition model Emax with an estimate of the IC₅₀ obtained using non-linear regression (IC Estimator version 1.2) and were reported as means calculated from three independent experiments [45].

3.2.2. Cytotoxicity Evaluation

A cytotoxicity evaluation was performed using the method reported by Mosmann [46] with slight modifications to determine the CC₅₀ and using doxorubicin as a cytotoxic reference compound. These assays were performed in human HepG2 cells purchased from ATCC (ref. HB-8065). These cells are a commonly used human hepatocarcinoma-derived cell line that has characteristics like those of primary hepatocytes. These cells express many hepatocyte-specific metabolic enzymes, thus enabling the cytotoxicity of tested product metabolites to be evaluated. Briefly, cells in 100 µL of complete RPMI medium (RPMI supplemented with 10% FCS, 1% L-glutamine (200 mM), penicillin (100 U/mL), and streptomycin (100 µg/mL)) were inoculated at 37 °C into each well of 96-well plates in a humidified chamber in 6% CO₂. After 24 h, 100 µL of medium, with the test compound at various concentrations dissolved in DMSO (final concentration less than 0.5% v/v), were added, and the plates were incubated for 72 h at 37 °C. Duplicate assays were performed for each sample. Each well was microscopically examined for precipitate formation before the medium was aspirated from the wells. After aspiration, 100 µL of MTT solution (0.5 mg/mL in medium without FCS) was then added to each well. Cells were incubated for 2 h at 37 °C. The MTT solution was removed and DMSO (100 µL) was added to dissolve the resulting blue formazan crystals. Plates were shaken vigorously (300 rpm) for 5 min. The absorbance was measured at 570 nm with 630 nm as the reference wavelength in a BIO-TEK ELx808 Absorbance Microplate Reader. DMSO was used as blank and doxorubicin (Sigma-Aldrich) as the positive control. Cell viability was calculated as percentage of control (cells incubated without compound). The CC₅₀ was determined from the dose–response curve using TableCurve 2D V5.0 software (Systat Software, Palo Alto, CA, USA).

3.3. FRET Melting Experiments

Compounds 1–2 were tested in subsequent FRET melting experiments. These were performed with dual-labeled oligonucleotides mimicking the *Plasmodium* telomeric sequences FPf1T (FAM-5'(GGGTTTA)3-GGG3'-TAMRA) and FPf8T [FAM-5'(GGGTTCA)3GGG3'-TAMRA], the human telomeric sequence F21T (FAM-(GGGTTA)3-GGG3'-TAMRA), and the human duplex sequence FdxT (FAM5'-TATAGCTATA-hexaethyleneglycol-TATAGCTATA3'-TAMRA) [25,26,47]. The oligonucleotides were pre-folded in 10 mM lithium cacodylate buffer (pH 7.2), with 10 mM KCl and 90 mM LiCl (K⁺ condition). The FAM emissions were recorded at 516 nm using a 492 nm excitation wavelength in the absence and presence of a single compound as a function of temperature (25 to 95 °C) in 96-well microplates by using a Stratagene MX3000P real-time PCR device at a rate of 1 °C·min⁻¹. Data were normalized between 0 and 1, and the required temperature for half denaturation of oligonucleotides, corresponding to an emission value of 0.5, was taken as the T_m. Each experiment was performed in duplicate with 0.2 µM of labelled oligonucleotide and 2 µM of compound under K⁺ condition. For each compound, three independent experiments were carried out.

4. Conclusions

In this research program report, we described the preparation, the antimalarial potentialities, and the in vitro cytotoxicity toward human cells of two novel series of 1,3-bis[(4-(substituted-aminomethyl)phenyl)methyl]benzene derivatives (1) and 1,3-bis[(4-(substituted-aminomethyl)phenoxy)methyl]benzene derivatives (2). These newly developed polyaromatic derivatives were evaluated for their in vitro antiprotozoal activity against both chloroquine-resistant (W2) and chloroquine-sensitive (3D7) strains of *Plasmodium falciparum*. Additionally, the cytotoxicity of these compounds was assessed in vitro using the human HepG2 cell line. Several of the synthesized aromatic nitrogen com-

pounds demonstrated promising in vitro antiplasmodial activity, with IC₅₀ values in the submicromolar to micromolar range against both W2 and 3D7 strains.

Among these, compounds **2k** and **1f** emerged as the most potent bioactive candidates against both the W2 and 3D7 strains. Notably, compound **1f** showed the most promising profile against the CQ-sensitive 3D7 strain with reduced sensitivity to MQ.

Previous studies have suggested that the telomeres of protozoan parasites could be considered as promising therapeutic targets. Therefore, we also explored the potential of these newly synthesized compounds to stabilize *Plasmodium* G-quadruplex (G4) sequences using FRET melting assays. Interestingly, while some derivatives demonstrated interesting stabilization of protozoal G-quadruplex structures, these did not correspond to the most effective antimalarial compounds. Consequently, no clear correlation was found between G-quadruplex stabilization and antimalarial efficacy for this series of compounds. It is unlikely that these 1,3-bis[(4-(substituted-aminomethyl)phenyl)methyl]benzene derivatives (**1**) and 1,3-bis[(4-(substituted-aminomethyl)phenoxy)methyl]benzene derivatives (**2**) exert their antimalarial activity through specific G-quadruplex binding mechanisms. Indeed, the discrepancy between activity and stabilization of G-quadruplexes demonstrates the complexity of the mechanism of action, which requires further research.

Furthermore, it would be valuable to expand the pharmacological evaluation of our new derivatives by exploring their potential mechanisms of action through additional studies—such as assessing their ability to inhibit β -hematin formation, interfere with apicoplast functions, or induce resistance. In conclusion, these novel polyaromatic 1,3-bis[(4-(substituted-aminomethyl)phenyl)methyl]benzene and 1,3-bis[(4-(substituted-aminomethyl)phenoxy)methyl]benzene compounds, **1–2**, may pave the way for the development of new, valuable, and original scaffolds in antimalarial drug design.

Supplementary Materials: The following supporting informations can be downloaded at: <https://www.mdpi.com/article/10.3390/ddc4030039/s1>, Supplementary data (Figures S1a–c to S36a–c) related to this article (¹H NMR, ¹³C NMR and ESI-MS of derivatives **7–8-a-r**) are available online.

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