

### 3-Aryl-2-Quinolone Derivatives: Synthesis and Characterization of In Vitro and In Vivo Antitumor Effects with Emphasis on a New Therapeutical Target Connected with Cell Migration

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Among 25 3-aryl-2-quinolone derivatives synthesized, the antitumor activity of some of them was characterized both in vitro and in vivo. In this series, no compound appeared to be cytotoxic in vitro, as was known by the colorimetric MTT assay carried out on 12 distinct human cancer cell lines obtained from the American Type Culture Collection. Indeed, the concentration values decreasing the growth of the 12 cell lines by at least 50% (IC<sub>50</sub> index) were always higher than 10<sup>-5</sup> M. We then made use of a computer-assisted phase-contrast videomicroscopy system to quantitatively determine in vitro the level of migration of living MCF-7 human breast cancer cells. For example, at 10<sup>-7</sup> M, compounds **7**, **13**, **16**, and **28** markedly decreased the migration level of these MCF-7 human breast cancer cells. The in vivo determination of the maximum tolerated dose showed that all compounds tested were definitively nontoxic. When the nontoxic, antimigratory compound **16** was combined with either doxorubicin or etoposide, two cytotoxic compounds routinely used in the clinic, this led to additive in vivo benefits from this treatment (as compared to individual administrations of the drugs) when the MXT mouse mammary adenocarcinoma was used. Thus, nontoxic antimigratory compounds, including the 2-quinolone derivatives synthesized here, can actually improve the efficiency of antitumor treatment when combined with conventional cytotoxic agents.

#### Introduction

From a schematic point of view, the development of malignancy in a given tissue occurs as follows. Some genomic mutations perturb cell cycle kinetics by increasing cell proliferation or decreasing cell death (or both), and these features lead to unrestrained growth of the genomically transformed cell population.<sup>1</sup> Then, some cells from this transformed cell population switch to the angiogenic phenotype, and this enables them to recruit endothelial cells from the healthy tissue, leading to a neoangiogenic process enabling the sustained growth of the developing neoplastic tissue.<sup>2</sup> Then, some cells migrate from the tumor bulk and colonize new tissues (the metastatic process), using blood or lymphatic vessels as major routes of migration.<sup>3</sup> The first chemotherapeutic agents used against cancer in clinics were drugs that target processes including nucleoside synthesis, strand replication, etc., i.e., processes controlling DNA replication and therefore cell proliferation.<sup>4</sup>

The discovery of programmed cell death (apoptosis) by Kerr and colleagues in the early 1970s<sup>5</sup> was evidence that rapidly growing tumors are not always tumors exhibiting high levels of cell proliferation but often low levels of cell death as compared to the normal cell

population from which these tumor cells issue.<sup>6</sup> These findings thus led to the development of anticancer drugs activating the cell death of tumor cells rather than inhibiting their proliferation.<sup>7</sup> Most of these agents target topoisomerases, which play crucial roles in DNA duplication.<sup>8</sup> They include, for example, doxorubicin (DOX) and etoposide (ETO) as inhibitors of topoisomerase II and irinotecan and topotecan as inhibitors of topoisomerase I. Thus, most of the agents used today in hospitals to treat cancer patients are drugs that more or less directly target the cell kinetics (i.e., proliferation vs death) of the cancer to be combatted. Great hopes are placed in complementary strategies including antiangiogenic,<sup>9</sup> immunotherapeutic,<sup>10</sup> and gene therapy<sup>11</sup> approaches. The fact nevertheless remains that the great majority of the drugs used in the standard treatment of cancer are toxic to highly toxic, and this limits their clinical use to a relatively low number of administrations per patient. In addition, several of these compounds must be combined into polychemotherapeutic regimens in order to have any effect against cancer. By way of evidence, such anticancer drug combinations increase the toxicity of the treatment and once again limit the number of administrations that can be applied per patient. In addition, a hallmark of malignancy, which is as important as cell kinetics, is the migration of cancer cells escaping from the tumor bulk that first invade neighboring tissue and then establish metastases. Antitubulin compounds, one major class of antican-

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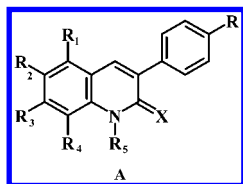
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cer drugs, already target tumor cell migration or at least partly.<sup>12</sup> While effective against human cancers, these compounds—including, for example, vincristine, vinorelbine, and paclitaxel—are nevertheless also toxic.

It has been known for many years that flavonols, as quercetin, are polyphenols, which act as free radical scavengers and could be significant anticarcinogenic substances (through food intake) due to not yet well-defined biological mechanisms.<sup>13</sup> Such flavonoids inhibit proliferation of the MCF-7 human breast cancer cell line.<sup>14</sup> Yoshida et al. have suggested that antiproliferative effects of quercetin were due to the specific arrest of the G<sub>1</sub> phase of the cell cycle.<sup>15</sup> However, the results of epidemiological studies reported so far do not show a clear protective effect of flavonol intake (through food and tea consumption) on cancer risk but a protective effect on selected cancers in a specific population cannot be ruled out. Because of that, hypotheses are proposed that a protective role of quercetin (from onions) in explaining the lower risk of stomach cancer might be due to interaction with early processes in tumor development (i.e., angiogenesis, cell migration, etc.). For this reason, we have considered the 2-quinolone template as a possible source of chemical modulation when looking at the benzopyrane-4-one ring. Moreover, variations on the 3-aryl moiety were designed with the variety of compounds known in the flavonoid/isoflavonoid families in mind.

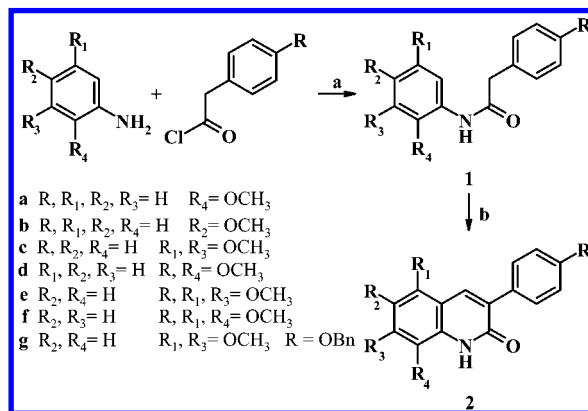
We have prepared 25 3-aryl-2-quinolone derivatives A with the aim of developing nontoxic antimigratory compounds that could complement the anticancer action of conventional drugs already used in hospitals. Some rationale as to why 3-aryl-2-quinolones can be antimigratory agents is given in the discussion.



## Chemistry

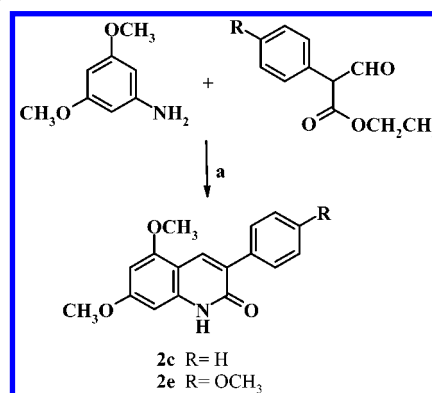
The syntheses of 3-aryl-2-quinolone derivatives have been accomplished as described in Schemes 1–6. According to the literature method,<sup>16,17</sup> the key 3-aryl-2-quinolones **2a–g** were synthesized. As described in Scheme 1, substituted anilines were acylated with phenylacetyl chloride, 4-methoxyphenylacetyl chloride, or 4-benzyloxyphenylacetyl chloride to give the corresponding amides **1a–g**,<sup>18</sup> which were then treated with phosphorus oxychloride and *N,N*-dimethylformamide (DMF) followed by an acid hydrolysis to give 3-aryl-2-quinolones **2a–g**. Compounds **2c,e** were also prepared through a one pot route (Scheme 2). Thus, the condensation of 3,5-dimethoxyaniline and ethyl 2-(phenyl or 4-methoxyphenyl)-2-formyl acetate<sup>19</sup> was carried out in the presence of polyphosphoric acid trimethylsilyl ester (PPSE)<sup>20</sup> at 110 °C for 2 h to give the desired compounds **2c,e**, respectively, in 16 and 32% yield. In an independent but closed work published in 1997, Bisagni et al. observed the same results by thermal cyclization of 3,5-dimethoxyaniline and ethyl 2-(4-methoxyphenyl)-2-formyl acetate.<sup>21</sup>

## Scheme 1<sup>a</sup>



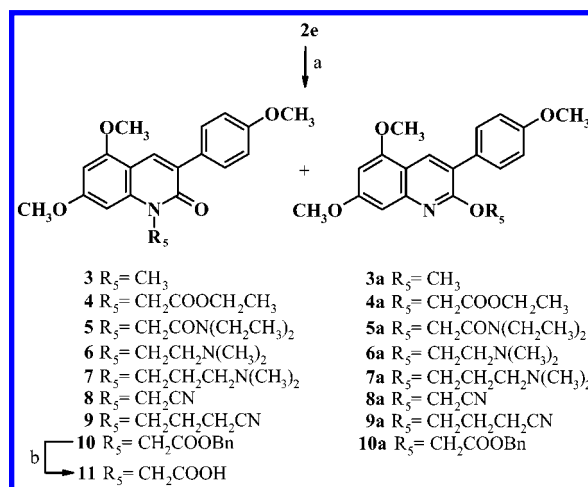
<sup>a</sup> Reagents and conditions: (a) Toluene, room temperature, 1 h. (b) (i) POCl<sub>3</sub>, DMF, –30 °C to 75 °C; (ii) AcOH/H<sub>2</sub>O, reflux 3 h.

## Scheme 2<sup>a</sup>



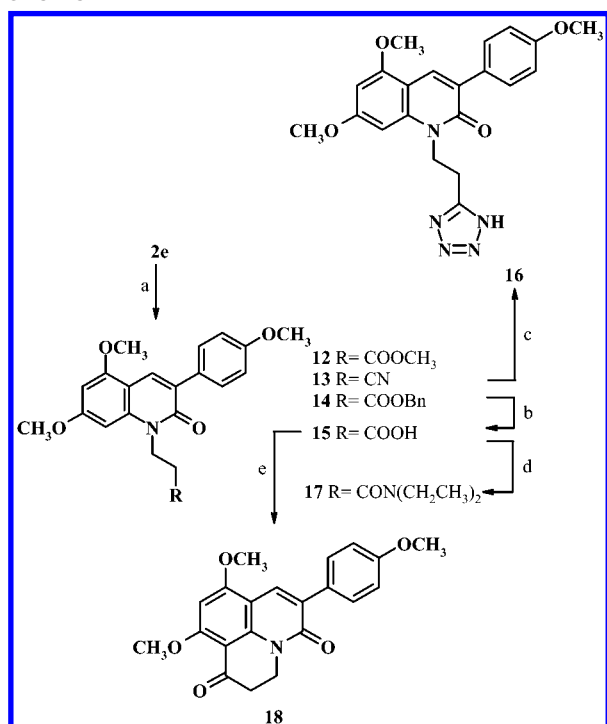
<sup>a</sup> Reagents and conditions: (a) PPSE, 110 °C, 2 h.

## Scheme 3<sup>a</sup>



<sup>a</sup> Reagents and conditions: (a) NaH, RX, DMF, 0 °C and then 90 °C. (b) H<sub>2</sub>, Pd–C 10%, 1,4-dioxane, room temperature, 4 h.

Alkylation of quinolone **2e** led to the preparation of target compounds **3–10** and *O*-alkylated derivatives **3a–10a** (Scheme 3). In all cases, the *N*-alkylated derivative was obtained as the major product. The structure of both series of compounds was unambiguously confirmed by <sup>13</sup>C nuclear magnetic resonance (NMR) (i.e., for **5**, δ 45.3 (NCH<sub>2</sub>) and for **5a**, δ 63.0 (OCH<sub>2</sub>)) and by analysis of the literature published on this topic.<sup>22</sup> Catalytic hydrogenolysis of **10** led to the acid **11**. Compounds **12–14** bearing a functionalized ethyl- enic chain were obtained via a Michael addition with

Scheme 4<sup>a</sup>

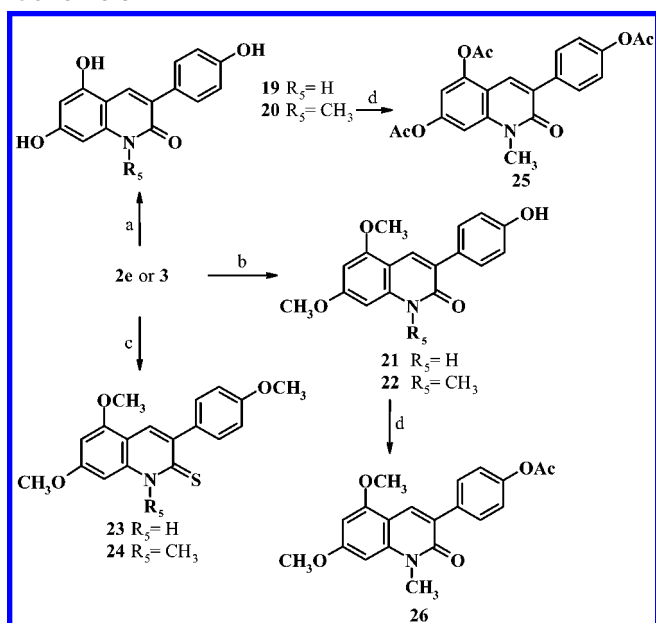
<sup>a</sup> Reagents and conditions: (a) CH<sub>2</sub>=CHR, Triton B, DMF, room temperature, 18 h. (b) H<sub>2</sub>, Pd/C 10%, 1,4-dioxane, room temperature, 48 h. (c) Bu<sub>3</sub>SnN<sub>3</sub>, toluene, 105 °C, 65 h. (d) DCC, HOBT, (Et)<sub>2</sub>NH, DMF, room temperature, 24 h. (e) PPA, P<sub>2</sub>O<sub>5</sub>, 110 °C, 45 min.

various acrylates (Scheme 4). Catalytic hydrogenolysis of **14** led to the acid **15**. Compound **13** was transformed into the 5-tetrazolyethyl derivative **16** by using tributyltin azide (Scheme 4). The dicyclohexylcarbodiimide/1-hydroxybenzotriazole coupling method was applied to **15** to give compound **17** in good yield (Scheme 4). Similarly, polyphosphoric acid cyclization of **15** led to the preparation of tricyclic target compound **18** (Scheme 4).

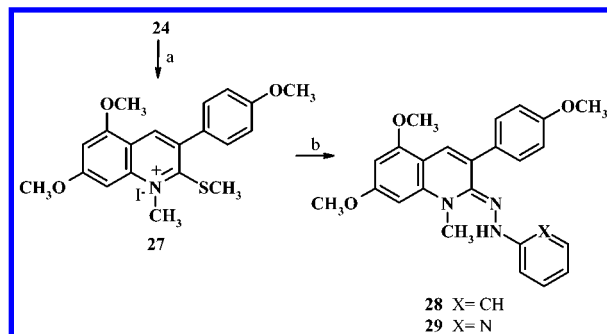
The *O*-demethylation of **2e** and **3** was performed with 48% HBr in acetic acid to give, respectively, **19** and **20** in 3 days or **21** and **22** in 5 h (Scheme 5). Compounds **2e** and **3** were converted into the corresponding 2-thioquinolones **23** and **24** by treatment with Lawesson's reagent in toluene (Scheme 5). Compounds **25** and **26** were generated by acetic anhydride–pyridine acetylation of **20** and **22** in 60% yield (Scheme 5). Treatment of **24** with iodomethane in tetrahydrofuran (THF) afforded the iodide salt **27** in 79% yield. Reaction of **27** with phenylhydrazine or 2-pyridinylhydrazine in refluxing ethanol gave hydrazones **28** and **29** in fair yields (Scheme 6). The yields and/or analytical data of compounds **1–26**, **28**, and **29** are summarized in Tables 1–3.

### Pharmacological Results and Discussion

The absence of in vitro cytotoxicity of 3-aryl-2-quinolone derivatives so prepared was demonstrated by means of the MTT colorimetric assay<sup>23</sup> on 12 distinct human cancer cell lines. The results show that when compared to the control value when assayed at 10<sup>−5</sup> M, 19 of the 25 2-quinolone derivatives synthesized significantly decreased the mean growth value of the 12 human cancer cell lines under study, while the remain-

Scheme 5<sup>a</sup>

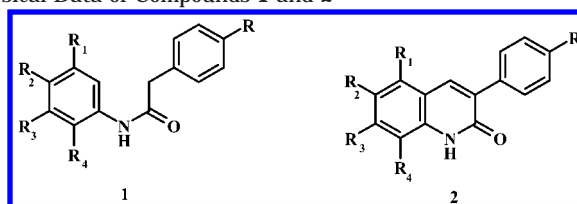
<sup>a</sup> Reagents and conditions: (a) 48% HBr, AcOH reflux, 3 days. (b) 48% HBr, AcOH, reflux, 5 h. (c) Lawesson's reagent, toluene, reflux, 18 h. (d) (Ac)<sub>2</sub>O, pyridine, room temperature, 18 h.

Scheme 6<sup>a</sup>

<sup>a</sup> Reagents and conditions: (a) ICH<sub>3</sub>, THF, room temperature, 12 h. (b) NH<sub>2</sub>NHPh or NH<sub>2</sub>NH-2-Pyr, EtOH, sealed tube, 90 °C.

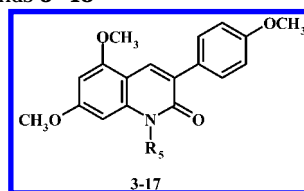
ing six compounds **2e**, **7**, **15**, **19**, **20**, and **23** were without any actual effect at this 10<sup>−5</sup> M dose. At the 10<sup>−6</sup> M dose, only few compounds significantly decreased the growth levels of human cancer cell populations. At the 10<sup>−7</sup> M dose, only two compounds (**17** and **21**) showed marginal cytotoxic effects. The determination of the maximum tolerated dose (MTD) index in vivo revealed that all quinolones prepared were found nontoxic at 160 mg/kg.

The effects of these quinolone derivatives were then characterized in vitro on MCF-7 human breast cancer cell motility by means of computer-assisted phase-contrast videomicroscopy.<sup>24</sup> The migration features of cancer cells involve two distinct but complementary processes, i.e., motility and invasion. Motility refers to the capacity of cells to move (including polymerization/depolymerization dynamics of the actin skeleton and integrin moving in adhesion complexes), while invasion refers to the capacity of cells to modulate their surrounding environment by proteolytic activity. The system that we set up thus enabled the motility level of living cells to be quantitatively determined in vitro. However, we showed with respect to human glioma cancer cells that their in vitro motility characteristics,

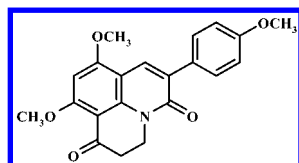
**Table 1.** Structures, Yields, and Physical Data of Compounds **1** and **2**

compd	R	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	yield (%)	mp (°C)	formula	anal
<b>1a</b>	H	H	H	H	OCH <sub>3</sub>	92	80–81 <sup>a,b</sup>	C <sub>15</sub> H <sub>15</sub> NO <sub>2</sub>	C, H, N
<b>1b</b>	H	H	OCH <sub>3</sub>	H	H	89	118–119 <sup>c,d</sup>	C <sub>15</sub> H <sub>15</sub> NO <sub>2</sub>	C, H, N
<b>1c</b>	H	OCH <sub>3</sub>	H	OCH <sub>3</sub>	H	87	109–111 <sup>a</sup>	C <sub>16</sub> H <sub>17</sub> NO <sub>3</sub>	C, H, N
<b>1d</b>	OCH <sub>3</sub>	H	H	H	OCH <sub>3</sub>	91	47–48 <sup>a</sup>	C <sub>16</sub> H <sub>17</sub> NO <sub>3</sub>	C, H, N
<b>1e</b>	OCH <sub>3</sub>	OCH <sub>3</sub>	H	OCH <sub>3</sub>	H	82	135–137 <sup>a,e</sup>	C <sub>17</sub> H <sub>19</sub> NO <sub>4</sub>	C, H, N
<b>1f</b>	OCH <sub>3</sub>	OCH <sub>3</sub>	H	H	OCH <sub>3</sub>	93	89–90 <sup>a</sup>	C <sub>17</sub> H <sub>19</sub> NO <sub>4</sub>	C, H, N
<b>1g</b>	OBn	OCH <sub>3</sub>	H	OCH <sub>3</sub>	H	81	122–123 <sup>c</sup>	C <sub>23</sub> H <sub>23</sub> NO <sub>4</sub>	C, H, N
<b>2a</b>	H	H	H	H	OCH <sub>3</sub>	10	188–189 <sup>f</sup>	C <sub>16</sub> H <sub>13</sub> NO <sub>2</sub>	C, H, N
<b>2b</b>	H	H	OCH <sub>3</sub>	H	H	28	243–244 <sup>f</sup>	C <sub>16</sub> H <sub>13</sub> NO <sub>2</sub>	C, H, N
<b>2c</b>	H	OCH <sub>3</sub>	H	OCH <sub>3</sub>	H	16 <sup>Meth.A,B</sup>	257–258 <sup>f,g</sup>	C <sub>17</sub> H <sub>15</sub> NO <sub>3</sub>	C, H, N
<b>2d</b>	OCH <sub>3</sub>	H	H	H	OCH <sub>3</sub>	10	148–149 <sup>f</sup>	C <sub>17</sub> H <sub>15</sub> NO <sub>3</sub>	C, H, N
<b>2e</b>	OCH <sub>3</sub>	OCH <sub>3</sub>	H	OCH <sub>3</sub>	H	22 <sup>Meth.A</sup> , 32 <sup>Meth.B</sup>	254–255 <sup>f,h</sup>	C <sub>18</sub> H <sub>17</sub> NO <sub>4</sub>	C, H, N
<b>2f</b>	OCH <sub>3</sub>	OCH <sub>3</sub>	H	H	OCH <sub>3</sub>	45	186–187 <sup>f</sup>	C <sub>18</sub> H <sub>17</sub> NO <sub>4</sub>	C, H, N
<b>2g</b>	OBn	OCH <sub>3</sub>	H	OCH <sub>3</sub>	H	20	234–235 <sup>f</sup>	C <sub>24</sub> H <sub>21</sub> NO <sub>4</sub>	C, H, N

<sup>a</sup> Toluene (recrystallization solvent). <sup>b</sup> Literature<sup>18</sup> 85 °C. <sup>c</sup> EtOAc/PE (recrystallization solvent). <sup>d</sup> Literature<sup>18</sup> 123 °C. <sup>e</sup> Literature<sup>21</sup> 150 °C. <sup>f</sup> EtOAc (recrystallization solvent). <sup>g</sup> Literature<sup>17</sup> 243 °C. <sup>h</sup> Literature<sup>21</sup> 260 °C; Meth. A, Meth. B, see experimental section.

**Table 2.** Structures and Physical Data of Compounds **3–18**

compd	R <sub>5</sub>	mp (°C)	formula	anal
<b>3</b>	CH <sub>3</sub>	125–126 <sup>a</sup>	C <sub>19</sub> H <sub>19</sub> NO <sub>4</sub>	C, H, N
<b>4</b>	CH <sub>2</sub> COOCH <sub>2</sub> CH <sub>3</sub>	160–161 <sup>a</sup>	C <sub>22</sub> H <sub>23</sub> NO <sub>6</sub>	C, H, N
<b>5</b>	CH <sub>2</sub> CON(CH <sub>2</sub> CH <sub>3</sub> ) <sub>2</sub>	178–179 <sup>b</sup>	C <sub>24</sub> H <sub>28</sub> N <sub>2</sub> O <sub>5</sub>	C, H, N
<b>6</b>	CH <sub>2</sub> CH <sub>2</sub> N(CH <sub>3</sub> ) <sub>2</sub>	113–114 <sup>c</sup>	C <sub>22</sub> H <sub>26</sub> N <sub>2</sub> O <sub>4</sub>	C, H, N
<b>7</b>	CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> N(CH <sub>3</sub> ) <sub>2</sub>	94–95 <sup>c</sup>	C <sub>23</sub> H <sub>28</sub> N <sub>2</sub> O <sub>4</sub>	C, H, N
<b>8</b>	CH <sub>2</sub> CN	208–209 <sup>b</sup>	C <sub>20</sub> H <sub>18</sub> N <sub>2</sub> O <sub>4</sub>	C, H, N
<b>9</b>	CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CN	157–158 <sup>b</sup>	C <sub>22</sub> H <sub>22</sub> N <sub>2</sub> O <sub>4</sub>	C, H, N
<b>10</b>	CH <sub>2</sub> COOBn	199–200 <sup>c</sup>	C <sub>27</sub> H <sub>25</sub> NO <sub>6</sub>	C, H, N
<b>11</b>	CH <sub>2</sub> COOH	179–180 <sup>c</sup>	C <sub>20</sub> H <sub>19</sub> NO <sub>6</sub>	C, H, N
<b>12</b>	CH <sub>2</sub> CH <sub>2</sub> COOCH <sub>3</sub>	100–101 <sup>b</sup>	C <sub>22</sub> H <sub>23</sub> NO <sub>6</sub>	C, H, N
<b>13</b>	CH <sub>2</sub> CH <sub>2</sub> CN	156–157 <sup>a</sup>	C <sub>21</sub> H <sub>20</sub> N <sub>2</sub> O <sub>4</sub>	C, H, N
<b>14</b>	CH <sub>2</sub> CH <sub>2</sub> COOBn	124–125 <sup>d</sup>	C <sub>28</sub> H <sub>27</sub> NO <sub>6</sub>	C, H, N
<b>15</b>	CH <sub>2</sub> CH <sub>2</sub> COOH	194–195 <sup>c</sup>	C <sub>21</sub> H <sub>21</sub> NO <sub>6</sub>	C, H, N
<b>16</b>	5-tetrazolyethyl	234–235 <sup>c</sup>	C <sub>21</sub> H <sub>21</sub> N <sub>5</sub> O <sub>4</sub>	C, H, N
<b>17</b>	CH <sub>2</sub> CH <sub>2</sub> CON(CH <sub>2</sub> CH <sub>3</sub> ) <sub>2</sub>	137–138 <sup>b</sup>	C <sub>25</sub> H <sub>30</sub> N <sub>2</sub> O <sub>5</sub>	C, H, N

**18**240–241<sup>e</sup>C<sub>21</sub>H<sub>19</sub>NO<sub>5</sub>

C, H, N

<sup>a</sup> Recrystallization solvent: EtOAc/PE. <sup>b</sup> EtOAc. <sup>c</sup> Et<sub>2</sub>O. <sup>d</sup> Et<sub>2</sub>O/PE. <sup>e</sup> CH<sub>2</sub>Cl<sub>2</sub>/PE.

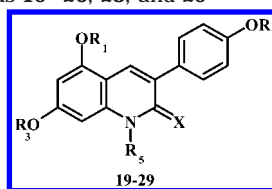
as determined by the system that we set up, correlate with their in vivo invasive capacities.<sup>24,25</sup> The fact that antimigratory effects observed in vitro for a given compound can relate to antimetastatic potential is already demonstrated for inhibitors of hepatocyte growth factor receptor.<sup>26,27</sup>

Preliminary data indicated that this first series of quinolone derivatives exhibits interacting effects at the cell migration level (data not shown) with inhibition of migration effects seen for various chemical types of R<sub>5</sub> or X radicals: for example, compounds **7** (X = O, R<sub>5</sub> = –(CH<sub>2</sub>)<sub>3</sub>–N(CH<sub>3</sub>)<sub>2</sub>), **13** (X = O, R<sub>5</sub> = –(CH<sub>2</sub>)<sub>2</sub>–CN), **16** (X = O, R<sub>5</sub> = 5-tetrazolyethyl), and **28** (X = NNHPh,

R<sub>5</sub> = –CH<sub>3</sub>). Figure 1 illustrates the antimigratory effects obtained with respect to compounds **13** and **16**. Both compounds induce a significant decrease in MCF-7 human breast cancer cell migration, with more marked and dose-dependent effects for compound **16** than **13**. Compound **16** had a significant antimigratory effect on the MCF-7 cells at the definitively noncytotoxic 10<sup>–7</sup> M dose. At the lowest dose tested for compound **16**, i.e., 10<sup>–8</sup> M, a slight stimulation of cell migration was observed (Figure 1B).

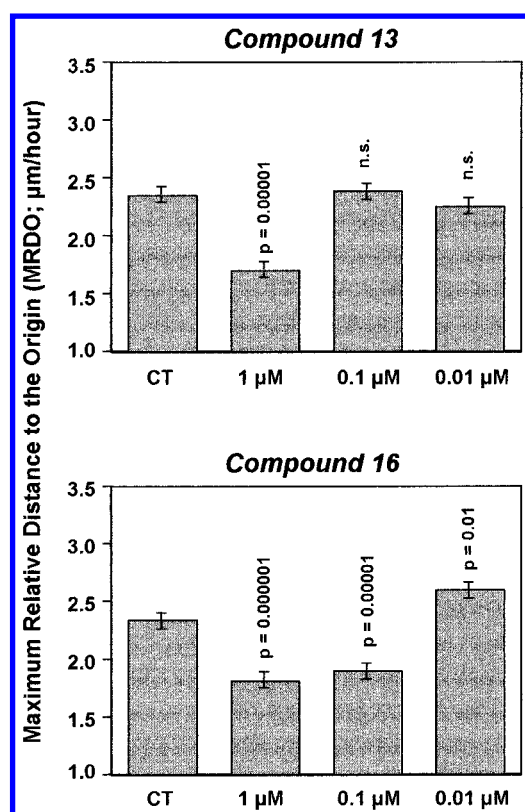
3-Aryl-2-quinolones are reported to be inhibitors of arachidonic acid-induced platelet aggregation<sup>28</sup> and also related to 3-arylpyridopyrimidines that are reported to



**Table 3.** Structures and Physical Data of Compounds **19–26**, **28**, and **29**

compd	R	R <sub>1</sub>	R <sub>3</sub>	R <sub>5</sub>	X	mp (°C)	formula	anal
<b>19</b>	H	H	H	H	O	>280 <sup>a,b</sup>	C <sub>15</sub> H <sub>11</sub> NO <sub>4</sub>	C, H, N
<b>20</b>	H	H	H	CH <sub>3</sub>	O	>280 <sup>b</sup>	C <sub>16</sub> H <sub>13</sub> NO <sub>4</sub>	C, H, N
<b>21</b>	H	CH <sub>3</sub>	CH <sub>3</sub>	H	O	275–276 <sup>b</sup>	C <sub>17</sub> H <sub>15</sub> NO <sub>4</sub>	C, H, N
<b>22</b>	H	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	O	204–205 <sup>b</sup>	C <sub>18</sub> H <sub>17</sub> NO <sub>4</sub>	C, H, N
<b>23</b>	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	H	S	229–230 <sup>c</sup>	C <sub>18</sub> H <sub>17</sub> NO <sub>3</sub> S	C, H, N
<b>24</b>	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	S	176–177 <sup>d</sup>	C <sub>19</sub> H <sub>19</sub> NO <sub>3</sub> S	C, H, N
<b>25</b>	Ac	Ac	Ac	CH <sub>3</sub>	O	206–207 <sup>b</sup>	C <sub>22</sub> H <sub>19</sub> NO <sub>7</sub>	C, H, N
<b>26</b>	Ac	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	O	148–149 <sup>d</sup>	C <sub>20</sub> H <sub>19</sub> NO <sub>5</sub>	C, H, N
<b>28</b>	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	NNHPh	148–149 <sup>e</sup>	C <sub>25</sub> H <sub>25</sub> N <sub>3</sub> O <sub>3</sub>	C, H, N
<b>29</b>	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	NNH-2-Pyr	140–141 <sup>e</sup>	C <sub>24</sub> H <sub>24</sub> N <sub>4</sub> O <sub>3</sub>	C, H, N

<sup>a</sup> Literature<sup>21</sup> >280 °C. <sup>b</sup> Recrystallization solvent: EtOAc. <sup>c</sup> Et<sub>2</sub>O. <sup>d</sup> EtOAc/PE. <sup>e</sup> MeOH.



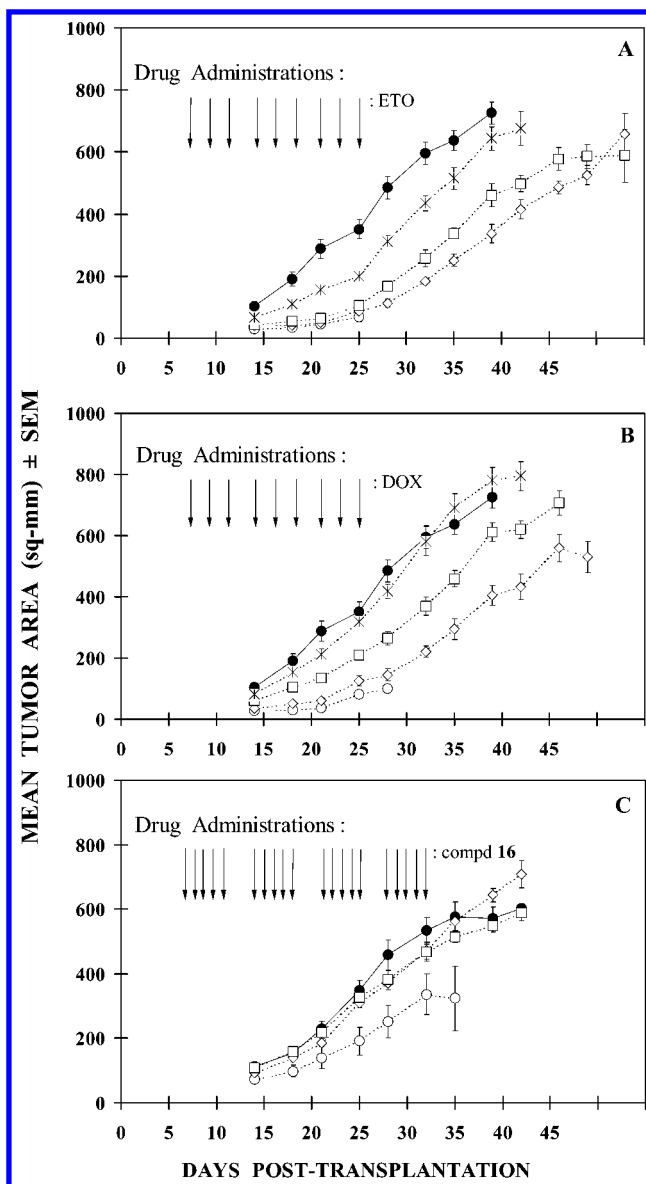
**Figure 1.** Characterization of the antimigratory effects induced by compounds **13** (A) and **16** (B) on the migration level of the human MCF-7 breast cancer cells. The migration level was quantitatively determined by means of computer-assisted phase-contrast videomicroscopy on several hundreds of cells per experimental condition. The data are reported as mean  $\pm$  SEM.

be both kinase receptor inhibitors<sup>29</sup> and angiogenesis inhibitors.<sup>30</sup> These data already published in the literature can have some relevance with our data and can therefore suggest potential 3-aryl-2-quinolone derivative-mediated effects on distinct types of kinase receptors that in turn will significantly decrease the motility levels of human breast cancer cells.

Compound **16** was then combined with either DOX or ETO (which are two clinically active anticancer drugs) in order to investigate in vivo the MXT mouse mammary adenocarcinoma,<sup>31,32</sup> which closely mimics

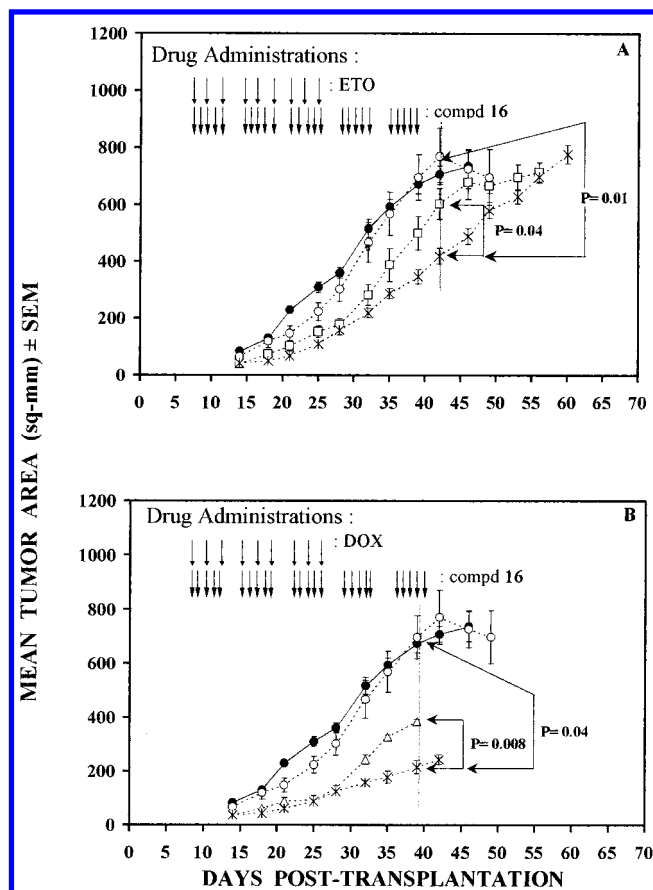
human breast cancer,<sup>33,34</sup> and whether any benefit could be obtained from additive treatment with such combinations. We chose the MXT mouse mammary adenocarcinoma model originating from ductal structures<sup>33,34</sup> for the following reasons. The first concerns the fact that up to 1986 leukemia models were the NCI's preferred models for testing new investigational agents in vivo, but these models were then abandoned because they are too chemosensitive and consequently fail to reflect clinical reality.<sup>35</sup> We thus preferred to make use of a solid tumor. Second, the MXT mouse mammary cancer was selected because more than 80% of female breast cancers are invasive intraduct carcinomas, i.e., not otherwise specified breast cancers,<sup>33</sup> while most of the experimental murine mammary tumors originate from the glandular acini.<sup>33</sup> The biological characteristics of mammary tumors from ductal as opposed to acinar structures differ markedly.<sup>36</sup> Figure 2 illustrates the effects obtained with ETO (A), DOX (B), and compound **16** (C). The data indicate that ETO significantly ( $P < 0.05$  to  $P < 0.001$  as compared to control at the different days postgraft) decreased the MXT tumor growth when tested at 20 (MTD/4) or 10 (MTD/8) mg/kg (Figure 2A). At 40 mg/kg (MTD/2), ETO showed itself to be highly toxic, while at 5 mg/kg (MTD/16), it became ineffective (Figure 2A). We chose the 10 mg/kg ETO dose for a combined treatment with compound **16** (Figure 3A). The same experimental approach was used with respect to DOX, which was tested at MTD/2 (5 mg/kg), MTD/4 (2.5 mg/kg), MTD/8 (1.25 mg/kg), and MTD/16 (0.63 mg/kg). The MTD/2 treatment appeared highly toxic, while the MTD/16 treatment was ineffective (Figure 2B). The MTD/4 treatment ( $P < 0.05$  to  $P < 0.01$ ) was more effective than the MTD/8 treatment ( $P < 0.05$  to  $P < 0.001$ ) and was chosen for the combined treatment with compound **16** (Figure 3B). This latter was assayed at MTD/2 (80 mg/kg), MTD/4 (40 mg/kg), and MTD/8 (20 mg/kg). Only 80 mg/kg, the highest dose tested, showed slight, but nevertheless significant ( $P < 0.05$ ), antitumor effects when compared to the control (Figure 2C). The 40 mg/kg dose (which exhibited no significant effects per se; Figure 2C) was the one chosen for combined treatment with either ETO (Figure 3A) or DOX (Figure 3B).

The data obtained revealed that real additive anticancer influences were observed for both compound **16**/



**Figure 2.** Characterization of the anticancer influence of ETO (A), DOX (B), and **16** (C) on the growth of the MXT mouse mammary adenocarcinoma. ETO was tested at 40 (MTD/2; ○), 20 (MTD/4; ◇), 10 (MTD/8; □), and 5 (MTD/16; ×) mg/kg. DOX was tested at 5 (MTD/2; ○), 2.5 (MTD/4; ◇), 1.25 (MTD/8; □), and 0.63 (MTD/16; ×) mg/kg. Compound **16** was tested at 80 (MTD/2; ○), 40 (MTD/4; ◇), and 20 (MTD/8; □). The control conditions are symbolized by black dots. ETO and DOX were administered i.p. (0.2 mL) nine times according to the experimental schedule depicted by the black arrows in the upper left-hand part of panels A and B. Derivative **16** was administered 20 times (see the black arrows in the upper left-hand part of panel C). There were nine mice per experimental group. The *P* levels of statistical significance were determined by means of the nonparametric Mann–Whitney test. The results are depicted as mean values (the symbols) ± their SEM (the bars).

ETO (Figure 3A) and compound **16**/DOX (Figure 3B) combinations, as was evidenced by the increases in the *P* levels of statistical significance depicted in Figure 3A,B. Indeed, a slight, but nevertheless significant, effect ( $P = 0.04$ ) was obtained when using 10 mg/kg of ETO with 40 mg/kg of compound **16**, with an actual increase observed in the survival periods of the MXT mammary cancer-bearing mice, which increased by 38% ( $P = 0.01$ ) as compared to the control (Figure 3A). The



**Figure 3.** Characterization of the anticancer influence exercised by ETO combined with **16** (× in A) and by DOX combined with **16** (× in B) on the growth of the MXT mouse mammary adenocarcinoma. ETO was assayed at 10 mg/kg, i.e., at MTD/8 (□ in A), DOX at MTD/4 (2.5 mg/kg; △ in B), and **16** at MTD/4 (40 mg/kg; ○ in A and B). The control conditions are symbolized by black dots. There were nine mice per experimental group. The *P* levels of statistical significance were determined by means of the nonparametric Mann–Whitney test. The results are depicted as mean values (the symbols) ± their SEM (the bars). The *P* values in A and B were calculated at the end of the experiments (see the vertical dotted line) when at least four mice survived in each experimental group.

combination of compound **16** (40 mg/kg) and DOX (2.5 mg/kg) also significantly ( $P = 0.008$ ) increased the antitumor effects observed with each compound tested individually (Figure 3B). The *P* values in Figure 3A,B were calculated at the end of the experiments (see the vertical dotted line) when at least four mice survived in each experimental group. These additive effects observed in terms of antitumor activities between compound **16** and DOX or ETO can result from a combined antimigratory effect brought by compound **16** on the tumor cells and an antiproliferative effect brought by ETO or DOX on the remaining tumor cells.

In conclusion, the data from the present study clearly indicate that an improvement in treatment was obtained in the case of the MXT mouse mammary tumor with a combination of the nontoxic antimigratory compound **16** (taken up as a representative of the group of study drugs) and the conventional cytotoxic drugs used routinely in hospitals, i.e., either ETO or DOX, which are two topoisomerase II inhibitors. The mechanism of action of compound **16** and congeners still remains to be elucidated. Further combinations with various other

anticancer drugs are currently being investigated against several other cancer cell lines.

## Experimental Section

**Chemistry.** Melting points were determined using a Büchi capillary instrument and are uncorrected. IR spectra were recorded on a Perkin-Elmer FTIR Paragon 1000 spectrophotometer. NMR spectra were recorded at 300 K on a Bruker Avance DPX 250 spectrometer. Chemical shifts are expressed in parts per million relative to tetramethylsilane (TMS), and spin multiplicities are given as s (singlet), br s (broad singlet), d (doublet), dd (double doublet), t (triplet), and m (multiplet). Mass spectra were recorded on a Perkin-Elmer SCIEX API 300 instrument using ionspray methodology. Elemental compositions of the compounds agreed to within 0.4% of the calculated value. Thin-layer chromatography (TLC) was run on precoated silica gel plates (Merck 60F<sub>254</sub>), and spots were visualized with a UV light at 254 nm. Column chromatography was carried out using Merck silica gel (230–400 mesh). Most chemicals and solvents were analytical grade and used without further purification. Ethyl 2-(phenyl or 4-methoxyphenyl)-2-formyl acetates were prepared according to ref 19. Commercial reagents were purchased from Acros Company.

**General Procedure for Preparation of Compound 1. N-(2-Methoxyphenyl)-2-phenylacetamide (1a).**<sup>18</sup> To a stirred solution of *o*-anisidine (1.37 mL, 10.0 mmol) in toluene (14 mL) at 0 °C was added dropwise a solution of phenylacetyl chloride (1.62 mL, 10.0 mmol) in toluene (5 mL). The reaction mixture was stirred at room temperature for 1 h and then treated with a saturated NaHCO<sub>3</sub> solution. The biphasic solution was vigorously stirred for 30 min, then decanted, and finally separated. The collected aqueous phase was extracted with EtOAc (2 × 10 mL). The combined organic layer was dried over MgSO<sub>4</sub> and evaporated. The crude oily residue was crystallized from toluene to yield **1a**. IR (KBr):  $\nu$  3287, 1652, 1598 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, CDCl<sub>3</sub>):  $\delta$  3.72 (s, 3H, CH<sub>3</sub>), 3.76 (s, 2H, CH<sub>2</sub>), 6.81 (dd, 1H, *J* = 1.8, 8.0 Hz, H<sub>Ar</sub>), 6.91–7.05 (m, 2H, H<sub>Ar</sub>), 7.21–7.37 (m, 5H, H<sub>Ar</sub>), 7.80 (br s, 1H, NH), 8.35 (dd, 1H, *J* = 1.8, 8.0 Hz, H<sub>Ar</sub>). <sup>13</sup>C NMR (62.90 MHz, CDCl<sub>3</sub>):  $\delta$  45.2, 55.7, 109.9, 119.5, 121.1, 123.7, 127.4, 127.6, 129.0 (2), 129.6 (2), 134.6, 147.8, 168.8. MS: *m/z* 242 (M<sup>+</sup> + 1).

**N-(4-Methoxyphenyl)-2-phenylacetamide (1b).**<sup>18</sup> Starting from *p*-anisidine and phenylacetyl chloride, compound **1b** was obtained according to the procedure described for **1a**. IR (KBr):  $\nu$  3290, 1650, 1603 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, CDCl<sub>3</sub>):  $\delta$  3.72 (s, 2H, CH<sub>2</sub>), 3.77 (s, 3H, CH<sub>3</sub>), 6.81 (d, 2H, *J* = 9.0 Hz, H<sub>Ar</sub>), 7.00 (br s, 1H, NH), 7.28–7.43 (m, 7H, H<sub>Ar</sub>). <sup>13</sup>C NMR (62.90 MHz, CDCl<sub>3</sub>):  $\delta$  44.8, 55.6, 114.2 (2), 121.9 (2), 127.7, 129.3 (2), 129.7 (2), 130.8, 134.7, 156.7, 169.1. MS: *m/z* 242 (M<sup>+</sup> + 1).

**N-(3,5-Dimethoxyphenyl)-2-phenylacetamide (1c).** Starting from 3,5-dimethoxyaniline and phenylacetyl chloride, compound **1c** was obtained according to the procedure described for **1a**. IR (KBr):  $\nu$  3286, 1657, 1616 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, CDCl<sub>3</sub>):  $\delta$  3.70 (s, 2H, CH<sub>2</sub>), 3.74 (s, 6H, CH<sub>3</sub>), 6.21 (t, 1H, *J* = 2.2 Hz, H<sub>Ar</sub>), 6.66 (d, 2H, *J* = 2.2 Hz, H<sub>Ar</sub>), 7.09 (br s, 1H, NH), 7.30–7.40 (m, 5H, H<sub>Ar</sub>). <sup>13</sup>C NMR (62.90 MHz, CDCl<sub>3</sub>):  $\delta$  44.9, 55.4 (2), 96.8, 97.9 (2), 127.7, 129.2 (2), 129.5 (2), 134.3, 139.4, 161.0 (2), 169.1. MS: *m/z* 272 (M<sup>+</sup> + 1).

**N-(2-Methoxyphenyl)-2-(4-methoxyphenyl)acetamide (1d).** Starting from *o*-anisidine and 4-methoxyphenylacetyl chloride, compound **1d** was obtained according to the procedure described for **1a**. IR (KBr):  $\nu$  3375, 1667, 1597 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, CDCl<sub>3</sub>):  $\delta$  3.68 (s, 2H, CH<sub>2</sub>), 3.72 (s, 3H, CH<sub>3</sub>), 3.82 (s, 3H, CH<sub>3</sub>), 6.79 (dd, 1H, *J* = 1.5, 8.0 Hz, H<sub>Ar</sub>), 6.89–7.03 (m, 4H, H<sub>Ar</sub>), 7.25 (d, 2H, *J* = 8.8 Hz, H<sub>Ar</sub>), 7.79 (br s, 1H, NH), 8.33 (dd, 1H, *J* = 1.5, 8.0 Hz, H<sub>Ar</sub>). <sup>13</sup>C NMR (62.90 MHz, CDCl<sub>3</sub>):  $\delta$  44.2, 55.3, 55.7, 109.9, 114.4 (2), 119.5, 121.0, 123.6, 126.6, 127.6, 130.7 (2), 147.8, 158.9, 169.3. MS: *m/z* 272 (M<sup>+</sup> + 1).

**N-(3,5-Methoxyphenyl)-2-(4-methoxyphenyl)acetamide (1e).**<sup>21</sup> Starting from 3,5-dimethoxyaniline and 4-methoxyphenylacetyl chloride, compound **1e** was obtained according

to the procedure described for **1a**. IR (KBr):  $\nu$  3292, 1658, 1615 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, CDCl<sub>3</sub>):  $\delta$  3.66 (s, 2H, CH<sub>2</sub>), 3.74 (s, 6H, CH<sub>3</sub>), 3.82 (s, 3H, CH<sub>3</sub>), 6.20 (t, 1H, *J* = 2.2 Hz, H<sub>Ar</sub>), 6.62–6.66 (m, 2H, H<sub>Ar</sub>), 6.95 (d, 2H, *J* = 7.5 Hz, H<sub>Ar</sub>), 6.97 (br s, 1H, NH), 7.23 (d, 2H, *J* = 7.5 Hz, H<sub>Ar</sub>). <sup>13</sup>C NMR (62.90 MHz, CDCl<sub>3</sub>):  $\delta$  44.0, 55.3, 55.4 (2), 96.7, 97.9 (2), 114.4 (2), 126.2, 130.7 (2), 139.4, 159.0, 161.0 (2), 169.5. MS: *m/z* 302 (M<sup>+</sup> + 1).

**N-(2,5-Dimethoxyphenyl)-2-(4-methoxyphenyl)acetamide (1f).** Starting from 2,5-dimethoxyaniline and 4-methoxyphenylacetyl chloride, compound **1f** was obtained according to the procedure described for **1a**. IR (KBr):  $\nu$  3292, 1659, 1614 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, CDCl<sub>3</sub>):  $\delta$  3.67 (s, 3H, CH<sub>3</sub>), 3.68 (s, 2H, CH<sub>2</sub>), 3.75 (s, 3H, CH<sub>3</sub>), 3.81 (s, 3H, CH<sub>3</sub>), 6.53 (dd, 1H, *J* = 3.0, 9.0 Hz, H<sub>Ar</sub>), 6.71 (d, 1H, *J* = 9.0 Hz, H<sub>Ar</sub>), 6.92 (d, 2H, *J* = 8.8 Hz, H<sub>Ar</sub>), 7.25 (d, 2H, *J* = 8.8 Hz, H<sub>Ar</sub>), 7.82 (br s, 1H, NH), 8.80 (d, 1H, *J* = 3.0 Hz, H<sub>Ar</sub>). <sup>13</sup>C NMR (62.90 MHz, CDCl<sub>3</sub>):  $\delta$  44.2, 55.3, 55.7, 56.3, 105.5, 108.6, 110.9, 114.4 (2), 126.4, 128.3, 130.6 (2), 142.0, 153.9, 158.9, 169.3. MS: *m/z* 302 (M<sup>+</sup> + 1).

**N-(3,5-Dimethoxyphenyl)-2-(4-benzyloxyphenyl)acetamide (1g).** Starting from 3,5-dimethoxyaniline and 4-benzyloxyphenylacetyl chloride, compound **1g** was obtained following a procedure as described for **1a**. The crude residue was purified by column chromatography (eluent ethyl acetate/petroleum ether 6:4) to afford **1g**. IR (KBr):  $\nu$  3291, 1659, 1610 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, CDCl<sub>3</sub>):  $\delta$  3.66 (s, 2H, CH<sub>2</sub>), 3.74 (s, 3H, CH<sub>3</sub>), 3.75 (s, 3H, CH<sub>3</sub>), 5.08 (s, 2H, CH<sub>2</sub>), 6.21 (t, 1H, *J* = 2.2 Hz, H<sub>Ar</sub>), 6.66 (d, 2H, *J* = 2.2 Hz, H<sub>Ar</sub>), 7.00 (d, 2H, *J* = 8.8 Hz, H<sub>Ar</sub>), 7.08 (br s, 1H, NH), 7.24 (d, 2H, *J* = 8.8 Hz, H<sub>Ar</sub>), 7.33–7.46 (m, 5H, H<sub>Ar</sub>). <sup>13</sup>C NMR (62.90 MHz, CDCl<sub>3</sub>):  $\delta$  44.2, 55.5 (2), 70.2, 96.9, 98.0 (2), 115.7 (2), 126.6, 127.6 (2), 128.2, 128.8 (2), 130.8 (2), 136.9, 139.6, 158.4, 161.1 (2), 169.7. MS: *m/z* 378 (M<sup>+</sup> + 1).

**General Procedure for Preparation of Compound 2. 8-Methoxy-3-phenyl-1,2-dihydro-2-quinolinone (2a).** To a solution of POCl<sub>3</sub> (1.75 mL, 19 mmol) at –30 °C was dropwise added anhydrous DMF (0.31 mL, 4.0 mmol). The solution was stirred for 15 min at –30 °C, and then, compound **1a** (2.7 mmol) was dropwise added. Under vigorous stirring, the reaction mixture was allowed to reach room temperature and was then heated at 75 °C for 1.5 h. The solution was poured into ice, neutralized with 30% aqueous NH<sub>4</sub>OH, and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The organic layer was dried over MgSO<sub>4</sub> and then evaporated in vacuo. The crude residue obtained was dissolved in glacial acetic acid (4.75 mL) and H<sub>2</sub>O (0.15 mL). The final solution was stirred at reflux for 3 h. After it was cooled, the solvent was evaporated in vacuo. The crude residue was diluted in H<sub>2</sub>O, neutralized with 25% NaOH, and finally extracted with CH<sub>2</sub>Cl<sub>2</sub>. The organic layer was dried over MgSO<sub>4</sub> and then evaporated in vacuo. The crude residue was crystallized from EtOAc. Crystals were filtered and washed twice with EtOAc to give **2a**. IR (KBr):  $\nu$  1646, 1607 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  3.92 (s, 3H, CH<sub>3</sub>), 7.13–7.16 (m, 2H, H<sub>Ar</sub>), 7.30–7.46 (m, 4H, H<sub>Ar</sub>), 7.74–7.77 (m, 2H, H<sub>Ar</sub>), 8.09 (s, 1H, =CH), 10.98 (br s, 1H, NH). <sup>13</sup>C NMR (62.90 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  56.5, 111.3, 120.3, 120.4, 128.3 (2), 128.4, 128.7, 129.2 (2), 132.6, 136.7, 138.2, 145.9, 161.1. MS: *m/z* 252 (M<sup>+</sup> + 1).

**6-Methoxy-3-phenyl-1,2-dihydro-2-quinolinone (2b).** According to the procedure described for **2a**, the 2-quinolone **2b** was prepared from **1b**. IR (KBr):  $\nu$  1645, 1618 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  3.80 (s, 3H, CH<sub>3</sub>), 7.16 (dd, 1H, *J* = 2.5, 8.9 Hz, H<sub>Ar</sub>), 7.28 (d, 1H, *J* = 8.9 Hz, H<sub>Ar</sub>), 7.29 (d, 1H, *J* = 2.5 Hz, H<sub>Ar</sub>), 7.34–7.47 (m, 3H, H<sub>Ar</sub>), 7.76 (d, 2H, *J* = 6.8 Hz, H<sub>Ar</sub>), 8.06 (s, 1H, =CH), 11.85 (br s, 1H, NH). <sup>13</sup>C NMR (62.90 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  55.4, 109.4, 116.0, 119.5, 120.1, 127.8, 127.9 (2), 128.7 (2), 131.9, 132.9, 136.4, 137.2, 154.2, 160.6. MS: *m/z* 252 (M<sup>+</sup> + 1).

**5,7-Dimethoxy-3-phenyl-1,2-dihydro-2-quinolinone (2c).**<sup>17</sup> **Method A.** According to the procedure described for **2a**, the 2-quinolone **2c** was prepared from **1c**. **Method B.** To a solution of 2-phenyl-2-formyl acetate (7.37 g, 38.4 mmol) and 3,5-dimethoxyaniline (5.0 g, 32 mmol) stirred at room temperature



for 30 min was added a freshly prepared solution of PPSE (15.0 g of phosphorus pentoxide and 36 mL of hexamethyldisiloxane in 1,2-dichloroethane stirred at 90 °C until complete dissolution of  $P_2O_5$  and then evaporation of solvent). The reaction mixture was stirred at 100 °C for 1 h. After the mixture was cooled,  $CH_2Cl_2$  (1000 mL) and  $H_2O$  (500 mL) were added to the solution. The biphasic solution was vigorously stirred overnight, then decanted, and finally separated. The collected aqueous phase was extracted with  $CH_2Cl_2$  ( $2 \times 100$  mL). The combined organic layer was dried over  $MgSO_4$  and evaporated. The crude oily residue was crystallized from EtOAc to give **2c**. IR (KBr):  $\nu$  1668, 1631  $cm^{-1}$ .  $^1H$  NMR (250 MHz,  $DMSO-d_6$ ):  $\delta$  3.81 (s, 3H,  $OCH_3$ ), 3.89 (s, 3H,  $OCH_3$ ), 6.36 (d, 1H,  $J = 1.8$  Hz,  $H_{Ar}$ ), 6.45 (d, 1H,  $J = 1.8$  Hz,  $H_{Ar}$ ), 7.28–7.42 (m, 3H,  $H_{Ar}$ ), 7.69 (d, 2H,  $J = 7.0$  Hz,  $H_{Ar}$ ), 8.00 (s, 1H, =CH), 11.81 (br s, 1H, NH).  $^{13}C$  NMR (62.90 MHz,  $DMSO-d_6$ ):  $\delta$  55.5, 56.0, 90.0, 93.1, 104.5, 126.9, 127.3, 127.9 (2), 128.4 (2), 131.4, 136.7, 140.8, 156.8, 161.4, 162.2. MS:  $m/z$  282 ( $M^+ + 1$ ).

**8-Methoxy-3-(4-methoxyphenyl)-1,2-dihydro-2-quinolinone (2d)**. According to the procedure described for **2a**, the 2-quinolone **2d** was prepared from **1d**. IR (KBr):  $\nu$  1652, 1625  $cm^{-1}$ .  $^1H$  NMR (250 MHz,  $DMSO-d_6$ ):  $\delta$  3.79 (s, 3H,  $CH_3$ ), 3.90 (s, 3H,  $CH_3$ ), 6.99 (d, 2H,  $J = 8.8$  Hz,  $H_{Ar}$ ), 7.09–7.15 (m, 2H,  $H_{Ar}$ ), 7.29 (dd, 1H,  $J = 3.2$ , 6.0 Hz,  $H_{Ar}$ ), 7.74 (d, 2H,  $J = 8.8$  Hz,  $H_{Ar}$ ), 8.03 (s, 1H, =CH), 10.90 (br s, 1H, NH).  $^{13}C$  NMR (62.90 MHz,  $DMSO-d_6$ ):  $\delta$  55.2, 56.0, 110.5, 113.4 (2), 119.6, 120.0, 121.8, 127.9, 128.5, 129.9 (2), 131.6, 136.4, 145.3, 159.1, 160.7. MS:  $m/z$  282 ( $M^+ + 1$ ).

**5,7-Dimethoxy-3-(4-methoxyphenyl)-1,2-dihydro-2-quinolinone (2e)**.<sup>21</sup> According to the procedures described for **2c**, the 2-quinolone **2e** was prepared from **1e** (method A) or 2-(4-methoxyphenyl)-2-formyl acetate and 3,5-dimethoxyaniline (method B). IR (KBr):  $\nu$  1664, 1628  $cm^{-1}$ .  $^1H$  NMR (250 MHz,  $DMSO-d_6$ ):  $\delta$  3.78 (s, 3H,  $CH_3$ ), 3.81 (s, 3H,  $CH_3$ ), 3.89 (s, 3H,  $CH_3$ ), 6.35 (d, 1H,  $J = 2.0$  Hz,  $H_{Ar}$ ), 6.45 (d, 1H,  $J = 2.0$  Hz,  $H_{Ar}$ ), 6.95 (d, 2H,  $J = 7.5$  Hz,  $H_{Ar}$ ), 7.66 (d, 2H,  $J = 7.5$  Hz,  $H_{Ar}$ ), 7.96 (s, 1H, =CH), 11.76 (br s, 1H, NH).  $^{13}C$  NMR (62.90 MHz,  $DMSO-d_6$ ):  $\delta$  55.1, 55.4, 55.9, 90.0, 93.0, 104.6, 113.3 (2), 126.5, 129.0, 129.6 (2), 130.2, 140.5, 156.6, 158.7, 161.5, 161.9. MS:  $m/z$  312 ( $M^+ + 1$ ).

**5,8-Dimethoxy-3-(4-methoxyphenyl)-1,2-dihydro-2-quinolinone (2f)**. According to the procedure described for **2a**, the 2-quinolone **2f** was prepared from **1f**. IR (KBr):  $\nu$  1639, 1571  $cm^{-1}$ .  $^1H$  NMR (250 MHz,  $CDCl_3$ ):  $\delta$  3.85 (s, 3H,  $CH_3$ ), 3.90 (s, 3H,  $CH_3$ ), 3.92 (s, 3H,  $CH_3$ ), 6.49 (d, 1H,  $J = 8.7$  Hz,  $H_{Ar}$ ), 6.84 (d, 1H,  $J = 8.7$  Hz,  $H_{Ar}$ ), 6.97 (d, 2H,  $J = 8.8$  Hz,  $H_{Ar}$ ), 7.74 (d, 2H,  $J = 8.8$  Hz,  $H_{Ar}$ ), 8.19 (s, 1H, =CH), 9.25 (br s, 1H, NH).  $^{13}C$  NMR (62.90 MHz,  $CDCl_3$ ):  $\delta$  55.2, 55.8, 56.2, 101.1, 109.7, 111.4, 113.6 (2), 128.7, 128.8, 130.0 (2), 131.4, 131.7, 139.4, 149.8, 159.5, 161.3. MS:  $m/z$  312 ( $M^+ + 1$ ).

**5,7-Dimethoxy-3-(4-benzoyloxyphenyl)-1,2-dihydro-2-quinolinone (2g)**. According to the procedure described for **2a**, the 2-quinolone **2g** was prepared from **1g**. IR (KBr):  $\nu$  1629, 1608  $cm^{-1}$ .  $^1H$  NMR (250 MHz,  $DMSO-d_6$ ):  $\delta$  3.81 (s, 3H,  $CH_3$ ), 3.90 (s, 3H,  $CH_3$ ), 5.15 (s, 2H,  $CH_2$ ), 6.37 (s, 1H,  $H_{Ar}$ ), 6.45 (s, 1H,  $H_{Ar}$ ), 7.04 (d, 2H,  $J = 8.8$  Hz,  $H_{Ar}$ ), 7.31–7.49 (m, 5H,  $H_{Ar}$ ), 7.69 (d, 2H,  $J = 7.5$  Hz,  $H_{Ar}$ ), 7.97 (s, 1H, =CH), 11.76 (br s, 1H, NH).  $^{13}C$  NMR (62.90 MHz,  $DMSO-d_6$ ):  $\delta$  55.4, 55.9, 69.2, 90.0, 93.0, 104.6, 114.3 (2), 126.4, 127.6 (2), 127.8, 128.4 (2), 129.2, 129.6 (2), 130.2, 137.1, 140.5, 156.6, 157.7, 161.5, 161.9. MS:  $m/z$  388 ( $M^+ + 1$ ).

**5,7-Dimethoxy-3-(4-methoxyphenyl)-1-methyl-1,2-dihydro-2-quinolinone (3)**. At 0 °C under argon, sodium hydride (93 mg, 3.86 mmol, 60% oil dispersion) was added portionwise to a solution of **2e** (600 mg, 1.93 mmol) in anhydrous DMF (30 mL). The mixture was stirred for 15 min at 0 °C, and then, iodomethane (0.48 mL, 7.72 mmol) diluted in anhydrous DMF (5 mL) was added. The final solution was stirred at 90 °C and monitored by TLC (reaction time 18 h). The cooled mixture was partitioned between  $H_2O$  (10 mL) and  $CH_2Cl_2$  (10 mL). The organic layers were dried over  $MgSO_4$  and concentrated in vacuo. The crude residue was purified by column chromatography (eluent  $CH_2Cl_2$ /EtOAc 8:2) to give successively **3** (408 mg, 68%) and **3a** (157 mg, 25%). IR (KBr):

$\nu$  1635, 1596  $cm^{-1}$ .  $^1H$  NMR (250 MHz,  $CDCl_3$ ):  $\delta$  3.73 (s, 3H,  $CH_3$ ), 3.83 (s, 3H,  $OCH_3$ ), 3.91 (s, 6H,  $OCH_3$ ), 6.30 (d, 1H,  $J = 2.0$  Hz,  $H_{Ar}$ ), 6.37 (d, 1H,  $J = 2.0$  Hz,  $H_{Ar}$ ), 6.94 (d, 2H,  $J = 7.5$  Hz,  $H_{Ar}$ ), 7.67 (d, 2H,  $J = 7.5$  Hz,  $H_{Ar}$ ), 8.12 (s, 1H, =CH).  $^{13}C$  NMR (62.90 MHz,  $CDCl_3$ ):  $\delta$  30.3, 55.3, 55.5, 55.8, 90.2, 92.6, 106.1, 113.5 (2), 127.0, 130.0, 130.1 (2), 130.2, 141.7, 157.6, 159.1, 162.2 (2). MS:  $m/z$  326 ( $M^+ + 1$ ).

**2,5,7-Trimethoxy-3-(4-methoxyphenyl)quinoline (3a)**. mp 106–107 °C (EtOAc/PE). IR (KBr):  $\nu$  1621, 1515  $cm^{-1}$ .  $^1H$  NMR (250 MHz,  $CDCl_3$ ):  $\delta$  3.86 (s, 3H,  $OCH_3$ ), 3.94 (s, 6H,  $OCH_3$ ), 4.08 (s, 3H,  $OCH_3$ ), 6.40 (d, 1H,  $J = 1.8$  Hz,  $H_{Ar}$ ), 6.85 (d, 1H,  $J = 1.8$  Hz,  $H_{Ar}$ ), 6.97 (d, 2H,  $J = 8.8$  Hz,  $H_{Ar}$ ), 7.57 (d, 2H,  $J = 8.8$  Hz,  $H_{Ar}$ ), 8.26 (s, 1H, =CH).  $^{13}C$  NMR (62.90 MHz,  $CDCl_3$ ):  $\delta$  53.5, 55.3, 55.5, 55.6, 95.9, 98.5, 112.8, 113.6 (2), 122.1, 129.6, 130.5 (2), 132.3, 147.9, 156.3, 158.9, 160.6, 161.3. MS:  $m/z$  326 ( $M^+ + 1$ ). Anal. ( $C_{19}H_{19}NO_4$ ) C, H, N.

**Ethyl 2-[5,7-Dimethoxy-3-(4-methoxyphenyl)-2-oxo-1,2-dihydro-1-quinolinyl]acetate (4)**. According to the procedure described for **3a**, the 2-quinolone **4** and compound **4a** were prepared starting from **2e** (1 equiv) and ethyl bromoacetate (2 equiv). Reaction time, 3 h; eluent chromatography  $CH_2Cl_2$ /EtOAc 7:3; yield, 70%. IR (KBr): 1735, 1647, 1609  $cm^{-1}$ .  $^1H$  NMR (250 MHz,  $CDCl_3$ ):  $\delta$  1.62 (t, 3H,  $J = 7.1$  Hz,  $CH_3$ ), 3.83 (s, 3H,  $OCH_3$ ), 3.87 (s, 3H,  $OCH_3$ ), 3.92 (s, 3H,  $OCH_3$ ), 4.22 (q, 2H,  $J = 7.1$  Hz,  $CH_2$ ), 5.10 (s, 2H,  $CH_2$ ), 6.14 (d, 1H,  $J = 2.0$  Hz,  $H_{Ar}$ ), 6.30 (d, 1H,  $J = 2.0$  Hz,  $H_{Ar}$ ), 6.94 (d, 2H,  $J = 7.5$  Hz,  $H_{Ar}$ ), 7.69 (d, 2H,  $J = 7.5$  Hz,  $H_{Ar}$ ), 8.18 (s, 1H, =CH).  $^{13}C$  NMR (62.90 MHz,  $CDCl_3$ ):  $\delta$  14.2, 44.8, 55.3, 55.5, 55.9, 61.6, 90.0, 92.8, 106.2, 113.5 (2), 126.6, 129.6, 130.1 (2), 131.0, 141.1, 157.8, 159.2, 161.9, 162.5, 168.4. MS:  $m/z$  398 ( $M^+ + 1$ ).

**Ethyl 2-[(5,7-Dimethoxy-3-(4-methoxyphenyl)-2-quinolinyl)oxy]acetate (4a)**. Yield, 25%; mp 95–96 °C (EtOAc/PE). IR (KBr):  $\nu$  1754, 1622, 1516  $cm^{-1}$ .  $^1H$  NMR (250 MHz,  $CDCl_3$ ):  $\delta$  1.27 (t, 3H,  $J = 7.1$  Hz,  $CH_3$ ), 3.86 (s, 3H,  $OCH_3$ ), 3.91 (s, 3H,  $OCH_3$ ), 3.93 (s, 3H,  $OCH_3$ ), 4.24 (q, 2H,  $J = 7.1$  Hz,  $CH_2$ ), 5.05 (s, 2H,  $CH_2$ ), 6.40 (d, 1H,  $J = 2.0$  Hz,  $H_{Ar}$ ), 6.76 (d, 1H,  $J = 2.0$  Hz,  $H_{Ar}$ ), 6.98 (d, 2H,  $J = 9.0$  Hz,  $H_{Ar}$ ), 7.68 (d, 2H,  $J = 9.0$  Hz,  $H_{Ar}$ ), 8.30 (s, 1H, =CH).  $^{13}C$  NMR (62.90 MHz,  $CDCl_3$ ):  $\delta$  14.2, 55.3, 55.5, 55.7, 60.9, 62.7, 96.2, 98.6, 113.4, 113.7 (2), 121.7, 129.2, 130.6 (2), 132.8, 147.3, 156.3, 158.8, 159.1, 161.4, 169.5. MS:  $m/z$  398 ( $M^+ + 1$ ). Anal. ( $C_{22}H_{23}NO_6$ ) C, H, N.

***N,N*-Diethyl-2-[5,7-dimethoxy-3-(4-methoxyphenyl)-2-oxo-1,2-dihydro-1-quinolinyl]acetamide (5)**. According to the procedure described for **3**, the 2-quinolone **5** and compound **5a** were prepared starting from **2e** (1 equiv) and 2-chloro-*N,N*-diethylacetamide (1.5 equiv). Reaction time, 3 h; eluent chromatography  $CH_2Cl_2$ /EtOAc 7:3; yield, 61%. IR (KBr): 1642, 1617, 1601  $cm^{-1}$ .  $^1H$  NMR (250 MHz,  $CDCl_3$ ):  $\delta$  1.10–1.23 (m, 6H,  $CH_3$ ), 3.38–3.49 (m, 4H,  $CH_2$ ), 3.83 (s, 3H,  $OCH_3$ ), 3.86 (s, 3H,  $OCH_3$ ), 3.91 (s, 3H,  $OCH_3$ ), 5.17 (s, 2H,  $CH_2$ ), 6.28 (d, 1H,  $J = 2.0$  Hz,  $H_{Ar}$ ), 6.34 (d, 1H,  $J = 2.0$  Hz,  $H_{Ar}$ ), 6.93 (d, 2H,  $J = 7.0$  Hz,  $H_{Ar}$ ), 7.66 (d, 2H,  $J = 7.0$  Hz,  $H_{Ar}$ ), 8.17 (s, 1H, =CH).  $^{13}C$  NMR (62.90 MHz,  $CDCl_3$ ):  $\delta$  13.0, 14.2, 40.9, 41.6, 45.3, 55.3, 55.5, 55.8, 90.8, 92.8, 106.3, 113.4 (2), 126.6, 129.9, 130.1 (2), 131.0, 141.7, 157.6, 159.0, 161.9, 162.3, 166.3. MS:  $m/z$  425 ( $M^+ + 1$ ).

***N,N*-Diethyl-2-[(5,7-dimethoxy-3-(4-methoxyphenyl)-2-quinolinyl)oxy]acetamide (5a)**. Yield, 30%; mp 146–147 °C (EtOAc/PE). IR (KBr):  $\nu$  1654, 1624, 1517  $cm^{-1}$ .  $^1H$  NMR (250 MHz,  $CDCl_3$ ):  $\delta$  1.14 (t, 3H,  $J = 7.5$  Hz,  $CH_3$ ), 1.28 (t, 3H,  $J = 7.5$  Hz,  $CH_3$ ), 3.36–3.47 (m, 4H,  $CH_2$ ), 3.85 (s, 3H,  $OCH_3$ ), 3.91 (s, 3H,  $OCH_3$ ), 3.93 (s, 3H,  $OCH_3$ ), 5.17 (s, 2H,  $CH_2$ ), 6.38 (d, 1H,  $J = 2.0$  Hz,  $H_{Ar}$ ), 6.73 (d, 1H,  $J = 2.0$  Hz,  $H_{Ar}$ ), 6.97 (d, 2H,  $J = 9.0$  Hz,  $H_{Ar}$ ), 7.75 (d, 2H,  $J = 9.0$  Hz,  $H_{Ar}$ ), 8.28 (s, 1H, =CH).  $^{13}C$  NMR (62.90 MHz,  $CDCl_3$ ):  $\delta$  12.9, 14.2, 40.2, 55.2, 55.4, 55.6, 63.0, 95.8, 98.4, 113.3, 113.5 (2), 121.9, 129.2, 130.6 (2), 132.5, 147.3, 156.2, 158.9, 161.1, 167.3. MS:  $m/z$  425 ( $M^+ + 1$ ). Anal. ( $C_{24}H_{28}N_2O_5$ ) C, H, N.

**1-[2-(Dimethylamino)ethyl]-5,7-dimethoxy-3-(4-methoxyphenyl)-1,2-dihydro-2-quinolinone (6)**. According to the procedure described for **3**, the 2-quinolone **6** and compound **6a** were prepared starting from **2e** (1 equiv) and 2-(dimethylamino)ethyl chloride (3 equiv). Reaction time, 3 h; eluent



chromatography Et<sub>2</sub>O/EtOAc 3:7 and then CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1; yield, 67%. IR (KBr):  $\nu$  1645, 1617 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, CDCl<sub>3</sub>):  $\delta$  2.39 (s, 6H, CH<sub>3</sub>), 2.65 (t, 2H,  $J$  = 7.8 Hz, CH<sub>2</sub>), 3.82 (s, 3H, OCH<sub>3</sub>), 3.90 (s, 3H, OCH<sub>3</sub>), 3.91 (s, 3H, OCH<sub>3</sub>), 4.44 (t, 2H,  $J$  = 7.8 Hz, CH<sub>2</sub>), 6.28 (d, 1H,  $J$  = 2.0 Hz, H<sub>Ar</sub>), 6.49 (d, 1H,  $J$  = 2.0 Hz, H<sub>Ar</sub>), 6.94 (d, 2H,  $J$  = 8.8 Hz, H<sub>Ar</sub>), 7.67 (d, 2H,  $J$  = 8.8 Hz, H<sub>Ar</sub>), 8.12 (s, 1H, =CH). <sup>13</sup>C NMR (62.90 MHz, CDCl<sub>3</sub>):  $\delta$  41.6, 45.8 (2), 55.3, 55.6, 55.8 (2), 90.1, 92.7, 106.4, 113.5 (2), 126.9, 129.8, 130.1 (2), 130.4, 141.1, 157.7, 159.1, 161.9, 162.4. MS:  $m/z$  383 (M<sup>+</sup> + 1).

***N,N*-Dimethyl-2-[5,7-dimethoxy-3-(4-methoxyphenyl)-2-quinolyl]oxy-1-ethanamine (6a).** Yield, 30%; mp 49–50 °C (Et<sub>2</sub>O). IR (KBr):  $\nu$  1621, 1584 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, CDCl<sub>3</sub>):  $\delta$  2.31 (s, 6H, NCH<sub>3</sub>), 2.77 (t, 2H,  $J$  = 6.0 Hz, CH<sub>2</sub>), 3.85 (s, 3H, OCH<sub>3</sub>), 3.93 (s, 6H, OCH<sub>3</sub>), 4.62 (t, 2H,  $J$  = 6.0 Hz, CH<sub>2</sub>), 6.39 (d, 1H,  $J$  = 2.0 Hz, H<sub>Ar</sub>), 6.81 (d, 1H,  $J$  = 2.0 Hz, H<sub>Ar</sub>), 6.94 (d, 2H,  $J$  = 8.8 Hz, H<sub>Ar</sub>), 7.58 (d, 2H,  $J$  = 8.8 Hz, H<sub>Ar</sub>), 8.25 (s, 1H, =CH). <sup>13</sup>C NMR (62.90 MHz, CDCl<sub>3</sub>):  $\delta$  45.7 (2), 55.3, 55.5 (2), 57.8, 63.8, 95.9, 98.5, 112.9, 113.4 (2), 122.0, 129.5, 130.6 (2), 132.3, 147.8, 156.3, 158.9, 160.0, 161.3. MS:  $m/z$  383 (M<sup>+</sup> + 1). Anal. (C<sub>22</sub>H<sub>26</sub>N<sub>2</sub>O<sub>4</sub>) C, H, N.

**1-[3-(Dimethylamino)propyl]-5,7-dimethoxy-3-(4-methoxyphenyl)-1,2-dihydro-2-quinolinone (7).** According to the procedure described for **3**, the 2-quinolone **7** and compound **7a** were prepared starting from **2e** (1 equiv) and 3-(dimethylamino)propyl chloride (2.2 equiv). Reaction time, 3 h; eluent chromatography Et<sub>2</sub>O/MeOH 8:2 and then CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1; yield, 70%. IR (KBr):  $\nu$  1635, 1617, 1598 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, CDCl<sub>3</sub>):  $\delta$  1.91–2.04 (m, 2H, CH<sub>2</sub>), 2.28 (s, 6H, NCH<sub>3</sub>), 2.46 (t, 2H,  $J$  = 7.2 Hz, CH<sub>2</sub>), 3.83 (s, 3H, OCH<sub>3</sub>), 3.91 (s, 3H, OCH<sub>3</sub>), 3.93 (s, 3H, OCH<sub>3</sub>), 4.36 (t, 2H,  $J$  = 7.2 Hz, CH<sub>2</sub>), 6.29 (d, 1H,  $J$  = 2.0 Hz, H<sub>Ar</sub>), 6.54 (d, 1H,  $J$  = 2.0 Hz, H<sub>Ar</sub>), 6.94 (d, 2H,  $J$  = 8.8 Hz, H<sub>Ar</sub>), 7.68 (d, 2H,  $J$  = 8.8 Hz, H<sub>Ar</sub>), 8.13 (s, 1H, =CH). <sup>13</sup>C NMR (62.90 MHz, CDCl<sub>3</sub>):  $\delta$  25.5, 41.8, 45.6 (2), 55.4, 55.5, 55.8, 57.1, 90.3, 92.6, 106.4, 113.5 (2), 126.9, 130.0, 130.1 (2), 130.3, 141.1, 157.6, 159.1, 162.0, 162. MS:  $m/z$  397 (M<sup>+</sup> + 1).

***N,N*-Dimethyl-2-[5,7-dimethoxy-3-(4-methoxyphenyl)-2-quinolyl]oxy-1-propanamine (7a).** Yield, 25%; mp 54–55 °C (Et<sub>2</sub>O). IR (KBr):  $\nu$  1621, 1584 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, CDCl<sub>3</sub>):  $\delta$  1.96–2.07 (m, 2H, CH<sub>2</sub>), 2.26 (s, 6H, NCH<sub>3</sub>), 2.47 (t, 2H,  $J$  = 6.5 Hz, CH<sub>2</sub>), 3.84 (s, 3H, OCH<sub>3</sub>), 3.92 (s, 6H, OCH<sub>3</sub>), 4.53 (t, 2H,  $J$  = 6.5 Hz, CH<sub>2</sub>), 6.39 (d, 1H,  $J$  = 2.2 Hz, H<sub>Ar</sub>), 6.82 (d, 1H,  $J$  = 2.2 Hz, H<sub>Ar</sub>), 6.96 (d, 2H,  $J$  = 8.8 Hz, H<sub>Ar</sub>), 7.57 (d, 2H,  $J$  = 8.8 Hz, H<sub>Ar</sub>), 8.26 (s, 1H, =CH). <sup>13</sup>C NMR (62.90 MHz, CDCl<sub>3</sub>):  $\delta$  27.0, 45.4 (2), 55.2, 55.5 (2), 56.6, 64.2, 95.8, 98.5, 112.7, 113.4 (2), 121.9, 129.6, 130.5 (2), 132.1, 147.9, 156.2, 158.8, 160.2, 161.2. MS:  $m/z$  397 (M<sup>+</sup> + 1). Anal. (C<sub>23</sub>H<sub>28</sub>N<sub>2</sub>O<sub>4</sub>) C, H, N.

**[5,7-Dimethoxy-3-(4-methoxyphenyl)-2-oxo-1,2-dihydro-1-quinolinyl]acetonitrile (8).** According to the procedure described for **3**, the 2-quinolone **8** and compound **8a** were prepared starting from **2e** (1 equiv) and bromoacetonitrile (2 equiv). Reaction time, 3 h; eluent chromatography CH<sub>2</sub>Cl<sub>2</sub>/EtOAc 7:3; yield, 61%. IR (KBr):  $\nu$  2216, 1660, 1607 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, CDCl<sub>3</sub>):  $\delta$  3.84 (s, 3H, OCH<sub>3</sub>), 3.94 (s, 3H, OCH<sub>3</sub>), 3.95 (s, 3H, OCH<sub>3</sub>), 5.29 (s, 2H, CH<sub>2</sub>), 6.36 (s, 2H, H<sub>Ar</sub>), 6.95 (d, 2H,  $J$  = 8.8 Hz, H<sub>Ar</sub>), 7.65 (d, 2H,  $J$  = 8.8 Hz, H<sub>Ar</sub>), 8.17 (s, 1H, =CH). <sup>13</sup>C NMR (62.90 MHz, CDCl<sub>3</sub>):  $\delta$  30.4, 55.3, 55.8, 56.0, 90.0, 93.5, 106.2, 113.6 (2), 114.8, 126.3, 129.0, 130.0 (2), 131.7, 139.8, 158.1, 159.4, 161.1, 163.0. MS:  $m/z$  351 (M<sup>+</sup> + 1).

**2-[5,7-Dimethoxy-3-(4-methoxyphenyl)-2-quinolinyl]-oxy]acetonitrile (8a).** Yield, 30%; mp 149–150 °C (Et<sub>2</sub>O). IR (KBr):  $\nu$  1623, 1586 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, CDCl<sub>3</sub>):  $\delta$  3.87 (s, 3H, OCH<sub>3</sub>), 3.95 (s, 6H, OCH<sub>3</sub>), 5.17 (s, 2H, CH<sub>2</sub>), 6.45 (d, 1H,  $J$  = 2.0 Hz, H<sub>Ar</sub>), 6.87 (d, 1H,  $J$  = 2.0 Hz, H<sub>Ar</sub>), 7.99 (d, 2H,  $J$  = 9.0 Hz, H<sub>Ar</sub>), 7.54 (d, 2H,  $J$  = 9.0 Hz, H<sub>Ar</sub>), 8.34 (s, 1H, =CH). <sup>13</sup>C NMR (62.90 MHz, CDCl<sub>3</sub>):  $\delta$  50.1, 55.3, 55.6, 55.7, 96.9, 98.6, 113.8 (2), 113.9, 116.1, 121.3, 128.4, 130.5 (2), 133.5, 147.1, 156.3, 157.2, 129.3, 161.8. MS:  $m/z$  351 (M<sup>+</sup> + 1). Anal. (C<sub>20</sub>H<sub>18</sub>N<sub>2</sub>O<sub>4</sub>) C, H, N.

**3-[5,7-Dimethoxy-3-(4-methoxyphenyl)-2-oxo-1,2-dihydro-1-quinolinyl]butanenitrile (9).** According to the pro-

cedure described for **3**, the 2-quinolone **9** and compound **9a** were prepared starting from **2e** (1 equiv) and 4-chlorobutyronitrile (2 equiv). Reaction time, 3 h; eluent CH<sub>2</sub>Cl<sub>2</sub>/EtOAc 9:1; yield, 33%. IR (KBr):  $\nu$  2247, 1639, 1609, 1597 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, CDCl<sub>3</sub>):  $\delta$  2.10–2.21 (m, 2H, CH<sub>2</sub>), 2.52 (t, 2H,  $J$  = 7.2 Hz, CH<sub>2</sub>), 3.83 (s, 3H, OCH<sub>3</sub>), 3.92 (s, 3H, OCH<sub>3</sub>), 3.93 (s, 3H, OCH<sub>3</sub>), 4.43 (t, 2H,  $J$  = 7.2 Hz, CH<sub>2</sub>), 6.31 (d, 1H,  $J$  = 2.2 Hz, H<sub>Ar</sub>), 6.42 (d, 1H,  $J$  = 2.2 Hz, H<sub>Ar</sub>), 6.94 (d, 2H,  $J$  = 8.8 Hz, H<sub>Ar</sub>), 7.66 (d, 2H,  $J$  = 8.8 Hz, H<sub>Ar</sub>), 8.15 (s, 1H, =CH). <sup>13</sup>C NMR (62.90 MHz, CDCl<sub>3</sub>):  $\delta$  15.3, 23.6, 41.8, 55.4, 55.9, 56.0, 89.6, 93.1, 106.4, 113.6 (2), 119.5, 126.7, 129.6, 130.1 (2), 130.8, 140.7, 157.9, 159.3, 161.2, 162.8. MS:  $m/z$  379 (M<sup>+</sup> + 1).

**2-[5,7-Dimethoxy-3-(4-methoxyphenyl)-2-quinolinyl]-oxy]butanenitrile (9a).** Yield, 33%; mp 89–90 °C (EtOAc). IR (KBr):  $\nu$  2247, 1624, 1607 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, CDCl<sub>3</sub>):  $\delta$  2.12–2.22 (m, 2H, CH<sub>2</sub>), 2.50 (t, 2H,  $J$  = 7.5 Hz, CH<sub>2</sub>), 3.87 (s, 3H, OCH<sub>3</sub>), 3.94 (s, 6H, OCH<sub>3</sub>), 4.61 (t, 2H,  $J$  = 7.5 Hz, CH<sub>2</sub>), 6.40 (d, 1H,  $J$  = 2.2 Hz, H<sub>Ar</sub>), 6.81 (d, 1H,  $J$  = 2.2 Hz, H<sub>Ar</sub>), 6.97 (d, 2H,  $J$  = 8.8 Hz, H<sub>Ar</sub>), 7.53 (d, 2H,  $J$  = 8.8 Hz, H<sub>Ar</sub>), 8.27 (s, 1H, =CH). <sup>13</sup>C NMR (62.90 MHz, CDCl<sub>3</sub>):  $\delta$  14.6, 25.4, 55.4, 55.7, 55.8, 63.6, 96.2, 98.6, 113.1, 113.7 (2), 119.5, 122.0, 129.5, 130.5 (2), 132.8, 147.9, 156.4, 159.1, 159.7, 161.6. MS:  $m/z$  379 (M<sup>+</sup> + 1). Anal. (C<sub>22</sub>H<sub>22</sub>N<sub>2</sub>O<sub>4</sub>) C, H, N.

**Benzyl 2-[5,7-Dimethoxy-3-(4-methoxyphenyl)-2-oxo-1,2-dihydro-1-quinolinyl]acetate (10).** According to the procedure described for **3**, the 2-quinolone **10** and compound **10a** were prepared starting from **2e** (1 equiv) and benzyl bromoacetate (2 equiv). Reaction time, 2 h; eluent chromatography CH<sub>2</sub>Cl<sub>2</sub>; yield, 72%. IR (KBr): 1748, 1642, 1617 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, CDCl<sub>3</sub>):  $\delta$  3.68 (s, 3H, OCH<sub>3</sub>), 3.84 (s, 3H, OCH<sub>3</sub>), 3.92 (s, 3H, OCH<sub>3</sub>), 5.16 (s, 2H, CH<sub>2</sub>), 5.21 (s, 2H, CH<sub>2</sub>), 6.03 (d, 1H,  $J$  = 2.0 Hz, H<sub>Ar</sub>), 6.27 (d, 1H,  $J$  = 2.0 Hz, H<sub>Ar</sub>), 6.94 (d, 2H,  $J$  = 7.5 Hz, H<sub>Ar</sub>), 7.25–7.32 (m, 5H, H<sub>Ar</sub>), 7.68 (d, 2H,  $J$  = 7.5 Hz, H<sub>Ar</sub>), 8.17 (s, 1H, =CH). <sup>13</sup>C NMR (62.90 MHz, CDCl<sub>3</sub>):  $\delta$  44.7, 55.3, 55.4, 55.8, 67.1, 89.8, 93.0, 106.2, 113.5 (2), 126.5, 128.3 (2), 128.4, 128.5 (2), 129.5, 130.1 (2), 131.1, 135.3, 141.0, 157.8, 159.2, 161.8, 162.4, 168.3. MS:  $m/z$  460 (M<sup>+</sup> + 1).

**Benzyl 2-[5,7-Dimethoxy-3-(4-methoxyphenyl)-2-quinolinyl]oxy]acetate (10a).** Yield, 20%; mp 114–115 °C (Et<sub>2</sub>O). IR (KBr):  $\nu$  1761, 1621, 1517 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, CDCl<sub>3</sub>):  $\delta$  3.87 (s, 3H, OCH<sub>3</sub>), 3.91 (s, 3H, OCH<sub>3</sub>), 3.95 (s, 3H, OCH<sub>3</sub>), 5.17 (s, 2H, CH<sub>2</sub>), 5.27 (s, 2H, CH<sub>2</sub>), 6.44 (d, 1H,  $J$  = 2.0 Hz, H<sub>Ar</sub>), 6.76 (d, 1H,  $J$  = 2.0 Hz, H<sub>Ar</sub>), 7.00 (d, 2H,  $J$  = 8.0 Hz, H<sub>Ar</sub>), 7.26–7.38 (m, 5H, H<sub>Ar</sub>), 7.71 (d, 2H,  $J$  = 8.0 Hz, H<sub>Ar</sub>), 8.36 (s, 1H, =CH). <sup>13</sup>C NMR (62.90 MHz, CDCl<sub>3</sub>):  $\delta$  55.2, 55.4, 55.5, 62.6, 66.4, 96.2, 98.5, 113.4, 113.6 (2), 121.6, 128.0 (2), 128.4 (3), 129.0, 130.5 (2), 132.7, 135.6, 147.2, 156.1, 158.6, 159.0, 161.3, 169.3. MS:  $m/z$  460 (M<sup>+</sup> + 1). Anal. (C<sub>27</sub>H<sub>25</sub>NO<sub>6</sub>) C, H, N.

**2-[5,7-Dimethoxy-3-(4-methoxyphenyl)-2-oxo-1,2-dihydro-1-quinolinyl]acetic Acid (11).** A solution of **10** (1.0 g, 2.2 mmol) in dry 1,4-dioxane (30 mL) was stirred under 40 psi of hydrogen for 4 h at room temperature in the presence of 10% Pd–C (100 mg) and then filtered. The filtrate was evaporated to dryness leaving a solid that was washed with Et<sub>2</sub>O to give **11** (764 mg, 95%). IR (KBr):  $\nu$  1732, 1614, 1583 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub> + D<sub>2</sub>O):  $\delta$  3.77 (s, 3H, OCH<sub>3</sub>), 3.85 (s, 3H, OCH<sub>3</sub>), 3.91 (s, 3H, OCH<sub>3</sub>), 5.02 (s, 2H, CH<sub>2</sub>), 6.46 (s, 1H, H<sub>Ar</sub>), 6.47 (s, 1H, H<sub>Ar</sub>), 6.93 (d, 2H,  $J$  = 9.0 Hz, H<sub>Ar</sub>), 7.62 (d, 2H,  $J$  = 9.0 Hz, H<sub>Ar</sub>), 8.03 (s, 1H, =CH). <sup>13</sup>C NMR (62.90 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  44.9, 55.5, 56.1, 56.6, 91.3, 93.3, 105.3, 113.8 (2), 125.7, 129.4, 130.1 (2), 130.4, 141.3, 157.6, 159.1, 161.3, 162.8, 170.1. MS:  $m/z$  370 (M<sup>+</sup> + 1).

**Methyl 3-[5,7-Dimethoxy-3-(4-hydroxyphenyl)-2-oxo-1,2-dihydro-1-quinolinyl]propanoate (12).** To a solution of **2e** (1.00 g, 3.2 mmol) and methyl acrylate (2.88 mL, 32.0 mmol) in anhydrous DMF (10 mL) at 0 °C was added Triton B (0.06 mL, 0.33 mmol). The final solution was stirred for 18 h at room temperature, and then, the solvent was removed in vacuo. The crude residue was partitioned between H<sub>2</sub>O and EtOAc and extracted. The organic layer was washed with H<sub>2</sub>O, dried over MgSO<sub>4</sub>, and concentrated in vacuo. The crude residue was

purified by column chromatography (eluent  $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  8:2) to give **12** (1.06 g, 83%). IR (KBr):  $\nu$  1725, 1638  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (250 MHz,  $\text{CDCl}_3$ ):  $\delta$  2.78 (t, 2H,  $J$  = 8.0 Hz,  $\text{CH}_2$ ), 3.68 (s, 3H,  $\text{CH}_3$ ), 3.80 (s, 3H,  $\text{OCH}_3$ ), 3.88 (s, 6H,  $\text{OCH}_3$ ), 4.57 (t, 2H,  $J$  = 8.0 Hz,  $\text{CH}_2$ ), 6.26 (d, 1H,  $J$  = 2.0 Hz,  $\text{H}_{\text{Ar}}$ ), 6.46 (d, 1H,  $J$  = 2.0 Hz,  $\text{H}_{\text{Ar}}$ ), 6.92 (d, 2H,  $J$  = 8.8 Hz,  $\text{H}_{\text{Ar}}$ ), 7.66 (d, 2H,  $J$  = 8.8 Hz,  $\text{H}_{\text{Ar}}$ ), 8.11 (s, 1H, =CH).  $^{13}\text{C}$  NMR (62.90 MHz,  $\text{CDCl}_3$ ):  $\delta$  31.9, 39.1, 51.8, 55.2, 55.5, 55.7, 89.8, 92.6, 106.2, 113.4 (2), 126.5, 129.5, 129.9 (2), 130.3, 140.5, 157.6, 159.0, 161.7, 162.4, 172.0. MS:  $m/z$  398 ( $\text{M}^+ + 1$ ).

**3-[5,7-Dimethoxy-3-(4-methoxyphenyl)-2-oxo-1,2-dihydro-1-quinolinyl]propanenitrile (13).** According to the procedure described for **12**, the 2-quinolone **13** was prepared starting from **2e** (1 equiv) and acrylonitrile (10 equiv). Reaction time, 2 h; eluent chromatography  $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  7:3; yield, 63%. IR (KBr):  $\nu$  2241, 1639  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (250 MHz,  $\text{CDCl}_3$ ):  $\delta$  2.88 (t, 2H,  $J$  = 7.1 Hz,  $\text{CH}_2$ ), 3.84 (s, 3H,  $\text{OCH}_3$ ), 3.93 (s, 3H,  $\text{OCH}_3$ ), 3.94 (s, 3H,  $\text{OCH}_3$ ), 4.59 (t, 2H,  $J$  = 7.1 Hz,  $\text{CH}_2$ ), 6.33 (d, 1H,  $J$  = 1.8 Hz,  $\text{H}_{\text{Ar}}$ ), 6.45 (d, 1H,  $J$  = 1.8 Hz,  $\text{H}_{\text{Ar}}$ ), 6.95 (d, 2H,  $J$  = 9.0 Hz,  $\text{H}_{\text{Ar}}$ ), 7.66 (d, 2H,  $J$  = 9.0 Hz,  $\text{H}_{\text{Ar}}$ ), 8.17 (s, 1H, =CH).  $^{13}\text{C}$  NMR (62.90 MHz,  $\text{CDCl}_3$ ):  $\delta$  15.9, 39.4, 55.3, 55.7, 55.9, 89.9, 93.0, 106.4, 113.6 (2), 117.7, 126.6, 129.2, 130.0 (2), 131.1, 140.5, 158.0, 159.3, 161.8, 162.7. MS:  $m/z$  365 ( $\text{M}^+ + 1$ ).

**Benzyl 3-[5,7-Dimethoxy-3-(4-methoxyphenyl)-2-oxo-1,2-dihydro-1-quinolinyl]propanoate (14).** According to the procedure described for **12**, the 2-quinolone **14** was prepared starting from **2e** (1 equiv) and benzyl acrylate (10 equiv). Reaction time, 18 h; eluent chromatography  $\text{EtOAc}/\text{PE}$  4:6; yield, 88%. IR (KBr):  $\nu$  1731, 1635, 1600  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (250 MHz,  $\text{CDCl}_3$ ):  $\delta$  2.86 (t, 2H,  $J$  = 8.0 Hz,  $\text{CH}_2$ ), 3.84 (s, 3H,  $\text{OCH}_3$ ), 3.85 (s, 3H,  $\text{OCH}_3$ ), 3.92 (s, 3H,  $\text{OCH}_3$ ), 4.63 (t, 2H,  $J$  = 8.0 Hz,  $\text{CH}_2$ ), 5.14 (s, 2H,  $\text{CH}_2$ ), 6.29 (d, 1H,  $J$  = 2.0 Hz,  $\text{H}_{\text{Ar}}$ ), 6.49 (d, 1H,  $J$  = 2.0 Hz,  $\text{H}_{\text{Ar}}$ ), 6.94 (d, 2H,  $J$  = 7.5 Hz,  $\text{H}_{\text{Ar}}$ ), 7.30–7.35 (m, 5H,  $\text{H}_{\text{Ar}}$ ), 7.68 (d, 2H,  $J$  = 7.5 Hz,  $\text{H}_{\text{Ar}}$ ), 8.13 (s, 1H, =CH).  $^{13}\text{C}$  NMR (62.90 MHz,  $\text{CDCl}_3$ ):  $\delta$  32.2, 39.1, 55.2, 55.4, 55.7, 66.5, 89.7, 92.6, 106.2, 113.4 (2), 126.5, 128.1 (2), 128.2, 128.4 (2), 129.5, 129.9 (2), 130.4, 135.5, 140.5, 157.6, 159.0, 161.7, 162.4, 171.3. MS:  $m/z$  474 ( $\text{M}^+ + 1$ ).

**3-[5,7-Dimethoxy-3-(4-methoxyphenyl)-2-oxo-1,2-dihydro-1-quinolinyl]propanoic Acid (15).** A solution of **14** (1.0 g, 2.1 mmol) in dry 1,4-dioxane (30 mL) was stirred under 40 psi of hydrogen for 48 h at room temperature in the presence of 10% Pd–C (100 mg) and then filtered. The filtrate was evaporated to dryness leaving a solid that was washed with  $\text{Et}_2\text{O}$  to give **15** (780 mg, 97%). IR (KBr):  $\nu$  1724, 1637, 1612, 1604  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (250 MHz,  $\text{DMSO}-d_6 + \text{D}_2\text{O}$ ):  $\delta$  2.60 (t, 2H,  $J$  = 7.5 Hz,  $\text{CH}_2$ ), 3.78 (s, 3H,  $\text{OCH}_3$ ), 3.90 (s, 3H,  $\text{OCH}_3$ ), 3.92 (s, 3H,  $\text{OCH}_3$ ), 4.48 (t, 2H,  $J$  = 7.5 Hz,  $\text{CH}_2$ ), 6.48 (d, 1H,  $J$  = 1.8 Hz,  $\text{H}_{\text{Ar}}$ ), 6.62 (d, 1H,  $J$  = 1.8 Hz,  $\text{H}_{\text{Ar}}$ ), 6.95 (d, 2H,  $J$  = 8.8 Hz,  $\text{H}_{\text{Ar}}$ ), 7.62 (d, 2H,  $J$  = 8.8 Hz,  $\text{H}_{\text{Ar}}$ ), 8.00 (s, 1H, =CH).  $^{13}\text{C}$  NMR (62.90 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  32.0, 38.9, 55.1, 55.7, 56.1, 90.6, 93.0, 105.0, 113.3 (2), 125.6, 129.3, 129.5, 129.8 (2), 140.5, 157.2, 158.7, 160.6, 162.4, 172.5. MS:  $m/z$  384 ( $\text{M}^+ + 1$ ).

**1-[2-(1H-1,2,3,4-Tetrazol-5-yl)ethyl]-5,7-dimethoxy-3-(4-methoxyphenyl)-1,2-dihydro-2-quinolinone (16).** A solution of **13** (350 mg, 0.9 mmol) and tributyltin azide (0.42 mL, 1.53 mmol) in anhydrous toluene (20 mL) was stirred at 105 °C for 65 h. After it was cooled, the solvent was removed in vacuo. The crude residue was purified by column chromatography (eluent  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  9:1) to give **16** (333 mg, 85%). IR (KBr):  $\nu$  1618, 1594  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (250 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  3.33 (t, 2H,  $J$  = 6.0 Hz,  $\text{CH}_2$ ), 3.79 (s, 3H,  $\text{OCH}_3$ ), 3.89 (s, 3H,  $\text{OCH}_3$ ), 3.93 (s, 3H,  $\text{OCH}_3$ ), 4.67 (t, 2H,  $J$  = 6.0 Hz,  $\text{CH}_2$ ), 6.48 (s, 1H,  $\text{H}_{\text{Ar}}$ ), 6.52 (s, 1H,  $\text{H}_{\text{Ar}}$ ), 6.96 (d, 2H,  $J$  = 9.0 Hz,  $\text{H}_{\text{Ar}}$ ), 7.59 (d, 2H,  $J$  = 9.0 Hz,  $\text{H}_{\text{Ar}}$ ), 8.01 (s, 1H, =CH).  $^{13}\text{C}$  NMR (62.90 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  21.4, 40.8, 55.1, 55.6, 56.1, 90.4, 93.0, 105.0, 113.3 (2), 125.5, 129.3, 129.6, 129.7 (2), 140.4, 153.7, 157.2, 158.7, 160.7, 162.4. MS:  $m/z$  408 ( $\text{M}^+ + 1$ ).

**N,N-Diethyl-3-[5,7-dimethoxy-3-(4-methoxyphenyl)-2-oxo-1,2-dihydro-1-quinolinyl]propanamide (17).** To a solution of **15** (1.0 g, 2.6 mmol) in anhydrous DMF (25 mL) were added hydroxybenzotriazole (353 mg, 2.6 mmol) and dicyclo-

hexylcarbodiimide (540 mg, 2.6 mmol) at 0 °C. After the mixture was stirred at 0 °C for 10 min, *N,N*-diethylamine (0.26 mL, 2.6 mmol) was added and the final mixture was stirred at 0 °C for 2 h and at room temperature for 24 h. The white precipitate of dicyclohexylurea was removed by filtration. The solvent was evaporated in vacuo. The crude residue was purified by column chromatography (eluent  $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  7:3) to afford **17** (680 mg, 60%). IR (KBr):  $\nu$  1636, 1617  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (250 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.11 (t, 3H,  $J$  = 7.0 Hz,  $\text{CH}_3$ ), 1.13 (t, 3H,  $J$  = 7.0 Hz,  $\text{CH}_3$ ), 2.78 (t, 2H,  $J$  = 8.0 Hz,  $\text{CH}_2$ ), 3.30 (q, 2H,  $J$  = 7.0 Hz,  $\text{CH}_2$ ), 3.39 (q, 2H,  $J$  = 7.0 Hz,  $\text{CH}_2$ ), 3.84 (s, 3H,  $\text{OCH}_3$ ), 3.91 (s, 3H,  $\text{OCH}_3$ ), 3.94 (s, 3H,  $\text{OCH}_3$ ), 4.65 (t, 2H,  $J$  = 8.0 Hz,  $\text{CH}_2$ ), 6.29 (d, 1H,  $J$  = 2.0 Hz,  $\text{H}_{\text{Ar}}$ ), 6.73 (d, 1H,  $J$  = 2.0 Hz,  $\text{H}_{\text{Ar}}$ ), 6.94 (d, 2H,  $J$  = 7.5 Hz,  $\text{H}_{\text{Ar}}$ ), 7.67 (d, 2H,  $J$  = 7.5 Hz,  $\text{H}_{\text{Ar}}$ ), 8.15 (s, 1H, =CH).  $^{13}\text{C}$  NMR (62.90 MHz,  $\text{CDCl}_3$ ):  $\delta$  13.1, 14.4, 31.0, 40.1, 40.5, 42.2, 55.3, 55.8 (2), 89.9, 93.1, 106.3, 113.5 (2), 126.6, 129.7, 130.0 (2), 130.5, 140.9, 157.6, 159.1, 162.1, 162.6, 169.9. MS:  $m/z$  439 ( $\text{M}^+ + 1$ ).

**8,10-Dimethoxy-6-(4-methoxyphenyl)-2,3-dihydro-1H,5H-pyrido[3,2,1-*ij*]quinoline-1,5-dione (18).** In a dry round flask,  $\text{P}_2\text{O}_5$  (63 mg) and PPA (500 mg) were stirred at 110 °C until complete dissolution of  $\text{P}_2\text{O}_5$ . Acid **15** (100 mg, 0.26 mmol) was added, and the final mixture was stirred at 110 °C for 45 min. After it was cooled, the mixture was poured into ice and neutralized by 2 N NaOH.  $\text{CH}_2\text{Cl}_2$  (200 mL) was added, and the mixture was stirred overnight. After extraction, the aqueous layer was extracted with  $\text{CH}_2\text{Cl}_2$  twice ( $2 \times 50$  mL). The organic phase was dried over  $\text{MgSO}_4$  and evaporated in vacuo. The crude residue was purified by column chromatography (eluent  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  98:2) to give **18** (62 mg, 65%). IR (KBr):  $\nu$  1678, 1649, 1634  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (250 MHz,  $\text{CDCl}_3$ ):  $\delta$  2.83 (t, 2H,  $J$  = 7.0 Hz,  $\text{CH}_2$ ), 3.85 (s, 3H,  $\text{OCH}_3$ ), 4.04 (s, 6H,  $\text{OCH}_3$ ), 4.54 (d, 2H,  $J$  = 7.0 Hz,  $\text{CH}_2$ ), 6.32 (s, 1H,  $\text{H}_{\text{Ar}}$ ), 6.96 (d, 2H,  $J$  = 7.5 Hz,  $\text{H}_{\text{Ar}}$ ), 7.68 (d, 2H,  $J$  = 7.5 Hz,  $\text{H}_{\text{Ar}}$ ), 8.12 (s, 1H, =CH).  $^{13}\text{C}$  NMR (62.90 MHz,  $\text{CDCl}_3$ ):  $\delta$  37.6, 40.3, 55.3, 56.1, 56.5, 88.9, 103.5, 104.8, 113.6 (2), 127.6, 129.0, 129.8, 130.0 (2), 142.9, 159.4, 161.6, 161.7, 163.7, 190.3. MS:  $m/z$  366 ( $\text{M}^+ + 1$ ).

**5,7-Dihydroxy-3-(4-hydroxyphenyl)-1,2-dihydro-2-quinolinone (19).**<sup>21</sup> To a solution of **2e** (1.0 g, 3.2 mmol) in AcOH (15 mL) was dropwise added 48% HBr in  $\text{H}_2\text{O}$  (5 mL). The final solution was stirred at reflux for 3 days. The cooled mixture was diluted with  $\text{H}_2\text{O}$ , neutralized with 10% aqueous NaOH (pH 6–7), and extracted with  $\text{CH}_2\text{Cl}_2$  twice. The organic layer was dried over  $\text{MgSO}_4$  and then evaporated in vacuo. The crude residue was purified by column chromatography (eluent  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  9:1) to give **19** (320 mg, 37%).  $^1\text{H}$  NMR (250 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  6.11 (d, 1H,  $J$  = 2.0 Hz,  $\text{H}_{\text{Ar}}$ ), 6.18 (d, 1H,  $J$  = 2.0 Hz,  $\text{H}_{\text{Ar}}$ ), 6.76 (d, 2H,  $J$  = 8.6 Hz,  $\text{H}_{\text{Ar}}$ ), 7.52 (d, 2H,  $J$  = 8.6 Hz,  $\text{H}_{\text{Ar}}$ ), 7.89 (s, 1H, =CH), 9.42 (s, 1H, OH), 9.84 (s, 1H, OH), 10.21 (s, 1H, OH), 11.46 (br s, 1H, NH).  $^{13}\text{C}$  NMR (62.90 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  91.3, 96.4, 103.5, 114.7 (2), 125.1, 127.8, 129.4 (2), 130.4, 140.7, 155.3, 156.6, 160.1, 161.8. MS:  $m/z$  270 ( $\text{M}^+ + 1$ ).

**5,7-Dihydroxy-3-(4-hydroxyphenyl)-1-methyl-1,2-dihydro-2-quinolinone (20).** According to the procedure described for **19**, the 2-quinolone **20** was prepared starting from **3** (eluent  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  9:1); yield: 35%.  $^1\text{H}$  NMR (250 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  3.53 (s, 3H,  $\text{NCH}_3$ ), 6.25 (s, 2H,  $\text{H}_{\text{Ar}}$ ), 6.76 (d, 2H,  $J$  = 8.8 Hz,  $\text{H}_{\text{Ar}}$ ), 7.47 (d, 2H,  $J$  = 8.8 Hz,  $\text{H}_{\text{Ar}}$ ), 7.92 (s, 1H, =CH), 9.42 (s, 1H, OH), 10.00 (s, 1H, OH), 10.35 (s, 1H, OH).  $^{13}\text{C}$  NMR (62.90 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  29.7, 91.8, 96.5, 103.5, 114.7 (2), 124.3, 128.4, 129.7 (3), 141.7, 155.9, 156.7, 160.6, 161.1. MS:  $m/z$  284 ( $\text{M}^+ + 1$ ).

**5,7-Dimethoxy-3-(4-hydroxyphenyl)-1,2-dihydro-2-quinolinone (21).** To a solution of **2e** (530 mg, 1.70 mmol) in AcOH (15 mL) was dropwise added 48% HBr in  $\text{H}_2\text{O}$  (2.7 mL). The final solution was stirred at reflux for 5 h. The cooled mixture was diluted with  $\text{H}_2\text{O}$ , neutralized with 10% aqueous NaOH (pH 6–7), and extracted with  $\text{CH}_2\text{Cl}_2$  twice. The organic layer was dried over  $\text{MgSO}_4$  and then evaporated in vacuo. The crude residue was purified by column chromatography (eluent  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  9:1) to give **21** (175 mg, 35%). IR (KBr):  $\nu$  1628, 1604, 1558  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (250 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  3.80 (s,



3H, OCH<sub>3</sub>), 3.89 (s, 3H, OCH<sub>3</sub>), 6.35 (d, 1H, *J* = 2.5 Hz, H<sub>Ar</sub>), 6.43 (d, 1H, *J* = 2.5 Hz, H<sub>Ar</sub>), 6.78 (d, 2H, *J* = 7.5 Hz, H<sub>Ar</sub>), 7.55 (d, 2H, *J* = 7.5 Hz, H<sub>Ar</sub>), 7.92 (s, 1H, =CH), 9.48 (s, 1H, OH), 11.72 (br s, 1H, NH). <sup>13</sup>C NMR (62.90 MHz, DMSO-*d*<sub>6</sub>): δ 55.4, 55.9, 90.0, 93.0, 104.7, 114.8 (2), 126.9, 127.4, 129.6, 129.8 (2), 140.4, 156.6, 156.9, 161.6, 161.8. MS: *m/z* 298 (M<sup>+</sup> + 1).

**5,7-Dimethoxy-3-(4-hydroxyphenyl)-1-methyl-1,2-dihydro-2-quinolinone (22).** According to the procedure described for **21**, the 2-quinolone **22** was prepared starting from **3** (eluent CH<sub>2</sub>Cl<sub>2</sub>/EtOAc 7:3); yield, 56%. <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>): δ 3.63 (s, 3H, NCH<sub>3</sub>), 3.89 (s, 3H, OCH<sub>3</sub>), 3.90 (s, 3H, OCH<sub>3</sub>), 6.46 (d, 1H, *J* = 1.9 Hz, H<sub>Ar</sub>), 6.54 (d, 1H, *J* = 1.9 Hz, H<sub>Ar</sub>), 6.76 (d, 2H, *J* = 8.5 Hz, H<sub>Ar</sub>), 7.48 (d, 2H, *J* = 8.5 Hz, H<sub>Ar</sub>), 7.93 (s, 1H, =CH), 9.47 (s, 1H, OH). <sup>13</sup>C NMR (62.90 MHz, DMSO-*d*<sub>6</sub>): δ 30.5, 56.1, 56.5, 91.3, 93.4, 105.3, 115.2 (2), 126.5, 128.4, 129.2, 130.2 (2), 141.7, 157.4 (2), 161.4, 162.5. MS: *m/z* 312 (M<sup>+</sup> + 1).

**5,7-Dimethoxy-3-(4-methoxyphenyl)-1,2-dihydro-2-quinolinethione (23).** A mixture of **2e** (100 mg, 0.32 mmol) and Lawesson's reagent (260 mg, 0.64 mmol) in anhydrous toluene (15 mL) was stirred at reflux for 18 h. After it was cooled, the solvent was removed in vacuo and the residue was purified by silica gel chromatography (eluent CH<sub>2</sub>Cl<sub>2</sub>/EtOAc 9:1) to give **23** (81 mg, 77%). IR (KBr): ν 1636, 1610, 1524 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>): δ 3.78 (s, 3H, OCH<sub>3</sub>), 3.83 (s, 3H, OCH<sub>3</sub>), 3.89 (s, 3H, OCH<sub>3</sub>), 6.49 (d, 1H, *J* = 1.9 Hz, H<sub>Ar</sub>), 6.79 (d, 1H, *J* = 1.9 Hz, H<sub>Ar</sub>), 6.92 (d, 2H, *J* = 8.7 Hz, H<sub>Ar</sub>), 7.49 (d, 2H, *J* = 8.7 Hz, H<sub>Ar</sub>), 7.78 (s, 1H, =CH), 13.50 (br s, 1H, NH). <sup>13</sup>C NMR (62.90 MHz, CDCl<sub>3</sub>): δ 55.1, 55.6, 56.1, 90.2, 95.2, 108.9, 112.9 (2), 128.2, 130.7 (2), 132.0, 136.3, 140.9, 156.4, 158.5, 162.5, 180.5. MS: *m/z* 328 (M<sup>+</sup> + 1).

**5,7-Dimethoxy-3-(4-methoxyphenyl)-1-methyl-1,2-dihydro-2-quinolinethione (24).** According to the procedure described for **23**, the 2-thioquinolone **24** was prepared starting from **3** (eluent EtOAc/PE 3:7); yield, 72%. IR (KBr): ν 1613, 1570, 1512 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, CDCl<sub>3</sub>): δ 3.84 (s, 3H, OCH<sub>3</sub>), 3.91 (s, 3H, OCH<sub>3</sub>), 3.95 (s, 3H, OCH<sub>3</sub>), 4.39 (s, 3H, NCH<sub>3</sub>), 6.39 (d, 1H, *J* = 2.0 Hz, H<sub>Ar</sub>), 6.56 (d, 1H, *J* = 2.0 Hz, H<sub>Ar</sub>), 6.93 (d, 2H, *J* = 7.5 Hz, H<sub>Ar</sub>), 7.43 (d, 2H, *J* = 7.5 Hz, H<sub>Ar</sub>), 7.97 (s, 1H, =CH). <sup>13</sup>C NMR (62.90 MHz, CDCl<sub>3</sub>): δ 39.5, 55.2, 55.6, 55.9, 91.1, 94.6, 109.9, 113.1 (2), 126.6, 130.8 (2), 134.3, 138.6, 142.7, 157.6, 158.8, 162.7, 184.5. MS: *m/z* 342 (M<sup>+</sup> + 1).

**5,7-Acetyl-3-(4-acetylphenyl)-1,2-dihydro-2-quinolinone (25).** A solution of **20** (200 mg, 0.71 mmol), anhydride acetic, and pyridine (8 mL, v/v) was stirred at room temperature for 18 h. H<sub>2</sub>O (10 mL) was added to the mixture and then extracted with CH<sub>2</sub>Cl<sub>2</sub> (2 × 10 mL). The organic layer was dried over MgSO<sub>4</sub> and then evaporated in vacuo. The crude residue was purified by column chromatography (eluent CH<sub>2</sub>Cl<sub>2</sub>/EtOAc 9:1) to give **25** (220 mg, 76%). IR (KBr): ν 1769, 1748, 1638, 1598 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, CDCl<sub>3</sub>): δ 2.31 (s, 3H, CH<sub>3</sub>), 2.33 (s, 3H, CH<sub>3</sub>), 2.40 (s, 3H, CH<sub>3</sub>), 3.72 (s, 3H, NCH<sub>3</sub>), 6.92 (d, 1H, *J* = 2.0 Hz, H<sub>Ar</sub>), 7.03 (d, 1H, *J* = 2.0 Hz, H<sub>Ar</sub>), 7.14 (d, 2H, *J* = 8.5 Hz, H<sub>Ar</sub>), 7.67 (d, 2H, *J* = 8.5 Hz, H<sub>Ar</sub>), 7.78 (s, 1H, =CH). <sup>13</sup>C NMR (62.90 MHz, CDCl<sub>3</sub>): δ 20.8, 21.0, 21.2, 30.5, 105.2, 110.2, 111.8, 121.4 (2), 129.7, 130.3 (2), 131.5, 134.2, 141.0, 148.8, 150.7, 151.9, 161.2, 168.6, 168.8, 169.6. MS: *m/z* 410 (M<sup>+</sup> + 1).

**5,7-Dimethoxy-3-(4-acetylphenyl)-1,2-dihydro-2-quinolinone (26).** According to the procedure described for **25**, the 2-quinolone **26** was prepared starting from **22**. Yield, 73%; reaction time, 2 h; eluent CH<sub>2</sub>Cl<sub>2</sub>/EtOAc 9:1. IR (KBr): ν 1751, 1639, 1601 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, CDCl<sub>3</sub>): δ 2.31 (s, 3H, CH<sub>3</sub>), 3.72 (s, 3H, NCH<sub>3</sub>), 3.90 (s, 6H, OCH<sub>3</sub>), 6.28 (d, 1H, *J* = 2.0 Hz, H<sub>Ar</sub>), 6.34 (d, 1H, *J* = 2.0 Hz, H<sub>Ar</sub>), 7.13 (d, 2H, *J* = 8.8 Hz, H<sub>Ar</sub>), 7.75 (d, 2H, *J* = 8.8 Hz, H<sub>Ar</sub>), 8.15 (s, 1H, =CH). <sup>13</sup>C NMR (62.90 MHz, CDCl<sub>3</sub>): δ 21.3, 30.4, 55.7, 55.9, 90.3, 92.7, 106.0, 121.1 (2), 126.3, 130.1 (3), 131.4, 142.1, 150.1, 157.8, 162.0, 162.7, 169.6. MS: *m/z* 354 (M<sup>+</sup> + 1).

**5,7-Dimethoxy-3-(4-methoxyphenyl)-1-methyl-2-(methylsulfanyl)quinolinium Iodide (27).** To a solution of **24** (682 mg, 2.0 mmol) in anhydrous THF (30 mL) was added io-

domethane (3.5 mL, 56 mmol) diluted in anhydrous THF (5 mL) at room temperature. The final solution was stirred overnight at room temperature. The red precipitate obtained was collected and washed with the same solvent to provide **27** (761 mg, 79%); mp 156–157 °C (THF washing). <sup>1</sup>H NMR (250 MHz, CDCl<sub>3</sub>): δ 2.44 (s, 3H, SCH<sub>3</sub>), 3.88 (s, 3H, OCH<sub>3</sub>), 4.02 (s, 3H, OCH<sub>3</sub>), 4.28 (s, 3H, OCH<sub>3</sub>), 5.03 (s, 3H, NCH<sub>3</sub>), 6.70 (d, 1H, *J* = 1.5 Hz, H<sub>Ar</sub>), 7.03 (d, 2H, *J* = 8.5 Hz, H<sub>Ar</sub>), 7.37 (br s, 1H, H<sub>Ar</sub>), 7.45 (d, 2H, *J* = 8.5 Hz, H<sub>Ar</sub>), 8.71 (s, 1H, =CH). <sup>13</sup>C NMR (62.90 MHz, CDCl<sub>3</sub>): δ 20.8, 46.5, 55.4, 56.7, 58.5, 92.9, 101.0, 114.4 (2), 117.6, 125.8, 128.9, 130.7 (2), 135.9, 138.8, 144.2, 157.4, 160.3, 167.7.

**5,7-Dimethoxy-3-(4-methoxyphenyl)-1-methyl-1,2-dihydro-2-quinolinone-2-phenylhydrazone (28).** In a sealed tube, a solution of **27** (200 mg, 0.4 mmol) and phenylhydrazine (0.28 mL, 2.8 mmol) in anhydrous EtOH (5 mL) was heated at 90 °C overnight. After it was cooled, the solvent was removed in vacuo. The residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (10 mL), and the organic solution was washed with H<sub>2</sub>O and then with saturated NaHCO<sub>3</sub> solution twice. The organic phase was dried over MgSO<sub>4</sub> and evaporated in vacuo. The crude residue was crystallized from MeOH to afford **28** (102 mg, 60%). IR (KBr): ν 3340, 1602, 1599 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, CDCl<sub>3</sub>): δ 3.58 (s, 3H, NCH<sub>3</sub>), 3.85 (s, 3H, OCH<sub>3</sub>), 3.86 (s, 3H, OCH<sub>3</sub>), 3.88 (s, 3H, OCH<sub>3</sub>), 6.04 (s, 1H, H<sub>Ar</sub>), 6.14 (s, 1H, H<sub>Ar</sub>), 6.48 (br s, 1H, NH), 6.56–6.67 (m, 3H, H<sub>Ar</sub>), 6.94 (d, 2H, *J* = 8.8 Hz, H<sub>Ar</sub>), 7.11 (t, 2H, *J* = 7.7 Hz, H<sub>Ar</sub>), 7.28 (s, 1H, =CH), 7.28–7.34 (m, 2H, H<sub>Ar</sub>). <sup>13</sup>C NMR (62.90 MHz, CDCl<sub>3</sub>): δ 33.3, 55.4 (2), 55.6, 89.6 (2), 111.6, 113.8 (2), 117.8, 125.9, 128.3, 128.9 (3), 129.5 (2), 130.5, 146.2, 157.2, 159.2 (2), 162.3. MS: *m/z* 416 (M<sup>+</sup> + 1).

**5,7-Dimethoxy-3-(4-methoxyphenyl)-1-methyl-1,2-dihydro-2-quinolinone-2-(2-pyridinyl)hydrazone (29).** Compound **29** was obtained according to the procedure described below but substituting the phenylhydrazine by 2-hydrazinopyridine; yield, 58%. IR (KBr): 3353, 1628, 1593 cm<sup>-1</sup>. <sup>1</sup>H NMR (250 MHz, CDCl<sub>3</sub>): δ 3.61 (s, 3H, NCH<sub>3</sub>), 3.87 (s, 6H, OCH<sub>3</sub>), 3.90 (s, 3H, OCH<sub>3</sub>), 6.07 (s, 1H, H<sub>Ar</sub>), 6.17 (s, 1H, H<sub>Ar</sub>), 6.51–6.55 (m, 1H, H<sub>Pyr</sub>), 6.94–7.01 (m, 3H, H<sub>Ar</sub> + H<sub>Pyr</sub>), 7.14 (br s, 1H, NH), 7.32–7.36 (m, 3H, =CH + H<sub>Ar</sub>), 7.48 (t, 1H, *J* = 7.3 Hz, H<sub>Pyr</sub>), 7.91 (d, 1H, *J* = 4.3 Hz, H<sub>Pyr</sub>). <sup>13</sup>C NMR (62.90 MHz, CDCl<sub>3</sub>): δ 33.2, 55.3, 55.4, 55.6, 89.7 (2), 104.7, 105.7, 113.4, 114.1 (2), 122.9, 129.0, 129.2 (2), 130.1, 137.5, 140.7, 143.7, 147.8, 157.3, 157.4, 159.2, 162.4. MS: *m/z* 417 (M<sup>+</sup> + 1).

**Pharmacology. In Vitro MTT Colorimetric Assay.** Twelve human tumor cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA). These included three glioblastomas (A-172, U-373 MG, and U-87 MG), two colon (HCT-15 and LoVo), two nonsmall cell lung (A549 and A-427), two bladder (J82 and T24), one prostate (PC-3), and two breast (T-47D and MCF-7) cancer models. The ATCC numbers of these cell lines are CRL1620 (A-172), HTB 14 (U-87 MG), HTB 17 (U-373 MG), CCL225 (HCT-15), CCL229 (LoVo), CCL 185 (A549), HBT 53 (A-427), HTB1 (J82), HTB4 (T24), HTB133 (T-47D), HTB22 (MCF-7), and CRL1435 (PC-3). The cells were cultured at 37 °C in sealed (airtight) Falcon plastic dishes (Nunc, Gibco, Belgium) containing Eagle's minimal essential medium (MEM, Gibco) supplemented with 5% fetal calf serum (FCS). All of the media were supplemented with a mixture of 0.6 mg/mL glutamine (Gibco), 200 IU/mL penicillin (Gibco), 200 IU/mL streptomycin (Gibco), and 0.1 mg/mL gentamycin (Gibco). The FCS was heat-inactivated for 1 h at 56 °C.

The twelve cell lines were incubated for 24 h in 96 microwell plates (at a concentration of 40 000 cells/mL culture medium) to ensure adequate plating prior to cell growth determination, which was carried out by means of the colorimetric MTT assay as detailed previously.<sup>23</sup> Six concentrations ranging from 10<sup>-5</sup> to 10<sup>-9</sup> M were assayed for each of the 25 drugs under study.

**In Vitro Cell Migration Assay.** The cell motility level of the MCF-7 human breast cancer cells was quantitatively determined by means of a device detailed elsewhere.<sup>24</sup> Briefly, this device consists of an inverted-phase contrast microscope equipped with a black and white CCD camera, an incubator



maintaining the temperature at 37 °C, a computer containing a frame grabber, and an image-processing software that analyzes digitized frames. The software that we set up thus enabled each living cell in the culture under study to be isolated automatically on the basis of specific morphological characteristics that in turn enables cells to be identified against their background (the plastic Falcon dishes). Once the segmentation procedure is complete, each cell is transformed into its center of gravity. An image is digitized every 4 min by the computer, and the video system is thus able to track the trajectory of each cell by analyzing the movement of its center of gravity. From these trajectories, the maximum relative distance to the origin (the MRDO quantitative variable) of the cell was calculated for each cell under analysis. This variable thus describes the greatest linear distance found between the original and the subsequent positions of the cell divided by the observation time.<sup>24</sup> All of the experiments were performed over 48 h at a cell concentration of 10 000 MCF-7 cells/mL MEM, with one image recorded every 4 min. The MCF-7 cells were grown in a standard MEM medium (see above for the MTT colorimetric assay) in control or in culture media containing either compound **13** or **16** at 10<sup>-6</sup>, 10<sup>-7</sup>, or 10<sup>-8</sup> M concentration. Each experimental condition was carried out in triplicate. At the beginning of the experiment, the minimal number of cells in a given experimental condition was 34, and the maximal number at the end of the experiments was 402 cells. According to the analyses in triplicate, the trajectories of a minimum of 719 and a maximum of 1002 MCF-7 cells were analyzed in each experimental condition.

**In Vivo Determination of the MTD.** We determined the MTD for each of 25 quinolone derivatives under study by defining the maximum dose of the drug that can be administered acutely (i.e., in one i.p. single dose) to healthy animals (B6D2F1, Iffa Credo), i.e., not grafted with tumors. The survival and weight of the animals were recorded for up to 28 days postinjection. Six different doses of each drug (5, 10, 20, 40, 80, and 160 mg/kg) were used for the determination of the MTD index, with each experimental group composed of three mice for this purpose.

**In Vivo Determination of Antitumor Activity.** The hormone-sensitive MXT (MXT-HS) mammary cancer model was set up experimentally at Baylor College by the Clark group.<sup>37</sup> We obtained the MXT-HS model in 1983 from Dr. D. Bogden (Mason Research Institute, Worcester, MA). The MXT-HS strain died out in our laboratory after a number of years, and only the hormone-insensitive (MXT-HI) one continued.<sup>36</sup> An experimental protocol was thus developed in our laboratory to differentiate hormone-insensitive MXT-HI tumor strains into hormone-sensitive MXT-HS ones.<sup>32</sup>

The MXT-HS tumors that we set up from the MXT-HI ones are maintained in our laboratory by monthly s.c. transplantations into 6 week old female B6D2F1 mice (Iffa Credo). Tumor size was measured weekly by means of a caliper and expressed as an area (mm<sup>2</sup>) by multiplying together the two largest perpendicular diameters.

All of the animals were kept in plastic cages in a room with a controlled temperature (22 ± 1 °C), light exposure (from 6:00 am to 6:00 pm), and 40–70% relative humidity. Food (AO4, UAR, Villemoisson, France) and water were provided ad libitum.

**Statistical Analysis.** The results are presented as the mean ± the standard error of the mean (SEM). The statistical comparisons of the data were carried out by means of the Fisher F (one way variance analysis for more than two groups) or the Student *t* (for two groups) tests after a check of the homogeneity of variance by means of the Levene test and of the normal distribution fitting of the data by means of the  $\chi$  squared test of goodness-of-fit. When these parametric conditions were not satisfied, the nonparametric Kruskal–Wallis (for more than two groups) or the Mann–Whitney (for two groups) tests were carried out. All of the statistical analyses were carried out using Statistica (Statsoft, Tulsa, OK).

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