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Synthesis of 3- and 5'-substituted flavone-8-carboxylic acids as 'three-armed' leukotriene *CysLT$_1$* receptor antagonists

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Abstract – Molecular modelling of leukotriene *CysLT$_1$* receptor antagonists have suggested that in addition to the two binding sites for a lipophilic and an acidic group, the receptor has a 'third pocket' to accommodate 'three-armed' ligands such as montelukast **1**. Based on the most rigid *CysLT$_1$* receptor antagonist 3'-[2-(2-quinolinyl)ethenyl]flavone-8-carboxylic acid **2**, we have synthesised 3- and 5'-substituted flavone derivatives to probe this additional binding pocket. Introduction of large substituents, e.g. 2-quinolinylmethoxy, to the C5' position of the flavone skeleton abolished the *CysLT$_1$* receptor affinity whereas the same modification at the C3 position yielded a potent *CysLT$_1$* antagonist. This observation implies that the third binding pocket of the receptor has considerable steric tolerance, probably corresponding to the substituents at C3 of the flavone skeleton. Further modification by introducing a C3 substituent containing a basic nitrogen resulted in compound **6g** with potent H_1 antihistaminic activity although the *CysLT$_1$* antagonistic activity was much reduced. Further study on the *CysLT$_1$* receptor recognition of three-armed antagonists may facilitate the design of more effective antiasthmatic agents, e.g. dual antagonists of histamine H_1 and leukotriene *CysLT$_1$* receptors. © Elsevier, Paris

antiasthmatic agents / histamine H_1 antagonists / leukotriene D_4 antagonists / molecular modelling

1. Introduction

Over the past decade evidence has accumulated showing that leukotrienes play an important role in the pathogenesis of asthma [1–6]. Structurally leukotrienes are divided into two groups: the hydroxyleukotriene LTB_4 and the cysteinyl leukotrienes (CysLTs) LTC_4, LTD_4 and LTE_4. CysLTs appear to be more important mediators than LTB_4 in asthma, especially as bronchoconstrictors. CysLTs also cause mucus secretion, increase vascular permeability, and are involved in the inflammation and hyperresponsiveness of human airways. Asthmatic subjects are more sensitive to the actions of CysLTs [7] and elevated levels of these endogenous substances have been detected during asthmatic attacks [8, 9]. Recent clinical success with CysLT antagonists, e.g. zafirlukast, pranlukast, montelukast [3, 10–12], represents the most significant advance in the acquisition of antiasthmatic agents for the last 20 years.

CysLTs produce their effects by stimulation of specific membrane receptors belonging to the G-protein-coupled superfamily [13, 14]. Two subtypes have been identified to bind specifically to CysLTs: *CysLT$_1$* for LTD_4 and LTE_4, and *CysLT$_2$* for LTC_4. Stimulation of the *CysLT$_1$* receptor leads to the characteristic symptoms of asthma whereas the consequences of selective activation of the *CysLT$_2$* receptor remain to be clarified.

In our search for effective antiasthmatic agents we became interested in *CysLT$_1$* receptor antagonists [15, 16]. Our attempts to define antagonist recognition of *CysLT$_1$* receptors started with the synthesis of a series of carboxylated flavonoids, which resulted in the discovery of two new leads 4-hydroxy-3-[1-oxo-2-propenyl-3-(2-quinolinyl)]benzoic acid and 6-bromo-8-carboxy-4-oxo-2-(2-quinolinyl)-1-4H-benzopyran with weak *CysLT$_1$* receptor affinity [16]. Further modification afforded a series of carboxylated chalcones [17] and flavones [18] with potent *CysLT$_1$* antagonistic activity. The flavone series contains the most rigid *CysLT$_1$* receptor antagonists known to date [18], for example 3'-(2-(2-quinolinyl)ethenyl)flavone-8-carboxylic acid (**2**, VUF 5017). Consequently we have developed a 3D model of *CysLT$_1$* antagonists using **2** as templates for the superimposition of various known *CysLT$_1$* antagonists [18].

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Based on this model we have shown that in addition to the two characteristic binding sites for a lipophilic group and an acidic function, respectively, there is a third pocket in the receptor cavity to accommodate the 'third-arm' of antagonists such as montelukast **1** and sulukast [10] (*figure 1*). Computer-aided superimposition (*figure 1*) of **1** and **2** revealed that this additional binding pocket might correspond to a substituent at either the C3 or the C5' position of the flavone skeleton. We therefore prepared derivatives of **2** by introducing various substituents at the C3 and C5' positions in order to probe this third binding pocket of the $CysLT_1$ receptor. In this paper we wish to report the synthesis and in vitro activity of these new flavone derivatives containing 3- or 5'-substituents. Preliminary attempts to design dual antagonists of histamine H_1 and leukotriene $CysLT_1$ receptors based on the above modification is also included. It has been proposed that such dual antagonists may be more effective antiasthmatic agents than selective $CysLT_1$ antagonists [15, 19].

2. Chemistry

For the synthesis of 'three-armed' flavones it would be most convenient to introduce a wide range of substituents at a late stage. Since alkylation of 3- or 5'-hydroxyflavones provides a facile route to various substituents having different physicochemical properties, we chose 3- and 5'-alkyloxy derivatives as our targets. The 3-hydroxyflavones **4**, the key intermediates for a wide range of 3-substituted flavone derivatives, were obtained from the corresponding chalcones **3** [17] in a one-step procedure (Algar–Flynn–Oyamada oxidation [21, 22]) (*figure 2*) in about 50% yield. Alkylation of **4** with an appropriate alkyl halide and the subsequent hydrolysis of the resulting esters **5** afforded the 3-substituted flavones **6**.

Although it seemed straightforward, alkylation of **4** in some cases may be troublesome. For instance, alkylation with 1,3-dibromopropane, 1-bromo-3-chloropropane or 3-bromo ethyl propionate gave unidentified mixtures of products. Hydrolysis of the esters **5**

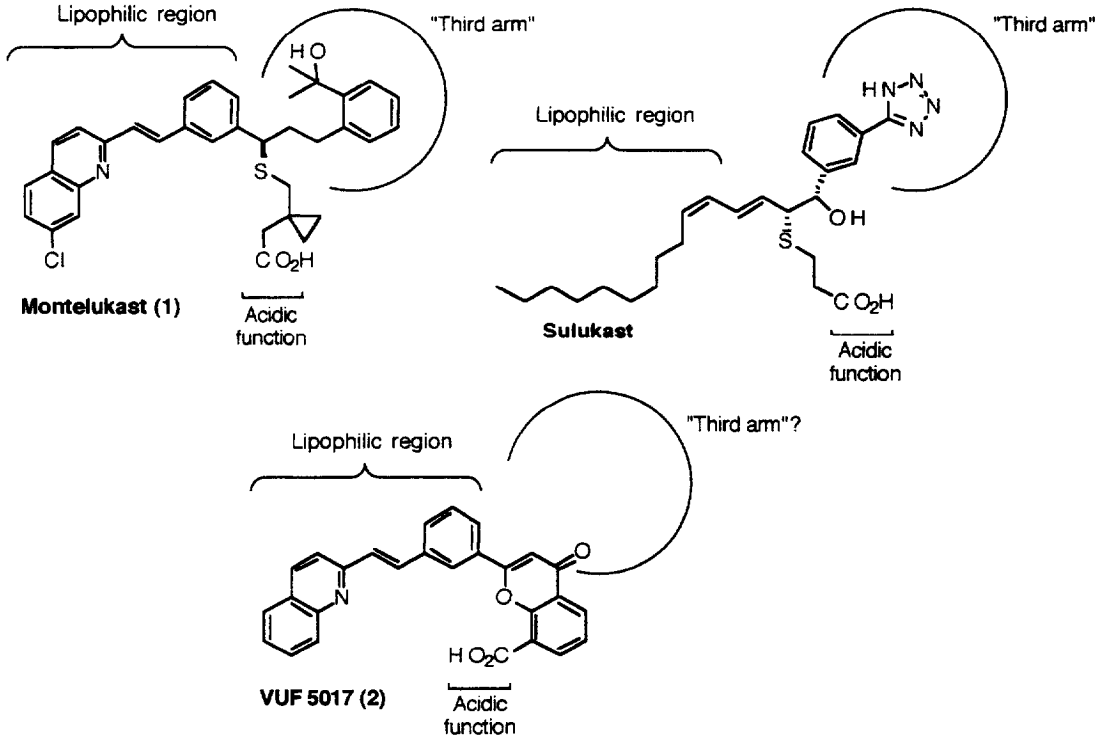


Figure 1. Correspondence of the 'third arms' of $CysLT_1$ receptor antagonists.

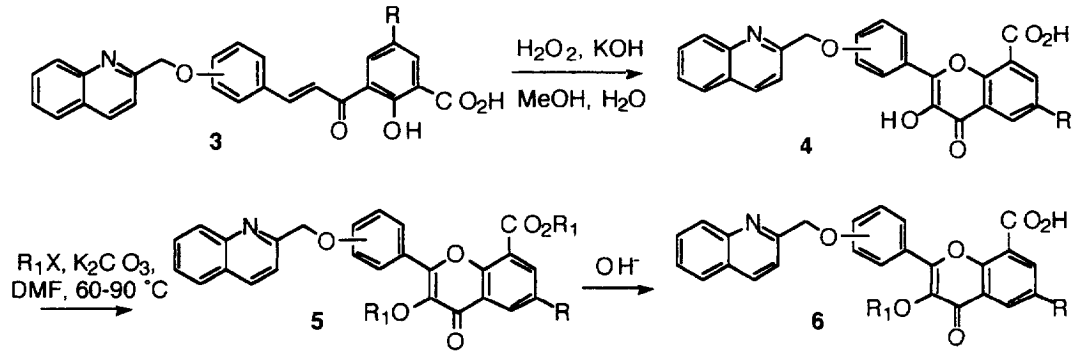


Figure 2. Synthesis of 3-substituted flavones **6**.

generally proceeded smoothly under the influence of sodium- or potassium hydroxide in a mixture of water and ethanol. In some cases THF was added to improve the solubility of the reactants. Target compounds **6** were obtained in 10–15% overall yield.

Compound **6g** was synthesised by reductive alkylation [23] of the primary amine **6f** which was obtained from the phthalimide **6e** with hydrazine (*figure 3*).

For the synthesis of 5'-substituted flavones, 5'-hydroxy-3'-(2-quinolinylmethoxy)flavone was considered as a useful precursor. The synthetic approach depicted in *figure 4* was used for obtaining this compound [24–26]. This route involved the coupling of **7** and acetophenone **8** to form the 1,3-diketone **9**. This intermediate was cyclodehydrated to flavone **10** which upon demethylation with boron tribromide in dichloromethane afforded 3',5'-dihydroxyflavone **11** in good yield. Aluminium chloride or bromide were found not suitable for the demethylation of **10**. Esterification of **11** provided compound **12**.

Alkylation of **12** in the presence of one equivalent 2-chloromethylquinoline resulted in the isolation of the dialkylated product **13** only, instead of the desired mono-substituted flavone. Apparently, the increased electron-donating effect of the 2-quinolinylmethoxy group in the monoalkylated flavone resulted in an increased reactivity of the second hydroxyl group to such an extent that the monoalkylated flavone is much more reactive towards the alkylating agent than the dihydroxyflavone **12**. Hydrolysis of **13** in a mixture of DMF-water in the presence of potassium hydroxide yielded 3',5'-bis(2-quinolinemethoxy) **14**.

3. Results and discussion

The *CysLT*$_1$ receptor affinities of 3- and 5'-substituted flavones are shown in *table I*, together with the receptor affinities of the reference compounds VUF 4918, VUF 4871 and VUF 5165 [18]. Compared with VUF 4918, the 3-hydroxyl analogue **4a** showed a 25-fold decreased affinity and the 3-carboxymethoxy analogue **6a** had even lower affinity. This seems to suggest that small and hydrophilic substituents are not

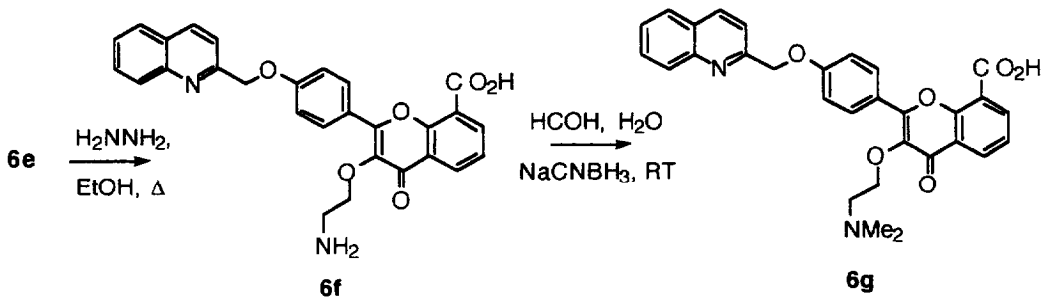


Figure 3. Synthesis of **6g**.

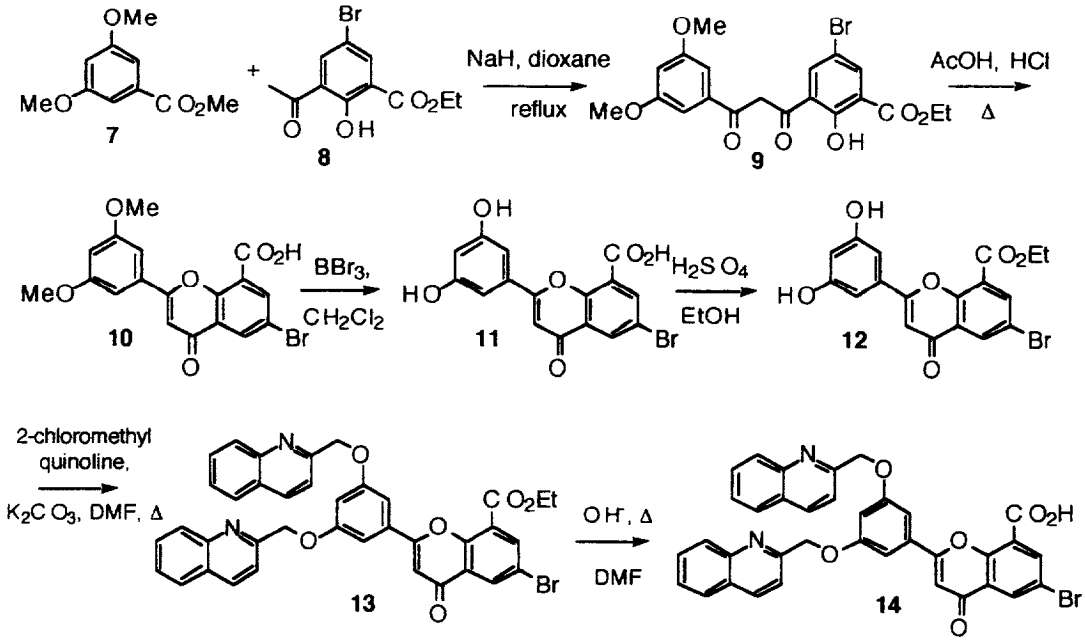
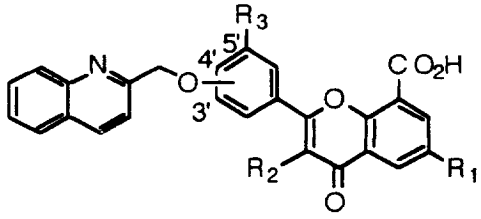


Figure 4. Synthesis of 5'-substituted flavone **14**.

Table I. *CysLT*$_1$ receptor affinity of 3- and 5'-substituted flavones.



Compound	Sub. pos.	R_1	R_2	R_3	K_D (nM)[a], or (% inhib. at 10^{-5} M)
VUF 4918	3'	H	H	H	17 ± 3
VUF 4871	4'	H	H	H	526 ± 88
VUF 5165	3'	Br	H	H'	31 ± 9
4a	3'	H	OH	H	452 ± 97
4b	3'	Br	OH	H	38 ± 9
4c	4'	H	OH	H	805 ± 130
6a	3'	H	OCH_2CO_2H	H	639 ± 78
6b	3'	Br	OCH_2CO_2H	H	40 ± 7
6c	3'	Br	$OCH_2CH_2CH_3$	H	44 ± 2
6d	3'	Br	2-quinolinylmethoxy	H	63 ± 3
6e	4'	H	$O(CH_2)_2$phthalimide	H	916 ± 166
6g	4'	H	$O(CH_2)_2NMe_2$	H	4020 ± 430
14	3'	Br	H	2-quinolinylmethoxy	(5%)

[a]The affinity data were obtained from binding assays in guinea pig lung membranes versus [^{3}H]LTD$_4$. The data are means ± S.E.M. of three determinations.

favourable at this position. However, when compared with VUF 4871, the corresponding 3-hydroxyl analogue **4c** was similarly active, maybe due to the low sensitivity of less active compounds towards modification (VUF 4817 and **4c** are much less active than their 3'-(2-quinolinemethoxy) counterparts VUF 4918 and **4a**).

Among the 6-bromo derivatives, the sensitivity towards 3-hydroxylation was virtually invisible and the 3-hydroxyflavone **4b** was as active as the non-hydroxy VUF 5165. This insensitivity towards substitution at C3 continued with carboxymethoxy **6b**, *n*-propoxy **6c** and 2-quinolinemethoxy **6d**. When compared with the non-brominated analogues VUF 4918, **4a** and **6a**, this insensitivity towards substitution at C3 seems to result from the bromine atom at position 6. Whether this is an electronic or lipophilic factor remains to be further investigated.

The most interesting structure–activity relationship within this series are the different effects of the introduction of a 2-quinolinemethoxy group to the C3 and C5' positions. The 3-substituted compound **6d** showed an affinity value (K_D) of 63 nM but the 5'-substituted compound **14** was virtually inactive. This observation implies that the third binding pocket of the *CysLT*$_1$ receptor corresponds to the substituent at position 3 of the flavone skeleton and has considerable steric tolerance.

In addition to the *CysLT*$_1$ receptor affinity discussed above, we have noticed that **6g** possessed some structural characteristics of histamine H_1 antagonists. Indeed the compound exhibited high affinity to histamine H_1 receptor with a K_D value of 10 nM versus [^{3}H]mepyramine in guinea pig lung membrane. Histamine H_1-receptor antagonists are very useful therapeutic agents for many allergic disorders [27]. Due to the complementary actions of CysLTs and histamine during inflammatory and allergic responses [28–30], compounds with dual antagonism of *CysLT*$_1$ and H_1 receptors may be more effective than any single mediator antagonists in the treatment of asthma [15, 19]. Further modification of **6g** towards a better balanced dual antagonism of H_1 and *CysLT*$_1$ receptors may provide eventually more effective antiasthmatic agents.

4. Conclusions

Based on the superimposition of **1** (montelukast) and **2** as incorporated in our 3D *CysLT*$_1$ antagonist model [18], we have suggested that a 'third arm' may be introduced into the flavone skeleton. Introduction of a 2-quinolinemethoxy substituent revealed that the antagonist binding site differentiated the substitutions at the 3- and 5'-positions, with the additional interaction point probably locating at a place corresponding to that of the C3 substituent of the flavone skeleton. Further modification on the C3 position, e.g. introduction of a nitrogen-containing group, may result in compounds with better antiasthmatic profiles, e.g. dual antagonism of histamine H_1 and leukotriene *CysLT*$_1$ receptors [15, 19].

5. Experimental protocols

5.1. Pharmacology

5.1.1. Radioligand binding assay with [^{3}H]LTD$_4$

The method is very similar to that described previously [15]. Briefly, a mixture of total volume of 0.3 mL containing 0.2 nM [^{3}H]LTD$_4$, guinea-pig lung membrane fractions (± 170 µg/mL) and the testing compound in a 10 mM piperazine-N,N'-bis(2-ethanesulphonic) acid buffer (pH 7.5) was incubated at 22 °C for 30 min. The piperazine-N,N'-bis(2-ethanesulphonic) acid buffer contains 10 mM $CaCl_2$, 10 mM $MgCl_2$, 50 mM NaCl, 2 mM cysteine and 2 mM glycine. The reaction was terminated by the addition of 5 mL ice-cold Tris-HCl/NaCl buffer (10 mM/100 mM, pH 7.5). The mixture was immediately filtered under vacuum (Whatman GF/C filters) and the filters were washed once with 20 mL ice-cold buffer. The retained radioactivity was determined by a liquid scintillation counter. In the saturation experiment, 2 µM LTD_4 was used to define the non-specific binding. A single, saturable binding site with B_{max} = 988 fmol/mg protein was found from saturation experiments. The K_D of [^{3}H]LTD$_4$ was established to be 2.16 x 10^{-10} M and no cooperativity was detected when the data were analysed by Hill plots (slope = 0.99).

5.1.2. In vitro inhibition of [^{3}H]mepyramine binding to guinea-pig lung membranes

The method is based on that described previously [31]. Briefly, a mixture of a total volume of 1.0 mL containing 0.5 nM [^{3}H]mepyramine (specific activity 21 Ci/mmol), guinea pig lung membrane proteins (± 370 mg/mL) and the testing compound in 50 mM Na–K phosphate buffer (pH 7.5) was incubated at 37 °C for 30 min. The reaction was stopped by the addition of 5 mL ice-cold phosphate buffer and followed by immediate filtration through Whatman GF/C filters. The filters were washed twice with about 20 mL cold buffer. The retained radioactivity was determined by a liquid scintillation counter after addition of 5 mL scintillation liquid.

In the saturation experiment, 10^{-4} M *R*-(–)-dimethindene was used to define the non-specific binding. A single, saturable binding site with B_{max} = 278 ± 24 fmol/mg protein was found from the saturation experiment. The K_D of [^{3}H]mepyramine was found to be 3.30 ± 0.26 x 10^{-9} M and no cooperativity was detected when the data were analysed by Hill plots (slope = 1.005).

5.2. Chemistry

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker AC 200 (^{1}H-NMR: 200.1 MHz, ^{13}C-NMR: 50.29 MHz) or a Bruker 400 MSL spectrometer (^{1}H-NMR: 400.1 MHz, ^{13}C-NMR: 100.63 MHz). ^{1}H-NMR chemical shifts (δ) are reported in ppm relative to $CDCl_3$ (δ = 7.25 ppm) or DMSO-d_5 (δ = 2.5 ppm). ^{13}C-NMR chemical shifts (δ) are reported in ppm relative to $CDCl_3$ (δ = 77.0 ppm) or DMSO-d_6 (δ =

39.5 ppm). Coupling constants are given in Hz. 2D-NMR (H–H and C–H-COSY) techniques were frequently used to support the interpretation of the 1D spectra. NOE-difference spectra were recorded on a Bruker 400 MSL spectrometer. The multiplicity of the carbon signals was determined by DEPT- or APT-spectra, or by a combination of a normal decoupled carbon spectrum in combination with a CH-correlation. The symbols used are (p) for primary, (s) for secondary, (t) for tertiary and (q) for quaternary carbon signals. The spectra were collected at room temperature at 200 MHz, unless stated otherwise.

FAB (HRMS) measurements were performed on a Finnigan MAT 90 spectrometer equipped with a WATV Cs ion gun, operated at a beam current of approximately 2 μA at 25 kV. High-resolution mass spectra were recorded on a Finnigan MAT-90. Melting points were measured on a Mettler FP-5 + FP-52 apparatus equipped with a microscope and are uncorrected.

Starting materials were commercially available. All end products had elemental analysis within 0.4% of theoretical values. However, compounds obtained in too small quantities to perform the elemental analysis or having an elemental analysis slightly outside of this range were found to be pure by both spectroscopic and chromatographic criteria.

5.2.1. General procedure for the synthesis of 3-hydroxyflavones

The 2'-hydroxy-(2-quinolinylmethoxy)chalcones **3** [17] (1.2 mmol) were dissolved in 35 mL methanol and 2 mL 16% KOH in water was added. The solution was cooled on ice and 2.0 mL 15% H_2O_2 was added dropwise. The reaction mixture was stored at 4 °C for 24 h and acidified with 1 N HCl. The precipitate was filtered and recrystallised from DMF to yield the pure products.

5.2.1.1. 3-Hydroxy-3'-(2-quinolinylmethoxy)flavone-8-carboxylic acid ***4a****:* Mp 272.2–274.2 °C, yield 13%. Recrystallisation from DMF/ethanol. ^{1}H-NMR (400 MHz, DMSO): δ 5.45 (s, 2H, CH_2O), 7.23 (dd, 1H, ^3J = 8.1 Hz, ^4J = 2.2 Hz, H4'), 7.50 (t, 1H, ^3J = 8.1 Hz, H5'), 7.55 (t, 1H, ^3J = 7.7 Hz, H6), 7.61–7.65 (m, 1H, H6-quinoline), 7.76 (d, 1H, ^3J = 8.5 Hz, H3-quinoline), 7.78–7.82 (m, 1H, H7-quinoline), 8.02 (d, 1H, ^3J = 8.1 Hz, H5-quinoline), 8.05 (d, 1H, ^3J = 8.5 Hz, H8-quinoline), 8.10 (d, 1H, ^3J = 7.9 Hz, H6'), 8.20 (s, 1H, H2'), 8.30 (d, 1H, ^3J = 7.4 Hz, H5/7), 8.35 (d, 1H, ^3J = 7.3 Hz, H5/7), 8.45 (d, 1H, ^3J = 8.5 Hz, H4-quinoline), 9.97 (br s, 1H, 3-OH). ^{13}C-NMR (50 MHz, DMSO): δ 70.72 (s), 113.63 (t), 116.20 (t), 119.72 (t), 120.76 (t), 122.04 (q), 123.79 (t), 126.48 (t), 127.02 (q), 127.79 (t), 128.32 (t), 129.33 (t), 129.57 (t), 129.74 (t), 132.58 (q), 135.95 (t), 136.96 (t), 139.32 (q), 144.44 (q), 144.57 (q), 146.69 (q), 152.42 (q), 157.01 (q), 158.03 (q), 165.15 (q), 172.50 (q). MH^+ 440 (base peak) for $C_{26}H_{17}NO_6$. Anal. $C_{26}H_{17}NO_6$•NaCl: C, H, N.

5.2.1.2. 6-Bromo-3-hydroxy-3'-(2-quinolinylmethoxy)flavone-8-carboxylic acid ***4b****:* Mp 293.2–294.3 °C, yield 45%. Recrystallisation from DMF/ethanol. ^{1}H-NMR (200 MHz, DMSO): δ 5.44 (s, 2H, CH_2O), 7.19–7.22 (m, 1H, H4'), 7.49 (t, 1H, ^3J = 8.1 Hz, H5'), 7.59–7.65 (m, 1H, H6-quinoline), 7.75 (d, 1H, ^3J = 8.4 Hz, H3-quinoline), 7.77–7.83 (m, 1H, H7-quinoline), 7.99–8.09 (m, 3H, H5-quinoline, H8-quinoline, H6'), 8.16 (s, 1H, H2'), 8.32 (d, 1H, ^4J = 2.5 Hz, H5/7), 8.38 (d, 1H, ^4J = 2.5 Hz, H5/7), 8.44 (d, 1H, ^3J = 8.5 Hz, H4-quinoline), 10.18 (br s, 1H, 3-OH). ^{13}C-NMR (50 MHz, DMSO): δ 70.71 (s), 113.64 (t), 115.65 (q), 116.36 (t), 119.67 (t), 120.77 (t), 123.67 (q), 123.77 (q), 126.45 (t), 127.00 (q), 127.76 (t), 128.34 (t), 129.60 (t), 129.70 (t), 130.86 (t), 132.31 (q), 136.89 (t), 137.66 (t), 139.49 (q), 144.92 (q), 146.71 (q), 151.28 (q), 156.97 (q), 158.00 (q), 163.87 (q), 171.38 (q). MH^+ 519 (base peak for $C_{26}H_{16}BrNO_6$. Anal. $C_{26}H_{16}BrNO_6$•0.5HCl: C, H, N.

5.2.1.3. 3-Hydroxy-4'-[(2-quinolinyl)methoxy]flavone-8-carboxylic acid ***4c****:* Mp 226.5–227.8 °C, yield 49%. ^{1}H-NMR (400 MHz, DMSO): δ 5.47 (s, 2H, CH_2O), 7.24 (d, 2H, H3', H5'), 7.30 (t, 1H, ^3J = 7.6 Hz, H6), 7.61–7.64 (m, 1H, H6-quinoline), 7.72 (d, 1H, ^3J = 8.5 Hz, H3-quinoline), 7.78–7.82 (m, 2H, H7-quinoline. H5/7), 7.94 (dd, 1H, ^3J = 7.9 Hz, ^4J = 1.6 Hz, H5/7), 8.01 (d, 1H, ^3J = 8.4 Hz, H5-quinoline), 8.05 (d, 1H, ^3J = 8.5 Hz, H8-quinoline), 8.44 (d, 1H, ^3J = 8.5 Hz, H4-quinoline), 8.44 (d, 2H, ^3J = 9.0 Hz, H2', H6'). ^{13}C-NMR (50 MHz, DMSO): δ 70.68 (s), 114.21 (t), 114.58 (t), 119.47 (t), 121.33 (q), 123.34 (t), 123.62 (t), 124.46 (q), 126.44 (t), 127.00 (q), 127.77 (t), 128.37 (t), 129.67 (q), 132.42 (q), 132.69 (t), 136.92 (t), 137.61 (q), 144.84 (q), 146.75 (q), 151.31 (q), 156.98 (q), 158.00 (q), 166.71 (q), 171.94 (q), 172.63 (q).

5.2.2. General procedure for the preparation of 3-alkyloxyflavone-8-carboxylic acids ***6***

A solution of 1 equiv 3-hydroxyflavones **4**, 2.2 equiv of an appropriate alkylhalide and 2.2 equiv of potassium carbonate in DMF was stirred at 50–90 °C until completion of the reaction. The DMF was removed in vacuo, and water was added. The product was extracted with ethyl acetate, the combined organic layers were washed with water, 1 N NaOH and brine, and dried over sodium sulphate. Removal of the solvent yielded the crude product **5** which were further purified by crystallisation from ethanol.

The esters **5** were dissolved in a mixture of THF, methanol and 5% aq. LiOH (1:1:1). The reaction mixture was stirred at room temperature until completion of the hydrolysis (TLC: ethyl acetate). The reaction mixture was then acidified, and the precipitate was collected by filtration. The crude acids **6** were further purified by recrystallization from DMF.

5.2.2.1. 3-Hydroxycarbonylmethoxy-3'-(2-quinolinylmethoxy)-flavone-8-carboxylic acid ***6a****:* Mp 230.8–232.6 °C (DMF/EtOH), yield 58%. ^{1}H-NMR (200 MHz, DMSO): δ 4.89 (s, 2H, CH_2CO_2H), 5.48 (s, 2H, CH_2O), 7.27–7.31 (m, 1H, H4'), 7.50 (t, 1H, ^3J = 8.0 Hz, H5'), 7.54 (t, 1H, ^3J = 7.9 Hz, H6), 7.62–7.66 (m, 1H, H6-quinoline), 7.75 (d, 1H, ^3J = 8.4 Hz, H3-quinoline), 7.78–7.82 (m, 1H, H7-quinoline), 8.03–8.06 (m, 3H, H5-quinoline, H8-quinoline. H6'), 8.20 (br s, 1H, H2'), 8.30 (d, 2H, ^3J = 7.7 Hz, H5, H7), 8.45 (d, 1H, ^3J = 8.3, H4-quinoline), 13.20 (br s, 1H, $ArCO_2H$). ^{13}C-NMR (50 MHz, DMSO): δ 67.32 (s), 70.76 (s), 114.50 (t), 117.76 (t), 119.66 (t), 121.37 (q), 121.60 (t), 124.00 (q), 124.41 (t), 126.46 (t), 127.01 (q), 127.78 (t), 128.34 (t), 129.27 (t), 129.55 (t), 129.73 (t), 131.57 (q), 136.11 (t), 136.90 (t), 139.21 (q), 146.74 (q), 152.42 (q), 153.00 (q), 157.04 (q), 157.93 (q), 165.02 (q), 169.81 (q), 173.24 (q). MS (*m/z*): $[M + H]^+$ 498 (base peak) for $C_{28}H_{19}NO_8$. Anal. $C_{28}H_{19}NO_8$•0.25,HCl: C, H, N.

5.2.2.2. 6-Bromo-3-hydroxycarbonylmethoxy-3'-(2-quinolinylmethoxy)flavone-8-carboxylic acid ***6b****:* Mp 252.5–252.6 °C (DMF), yield 50%. ^{1}H-NMR (200 MHz, DMSO): δ 4.88 (s, 2H, CH_2CO_2H), 5.49 (s, 2H, CH_2O), 7.30 (d, 1H, ^3J = 8.1 Hz, H4'), 7.51 (t, 1H, ^3J = 8.0 Hz, H5'), 7.62–7.68 (m, 1H, H6-quinoline), 7.76–7.87 (m, 2H, H3-quinoline, H7-quinoline), 8.02–8.09 (m, 3H, H6', H5-quinoline, H8-quinoline), 8.19 (s, 1H, H2'), 8.35 (s, 2H, H5, H7), 8.49 (d, 1H, ^3J = 8.6 Hz, H4-quinoline). ^{13}C-NMR (50 MHz, DMSO): δ 67.32 (s), 70.56 (s),

114.52 (t), 116.38 (q), 117.94 (t), 119.67 (t), 121.65 (t), 123.71 (q), 125.69 (q), 126.58 (t), 127.03 (q), 127.83 (t), 128.03 (t), 129.59 (t), 129.92 (t), 130.95 (t), 131.31 (q), 137.28 (t), 137.94 (t), 139.31 (q), 146.37 (q), 151.35 (q), 153.26 (q), 156.92 (q), 157.88 (q), 163.70 (q), 169.72 (q), 172.07 (q). Anal. $C_{28}H_{18}BrNO_8$•0.25HCl: C, H N, Br.

5.2.2.3. 6-Bromo-3-propyloxy-3'-(2-quinolinylmethoxy)flavone-8-carboxylic acid ***6c****:* Mp 237.1–239.3 °C, yield 42%. ^{1}H-NMR (200 MHz, DMSO): δ 0.87 (t, 3H, ^3J = 7.4 Hz, $OCH_2CH_2CH_3$), 1.56–1.67 (m, 2H, $OCH_2CH_2CH_3$), 3.98 (t, 2H, ^3J = 6.7 Hz, $OCH_2CH_2CH_3$), 5.46 (s, 2H, CH_2O), 7.29 (dd, 1H, ^3J = 8.3 Hz, ^4J = 2.6 Hz, H4'), 7.51 (t, 1H, ^3J = 8.1 Hz, H5'), 7.59–7.63 (m, 1H, H6-quinoline), 7.72 (d, 1H, ^3J = 8.5 Hz, H3-quinoline), 7.67–7.70 (m, 1H, H7-quinoline), 792 (d, 1H, ^3J = 8.0 Hz, H5-quinoline), 7.99–8.05 (m, 3H, H8-quinoline H6', H2'), 8.32 (s, 2H, H5, H7), 8.44 (d, 1H, ^3J = 8.6 Hz, H4-quinoline). ^{13}C-NMR (50 MHz, DMSO): δ 10.05 (p), 22.53 (s), 70.70 (s), 73.26 (s), 114.30 (t), 116.25 (q), 117.73 (t), 119.43 (t), 121.31 (t), 123.94 (q), 125.88 (q), 126.44 (t), 127.00 (q), 127.76 (t), 128.32 (t), 129.67 (t), 129.72 (t), 130.89 (t), 131.43 (q), 136.89 (t), 137.73 (q), 140.07 (q), 146.73 (q), 151.47 (q), 154.12 (q), 156.98 (q), 157.93 (q), 163.77 (q), 172.30 (q). MH^+ 561 (base peak) for $C_{29}H_{23}BrNO_6$. Anal. $C_{29}H_{23}BrNO_6$: C, H, N, Br.

5.2.2.4. 6-Bromo-3,3'-di-(2-quinolinylmethoxy)flavone-8-carboxylic acid ***6d****:* Mp 229.6–229.7 °C, yield 51%. ^{1}H-NMR (200 MHz, DMSO): δ 5.34 (s, 2H, CH_2O), 5.40 (s, 2H, CH_2O), 7.26 (m, 1H, H4'), 7.44 (t, 1H, ^3J = 8.0 Hz, H5'), 7.56–7.79 (m, 6H, 2 H6-quinoline, 2 H7-quinoline, 2 H3-quinoline), 7.91–8.03 (m, 6H, H6', H2', 2 H5-quinoline, 2 H8-quinoline), 8.35 (d, 1H, J = 8.5 Hz, H4-quinoline), 8.37 (d, 1H, ^3J = 8.5 Hz, H4-quinoline), 8.36 (s, 2H, H5, H7). ^{13}C-NMR (50 MHz, DMSO): δ 70.66 (s), 74.38 (s), 114.50 (t), 116.44 (q), 117.74 (t), 119.45 (t), 120.08 (t), 121.1 (t), 123.89 (q), 125.91 (q), 126.39 (t), 126.42 (t), 126.95 (q), 127.61 (t), 127.73 (t), 128.32 (t), 128.41 (t), 129.40 (t), 129.58 (t), 129.69 (t), 130.99 (t), 131.16 (q), 136.53 (t), 136.82 (t), 137.00 (t), 139.76 (q), 146.55 (q), 146.68 (q), 151.53 (q), 154.59 (q), 156.54 (q), 156.82 (q), 157.85 (q) 163.73 (q), 172.28 (q). Anal. $C_{36}H_{23}BrN_2O_6$•0.8HCl: C, H, N, Br.

5.2.2.5. 3-(2-Phthalimidoethoxy)-4'-[(2-quinolinyl)methoxy]-flavone-8-carboxylic acid ***6e****:* Mp 170.2–173.3 °C, yield 89%. ^{1}H-NMR (400 MHz, DMSO): δ 3.54–3.55 (m, 2H, NCH_2CH_2O), 4.18–4.19 (m, 2H, NCH_2CH_2O), 5.44 (s, 2H, CH_2O), 7.20 (d, 2H, ^3J = 8.6 Hz, H3', H5'), 7.48–7.55 (m, 3H, H6, H5-phthalimide, H6-phthalimide), 7.61–7.65 (m, 1H, H6-quinoline), 7.68–7.70 (m, 2H, H4-phthalimide, H7-phthalimide), 7.80 (d, 1H, ^3J = 8.5 Hz, H3-quinoline), 7.78–7.82 (m, 1H, H7-quinoline), 7.99–8.05 (m, 2H, H5-quinoline, H8-quinoline), 8.22–8.27 (m, 2H, H5, H7), 8.35 (d, 2H, ^3J = 8.6 Hz, H2', H6'), 8.43 (d, 1H, ^3J = 8.5 Hz, H4-quinoline). ^{13}C-NMR (50 MHz, DMSO): δ 69.92 (s), 70.67 (s), 70.68 (s), 114.75 (t), 119.34 (t), 122.13 (q), 122.89 (q), 124.05 (q), 124.16 (t), 126.43 (t), 126.97 (q), 127.29 (t), 127.74 (t), 128.31 (t), 128.97 (t), 129.70 (t), 130.45 (t), 130.80 (t), 135.49 (t), 136.93 (t) 137.85 (q), 138.75 (q), 146.69 (q), 152.36 (q), 154.39 (q), 156.74 (q), 160.01 (q), 165.12 (q), 167.71 (q), 168.43 (q), 173.22 (q). Anal. $C_{36}H_{24}N_2O_8$•1.0HCl: C, H, N.

5.2.2.6. 3-[2-(N,N-Dimethylamino)ethoxy]-4'-(2-quinolinylmethoxy)flavone-8-carboxylic acid ***6g****:* A solution of 0.18 g (0.29 mmol) **6e** in 60 mL ethanol was heated under reflux for 1 h together with 10 mL hydrazine (1.1 equiv). The precipitate (**6f**) was filtered. The crude amine (70 mg) was stirred overnight together with 0.5 mL 37% formaldehyde and 10 mg sodium cyanoborohydride at room temperature. The solvents were removed, the crude product was dissolved in ethanol. A few drops of 1 N HCl were added and the salt (35 mg) was obtained after filtration of the precipitate. Yield 21%, mp > 300 °C. ^{1}H-NMR (200 MHz, DMSO): δ 2.94 (d, 6H, NCH_3), 3.48–3.53 (m, 2H, NCH_2CH_2O), 4.28–4.36 (m, 2H, NCH_2CH_2O), 5.36 (s, 2H, quinoline CH_2O), 7.31 (d, 2H, ^3J = 9.0 Hz, H3', H5'), 7.57 (t, 1H, ^3J = 7.7 Hz, H6), 7.68–7.75 (m, 1H, H6-quinoline), 7.83–7.93 (m, 2H, H3-quinoline, H7-quinoline), 8.16 (d, 1H, ^3J = 9.3 Hz, H8-quinoline), 8.28–8.35 (m, 5H, H2', H6', H5, H7, HCl), 8.66 (d, 1H, ^3J = 8.5 Hz, H4-quinoline), 10.99 (br s, 1H, $ArCO_2H$). ^{13}C-NMR (50 MHz, DMSO): δ 42.18 (p), 55.54 (s), 65.51 (s), 69.81 (s), 115.05 (t), 119.63 (t), 121.56 (q), 122.60 (q), 124.01 (q), 124.52 (t), 126.87 (t), 127.06 (t), 127.14 (q), 128.02 (t), 129.07 (t), 130.51 (t), 130.67 (t), 135.87 (t), 138.18 (q), 138.73 (t), 145.03 (q), 152.48 (q), 155.04 (q), 156.35 (q), 160.11 (q), 173.21 (q).

5.2.3. 1-(5-Bromo-3-ethoxycarbonyl-2-hydroxylphenyl)-3-(3,5-dimethoxyphenyl)propane-1,3-dione ***9***

A solution of 10.0 g (35.0 mmol) ethyl 3-acetyl-5-bromo-2-hydroxybenzoate **8** and 11.7 g (55.2 mmol) methyl 3,5-dimethoxybenzoate **7** in 100 mL dry dioxane was added dropwise to a suspension of 6.0 g 60% NaH in 80 mL dioxane. The reaction mixture was heated under reflux overnight, and the bulk of dioxane was removed in vacuo. Petroleum ether 40–60 °C (400 mL) was added, and the precipitate was filtered. The crude product was suspended in water and acidified with 3 M HCl. The yellow precipitate was filtered and used in the next step without further purification.

5.2.4. 6-Bromo-3',5'-dimethoxyflavone-8-carboxylic acid ***10***

A solution of the diketone **9** (16.62 g) in ethanol containing 2% of concentrated HCl was refluxed for 7 h. The bulk of ethanol was removed under reduced pressure, and the precipitate was collected by filtration. Recrystallisation from DMF yielded 6.06 g (15.0 mmol) of the pure product **10**. Mp 207.3–207.8 °C, yield 43%. ^{1}H-NMR (200 MHz, DMSO): δ 3.86 (s, 6H, 2 OCH_3), 6.75 (bs, 1H, H4'), 7.33–7.36 (m, 3H, H3, H2', H6'), 8.33–8.37 (m, 2H, H5, H7). MS (*m/z*): 405 $[M + H]^+$ for $C_{18}H_{13}BrO_6$.

5.2.5. 6-Bromo-3',5'-dihydroxyflavone-8-carboxylic acid ***11***

A solution (1 M) of BBr_3 in dichloromethane (88 mL, 88 mmol) was added dropwise to a suspension of **10** (5.77 g, 14.3 mmol) in 150 mL of dichloromethane. The reaction mixture was stirred at room temperature for 3 days, and poured out onto cracked ice and concentrated HCl. The yellow precipitate was collected by filtration, and washed with ethanol and petroleum ether 40–60 °C, yielding 4.98 g (13.2 mmol) of the dihydroxyflavone **11**. Mp > 300 °C, yield 93%. ^{1}H-NMR (200 MHz, DMSO): δ 6.51 (s, 1H, H3), 6.93 (s, 1H, H4), 6.99 (d, 2H, ^4J = 2.1 Hz, H2', H5'), 8.29 (d, 2H, ^4J = 2.2 Hz, H5, H7). ^{13}C-NMR (50 MHz, DMSO): δ 104.67 (t), 106.10 (t), 106.28 (t), 116.73 (q), 124.20 (q), 125.59 (q), 130.62 (t), 132.17 (q), 137.64 (t), 152.42 (q), 158.61 (2q), 163.17 (q), 163.67 (q), 175.06 (q). MS (*m/z*): 377 $[M + H]^+$ for $C_{16}H_9BrO_6$.

5.2.6. Ethyl 6-bromo-3',5'-dihydroxyflavone-8-carboxylate ***12***

A solution of 4.6 g (12.2 mmol) of **11** in 200 mL ethanol and 10 mL concentrated sulphuric acid was heated under reflux for 5 h. The reaction mixture was cooled to 4 °C, and the precipi-

tate was collected by filtration. Recrystallisation from DMF yielded 2.04 g (5.0 mmol) of pure compound **12**. Mp > 300 °C, yield 41%. ^{1}H-NMR (200 MHz, DMSO): δ 1.40 (t, 3H, ^3J = 7.1 Hz, OCH_2CH_3), 4.44 (q, 2H, ^3J = 7.1 Hz, OCH_2CH_3), 6.49 (t, 1H, ^4J = 2.0 Hz, H4'), 6.90 (s, 1H, H3), 6.95 (d, 2H, ^4J = 2.0 Hz, H2', H6'), 8.28 (d, 1H, ^4J = 2.6 Hz, H5/H7), 8.29 (d, 1H, ^4J = 2.6 Hz. H5/H7), 9.73 (s, 2H, ArOH). ^{13}C-NMR (50 MHz, DMSO): δ 13.76 (p), 61.83 (s), 104.58 (t), 106.11 (t), 106.39 (t), 116.72 (q), 122.83 (q), 125.62 (q), 131.18 (t), 132.09 (t), 137.78 (t), 152.39 (q), 158.64 (q), 162.22 (q), 163.14 (q), 174.90 (q). MS (*m/z*): 405 $[M + H]^+$ for $C_{18}H_{13}BrO_6$.

5.2.7. Ethyl 6-bromo-3',5'-bis(2-quinolinemethoxy)flavone-8-carboxylate ***13***

A solution of **12** (1.93 g, 4.8 mmol), 2-chloromethyl quinoline hydrochloric acid (1.05 g, 4.8 mmol) and potassium carbonate (1.45 g, 10.5 mmol) in 30 mL DMF was stirred over night at 80 °C. The DMF was removed under reduced pressure, and water was added. The crude product was extracted with ethyl acetate, and the combined organic layers were dried over sodium sulphate. After the removal of the solvent, the residue was crystallised from DMF yielding a mixture of two products, which were separated with Amberlite resin IR-45(OH). The neutral product was characterised as the dialkylated flavone **13** (0.57 g, 0.83 mmol). Mp 189.1–189.2 °C, yield 17%. ^{1}H-NMR (200 MHz, DMSO): δ 1.39 (t, 3H, ^3J = 7.1 Hz, OCH_2CH_3), 4.42 (q, 2H, ^3J = 7.0 Hz, OCH_2CH_3), 5.49 (s, 4H, 2 x OCH_2), 7.10 (bs, 1H, H4'), 7.34 (s, 1H, H3), 7.57–7.72 (m, 6H, 2 x H6-quinoline, 2 x H3-quinoline, H2', H6'), 7.77–7.83 (m, 2H, ^3J = 7.6 Hz, 2 x H7-quinoline), 7.98–8.04 (m, 4H, 2 x H5-quinoline, 2 x H8-quinoline), 8.30 (d, 1H, ^4J = 2.6 Hz, H5/7), 8.35 (d, 1H, ^4J = 2.6 Hz, H5/H7), 8.42 (d, 2H, ^3J = 8.6 Hz, H4-quinoline). ^{13}C-NMR (50 MHz, $CDCl_3$): 14.16 (p), 61.95 (s), 71.33 (s), 105.31 (t), 106.26 (t), 107.27 (t), 117.67 (q), 118.97 (t), 122.47 (q), 125.94 (q), 126.55 (t), 127.40 (q), 127.53 (t), 128.72 (t), 129.81 (t), 132.59 (t), 132.96 (q), 137.07 (t), 138.87 (t), 147.21 (q), 153.11 (q), 156.71 (q), 159.96 (q), 162.37 (q), 163.03 (q), 176.02 (q). MS (*m/z*): 687 $[M + H]^+$ for $C_{38}H_{27}BrN_2O_6$.

5.2.8. 6-Bromo-3',5'-bis(2-quinolinemethoxy)flavone-8-carboxylic acid ***14***

A solution of **13** (0.34 g, 0.49 mmol) in 30 mL DMF was heated to 60 °C, and 25 mL of 10% NaOH in water was then added. After stirring for 1 h, the reaction mixture was acidified with 3 M HCl and the precipitate was collected by filtration. Recrystallisation from DMF/ethanol yielded 0.24 g (0.36 mmol) of the pure product **14** as a white solid. Mp > 300 °C, yield 74%. ^{1}H-NMR (200 MHz, DMSO): δ 5.46 (s, 4H, OCH_2), 7.01 (s, 1H, H4'), 7.20 (s, 1H, H3), 7.57–7.81 (m, 8H, 2 H3-quinoline, 2 x H6-quinoline, 2 x H7-quinoline, H2', H6'), 7.94–8.03 (m, 6H, 2 x H5-quinoline, 2 x H8-quinoline, H5, H7), 8.39 (d, 2H, ^3J = 8.5 Hz, 2 x H4-quinoline). ^{13}C-NMR (50 MHz, DMSO): δ 70.96 (s), 105.20 (t), 105.89 (t), 106.13 (t), 116.91 (q), 119.70 (t), 125.00 (q), 125.49 (t), 126.40 (t), 126.94 (q), 127.71 (t), 128.35 (t), 129.63 (t), 133.07 (q), 136.14 (t), 136.80 (t), 146.71 (q), 151.49 (q), 156.81 (q), 159.49 (q), 161.89 (q), 164.48 (q), 176.11 (q). Anal. $C_{36}H_{23}BrN_2O_6 \cdot 2HCl$: C, H, N, Br.

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