Synthesis and Endothelin Receptor Binding Affinity of a Novel Class of 2-Substituted-4-aryl-3-quinolinecarboxylic Acid Derivatives[§]

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Abstract: The 21-amino acid peptide endothelin-1 (ET-1) is the predominant isoform of the endothelin peptide family, which includes ET-2, and ET-3. These peptides display a variety of physiological activities including vasoconstriction and the stimulation of cell proliferation in tissues both within and outside of the cardiovascular system. They exert their actions *via* activation of two distinct receptor subtypes, ET_A and ET_B, belonging to the G protein-coupled receptor (GPCR) superfamily. Ligands of these receptors have received numerous citations in the recent pharmaceutical literature. In particular receptor antagonists, both ET_A- and ET_B-selective, as well as non-selective, have been described due to their wide therapeutic potential. As a part of our program toward the development of selective ET_A ligands we have designed and we now report new molecules based on 2-substituted-4-aryl-3-quinolinecarboxylic acid moiety. Binding profile for some compounds (**40**, **44**, **46**, and **47**) of this class showed a reasonable affinity and selectivity for ET_A receptors.

Key Words: 2-Substituted-4-aryl-3-quinolinecarboxylic acids derivatives, endothelin receptors, G protein-coupled receptors, hypertension.

INTRODUCTION

The endothelins (ET-1, ET-2, and ET-3) are a family of 21-amino acid bicyclic peptides, isolated from mammalian cells, with ET-1 being the predominant form and the most potent endogenous vasoconstrictor reported to date [1, 2]. These peptides contain a conserved hydrophobic C-terminal sequence that is essential for activity [3]. The biology and characterization of ETs have been the subject, in the last decade, of various thousands of literature reports including many reviews [3-7]. ETs are known to exert their pharmacological effects by acting upon, at least, two specific G-protein coupled receptors, ET_A and ET_B, both of which are widely distributed in mammalian tissues [3]. For instance, ET_A is present primarily on vascular smooth muscle, lung, aorta, and heart; ET_B is present on endothelium, cerebral cortex, cerebellum, liver, kidney, lung, and placenta. ET_A receptors have high affinity for ET-1 whereas ET_B receptors have high affinity for all the ET peptides and the structurally related snake venom peptide sarafotoxin [3-5]. Activation of either receptor has been shown to mediate vasoconstriction, inotropism, modulation of nervous function, vasodilatation and mitogenesis. It is, therefore, not surprising to find that ETs are implicated in the pathophysiology of a number of diseases including hypertension, cerebral vasospasm, ischemia, renal failure, pulmonary hypertension, and different tumours. Thus, ET antagonists are promising new agents in the treatment of cardiovascular diseases [3-7].

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To date, it is still not clear if a selective ET_A receptor antagonist could be more useful than a mixed ET_A/ET_B antagonist [8-10]. The opposite effects of ET_A and ET_B receptors on blood vessels, and ET_B receptor mediated actions of ET-1 clearance and renal sodium handling, indicate that, for cardiovascular disorder such as hypertension, focusing on selective blockade of ET_A receptors rather than on simultaneous antagonism of both receptors, might be the most promising therapeutic strategy [8-12].

As mentioned before the endothelin system raised great interest among researchers for its physiopathologic role, but a careful survey of the literature on ET receptor ligands showed that only a few chemical classes are known to bind these receptors. Based on these premises and as a part of our program toward the development of selective ET_A ligands, the aim of this work was the identification of a novel class of derivatives with reasonable ET_A affinity and selectivity [13-15].

Different chemical features, emerged from literature reports, were taken into account for the design of the novel ET ligands and were supported onto a 3-quinolinecarboxylic acid to give a scaffold endowed with different diversity points [16-20]. A carboxylic acid group was fixed at the 3-position because an acidic group seems to be crucial for ET activity [16-20]. The presence of one or more aryl residues and their relative position to the carboxylic group seem to be critical for ET activity [16-20]. For this reason aromatic moieties variously substituted were introduced at the 2- and 4-position of the 3-quinolinecarboxylic acid scaffold. General formula for the novel synthesized compounds is reported in Fig. **1**.

CHEMISTRY

Synthetic routes for the novel compounds are outlined in Schemes **1**, **2**, and **3**. Briefly, the appropriate (2-aminophenyl)

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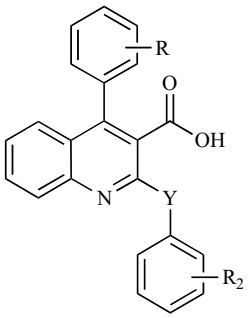


Fig. (1). General formula for 2-substituted-4-aryl-3-quinolinecarboxylic acid derivatives **34-47**.

arylmethanone (**1**, **2**) was reacted with 3-chloro-3-oxopropanoic acid alkyl ester (**3**, **4**) in anhydrous dichloromethane, to give, respectively, compounds **5-7** [21]. These intermediates were converted, in high yield, to the respective chloro derivatives **8-10** by reaction with phosphoric trichloride at reflux (Scheme **1**) [22]. Compound **2**, not commercially available, was synthesized in three steps as reported by Furstner *et al*. [23]. 2-Substituted-4-aryl-3-quinolinecarboxylic acid alkyl esters **14-19** and **26-33** were prepared as depicted in Scheme **2**. Briefly, aryl-aryl condensation for compounds **14-19** was achieved, in high yield, by standard Suzuki coupling between the appropriate phenyl boronic acid (**11-13**) and chloro derivatives **8** or **10** (Scheme **2**) [24]. Alternatively, compounds **26-29** or **32,** and **33** were obtained, starting from **9** or **10**, by the action of the appropriate thiophenol (**20, 21**) or phenol (**22, 23**), in the presence of NaH in *N,N*-dimethylformamide at room temperature. Condensation reaction with phenols, generally, required 8 h at 22 °C to go to completion. First attempts using thiophenols under the same reaction condition and using ethanol/water (1:1) during the work up phase lead to the isolation in low yield of 2-ethoxy-4-phenyl-3-quinolinecarboxylic acid ethyl ester [25]. The desired compounds **26**, **27**, **32**, and **33** were obtained by shortening the reaction duration from 8 h to 45 min. and avoiding the use of ethanol during the work up phase. Finally, when X = NH, the desired compounds **30** and **31** were obtained by direct fusion of the appropriate reactants at 160 °C for 2 h (Scheme **2**).

The obtained 2-substituted-4-aryl-3-quinolinecarboxylic acid alkyl esters, **14-19** and **26-33**, were hydrolyzed to give the final compounds **34-47** according to Scheme **3**. First attempts to use aqueous 2 N NaOH gave the desired final compounds in somewhat low yield. To overcome this limitation ethanolic KOH 15% (25 equivalents) was used at reflux for 8 h and yields were considerably higher (>80%).

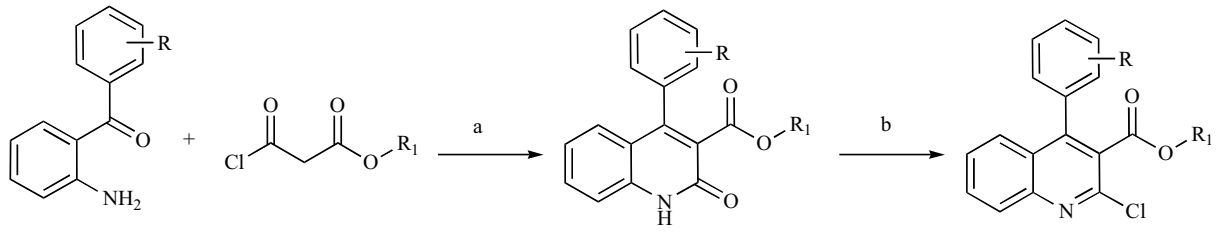
All the synthesized products were characterized by ^{1}H NMR spectra, IR, and elemental analysis and analytical data were consistent with the proposed structures.

RESULTS AND DISCUSSIONS

Receptor binding assays were performed on compounds **34-47**, using CHO-K_1 cells stably expressing human ET_A and ET_B receptors and using [^{125}I]Endothelin-1 as radioligand. All the synthesized compounds were tested and, among them, some derivatives showed a percentage of inhibition in the low-micromolar range. As a general trend, most of the tested molecules seem to bind preferentially the ET_{Ah} receptor subtype over the ET_{Bh} one (Table **1**). In particular, 4-(1,3-benzodioxol-5-yl)-2-(4-methoxyphenylthio)-3-quinolinecarboxylic acid (**46**) was the most interesting and selective among tested compounds. In fact, while completely inactive at ET_{Bh} receptors, analysis of its dose-inhibition curve for the ET_{Ah} receptor resulted in a K_i, obtained using the Cheng and Prusoff equation, of 0.728 ± 0.18 μM.

Binding results suggest that a sulfur bridge between the quinoline scaffold and the phenyl ring at the 2-position is optimal for affinity when compared with derivatives with no bridge showing no affinity on both ET_{Ah} and ET_{Bh} receptors (**46** *vs* **43**, **47** *vs* **45**). On the other hand a 1,3-benzodioxol-5-yl residue at the 4-position of the quinoline ring seems to be important for affinity (**44** *vs* **35**, **46** *vs* **37**, **47** *vs* **38**). Compounds **34-39**, **41-43**, and **45** showed a percentage of inhibition of specific binding lower than 10% at 10^{-5} M on both receptors.

In conclusion the new designed chemical class was explored preparing fourteen compounds and led to the discovery of ET_A selective 3-quinoline carboxylic acid derivatives, such as compounds **46** and **47**. These two derivatives, along with **40** and **44**, have been selected as *hit compounds* of this new series and a further study is in progress to investigate the effect on ET affinity of the substitutions on the quinoline ring at the 6- or 7-positions.



1, **2**

1: R = H
2: R = 3,4-OCH_2O-

3, **4**

3: $R_1 = CH_3$
4: $R_1 = CH_2CH_3$

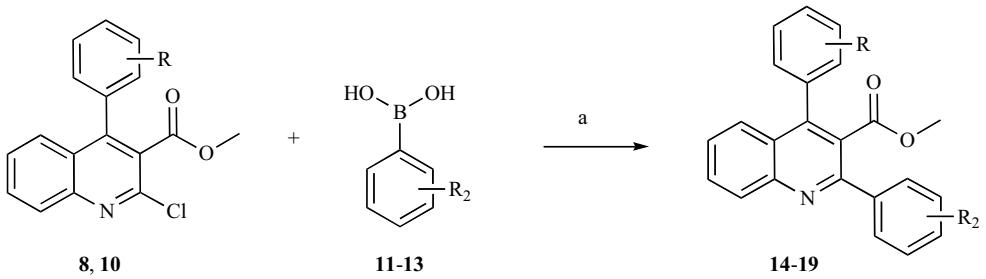
5-7

5: R = H, $R_1 = CH_3$
6: R = H, $R_1 = CH_2CH_3$
7: R = 3,4-OCH_2O-, $R_1 = CH_3$

8-10

8: R = H, $R_1 = CH_3$
9: R = H, $R_1 = CH_2CH_3$
10: R = 3,4-OCH_2O-, $R_1 = CH_3$

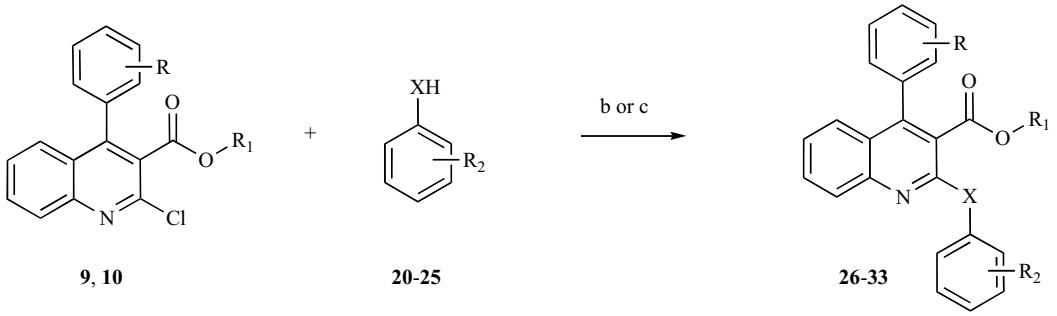
Scheme 1 Conditions: (a) Dichloromethane anhydrous, N_2, 0 °C, 1 h; then 22 °C, 1 h; then silica gel, 22 °C, 24 h; (b) $POCl_3$, reflux, 2 h.



8, **10** **11-13** **14-19**

11: R_2 = 4-OCH_3
12: R_2 = 3,4-OCH_2O-
13: R_2 = 3,4-$(OCH_3)_2$

14: R = H, R_2 = 4-OCH_3
15: R = H, R_2 = 3,4-OCH_2O-
16: R = H, R_2 = 3,4-$(OCH_3)_2$
17: R = 3,4-OCH_2O-, R_2 = 4-OCH_3
18: R = 3,4-OCH_2O-, R_2 = 3,4-OCH_2O-
19: R = 3,4-OCH_2O-, R_2 = 3,4-$(OCH_3)_2$

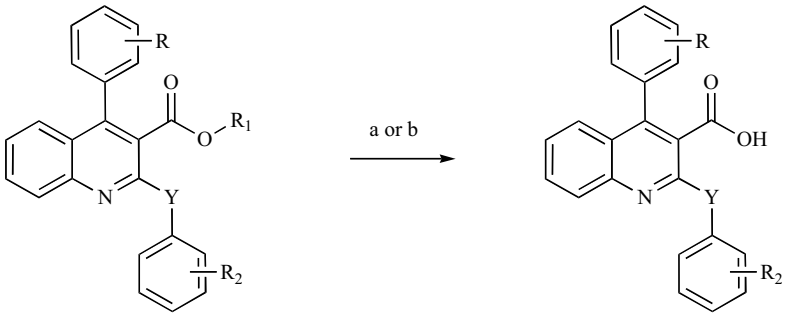


9, **10** **20-25** **26-33**

20: X = S, R_2 = 4-OCH_3
21: X = S, R_2 = 3,4-$(OCH_3)_2$
22: X = O, R_2 = 4-OCH_3
23: X = O, R_2 = 3,4-OCH_2O-
24: X = NH, R_2 = 4-OCH_3
25: X = NH, R_2 = 3,4-OCH_2O-

26: R = H, X = S, R_1 = CH_2CH_3, R_2 = 4-OCH_3
27: R = H, X = S, R_1 = CH_2CH_3, R_2 = 3,4-$(OCH_3)_2$
28: R = H, X = O, R_1 = CH_2CH_3, R_2 = 4-OCH_3
29: R = H, X = O, R_1 = CH_2CH_3, R_2 = 3,4-OCH_2O-
30: R = H, X = NH, R_1 = CH_2CH_3, R_2 = 4-OCH_3
31: R = H, X = NH, R_1 = CH_2CH_3, R_2 = 3,4-OCH_2O-
32: R = 3,4-OCH_2O-, X = S, R_1 = CH_3, R_2 = 4-OCH_3
33: R = 3,4-OCH_2O-, X = S, R_1 = CH_3, R_2 = 3,4-$(OCH_3)_2$

Scheme 2 Conditions: (a) K_2CO_3, $Pd(PPh_3)_4$, N_2, toluene/ethanol/water (10:1:1), reflux 48 h; (b) NaH 95%, DMF anhydrous, 0 °C, 15 min.; then 22 °C, 0.5-8 hrs; (c) 2 h, 160 °C.

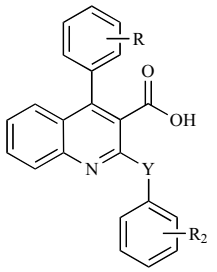


14-19, **26-33** **34-47**

34: R = H, Y = nihil, R_2 = 4-OCH_3
35: R = H, Y = nihil, R_2 = 3,4-OCH_2O-
36: R = H, Y = nihil, R_2 = 3,4-$(OCH_3)_2$
37: R = H, Y = S, R_2 = 4-OCH_3
38: R = H, Y = S, R_2 = 3,4-$(OCH_3)_2$
39: R = H, Y = O, R_2 = 4-OCH_3
40: R = H, Y = O, R_2 = 3,4-OCH_2O-
41: R = H, Y = NH, R_2 = 4-OCH_3
42: R = H, Y = NH, R_2 = 3,4-OCH_2O-
43: R = 3,4-OCH_2O-, Y = nihil, R_2 = 4-OCH_3
44: R = 3,4-OCH_2O-, Y = nihil, R_2 = 3,4-OCH_2O-
45: R = 3,4-OCH_2O-, Y = nihil, R_2 = 3,4-$(OCH_3)_2$
46: R = 3,4-OCH_2O-, Y = S, R_2 = 4-OCH_3
47: R = 3,4-OCH_2O-, Y = S, R_2 = 3,4-$(OCH_3)_2$

Scheme 3 Conditions (a) NaOH 2 N, ethanol/THF (1:1), reflux, 72 h; (b) KOH 15% in ethanol, water, reflux, 8 h.

Table 1. Binding Properties of Some 2-Substituted-4-aryl-3-quinolinecarboxylic Acid Derivatives



Compound	Y	R	R_2	ET_{Ah} ($K_i \pm$ sd, μM)[a]	ET_{Bh} ($K_i \pm$ sd, μM)[a]
40	O	H	3,4-OCH_2O-	5.54 ± 0.74	NA[b]
44	nihil	3,4-OCH_2O-	3,4-OCH_2O-	7.47 ± 1.00	NA[b]
46	S	3,4-OCH_2O-	4-OCH_3	0.728 ± 0.17	14.90 ± 2.74
47	S	3,4-OCH_2O-	3,4-$(OCH_3)_2$	1.63 ± 0.33	NA[b]
BQ-123				5.40 ± 0.77 (nM)	18[c]
BQ-788				280 (nM)[d]	7.14 ± 0.23 (nM)

[a] K_i values were taken as in ref 26 and are means (± SD) of three separate experiments; [b] NA: not active; [c] K_i value taken from Ishikawa and coworkers. (ref 29); [d] K_i value taken from Ishikawa and coworkers. (ref 30).

EXPERIMENTAL PROCEDURES

Melting points were determined in a Gallenkamp apparatus with a digital thermometer MFB-595 in glass capillary tubes and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer FTIR 1600 spectrometer in KBr disks. Elemental analyses for C, H, N, and S were within ± 0.4% of theoretical values and were performed on a Carlo Erba Elemental Analyzer Mod. 1108 apparatus. ^1H and ^{13}C NMR spectra were recorded at 200 MHz on a Varian Inova Unity 200 spectrometer in DMSO-d_6 solution. Chemical shifts are given in δ values (ppm), using tetramethylsilane as the internal standard; coupling constants (*J*) are given in hertz (Hz). Signal multiplicities are characterized as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad signal). All the synthesized compounds were tested for purity on TLC (aluminium sheet coated with silica gel 60 F_{254}, Merck) and visualized by UV (λ = 254 and 366 nm). All chemicals and solvents were reagent grade and were purchased from commercial vendors. Compounds **5**, **6**, **8**, and **9** are described in literature [22], analytical data are consistent and were synthesized by a slightly modified procedure as reported below.

SYNTHESIS

General Procedure for the Synthesis of 4-Aryl-1, 2-dihydro-2-oxo-3-quinolinecarboxylic Acid Alkyl Ester (5-7)

A solution of the appropriate (2-aminophenyl)arylmethanone (**1**, **2**) (0.030 mol) in anhydrous dichloromethane (40 mL) was cooled at 0 °C and the opportune 3-chloro-3-oxo-propanoic acid alkyl ester (**3**, **4**) (0.036 mol) was added dropwise, under nitrogen atmosphere. The resulting suspension was let warm and stirred for 1 h at 22 °C. After this period, silica gel (2 g) was added and the reaction mixture was stirred for 24 h at room temperature. The solid residue was separated by filtration. The organic layer was washed with $NaHCO_3$ 5% (1 × 50 mL), brine (1 × 50 mL), dried over sodium sulphate anhydrous and evaporated to give the desired products which were used into the next step without any further purification. Using this procedure the following compounds were obtained.

1,2-Dihydro-2-oxo-4-phenyl-3-quinolinecarboxylic Acid Methyl Ester (5)

The title compound was obtained as a white powder (66%): mp 216-218 °C; IR (KBr) cm^{-1} 2945, 1739, 1649, 1481, 1430, 1380, 1242, 1088, 758; ^1H NMR (DMSO-d_6) δ 12.32 (br s, 1 H, NH), 7.63-7.11 (m, 9 H, aromatic), 3.49 (s, 3 H, CH_3). Anal. ($C_{17}H_{13}NO_3$) C, H, N.

1,2-Dihydro-2-oxo-4-phenyl-3-quinolinecarboxylic Acid Ethyl Ester (6)

The title compound was obtained as a white powder (71%): mp 203-205 °C; IR (KBr) cm^{-1} 2951, 1735, 1653, 1480, 1433, 1379, 1239, 1088, 990, 867, 758; ^1H NMR (DMSO-d_6) δ 12.27 (br s, 1 H, NH), 7.63-6.91 (m, 9 H, aromatic), 3.95 (q, J = 7.0 Hz, 2 H, CH_2), 0.86 (t, J = 7.0 Hz, 3 H, CH_3). Anal. ($C_{18}H_{15}NO_3$) C, H, N.

4-(1,3-Benzodioxol-5-yl)-1,2-dihydro-2-oxo-3-quinolinecarboxylic Acid Ethyl Ester (7)

The title compound was obtained as a white powder (59%): mp 264-266 °C; IR (KBr) cm^{-1} 1738, 1649, 1451, 1438, 1379, 1242, 1091, 1036, 933, 764; ^1H NMR (DMSO-d_6) δ 12.28 (br s, 1 H, NH), 7.63-7.53 (m, 1 H, aromatic), 7.42-7.36 (m, 1 H, aromatic), 7.27-7.04 (m, 3 H, aromatic),

6.94-6.90 (m, 1 H, aromatic), 6.80-6.74 (m, 1 H, aromatic), 6.13 (s, 2 H, CH_2), 3.55 (s, 3 H, CH_3). Anal. ($C_{18}H_{13}NO_5$) C, H, N.

General Procedure for the Synthesis of 4-Aryl-2-chloro-3-quinolinecarboxylic Acid Alkyl Ester Derivatives (8-10)

A solution of the appropriate 4-aryl-1,2-dihydro-2-oxo-3-quinolinecarboxylic acid alkyl ester (**5-7**) (0.015 mol) in phosphoric trichloride (0.150 mol) was refluxed for 2 h, cooled and poured into ice-water. The obtained suspension was neutralized with NaOH 6 M, filtered, washed with water and dried. Recrystallization from ethanol gave the analytical samples. Using this procedure the following compounds were obtained.

2-Chloro-4-phenyl-3-quinolinecarboxylic Acid Methyl Ester (8)

The title compound was obtained as a white powder (92%): mp 131-133 °C; IR (KBr) cm^{-1} 3263, 1739, 1557, 1483, 1384, 1223, 1133, 1125, 1028, 901, 759; ^1H NMR (DMSO-d_6) δ 8.13-8.05 (m, 1 H, aromatic), 7.99-7.87 (m, 1 H, aromatic), 7.75-7.50 (m, 5 H, aromatic), 7.46-7.38 (m, 2 H, aromatic), 3.61 (s, 3 H, CH_3). Anal. ($C_{17}H_{12}ClNO_2$) C, H, N.

2-Chloro-4-phenyl-3-quinolinecarboxylic Acid Ethyl Ester (9)

The title compound was obtained as a pale yellow powder (97%): mp 93-95 °C; IR (KBr) cm^{-1} 3200, 1745, 1559, 1495, 1380, 1243, 1123, 1028, 901, 863, 759; ^1H NMR (DMSO-d_6) δ 8.10-8.05 (m, 1 H, aromatic), 7.98-7.88 (m, 1 H, aromatic), 7.72-7.52 (m, 5 H, aromatic), 7.42-7.37 (m, 2 H, aromatic), 4.08 (q, J = 7.0 Hz, 2 H, CH_2), 0.92 (t, J = 7.0 Hz, 3 H, CH_3). Anal. ($C_{18}H_{14}ClNO_2$) C, H, N.

4-(1,3-Benzodioxol-5-yl)-2-chloro-3-quinolinecarboxylic Acid Methyl Ester (10)

The title compound was obtained as a white powder (92%): mp 160-162 °C; IR (KBr) cm^{-1} 1732, 1567, 1482, 1440, 1386, 1237, 1125, 1037, 923, 770; ^1H NMR (DMSO-d_6) δ 8.10-8.06 (m, 1 H, aromatic), 7.97-7.89 (m, 1 H, aromatic), 7.70-7.67 (m, 2 H, aromatic), 7.14-7.01 (m, 2 H, aromatic), 6.88-6.83 (m, 1 H, aromatic), 6.17-6.15 (s, 2 H, CH_2), 3.68 (s, 3 H, CH_3). Anal. ($C_{18}H_{12}ClNO_4$) C, H, N.

General Procedure for the Synthesis of 2-Aryl-4-aryl-3-quinolinecarboxylic Acid Methyl Ester Derivatives (14-19)

To a solution of the appropriate 4-aryl-2-chloro-3-quinolinecarboxylic acid methyl ester **(8, 10)** (1.00 mmol) in toluene (20 mL) and ethanol (2 mL), were added the opportune boronic acid (**11-13**) (1.00 mmol) and an aqueous K_2CO_3 solution (3.00 mmol, 0.415 g in 3 mL of water). The reaction mixture was degassed with a nitrogen flow during 0.5 h. After adding $Pd(PPh_3)_4$ (0.058 g, 5%), the reaction mixture was degassed with nitrogen flow for a further 15 min. The reaction medium was stirred at reflux under nitrogen atmosphere during 48 h and then cooled to 22 °C. The solution was filtered through a plug of celite and toluene was evaporated. Then, diethyl ether (100 mL) was added to the resulting aqueous phase. After phase separation, the organic layer was washed with water (1 × 50 mL), brine (1 × 50 mL), and dried over sodium sulphate anhydrous. The solvent was evaporated under vacuum, and the residue was recrystallized from ethanol to afford the desired title compounds (**14-19**). Using this procedure the following compounds were obtained.

2-(4-Methoxyphenyl)-4-phenyl-3-quinolinecarboxylic Acid Methyl Ester (14)

The title compound was obtained as a white powder (62%): mp 108-110 °C; IR (KBr) cm^{-1} 1724, 1604, 1436, 1234, 1176, 1102, 1027, 830, 763, 699; ^1H NMR (DMSO-d_6) δ 8.17-8.13 (m, 1 H, aromatic), 7.91-7.83 (m, 1 H, aromatic), 7.68-7.36 (m, 9 H, aromatic), 7.11-7.06 (m, 2 H, aromatic), 3.83 (s, 3 H, OCH_3), 3.37 (s, 3 H, $COOCH_3$). Anal. ($C_{24}H_{19}NO_3$) C, H, N.

2-(1,3-Benzodioxol-5-yl)-4-phenyl-3-quinolinecarboxylic Acid Methyl Ester (15)

The title compound was obtained as a white powder (73%): mp 118-120 °C; IR (KBr) cm^{-1} 2954, 1720, 1614, 1450, 1213, 1174, 1108, 1027, 834, 763; ^1H NMR (DMSO-d_6) δ 8.17-8.13 (m, 1 H, aromatic), 7.91-7.83 (m, 1 H, aromatic), 7.65-7.03 (m, 10 H, aromatic), 6.13 (s, 2 H, CH_2), 3.38 (s, 3 H, CH_3). Anal. ($C_{24}H_{17}NO_4$) C, H, N.

2-(3,4-Dimethoxyphenyl)-4-phenyl-3-quinolinecarboxylic Acid Methyl Ester (16)

The title compound was obtained as a light yellow powder (68%): mp 115-117 °C; IR (KBr) cm^{-1} 2991, 1727, 1618, 1551, 1480, 1445, 1300, 1180, 1105, 984, 830, 751; ^1H NMR (DMSO-d_6) δ 8.19-8.14 (m, 1 H, aromatic), 7.91-7.84 (m, 1 H, aromatic), 7.63-7.22 (m, 9 H, aromatic), 7.12-7.08 (m, 1 H, aromatic), 3.83 (s, 3 H, OCH_3), 3.81 (s, 3 H, OCH_3), 3.38 (s, 3 H, $COOCH_3$). Anal. ($C_{25}H_{21}NO_4$) C, H, N.

4-(1,3-Benzodioxol-5-yl)-2-(4-methoxyphenyl)-3-quinolinecarboxylic Acid Methyl Ester (17)

The title compound was obtained as a light yellow powder (83%): mp 98-101 °C; IR (KBr) cm^{-1} 1727, 1608, 1548, 1481, 1438, 1299, 1235, 1178, 1105, 1034; ^1H NMR (DMSO-d_6) δ 8.15-8.11 (m, 1 H, aromatic), 7.90-7.81 (m, 1 H, aromatic), 7.68-7.60 (m, 4 H, aromatic), 7.11-7.00 (m, 4 H, aromatic), 6.84-6.79 (m, 1 H, aromatic), 6.16 (s, 2 H, CH_2), 3.83 (s, 3 H, OCH_3), 3.45 (s, 3 H, $COOCH_3$). Anal. ($C_{25}H_{19}NO_5$) C, H, N.

2-(1,3-Benzodioxol-5-yl)-4-(1,3-benzodioxol-5-yl)-3-quinolinecarboxylic Acid Methyl Ester (18)

The title compound was obtained as a light yellow powder (66%): mp 109-111 °C; IR (KBr) cm^{-1} 1727, 1601, 1481, 1440, 1238, 1036, 924, 802; ^1H NMR (DMSO-d_6) δ 8.15-8.10 (m, 1 H, aromatic), 7.89-7.84 (m, 1 H, aromatic), 7.63-7.60 (m, 2 H, aromatic), 7.23-6.98 (m, 5 H, aromatic), 6.83-6.78 (m, 1 H, aromatic), 6.12 (s, 2 H + 2 H, CH_2), 3.48 (s, 3 H, CH_3). Anal. ($C_{25}H_{17}NO_6$) C, H, N.

4-(1,3-Benzodioxol-5-yl)-2-(3,4-dimethoxyphenyl)-3-quinolinecarboxylic Acid Methyl Ester (19)

The title compound was obtained as a light yellow powder (62%): mp 125-127 °C; IR (KBr) cm^{-1} 2956, 1714, 1648,

1550, 1382, 1300, 1172, 1121, 1060, 953, 849, 762; ^{1}H NMR (DMSO-d_6) δ 8.17-8.11 (m, 1 H, aromatic), 7.89-7.81 (m, 1 H, aromatic), 7.63-7.59 (m, 2 H, aromatic), 7.30-7.22 (m, 2 H, aromatic), 7.11-6.98 (m, 3 H, aromatic), 6.83-6.77 (m, 1 H, aromatic), 6.15 (s, 2 H, CH_2), 3.83 (s, 3 H, OCH_3), 3.81 (s, 3 H, OCH_3), 3.45 (s, 3 H, $COOCH_3$); [^{13}C]NMR (DMSO-d_6) δ 168.43, 154.26, 149.72, 148.54, 147.57, 147.27, 146.37, 131.99, 130.98, 129.33, 128.24, 127.50, 126.56, 126.24, 124.85, 122.83, 120.95, 111.72, 111.50, 109.78, 108.39, 101.50, 55.55, 55.47, 52.27. Anal. ($C_{26}H_{21}NO_6$) C, H, N.

General Procedure for the Synthesis of 2-Arylthio or 2-Aryloxy-4-aryl-3-quinolinecarboxylic Acid Alkyl Ester (26-29, 32, 33)

A solution of the appropriate benzenethiol (**20**, **21**) or phenol (**22**, **23**) (3.00 mmol) in 2 mL of anhydrous *N,N*-dimethylformamide was cooled at 0 °C and sodium hydride 95% (6.00 mmol) was added. The obtained suspension was allowed to warm at 22 °C and stirred for 15 min. After this period, the opportune 4-aryl-2-chloro-3-quinolinecarboxylic acid alkyl ester (**9**, **10**) (1.00 mmol) was added and the resulting suspension was stirred at 22 °C for 45 min. for compounds **26**, **27**, **32**, and **33** and 8 h for compounds **28** and **29**. The reaction mixture was slowly dropped into 40 mL of water. The obtained precipitate was filtered, dried and crystallized from ethanol to give the desired title compounds as solids (**26-29**, **32**, **33**). Using this procedure the following compounds were obtained.

2-(4-Methoxyphenylthio)-4-phenyl-3-quinolinecarboxylic Acid Ethyl Ester (26)

The title compound was obtained as a white powder (58%): mp 134-136 °C; IR (KBr) cm^{-1} 3263, 2956, 2832, 1712, 1650, 1549, 1382, 1299, 1171, 1121, 1058, 953, 762; ^{1}H NMR (DMSO-d_6) δ 7.74-7.31 (m, 11 H, aromatic), 7.10-7.03 (m, 2 H, aromatic), 4.01 (q, J = 7.0 Hz, 2 H, CH_2CH$_3$), 3.83 (s, 3 H, OCH_3), 0.88 (t, J = 7.0 Hz, 3 H, CH$_2$CH_3). Anal. ($C_{25}H_{21}NO_3S$) C, H, N, S.

2-(3,4-Dimethoxyphenylthio)-4-phenyl-3-quinolinecarboxylic Acid Ethyl Ester (27)

The title compound was obtained as a white powder (80%): mp 119-121 °C; IR (KBr) cm^{-1} 2931, 1710, 1583, 1544, 1499, 1245, 1168, 1129, 1022, 756; ^{1}H NMR (DMSO-d_6) δ 7.79-7.70 (m, 2 H, aromatic), 7.58-7.34 (m, 7 H, aromatic), 7.20-6.93 (m, 3 H, aromatic), 4.03 (q, J = 7.0 Hz, 2 H, CH_2CH$_3$), 3.83 (s, 3 H, OCH_3), 3.77 (s, 3 H, OCH_3), 0.88 (t, J = 7.0 Hz, 3 H, CH$_2$CH_3). Anal. ($C_{26}H_{23}NO_4S$) C, H, N, S.

2-(4-Methoxyphenyloxy)-4-phenyl-3-quinolinecarboxylic Acid Ethyl Ester (28)

The title compound was obtained as a white powder (58%): mp 98-100 °C; IR (KBr) cm^{-1} 3002, 2964, 2835, 1734, 1573, 1502, 1396, 1303, 1223, 1198, 1074, 764, 702; ^{1}H NMR (DMSO-d_6) δ 7.74-7.39 (m, 9 H, aromatic), 7.23-7.18 (m, 2 H, aromatic), 7.05-6.99 (m, 2 H, aromatic), 4.08 (q, J = 7.0 Hz, 2 H, CH_2CH$_3$), 3.81 (s, 3 H, OCH_3), 0.91 (t, J = 7.0 Hz, 3 H, CH$_2$CH_3). Anal. ($C_{25}H_{21}NO_4$) C, H, N.

2-[(1,3-Benzodioxol-5-yl)oxy]-4-phenyl-3-quinolinecarboxylic Acid Ethyl Ester (29)

The title compound was obtained as a light yellow powder (57%): mp 75-76 °C; IR (KBr) cm^{-1} 1733, 1574, 1483, 1396, 1226, 1121, 1069, 1032, 766; ^{1}H NMR (DMSO-d_6) δ 7.74-7.39 (m, 9 H, aromatic), 7.01-6.94 (m, 2 H, aromatic), 6.73-6.68 (m, 1 H, aromatic), 6.11 (s, 2 H, OCH_2O), 4.08 (q, J = 7.0 Hz, 2 H, CH_2CH$_3$), 0.93 (t, J = 7.0 Hz, 3 H, CH$_2$CH_3). Anal. ($C_{25}H_{19}NO_5$) C, H, N.

4-(1,3-Benzodioxol-5-yl)-2-(4-methoxyphenylthio)-3-quinolinecarboxylic Acid Methyl Ester (32)

The title compound was obtained as a yellow powder (53%): mp 133-135 °C; IR (KBr) cm^{-1} 1725, 1590, 1484, 1440, 1293, 1239, 1034, 773; ^{1}H NMR (DMSO-d_6) δ 7.72-7.51 (m, 6 H, aromatic), 7.11-6.95 (m, 4 H, aromatic), 6.84-6.78 (m, 1 H, aromatic), 6.15 (s, 2 H, CH_2), 3.83 (s, 3 H, OCH_3), 3.65 (s, 3 H, $COOCH_3$). Anal. ($C_{25}H_{19}NO_5S$) C, H, N, S.

4-(1,3-Benzodioxol-5-yl)-2-(3,4-dimethoxyphenylthio)-3-quinolinecarboxylic Acid Methyl Ester (33)

The title compound was obtained as a light yellow powder (57%): mp 148-149 °C; IR (KBr) cm^{-1} 2940, 1721, 1582, 1481, 1438, 1232, 1030, 759; ^{1}H NMR (DMSO-d_6) δ 7.73-7.51 (m, 4 H, aromatic), 7.19-6.96 (m, 5 H, aromatic), 6.83-6.76 (m, 1 H, aromatic), 6.15 (s, 2 H, CH_2), 3.83 (s, 3 H, OCH_3), 3.77 (s, 3 H, OCH_3), 3.65 (s, 3 H, $COOCH_3$). Anal. ($C_{26}H_{21}NO_6S$) C, H, N, S.

General Procedure for the Synthesis of 2-Arylamino-4-aryl-3-quinolinecarboxylic Acid Ethyl Ester (30, 31)

A mixture of the appropriate benzeneamine (**24**, **25**) (1.10 mmol) and 4-phenyl-2-chloro-3-quinolinecarboxylic acid ethyl ester (**9**) (1.00 mmol) was heated in an oil bath for 2 h at 160 °C. After cooling, the reaction mixture was treated with 3 mL of warm ethanol and the precipitate was filtered off, washed with ethanol, and dried. Recrystallization from ethanol gave the desired products as solids (**30**, **31**). Using this procedure the following compounds were obtained.

2-[(4-Methoxyphenyl)amino]-4-phenyl-3-quinolinecarboxylic Acid Ethyl Ester (30)

The title compound was obtained as a yellow powder (70%): mp 155-157 °C; IR (KBr) cm^{-1} 3308, 3058, 2947, 2829, 1678, 1602, 1543, 1506, 1301, 1227, 1091, 762; ^{1}H NMR (DMSO-d_6) δ 8.55 (s, 1 H, NH), 7.57-7.51 (m, 7 H, aromatic), 7.35-7.22 (m, 4 H, aromatic), 6.98-6.93 (m, 2 H, aromatic), 3.99 (q, J = 7.0 Hz, 2 H, CH_2CH$_3$), 3.77 (s, 3 H, OCH_3), 0.75 (t, J = 7.0 Hz, 3 H, CH$_2$CH_3); [^{13}C]NMR (DMSO-d_6) δ 166.74, 154.95, 150.10, 148.69, 147.14, 136.14, 133.21, 131.00, 128.84, 128.25, 126.43, 123.28, 122.68, 122.39, 121.93, 116.13, 113.67, 61.19, 55.19, 13.09. Anal. ($C_{25}H_{22}N_2O_3$) C, H, N.

2-[(1,3-Benzodioxol-5-yl)amino]-4-phenyl-3-quinolinecarboxylic Acid Ethyl Ester (31)

The title compound was obtained as a yellow powder (53%): mp 123-125 °C; IR (KBr) cm^{-1} 3059, 2875, 1691, 1575, 1542, 1489, 1391, 1282, 1191, 758; ^{1}H NMR (DMSO-

d_6) δ 8.58 (s, 1 H, NH), 7.67-7.51 (m, 6 H, aromatic), 7.33-7.13 (m, 5 H, aromatic), 6.94-6.88 (m, 1 H, aromatic), 6.03 (s, 2 H, OCH_2O), 3.97 (q, J = 7.0 Hz, 2 H, CH_2CH_3), 0.75 (t, J = 7.0 Hz, 3 H, CH_2CH_3). Anal. ($C_{25}H_{20}N_2O_4$) C, H, N.

METHOD A: General Procedure for the Synthesis of 4-Phenyl-2-substituted-3-quinolinecarboxylic Acid Derivatives (39-42)

A solution of the appropriate 4-phenyl-2-substituted-3-quinolinecarboxylic acid ethyl ester (**28-31**) (0.50 mmol) in aqueous NaOH 2 N (5 mmol, 2.5 mL), tetrahydrofurane (2.5 mL) and ethanol (2.5 mL) was refluxed for 72 h. The crude mixture was concentrated under vacuum and acidified to pH = 3. The resulting suspension was diluted with water, and solids were filtered off, washed with water, and dried. The obtained crude material was purified by column chromatography (silica gel, 60 230-400 mesh, Merck) using cyclohexane/EtOAc/acetic acid (5:5:0.5) as eluent, to afford the title compounds as solids (**39-42**). Using this procedure the following compounds were obtained.

2-(4-Methoxyphenyloxy)-4-phenyl-3-quinolinecarboxylic Acid (39)

The title compound was obtained as a white powder (59%): mp 181-182 °C; IR (KBr) cm^{-1} 3061, 3001, 1958, 2835, 1707, 1576, 1503, 1347, 1198, 1080, 758; ^1H NMR (DMSO-d_6) δ 13.45 (br s, 1 H, COOH), 7.70-7.42 (m, 9 H, aromatic), 7.25-7.18 (m, 2 H, aromatic), 7.05-7.00 (m, 2 H, aromatic), 3.81 (s, 3 H, OCH_3); [^{13}C]NMR (DMSO-d_6) δ 166.62, 157.13, 156.46, 147.73, 146.19, 145.20, 134.48, 130.73, 129.23, 128.87, 128.49, 127.36, 126.16, 125.69, 123.94, 122.78, 114.62, 55.42. Anal. ($C_{23}H_{17}NO_4$) C, H, N.

2-[(1,3-Benzodioxol-5-yl)oxy]-4-phenyl-3-quinolinecarboxylic Acid (40)

The title compound was obtained as a white powder (60%): mp 178-180 °C; IR (KBr) cm^{-1} 1711, 1585, 1482, 1344, 1226, 1173, 1034, 767; ^1H NMR (DMSO-d_6) δ 13.43 (br s, 1 H, COOH), 7.73-7.42 (m, 9 H, aromatic), 7.02-6.94 (m, 2 H, aromatic), 6.75-6.67 (m, 1 H, aromatic), 6.11 (s, 2 H, CH_2). Anal. ($C_{23}H_{15}NO_5$) C, H, N.

2-[(4-Methoxyphenyl)amino]-4-phenyl-3-quinolinecarboxylic Acid (41)

The title compound was obtained as a yellow powder (54%): mp 123-125 °C; IR (KBr) cm^{-1} 3057, 2950, 2831, 1642, 1596, 1511, 1297, 1234, 1177, 1080, 759; ^1H NMR (DMSO-d_6) δ 8.74 (s, 1 H, NH), 7.79-7.50 (m, 7 H, aromatic), 7.38-7.18 (m, 4 H, aromatic), 6.98-6.92 (m, 2 H, aromatic), 3.77 (s, 3 H, CH_3); [^{13}C]NMR (DMSO-d_6) δ 168.58, 154.82, 150.35, 148.24, 146.93, 136.56, 133.38, 130.75, 128.95, 128.18, 126.45, 126.42, 123.17, 122.34, 122.28, 116.88, 113.72, 55.20. Anal. ($C_{23}H_{18}N_2O_3$) C, H, N.

2-[(1,3-Benzodioxol-5-yl)amino]-4-phenyl-3-quinolinecarboxylic Acid (42)

The title compound was obtained as a yellow powder (48%): mp 220-222 °C; IR (KBr) cm^{-1} 3055, 2950, 1649, 1596, 1483, 1293, 1221, 1036, 759; ^1H NMR (DMSO-d_6) δ 8.81 (s, 1 H, NH), 7.70-7.49 (m, 6 H, aromatic), 7.38-7.15 (m, 5 H, aromatic), 6.93-6.86 (m, 1 H, aromatic), 6.02 (s, 2 H, CH_2). Anal. ($C_{23}H_{16}N_2O_4$) C, H, N.

METHOD B: General Procedure for the Synthesis of 4-Aryl-2-substituted-3-quinolinecarboxylic Acid Derivatives (34-38, 43-47)

A solution of the appropriate 4-aryl-2-substituted-3-quinolinecarboxylic acid alkyl ester (**14-19**, **26, 27**, **32**, **33**) (0.50 mmol) was dissolved in ethanolic KOH solution 15% (20 mL), then water was added (2 mL) and the resulting reaction medium was refluxed for 8 h. The crude mixture was concentrated under vacuum and acidified to pH = 3. The resulting suspension was diluted with water, and solids were filtered off, washed abundantly with water, and dried. Recrystallization from ethyl acetate gave the desired products as solids (**34-38, 43-47**). Using this procedure the following compounds were obtained.

2-(4-Methoxyphenyl)-4-phenyl-3-quinolinecarboxylic Acid (34)

The title compound was obtained as a white powder (85%): mp 245-247 °C; IR (KBr) cm^{-1} 2956, 1710, 1650, 1549, 1377, 1299, 1175, 1101, 953, 899, 762; ^1H NMR (DMSO-d_6) δ 8.15-8.09 (m, 1 H, aromatic), 7.88-7.38 (m, 10 H, aromatic), 7.10-7.02 (m, 2 H, aromatic), 3.88 (s, 3 H, CH_3). Anal. ($C_{23}H_{17}NO_3$) C, H, N.

2-(1,3-Benzodioxol-5-yl)-4-phenyl-3-quinolinecarboxylic Acid (35)

The title compound was obtained as a light yellow powder (83%): mp 250-252 °C; IR (KBr) cm^{-1} 3010, 2981, 1710, 1585, 1471, 1350, 1226, 1170, 1034, 951, 767; ^1H NMR (DMSO-d_6) δ 8.15-8.09 (m, 1 H, aromatic), 7.88-7.79 (m, 1 H, aromatic), 7.62-7.27 (m, 9 H, aromatic), 7.09-7.03 (m, 1 H, aromatic), 6.13 (s, 2 H, CH_2). Anal. ($C_{23}H_{15}NO_4$) C, H, N.

2-(3,4-Dimethoxyphenyl)-4-phenyl-3-quinolinecarboxylic Acid (36)

The title compound was obtained as a light yellow powder (95%): mp 189-190 °C; IR (KBr) cm^{-1} 3007, 2941, 1642, 1596, 1509, 1294, 1234, 1180, 1080, 961, 759; ^1H NMR (DMSO-d_6) δ 8.16-8.10 (m, 1 H, aromatic), 7.88-7.82 (m, 1 H, aromatic), 7.80-7.35 (m, 9 H, aromatic), 7.12-7.06 (m, 1 H, aromatic), 3.83 (s, 3 H, CH_3), 3.81 (s, 3 H, CH_3). Anal. ($C_{24}H_{19}NO_4$) C, H, N.

2-(4-Methoxyphenylthio)-4-phenyl-3-quinolinecarboxylic Acid (37)

The title compound was obtained as a white powder (88%): mp 235-236 °C; IR (KBr) cm^{-1} 3062, 2955, 2831, 1691, 1546, 1492, 1381, 1298, 1171, 910, 766; ^1H NMR (DMSO-d_6) δ 7.69-7.35 (m, 11 H, aromatic), 7.09-7.04 (m, 2 H, aromatic), 3.83 (s, 3 H, CH_3). Anal. ($C_{23}H_{17}NO_3S$) C, H, N, S.

2-(3,4-Dimethoxyphenylthio)-4-phenyl-3-quinolinecarboxylic Acid (38)

The title compound was obtained as a white powder (90%): mp 210-212 °C; IR (KBr) cm^{-1} 3015, 2901, 1640, 1597, 1510, 1293, 1180, 961, 843, 759; ^1H NMR (DMSO-d_6) δ 7.70-7.67 (m, 2 H, aromatic), 7.56-7.35 (m, 7 H, aromatic),

7.23-7.04 (m, 3 H, aromatic), 3.84 (s, 3 H, CH_3), 3.78 (s, 3 H, CH_3). Anal. ($C_{24}H_{19}NO_4S$) C, H, N, S.

4-(1,3-Benzodioxol-5-yl)-2-(4-methoxyphenyl)-3-quinolinecarboxylic Acid (43)

The title compound was obtained as a yellow powder (87%): mp 158-160 °C; IR (KBr) cm^{-1} 2920, 1604, 1480, 1438, 1236, 1032, 832, 761, 649; ^1H NMR (DMSO-d_6) δ 13.25 (br s, 1 H, COOH), 8.13-8.07 (m, 1 H, aromatic), 7.84-7.54 (m, 5 H, aromatic), 7.11-6.97 (m, 4 H, aromatic), 6.87-6.81 (m, 1 H, aromatic), 6.14 (s, 2 H, CH_2), 3.83 (s, 3 H, CH_3). Anal. ($C_{24}H_{17}NO_5$) C, H, N.

2-(1,3-Benzodioxol-5-yl)-4-(1,3-benzodioxol-5-yl)-3-quinolinecarboxylic Acid (44)

The title compound was obtained as a yellow powder (91%): mp 184-186 °C; IR (KBr) cm^{-1} 1725, 1586, 1482, 1440, 1239, 1035, 928, 770; ^1H NMR (DMSO-d_6) δ 8.13-8.07 (m, 1 H, aromatic), 7.88-7.79 (m, 1 H, aromatic), 7.60-7.55 (m, 2 H, aromatic), 7.30-7.26 (m, 2 H, aromatic), 7.12-6.97 (m, 3 H, aromatic), 6.88-6.83 (m, 1 H, aromatic), 6.14 (s, 2 H, CH_2), 6.12 (s, 2 H, CH_2). Anal. ($C_{24}H_{15}NO_6$) C, H, N.

4-(1,3-Benzodioxol-5-yl)-2-(3,4-dimethoxyphenyl)-3-quinolinecarboxylic Acid (45)

The title compound was obtained as a yellow powder (89%): mp 175-177 °C; IR (KBr) cm^{-1} 2989, 1651, 1489, 1435, 1244, 1032, 972, 761, 674; ^1H NMR (DMSO-d_6) δ 13.24 (br s, 1 H, COOH), 8.15-8.08 (m, 1 H, aromatic), 7.88-7.79 (m, 1 H, aromatic), 7.60-7.54 (m, 2 H, aromatic), 7.42-7.32 (m, 2 H, aromatic), 7.12-7.04 (m, 3 H, aromatic), 6.88-6.82 (m, 1 H, aromatic), 6.14 (s, 2 H, CH_2), 3.83 (s, 3 H, CH_3), 3.81 (s, 3 H, CH_3); [^{13}C]NMR (DMSO-d_6) δ 169.40, 154.11, 149.64, 148.38, 147.49, 147.11, 146.89, 145.00, 132.28, 130.50, 129.25, 128.53, 128.32, 127.28, 126.12, 125.10, 123.19, 121.22, 112.15, 111.42, 110.05, 108.30, 101.45, 55.59, 55.43. Anal. ($C_{25}H_{19}NO_6$) C, H, N.

4-(1,3-Benzodioxol-5-yl)-2-(4-methoxyphenylthio)-3-quinolinecarboxylic Acid (46)

The title compound was obtained as a yellow powder (82%): mp 292-294 °C; IR (KBr) cm^{-1} 1589, 1484, 1441, 1243, 1032, 771; ^1H NMR (DMSO-d_6) δ 7.54-7.35 (m, 6 H, aromatic), 7.07-6.92 (m, 4 H, aromatic), 6.85-6.79 (m, 1 H, aromatic), 6.10 (s, 2 H, CH_2), 3.82 (s, 3 H, CH_3). Anal. ($C_{24}H_{17}NO_5S$) C, H, N, S.

4-(1,3-Benzodioxol-5-yl)-2-(3,4-dimethoxyphenylthio)-3-quinolinecarboxylic Acid (47)

The title compound was obtained as a yellow powder (83%): mp 135-137 °C; IR (KBr) cm^{-1} 2923, 1724, 1580, 1482, 1439, 1234, 1030, 931, 762; ^1H NMR (DMSO-d_6) δ 13.50 (br s, 1 H, COOH), 7.69-7.63 (m, 2 H, aromatic), 7.54-7.49 (m, 2 H, aromatic), 7.22-6.80 (m, 6 H, aromatic), 6.14 (s, 2 H, CH_2), 3.83 (s, 3 H, CH_3), 3.77 (s, 3 H, CH_3); [^{13}C]NMR (DMSO-d_6) δ 167.60, 154.83, 149.87, 148.85, 147.66, 147.21, 147.00, 144.44, 130.67, 128.19, 128.10, 127.39, 126.61, 126.29, 124.63, 123.16, 119.60, 118.49, 112.05, 109.90, 108.42, 101.50, 55.70, 55.54. Anal. ($C_{25}H_{19}NO_6S$) C, H, N, S.

PHARMACOLOGY

Binding of compounds **34-47** was determined using human recombinant ET_A or ET_B receptors (CHO-K1 cell line, Euroscreen). The cells were resuspended in Tris HCl, 50 mM, pH 7.5 containing 10 mM $MgCl_2$ and used at concentration of 0.08 µg/sample for ET_A receptors, and 0.7 µg/sample for ET_B receptors. Assay [26] was initiated by adding 25 µl of [^{125}I]Endothelin-1 (0.2-0.4 nM; specific activity 2000 Ci/mmole, Amersham) in 0.05% BSA protease free in a final volume of 100 µl (tubes minisorp, Nunc). Non-specific binding was obtained in the presence of 1 µM Endothelin-1 and 1 µM BQ-123 for ET_A receptors or 1 µM Endothelin-1 and 1 µM BQ-788 for ET_B receptors. Compounds were dissolved in DMSO and tested at 10^{-5} and 10^{-7} M in triplicate, inhibition curves of endothelin-1, BQ-123, BQ-788 and compounds **40**, **44**, **46**, and **47** were obtained using 5-7 different concentrations in triplicate. The incubation (30 °C, 60 min) was stopped by dilution with cold buffer (Tris HCl, 20 mM, pH 7.5 containing 10 mM $MgCl_2$) and filtration through GF/C filters presoaked in 0.1% BSA protease free. The filters were washed three times with the same buffer using a Brandel cell harvester and were counted in a gamma counter with a 90% efficiency. Inhibition curves were analyzed using the "Allfit" [27] program and the K_i values were derived from the IC_{50} values using the Cheng and Prusoff equation [28].

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ABBREVIATIONS

GPCR = G protein-coupled receptor

ET = Endothelin

ET-1 = Endothelin 1

ET-2 = Endothelin 2

ET-3 = Endothelin 3

ET_A = Endothelin receptor subtype A

ET_B = Endothelin receptor subtype B

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