



Published in final edited form as:

*J Med Chem.* 2009 April 9; 52(7): 1873–1884. doi:10.1021/jm801335z.

## Synthesis of Casimiroin and Optimization of Its Quinone Reductase 2 and Aromatase Inhibitory Activities

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### Abstract

An efficient method has been developed to synthesize casimiroin (**1**), a component of the edible fruit of *Casimiroa edulis*, on a multigram scale in good overall yield. The route was versatile enough to provide an array of compound **1** analogues that were evaluated as QR2 and aromatase inhibitors. In addition, X-ray crystallography studies of QR2 in complex with compound **1** and one of its more potent analogs has provided insight into the mechanism of action of this new series of QR2 inhibitors. The initial biological investigations suggest that compound **1** and its analogues merit further investigation as potential chemopreventive or chemotherapeutic agents.

### Introduction

The medicinal values of zapote blanco, an edible fruit of *Casimiroa edulis* Llave et Lex (Rutaceae), have been proposed for many years. Our continuous efforts to discover chemopreventive agents from natural products led us to perform a study on casimiroin (**1**), a key constituent of the seeds of zapote blanco, that showed the ability to inhibit mutagenicity induced by 7,12-dimethylbenz[*a*]anthracene (DMBA) in *Salmonella typhimurium* strain TM677, as well as inhibition of the formation of DMBA-induced preneoplastic lesions in mouse mammary gland organ culture (MMOC).<sup>1</sup> Compound **1**, therefore, has the potential to inhibit carcinogenesis. The goals of the present investigation were to devise and execute a practical synthesis of compound **1** that would afford larger quantities for advanced biological testing and also to optimize the potential of compound **1** analogues as inhibitors of QR2 and aromatase (CYP19A1).

Quinone reductase 2 (QR2), a cytosolic FAD-dependent flavoenzyme that catalyzes metabolic reduction of quinones with unexpected co-substrate specificity, is expressed in various organs including heart, liver, skeletal muscle and kidney.<sup>2</sup> It has been described as an enzyme of surprises and mysteries,<sup>3</sup> and recent studies have revealed that genetic polymorphisms of QR2 are associated with a number of neurological diseases such as Parkinson's, schizophrenia, and

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**Supporting Information** Available: Results from elemental analyses of intermediates and target compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

others.<sup>4–6</sup> QR2 also has a unique relationship with quinoline-containing antimalarial drugs,<sup>7</sup> and it binds melatonin, which has led to its classification as the third melatonin receptor binding site or MT<sub>3</sub>.<sup>8</sup> QR2 shares 49% sequence identity with the NAD(P)H-dependent quinone reductase 1 (QR1), but does not recognize NADH or NAD(P)H as co-substrates. Instead, it catalyses the oxidation of reduced *N*-alkyl- and *N*-ribosylnicotinamides by menadione and other quinones. Because of its high similarity in both primary amino acid sequence and tertiary structure to QR1, it is believed that QR2 might also serve as a detoxification enzyme that can produce hydroquinones that are less toxic to cells compared to the parent quinones. However, recent studies have indicated that QR2 may actually transform certain quinone substrates into more highly reactive species that are capable of causing more cellular damage.<sup>9–11</sup> Therefore, it is hypothesized that inhibition of QR2 in certain cases may lead to protection of cells against these reactive species. Melatonin, a known anti-oxidant, inhibits QR2 and its X-ray structure in complex with QR2 has been determined.<sup>6</sup> Coenzyme Q0, another anti-oxidant, has been shown to be an efficient substrate of the QR2-catalyzed reaction.<sup>12</sup> These observations suggest a mysterious link between QR2 and cellular anti-oxidant status. Several QR2 inhibitors have been reported in the literature.<sup>13</sup> QR2 is inhibited by a variety of natural polyphenols,<sup>12</sup> and the X-ray crystal structure of QR2 in complex with the putative chemopreventive agent resveratrol, a potent inhibitor of the enzyme, has been determined.<sup>6,14</sup> These latter observations motivated the present study.

Aromatase, a key cytochrome P450 enzyme, catalyzes the rate-limiting aromatization step in the conversion of androgens (testosterone and androstenedione) to estrogens (estradiol and estrone). Aromatase inhibitors decrease bioavailable estrogen and have been shown to have considerable antitumor activity in certain breast cancers.<sup>15</sup> Such compounds ultimately reduce estradiol receptor stimulation and reduce formation of genotoxic estrogen metabolites.<sup>16</sup> Certain nonsteroidal aromatase inhibitors are already in clinical use for the treatment of breast cancer, and several naturally occurring nonsteroidal aromatase inhibitors have shown promising chemopreventive activity.<sup>17</sup>

In our group, QR2 and aromatase have been targeted for identification of naturally occurring chemopreventive agents.<sup>6</sup> Our preliminary observation of the antimutagenic activity of compound **1**,<sup>1</sup> along with the limited quantities of compound **1** from natural sources, led us to synthesize compound **1** and its analogues for further biological testing. This report discloses a new and improved total synthesis of compound **1**, the discovery of its QR2 and aromatase inhibitory activities, and the optimization of these activities resulting in a new series of potent inhibitors of both enzymes. In addition, X-ray crystallography studies of QR2 in complex with compound **1** and a more potent analog are described. These studies have provided insight into the mechanism of QR2 inhibition by compound **1** and related compounds.

## Results and Discussion

### Chemistry

Although a synthesis of compound **1** has been reported in the literature, it has a month-long critical cyclization step that provides the desired intermediate as a minor product in only 10% yield, resulting in a very poor overall yield (0.67%) over 11 steps.<sup>18</sup> The final step involves several recrystallizations followed by sublimation to remove contaminating by-products.

A retrosynthetic analysis of compound **1** is shown in Scheme 1. The key step is the synthesis of intermediate **3b**, a degradation product of compound **1**, by a Curtius rearrangement. The final product **1** was planned to be made by acid-mediated intramolecular Friedel-Crafts acylation followed by a methylation of an enol.

In practice, the present synthesis of compound **1** was executed in a straightforward manner, without any unexpected difficulties, as outlined in Scheme 2. The degradation product **3b** of compound **1** was easily prepared in six steps from commercially available 2,3-dihydroxy benzoic acid (**4**) using a modified literature procedure<sup>19</sup> in 27% overall yield. Curtius rearrangement of dioxole benzoic acid **5a** using DPPA<sup>a</sup> and Et<sub>3</sub>N in refluxing benzene, followed by in situ quenching with benzyl alcohol, produced compound **6a**. N-Methylation of **6a** using NaH and MeI in THF, followed hydrogenolysis of the benzyl group and decarboxylation, yielded N-methylated amine **3b**. Acylation of the secondary amine **3b** with diethyl malonate afforded intermediate **8**, which was saponified using sodium hydroxide to provide the acid **2**. Polyphosphoric acid-mediated cyclization led to intermediate **9**. Methylation of **9** with TMS-diazomethane afforded compound **1** as a white powder in ~15% overall yield from commercially available acid **4**. The <sup>1</sup>H NMR, <sup>13</sup>C NMR, IR, mass spectral, and elemental analysis data of the synthetic compound **1** were consistent with those reported for the natural product. Synthetic compound **1** was used for various biological assays to determine its chemopreventive activity. The encouraging biological results motivated the synthesis of additional compound **1** congeners in order to gain insight into the structure-activity relationships and mechanism of action.

Compound **1** analogues **1a-q** (Scheme 3) were synthesized by treating the corresponding amines **3a-q** with diketene in refluxing benzene to yield ketoamides **10a-q**, followed by a polyphosphoric acid (PPA)-mediated cyclization that afforded very high yields, except for compounds **1j** and **1p**. PPA-mediated cyclization of compounds **10j** and **10p** produced quinolinones **1j** and **1p**, respectively, as minor products. The desired products **1j** and **1p** were therefore prepared in very high yield by methylating compounds **1i** and **1o**, respectively, using NaH and MeI in THF, as shown in Scheme 4. Quinolinone **1r** was also prepared from **1q** in a similar way. Compound **1a** was synthesized in 82% yield by treating ketoamide compound **10a** with trifluoroacetic acid at room temperature.

Most of the free amines or mono-methylated amines are commercially available. However, some of them had to be synthesized from the corresponding acids via Curtius rearrangement as shown in Scheme 5. Amines **3l** and **3q** were synthesized in good yields following literature procedures.<sup>20,21</sup> Quinolinone **1l** was used to synthesize quinone **12** following a previously reported literature procedure as outlined in Scheme 6.<sup>21</sup>

### Quinone Reductase 2 Inhibitory Activity

QR2 inhibition assays were performed on synthetic compound **1** and its various analogues, as described in the Experimental Section. The IC<sub>50</sub> values are reported in Table 1. Synthetic compound **1** was determined to have an IC<sub>50</sub> value of 54.1 ± 6.7 μM. Most of the compound **1** analogues displayed superior inhibitory potency compared with the natural product and were active in the lower micromolar range. In general, N-methylated analogues showed better potency than non-N-methylated quinolinones in inhibiting QR2. Compound **1j**, with IC<sub>50</sub> 1.9 ± 0.2 μM, is comparable in potency with most of the QR2 inhibitors reported in the literature. The replacement of the 6-methoxy group of compound **1** by a methyl group increases the inhibitory activity more than 8-fold (**1b**, IC<sub>50</sub> = 6.2 ± 0.28 μM). Non-N-methylated quinolinone **1a** (IC<sub>50</sub> = 10.8 ± 2.1 μM) showed a decrease in activity relative to **1b**, but still has better potency than the natural product. Complete removal of the dioxole ring from **1a** made the resulting compound **1c** inactive; however, N-methylated compound **1d** showed an IC<sub>50</sub> value

<sup>a</sup>Abbreviations: DPPA, diphenylphosphoryl azide; TMS, trimethylsilyl; FAD, flavin adenine dinucleotide; HRMS, high-resolution mass spectrometry; EIMS, electron-impact mass spectrometry; DMF, dimethylformamide; ESIMS, electrospray-ionization mass spectrometry; MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; BSA, bovine serum albumin; DMSO, dimethylsulfoxide; SER-CAT, Southeast Regional Collaborative Access Team; CCD, charge-coupled device; CCP4, Collaborative Computational Project Number 4; NADPH, the reduced form of nicotinamide adenine dinucleotide phosphate (NADP<sup>+</sup>).

of  $18.2 \pm 3.1 \mu\text{M}$ , an approximately 3-fold improvement of activity relative to compound **1**. The presence of an 8-methoxy group (**1e**, **1f**) improved the activity relative to the unsubstituted derivatives (**1c**, **1d**). Quinolinone **1f** had an  $\text{IC}_{50}$  value  $5.8 \pm 0.9 \mu\text{M}$ , which is an approximately 10-fold increase in potency compared with the natural product.

To analyze the effect of methoxy substitutions on the benzene ring, several dimethoxylated (**1g-l**), trimethoxylated (**1m-p**), and tetramethoxylated (**1q-r**) quinolinone derivatives were synthesized and evaluated. It was found that introduction of another methoxy group at the 7 position in 8-methoxylated quinolines (**1e**, **1d**) decreased the inhibitory potency (**1g**,  $\text{IC}_{50}$   $29.3 \pm 3.4 \mu\text{M}$ , **1h**,  $\text{IC}_{50}$   $9.3 \pm 2.3 \mu\text{M}$ ). In contrast, *N*-methylated 6,8-dimethoxy derivative **1j** ( $\text{IC}_{50}$   $1.9 \pm 0.2 \mu\text{M}$ ) and the non-*N*-methylated 6,8-dimethoxy compound **1i** ( $\text{IC}_{50}$   $8.8 \pm 1.1 \mu\text{M}$ ) have superior potency over other disubstituted analogues. Dimethoxy derivatives **1k** ( $\text{IC}_{50}$   $10.8 \pm 1.5 \mu\text{M}$ ), **1l** ( $\text{IC}_{50}$   $4.1 \pm 0.6 \mu\text{M}$ ) and trimethoxy derivative **1m** ( $\text{IC}_{50}$   $10.0 \pm 1.4 \mu\text{M}$ ) and **1n** ( $\text{IC}_{50}$   $7.0 \pm 1.1 \mu\text{M}$ ) revealed a similar pattern but were less potent than the 6,8-dimethoxy derivatives. Surprisingly, a change in pattern was observed for other trimethoxylated series. Non-*N*-methylated **1o** ( $\text{IC}_{50}$   $6.0 \pm 0.6 \mu\text{M}$ ) had better potency than *N*-methylated **1p** ( $\text{IC}_{50}$   $10.8 \pm 1.3 \mu\text{M}$ ). A decrease in inhibitory potency for *N*-methylated 6,7,8-trimethoxy derivatives relative to 5,6,8-trimethoxy analogues and completely inactive tetramethoxy analogues **1q** and **1r** suggests that the steric bulk of the consecutive methoxy groups may force them to be pointed out of the planar ring system, making the  $\pi$ - $\pi$  stacking interaction with FAD unfavorable.

### X-Ray Structure Analysis

In order to gain insight into the binding mode of this new series of QR2 inhibitors, the X-ray crystal structures of compound **1** and one of the most potent analogs, **1l**, from the series in complex with human QR2 were determined. Complete X-ray data sets were collected and processed and the final structural models were refined to  $1.84 \text{ \AA}$  and  $1.98 \text{ \AA}$  for the compound **1** and compound **1l** complexes, respectively. The final data collection and refinement statistics are summarized in Table 2. QR2 is a dimer in solution and it crystalizes as a dimer in the asymmetric unit with two nearly equivalent catalytic sites defined as site 1 for chain A and site 2 for chain B.<sup>6</sup> Crystals of QR2 in complex with compound **1** produced strong electron density in Fo-Fc difference maps consistent with the natural product being bound in two different orientations within each active site of the dimer (Figure 1a). In contrast, QR2 crystals complexed with compound **1l** produced strong electron density for only one orientation of the inhibitor within each active site of the dimer (Figure 1b).

Two molecules of compound **1** were manually built within the electron density of the QR2-compound **1** active sites, and the model was refined with the occupancy of each molecule to 50%. The two orientations of compound **1** within the active site of a single monomer (site 1) are nearly identical to the orientations within the active site of the second monomer (site 2). The two orientations of compound **1** within a single site are related to each other by a  $180^\circ$  flip. In the first orientation (Figure 1a, color code yellow), the methoxy group of compound **1** points towards Phe178, whereas in the second orientation, it orients towards solvent-exposed surface (Figure 1a, color code green). Despite this difference, the interactions of compound **1** within the QR2 active sites are similar and involve mainly a hydrogen bond between the carbonyl oxygen of compound **1** and the side-chain amide nitrogen of Asn161, and a series of hydrophobic contacts with surrounding side chains and the flavin ring of FAD. These interactions are maintained for both compound **1** and compound **1l**.

The single orientation of **1l** is nearly identical between sites 1 and 2 of the dimer (Figure 1b). A superposition of site 1 from the QR2-compound **1** structure with site 1 of the QR2-**1l** structure reveals that one of the compound **1** orientations is nearly identical to that of **1l** with respect to the quinolinone ring (Figure 2). In general, a careful analysis of the two crystal structures reveal

that these two inhibitors, **1** and **11**, bind within the active site and sit parallel to the plane of the isoalloxazine ring of the bound cofactor FAD, occupying very similar positions as menadione in the QR2-menadione complex,<sup>22</sup> resveratrol in the QR2-resveratrol complex,<sup>13</sup> CB1954 in the QR2-CB1954 complex,<sup>23</sup> and melatonin in the QR2-melatonin complex.<sup>6</sup> All of these molecules bind deeply within the hydrophobic cleft of QR2, which is surrounded by side chains of Tyr132', Phe178', Phe126', Met164' and Cys121' from one side of a QR2 monomer, and Tyr155, Phe106 and the isoalloxazine ring of FAD from the other monomer. The side chain of Trp105 forms the bottom of the hydrophobic cleft and on one end of the cleft reside the side chains of Asn161 and the hydroxyl groups of Tyr155 and Tyr132. These latter two amino acid side chains form a relatively hydrophilic surface at one end of the cleft and also the hydroxyl group of Thr71' and main-chain carbonyls from Gly68 and Asp117 all point towards the cavity on the other end. The positions of these amino acid residues indicate that both ends have numerous functional groups accessible for hydrogen bonding. The fourth side of this unique 17 Å long and 7 Å wide cavity is accessible to the solvent. Because of the narrow width of this cavity, the enzyme can typically only accommodate molecules that can adopt nearly flat conformations. The crystal structures indicate that the benzene rings of these quinolinones stack with the C ring of FAD, similar to the benzene ring of CB1954. Hydrophobic interactions with Trp105, Gly68, Phe126, and Phe178 help to stabilize inhibitor binding via interactions with the quinolinone ring and carbonyl group. These two inhibitors occupy about two-thirds of the active site.

The observation that the weaker inhibitor, compound **1**, binds in two orientations within the active site of QR2 whereas the 10-fold more potent inhibitor **11** binds in only one orientation is reminiscent of our seminal observation that weak binding inhibitors of QR2, such as melatonin, tend to bind in multiple orientations whereas their higher affinity analogs, such as iodomelatonin, tend to bind in only one orientation.<sup>6</sup> In that study, we found that melatonin, with an IC<sub>50</sub> of 11.3 μM, binds in at least three orientations depending on which of three crystal structures of this complex are analyzed, whereas iodomelatonin, with an IC<sub>50</sub> of 1.1 μM, binds in only one orientation in the active sites of two independently determined X-ray structures. Our observation that inhibitor binding to QR2 can be a stochastic process dependent on the strength of the interaction has recently been supported by X-ray structures of QR2 in complex with dopamine and andrenochrome, where dopamine was found to bind in two orientations.<sup>24</sup>

### Aromatase Inhibitory Activity

An aromatase inhibition assay was performed on synthetic compound **1** and its analogues, as described in the Experimental Section. The IC<sub>50</sub> values of the compounds are reported in Table 1. Synthetic compound **1** was determined to have an IC<sub>50</sub> value of 3.9 ± 0.67 μM. The analogues showed good inhibitory potency against aromatase except for a few N-nonmethylated analogues (**1a**, **1c**, **1e**, **1i**, **11**) and two N-methylated analogues (**1d** and **12**). The replacement of the 6-methoxy group of compound **1** by a methyl group increased the inhibitory activity (**1b**, IC<sub>50</sub> = 1.2 ± 0.03 μM). However, removal of dioxole ring made the resulting compounds **1c** and **1d** inactive. Introduction of an 8-methoxy restored the inhibitory potency of **1f** (IC<sub>50</sub> 2.0 ± 0.1 μM), however, analogue **1e** was not active. Interestingly, 7,8-dimethoxy analogue **1g** (IC<sub>50</sub> 31.5 ± 3.1 μM) showed moderate inhibitory activity, but in comparison with the 8-methoxy N-methylated analogue **1f**, the activity of **1h** was reduced (IC<sub>50</sub> 5.7 ± 0.07 μM). The 6,8-dimethoxy derivative **1i** was found to be inactive, but its congener **1j** (IC<sub>50</sub> 0.96 ± 0.02 μM) showed very potent inhibitory activity in the aromatase assay. All other N-nonmethylated analogues (**1k**, **1m**, **1o**, **1q**) and N-methylated analogues (**1l**, **1n**, **1p**, **1r**) showed a similar trend as we have seen for 7,8-dimethoxy analogues (**1g**, **1h**), with the N-methylated inhibitors being more potent. Inhibitory activity for **1j**, **1l** and **1r** are almost the same and the IC<sub>50</sub> values are in submicromolar range. 5,6,8-Trimethoxy-N-methylated analogue **1p** displayed the best

inhibitory potency, with an  $IC_{50}$  value of  $0.1 \pm 0.004 \mu M$ , which compares favorably with the clinically useful aromatase inhibitor aminoglutethimide. These observations show that N-methylated analogues are better aromatase inhibitors and methoxy substitution on the ring makes a significant contribution to the potency.

### Cytotoxicity Studies

Ideally, potential chemotherapeutic agents should not be highly cytotoxic. In order to evaluate this aspect, the cytotoxicities of the compounds were evaluated in four different cell lines, and the data are presented in Table 1. The results indicate that the present series of quinolinones are in general not significantly cytotoxic. Those expressing a low degree of cytotoxicity were compounds **1o**, **1p**, and **12**.

### Conclusion

An efficient total synthesis of compound **1** and a series of compound **1**-based QR2 and aromatase inhibitors have been successfully carried out. Structural modification of the parent natural product resulted in the most active compound **1j** vs. QR2 and **1p** vs. aromatase. The crystal structures of compound **1** and one of the potent analogues **1l** in complex with QR2 provide a detailed description of the enzyme active site that can be utilized in the further design of new QR2 inhibitors.

The present series of compounds inhibits both aromatase and QR2, two distinct targets for the development of chemopreventive agents. As is the case with combination drug therapy, dual targeting through a pleiotropic mechanism offers the potential to enhance efficacy if the two drug targets can function in a synergistic fashion.

### Experimental Section

NMR spectra were obtained at 300 MHz ( $^1H$ ) and 75 MHz ( $^{13}C$ ) in  $CDCl_3$  using  $CHCl_3$  as internal standard. Flash chromatography was performed with 230–400 mesh silica gel. TLC was carried out using commercially available precoated glass silica gel plates of 2.5 mm thickness. Melting points are uncorrected. Microanalyses were performed at the Purdue Microanalysis Laboratory. All yields refer to yields of isolated compounds. Unless otherwise stated, chemicals and solvents were of reagent grade and used as obtained from commercial sources without further purification. Tetrahydrofuran (THF) was freshly distilled from sodium/benzophenone ketyl radical, acetone from potassium carbonate, dichloromethane from  $CaH_2$ , benzene from  $P_2O_5$ , and triethylamine from KOH prior to use.

#### 6-Methoxy-9-methyl-[1,3]dioxolo[4,5-*h*]quinolin-8(9*H*)-one (**1**)

A suspension of hydroxyquinolone **9** (300 mg, 1.3 mmol) in ether (1 mL) at 0 °C was treated with excess TMS-diazomethane (2 M in hexanes, 10 mL) and the mixture was stirred for 20 min. Absolute methanol (10 mL) was added dropwise and the mixture was stirred for 6 h. An additional amount of TMS-diazomethane (2 M in hexanes, 5 mL) and absolute methanol (5 mL) were added successively to the mixture and the mixture was stirred for another 12 h. After completion of the reaction, the mixture was treated with a solution of dilute acetic acid (0.5 mL) to decompose the excess diazomethane. Evaporation of the solution and purification by silica gel column chromatography, eluting with an acetone-hexane (1:1) mixture, yielded compound **1** (240 mg, 78%) as a colorless solid: mp 199–201 °C (lit<sup>18</sup> mp 202–203 °C). IR (KBr) 3319, 1735, 1645, 1592, 1444, 1465, 1331, 1265, 1232, 1159, 1057, 940  $cm^{-1}$ ;  $^1H$  NMR ( $CDCl_3$ , 300 MHz)  $\delta$  7.51 (d,  $J = 8.4$  Hz, 1 H), 6.74 (d,  $J = 8.4$  Hz, 1 H), 6.01 (s, 2 H), 5.86 (s, 1 H), 3.87 (s, 3 H) 3.80 (s, 3 H);  $^{13}C$  NMR ( $CDCl_3$ , 75 MHz)  $\delta$  164.0, 162.6, 149.8, 133.5, 126.5, 118.0, 112.9, 104.2, 100.2, 94.5, 55.8, 31.9; EIMS ( $m/z$ , relative intensity) 233 ( $M^+$ ,

100), 218 (26), 190 (31), 176 (24), 160 (10), 146 (9), 132 (8), 117 (7), 69 (17); HRMS calcd for C<sub>12</sub>H<sub>11</sub>NO<sub>4</sub> 233.0688, found 233.0692. Anal. (C<sub>12</sub>H<sub>11</sub>NO<sub>4</sub>) C, H, N.

### General Procedures for Cyclization

**Method A: Using H<sub>2</sub>SO<sub>4</sub>**—A solution of the acetoacetamide (2 mmol) in concd sulfuric acid (5 mL) was stirred at room temperature for 30–60 min. The reaction mixture was poured on ice (1 g) and neutralized or slightly basified to pH ~8 with 28–30% aqueous ammonia (5–10 mL). The aqueous solution was extracted with chloroform (3 × 50 mL) and the organic extract was dried and evaporated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel, eluting with ethyl ether or ethyl acetate-hexane mixture to produce the pure product in moderate yield.

**Method B: Using Polyphosphoric Acid (PPA)**—The acetoacetamide (2 mmol) was added to polyphosphoric acid (5 g) at 95–100 °C and the mixture was allowed to stir for 2.5 h at the same temperature, and then poured into ice-water. After polyphosphoric acid had dissolved, the solution was neutralized with 4 N NaOH solution. The deposited solid was collected by filtration or extracted with chloroform and evaporated to dryness. Solid was purified by column chromatography on silica gel, eluting with ethyl ether or ethyl acetate-hexane mixture to yield the pure product in higher yield than obtained with method A.

**Method C: Using Trifluoroacetic Acid**—A solution of the acetoacetamide (2 mmol) in trifluoroacetic acid (5 mL) was stirred at room temperature for 24 h. The reaction mixture was evaporated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel, eluting with ethyl ether or ethyl acetate-hexane mixture to afford the pure product in good yield. This method was used when isolation of the product was very difficult with methods A and B because of solubility issues.

### 6-Methyl-[1,3]dioxolo[4,5-*h*]quinolin-8(9*H*)-one (1a)

This compound was prepared in 82% yield by following Method C. Colorless solid: mp 285 °C (dec). IR (KBr) 1655, 1645, 1573, 1477, 1435, 1405, 1395, 1292, 1242 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 7.31 (d, *J* = 8.7 Hz, 1 H), 6.94 (dd, *J* = 8.4, 0.9 Hz, 1 H), 6.49 (s, 1 H), 6.19 (d, *J* = 0.9 Hz, 2 H), 2.51 (s, 3 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) δ 163.8, 152.8, 149.6, 134.2, 121.7, 119.1, 117.6, 116.1, 106.5, 103.3, 19.7; EIMS (*m/z*, relative intensity) 203 (M<sup>+</sup>, 100), 174 (17), 161 (6), 146 (35), 117 (27), 90 (11), 69 (23), 57 (27); HRMS calcd for C<sub>11</sub>H<sub>9</sub>NO<sub>3</sub> 203.0582, found 203.0584. Anal. (C<sub>11</sub>H<sub>9</sub>NO<sub>3</sub>) C, H, N.

### 6,9-Dimethyl-[1,3]dioxolo[4,5-*h*]quinolin-8(9*H*)-one (1b)

This compound was prepared in 73% yield by following Method B. Colorless solid: 158–159 °C. IR (KBr) 1652, 1590, 1506, 1416, 1382, 1328, 1261, 1124, 1093, 1050, 1004, 939, 753 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 7.19 (d, *J* = 8.4 Hz, 1 H), 6.77 (d, *J* = 8.4 Hz, 1 H), 6.39 (s, 1 H), 6.01 (s, 2 H), 3.84 (s, 3 H), 2.35 (s, 3 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) δ 162.0, 149.0, 146.3, 133.5, 126.2, 119.5, 118.6, 117.9, 104.1, 100.8, 32.0, 19.4; EIMS (*m/z*, relative intensity) 217 (M<sup>+</sup>, 100), 202 (6), 188 (41), 174 (42), 160 (20), 146 (10), 130 (25), 116 (8), 89 (14), 63 (11); HRMS calcd for C<sub>12</sub>H<sub>11</sub>NO<sub>3</sub> 217.0739, found 217.0738. Anal. (C<sub>12</sub>H<sub>11</sub>NO<sub>3</sub>) C, H, N.

### 4-Methylquinolin-2(1*H*)-one (1c)

This compound was prepared in 62% yield as a colorless solid by following Method B: mp 214–216 °C (lit<sup>25</sup> mp 220–222 °C). IR (KBr) 3300, 2800, 1658, 1635, 1557, 1508, 1492, 1435, 1371, 1261 cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 300 MHz) δ 7.69 (d, *J* = 8.1 Hz, 1 H), 7.49 (m, 1 H), 7.29 (d, *J* = 8.1 Hz, 1 H), 7.18 (m, 1 H), 6.39 (s, 1 H), 2.41 (s, 3 H); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 75 MHz) δ 161.6, 147.9, 138.6, 130.2, 124.7, 121.6, 120.8, 119.5, 115.4, 18.4; EIMS (*m/z*, relative

intensity) 159 ( $M^+$ , 44), 130 (100), 103 (9), 89 (5), 77 (11), 63 (6), 51 (7); HRMS calcd for  $C_{10}H_9NO$  159.0684, found 159.0683. Anal. ( $C_{10}H_9NO$ ) C, H, N.

#### 1,4-Dimethylquinolin-2(1H)-one (1d)

This compound was prepared in 82% yield as a colorless solid by following Method B: mp 132–134 °C (lit<sup>26</sup> mp 130–132 °C). IR (KBr) 1652, 1615, 1592, 1562, 1454, 1370, 1321  $cm^{-1}$ ;  $^1H$  NMR ( $CDCl_3$ , 300 MHz)  $\delta$  7.68 (dd,  $J = 1.2, 7.8$  Hz, 1 H), 7.54 (dd,  $J = 1.2, 8.4$  Hz, 1 H), 7.34 (d,  $J = 8.4$  Hz, 1 H), 7.23 (dd,  $J = 1.2, 7.8$  Hz, 1 H), 6.57 (s, 1 H), 3.67 (s, 3 H), 2.43 (s, 3 H);  $^{13}C$  NMR ( $CDCl_3$ , 75 MHz)  $\delta$  162.0, 146.3, 139.7, 130.4, 125.1, 121.8, 121.3, 121.0, 114.3, 29.1, 18.9; EIMS ( $m/z$ , relative intensity) 173 ( $M^+$ , 82), 144 (100), 130 (71), 115 (15), 103 (12), 89 (7), 77 (17), 63 (8), 51 (9); HRMS calcd for  $C_{11}H_{11}NO$  173.0841, found 173.0841. Anal. ( $C_{11}H_{11}NO$ ) C, H, N.

#### 8-Methoxy-4-methylquinolin-2(1H)-one (1e)

This compound was prepared in 90% yield as a colorless solid by following Method B: mp 188–190 °C (lit<sup>27</sup> 187–189 °C). IR (KBr) 3400, 2800, 1649, 1605, 1473, 1463, 1390, 1266  $cm^{-1}$ ;  $^1H$  NMR ( $CDCl_3$ , 300 MHz)  $\delta$  9.33 (brs, 1 H), 7.25 (d,  $J = 8.1$  Hz, 1 H), 6.79 (m, 1 H), 6.97 (d,  $J = 8.1$  Hz, 1 H), 6.53 (s, 1 H), 3.96 (s, 3 H), 2.45 (s, 3 H);  $^{13}C$  NMR ( $CDCl_3$ , 75 MHz)  $\delta$  161.6, 148.7, 145.5, 128.1, 121.7, 121.2, 120.5, 116.1, 109.7, 55.9, 19.1; EIMS ( $m/z$ , relative intensity) 189 ( $M^+$ , 100), 174 (17), 160 (58), 146 (32), 130 (27), 118 (31), 91 (21), 65 (20), 51 (14); HRMS calcd for  $C_{11}H_{11}NO_2$  189.0790, found 189.0787. Anal. ( $C_{11}H_{11}NO_2$ ) C, H, N.

#### 8-Methoxy-1,4-dimethylquinolin-2(1H)-one (1f)

This compound was prepared in 72% yield as a yellowish solid by following Method B: mp 72–74 °C (lit<sup>28</sup> mp 69–70.5 °C). IR (KBr) 1657, 1611, 1595, 1459, 1385, 1260  $cm^{-1}$ ;  $^1H$  NMR ( $CDCl_3$ , 300 MHz)  $\delta$  7.36 (d,  $J = 8.1$  Hz, 1 H), 7.32 (m, 1 H), 7.11 (d,  $J = 8.1$  Hz, 1 H), 6.64 (s, 1 H), 3.97 (s, 3 H), 3.95 (s, 3 H), 2.47 (s, 3 H);  $^{13}C$  NMR ( $CDCl_3$ , 75 MHz)  $\delta$  163.2, 148.6, 145.9, 131.1, 123.4, 122.3, 121.2, 117.4, 113.5, 56.3, 35.2, 19.4; EIMS ( $m/z$ , relative intensity) 203 ( $M^+$ , 80), 188 (100), 173 (27), 160 (51), 144 (43), 130 (45), 103 (7), 69 (13), 55 (12); HRMS calcd for  $C_{12}H_{13}NO_2$  203.0946, found 203.0946. Anal. ( $C_{12}H_{13}NO_2$ ) C, H, N.

#### 7,8-Dimethoxy-4-methylquinolin-2(1H)-one (1g)

This compound was prepared in 55% yield as a colorless solid by following Method A: mp 187–189 °C. IR (KBr) 1651, 1620, 1600, 1515, 1469, 1396, 1284, 1104  $cm^{-1}$ ;  $^1H$  NMR ( $CDCl_3$ , 300 MHz)  $\delta$  9.26 (brs, 1 H), 7.28 (d,  $J = 8.1$  Hz, 1 H), 6.77 (d,  $J = 8.1$  Hz, 1 H), 6.61 (s, 1 H), 3.88 (s, 3 H), 3.87 (s, 3 H), 2.35 (s, 3 H);  $^{13}C$  NMR ( $CDCl_3$ , 75 MHz)  $\delta$  162.3, 152.5, 148.6, 133.6, 132.3, 119.9, 118.6, 115.3, 107.2, 60.8, 56.0, 18.9; EIMS ( $m/z$ , relative intensity) 219 ( $M^+$ , 100), 204 (43), 190 (19), 130 (12), 69 (17), 43 (16); HRMS calcd for  $C_{12}H_{13}NO_3$  219.0895, found 219.0895. Anal. ( $C_{12}H_{13}NO_3$ ) C, H, N.

#### 7,8-Dimethoxy-1,4-dimethylquinolin-2(1H)-one (1h)

This compound was prepared in 78% yield as a colorless solid by following Method B: mp 118–120 °C. IR (KBr) 1655, 1589, 1557, 1454, 1387, 1324, 1268, 1057, 795  $cm^{-1}$ ;  $^1H$  NMR ( $CDCl_3$ , 300 MHz)  $\delta$  7.35 (d,  $J = 9.0$  Hz, 1 H), 6.85 (d,  $J = 9.0$  Hz, 1 H), 6.40 (s, 1 H), 3.91 (s, 3 H), 3.87 (s, 3 H), 3.72 (s, 3 H), 2.34 (s, 3 H);  $^{13}C$  NMR ( $CDCl_3$ , 75 MHz)  $\delta$  163.8, 154.9, 145.9, 137.0, 135.0, 120.9, 118.9, 117.6, 107.0, 61.6, 56.1, 33.8, 19.2; EIMS ( $m/z$ , relative intensity) 233 ( $M^+$ , 61), 218 (93), 202 (35), 175 (100), 130 (20), 118 (18), 77 (22), 51 (17); HRMS calcd for  $C_{13}H_{15}NO_3$  233.1052, found 233.1057. Anal. ( $C_{13}H_{15}NO_3$ ) C, H, N.



**6,8-Dimethoxy-4-methylquinolin-2(1H)-one (1i)**

This compound was prepared in 67% yield as a light brown solid by following Method B: mp 232–234 °C (lit<sup>29</sup> mp 231–233 °C). IR (KBr) 1653, 1619, 1605, 1462, 1399, 1380, 1277, 1217 cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 300 MHz) δ 10.5 (brs, 1 H), 6.80 (s, 1 H), 6.72 (s, 1 H), 6.41 (s, 1 H), 3.87 (s, 3 H), 3.81 (s, 3 H), 2.40 (s, 3 H); EIMS (*m/z*, relative intensity) 219 (M<sup>+</sup>, 100), 203 (15), 190 (5), 176 (63), 161 (10), 133 (9), 105 (4), 69 (14), 55 (14); HRMS calcd for C<sub>12</sub>H<sub>13</sub>NO<sub>3</sub> 219.0895, found 219.0894. Anal. (C<sub>12</sub>H<sub>13</sub>NO<sub>3</sub>) C, H, N.

**6,8-Dimethoxy-1,4-dimethylquinolin-2(1H)-one (1j)**

This compound was prepared in 17% yield as a colorless solid by following Method B: mp 157–159 °C. IR (KBr) 1656, 1619, 1595, 1577, 1456, 1394, 1317, 1276, 1166 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 6.50 (m, 2 H), 6.43 (s, 1 H), 3.74 (s, 3 H), 3.73 (s, 3 H), 3.72 (s, 3 H), 2.23 (s, 3 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) δ 162.5, 154.5, 149.5, 145.2, 125.7, 123.4, 121.5, 102.6, 98.3, 56.0, 55.2, 34.8, 19.4; EIMS (*m/z*, relative intensity) 233 (M<sup>+</sup>, 100), 218 (70), 188 (68), 175 (35), 160 (23), 147 (26), 118 (7), 97 (10), 69 (36); HRMS calcd for C<sub>12</sub>H<sub>13</sub>NO<sub>3</sub> 233.1052, found 233.1048. Anal. (C<sub>12</sub>H<sub>13</sub>NO<sub>3</sub>) C, H, N.

**5,8-Dimethoxy-4-methylquinolin-2(1H)-one (1k)**

This compound was prepared in 84% yield as a light brown solid by following Method A: mp 187–189 °C (lit<sup>30</sup> mp 188–189 °C). IR (KBr) 3400, 2800, 1651, 1613, 1489, 1463, 1441, 1369, 1266 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 9.09 (brs, 1 H), 6.79 (d, *J* = 8.7 Hz, 1 H), 6.44 (d, *J* = 8.7 Hz, 1 H), 6.31 (s, 1 H), 3.84 (s, 3 H), 3.77 (s, 3 H), 2.56 (s, 3 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) δ 161.2, 151.9, 150.3, 139.6, 129.8, 121.4, 111.4, 109.9, 102.1, 56.1, 55.5, 24.5; EIMS (*m/z*, relative intensity) 219 (M<sup>+</sup>, 41), 204 (100), 190 (14), 176 (42), 161 (32), 148 (12), 133 (23), 120 (5), 104 (9), 89 (6), 77 (8); HRMS calcd for C<sub>12</sub>H<sub>13</sub>NO<sub>3</sub> 219.0895, found 219.0896. Anal. (C<sub>12</sub>H<sub>13</sub>NO<sub>3</sub>) C, H, N.

**5,8-Dimethoxy-1,4-dimethylquinolin-2(1H)-one (1l)**

This compound was prepared in 80% yield as a colorless solid by following Method B: mp 122–124 °C (lit<sup>20</sup> mp 122–123 °C). IR (KBr) 1644, 1593, 1478, 1459, 1437, 1398, 1263 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 6.98 (d, *J* = 8.7 Hz, 1 H), 6.58 (d, *J* = 8.7 Hz, 1 H), 6.41 (s, 1 H), 3.80 (s, 3 H), 3.79 (s, 3 H), 3.77 (s, 3 H), 2.55 (s, 3 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) δ 163.8, 152.7, 147.4, 143.2, 133.9, 121.9, 115.4, 104.1, 57.8, 55.8, 36.0, 25.1; EIMS (*m/z*, relative intensity) 233 (M<sup>+</sup>, 52), 218 (100), 203 (38), 190 (34), 175 (68), 147 (14), 132 (10), 118 (12), 91 (8), 77 (7); HRMS calcd for C<sub>13</sub>H<sub>15</sub>NO<sub>3</sub> 233.1052, found 233.1056. Anal. (C<sub>13</sub>H<sub>15</sub>NO<sub>3</sub>) C, H, N.

**6,7,8-Trimethoxy-4-methylquinolin-2(1H)-one (1m)**

This compound was prepared in 70% yield as a colorless solid by following Method B: mp 218–220 °C. IR (KBr) 1647, 1598, 1495, 1461, 1406, 1388, 1378, 1276, 1195, 1120, 1015, 981, 795 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 9.28 (brs, 1 H), 6.76 (s, 1 H), 6.44 (s, 1 H), 3.97 (s, 3 H), 3.91 (s, 3 H), 3.88 (s, 3 H), 2.39 (s, 3 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) δ 161.8, 149.0, 147.9, 143.7, 139.1, 127.0, 120.5, 115.9, 100.9, 61.3, 61.1, 56.2, 19.2; EIMS (*m/z*, relative intensity) 249 (M<sup>+</sup>, 100), 234 (88), 220 (24), 203 (16), 191 (50), 176 (7), 163 (23), 148 (19), 134 (4), 120 (16), 92 (6), 65 (7); HRMS calcd for C<sub>13</sub>H<sub>15</sub>NO<sub>4</sub> 249.1001, found 249.1005. Anal. (C<sub>13</sub>H<sub>15</sub>NO<sub>4</sub>) C, H, N.

**6,7,8-Trimethoxy-1,4-dimethylquinolin-2(1H)-one (1n)**

This compound was prepared by following Method B in 85% yield as a colorless solid: mp 144–146 °C. IR (KBr) 1655, 1611, 1596, 1554, 1421, 1452, 1392, 1280, 1114, 1045, 915, 795

$\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  6.73 (s, 1 H), 6.38 (s, 1 H), 3.84 (s, 3 H), 3.80 (s, 3 H), 3.75 (s, 3 H), 3.70 (s, 3 H), 2.26 (s, 3 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  162.8, 148.3, 145.5, 145.2, 142.5, 129.4, 120.1, 118.2, 102.0, 61.6, 60.8, 55.8, 33.5, 19.2; EIMS ( $m/z$ , relative intensity) 263 ( $\text{M}^+$ , 100), 248 (86), 205 (70), 162 (34), 133 (29), 124 (15), 93 (26), 69 (46); HRMS calcd for  $\text{C}_{14}\text{H}_{17}\text{NO}_4$  263.1058, found 263.1160; Anal. ( $\text{C}_{14}\text{H}_{17}\text{NO}_4$ ) C, H, N.

#### 5,6,8-Trimethoxy-4-methylquinolin-2(1H)-one (1o)

This compound was prepared as a colorless solid in 74% yield by following Method B: mp 149–151 °C (lit31 mp 150–152 °C). IR (KBr) 1649, 1605, 1567, 1472, 1381, 1363, 1245, 1203, 1165, 1077, 1050, 1020, 971, 795  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  9.09 (brs, 1 H), 6.64 (s, 1 H), 6.31 (s, 1 H), 3.83 (s, 3 H), 3.80 (s, 3 H), 3.75 (s, 3 H), 2.53 (s, 3 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  160.7, 148.7, 147.1, 141.4, 140.2, 123.1, 115.0, 99.7, 61.0, 57.0, 56.1, 23.1; EIMS ( $m/z$ , relative intensity) 249 ( $\text{M}^+$ , 76), 234 (100), 206 (56), 191 (25), 176 (5), 163 (10), 134 (7), 77 (6), 51 (4); HRMS calcd for  $\text{C}_{13}\text{H}_{15}\text{NO}_4$  249.1001, found 249.1004. Anal. ( $\text{C}_{13}\text{H}_{15}\text{NO}_4$ ) C, H, N.

#### 5,6,8-Trimethoxy-1,4-dimethylquinolin-2(1H)-one (1p)

Sodium hydride (60% oil dispersion, 10 mg, 0.25 mmol) was added to a solution of compound **1o** (50 mg, 0.2 mmol) in dry DMF (1 mL) and the mixture was stirred at 75–85 °C for 2 h. The reaction mixture was cooled to room temperature and methyl iodide (0.05 mL, 0.76 mmol) was added. After being stirred at 80 °C for 6 h, the reaction mixture was quenched with ice water and extracted with ether. The organic layer was dried and evaporated to dryness under reduced pressure. The crude product was purified by silica gel column chromatography, eluting with ethyl acetate-hexanes (1:1), to yield compound **1p** (40 mg, 74%) and 2,5,6,8-tetramethoxy-4-methylquinoline (5 mg, 10%). **1p**: yellowish solid, mp 128–130 °C (lit31 mp 130–131 °C). IR (KBr) 1657, 1606, 1463, 1362, 1260  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  6.78 (s, 1 H), 6.45 (s, 1 H), 3.88 (s, 3 H), 3.82 (s, 3 H), 3.77 (s, 3 H), 3.76 (s, 3 H), 2.57 (s, 3 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  162.9, 147.7, 146.1, 144.9, 141.1, 127.2, 123.6, 118.4, 103.0, 61.3, 57.6, 56.8, 35.8, 23.7; EIMS ( $m/z$ , relative intensity) 263 ( $\text{M}^+$ , 100), 248 (43), 233 (26), 220 (47), 205 (40), 190 (12), 176 (11), 162 (10), 134 (8), 91 (7), 69 (15); HRMS calcd for  $\text{C}_{14}\text{H}_{17}\text{NO}_4$  263.1158, found 263.1160. Anal. ( $\text{C}_{14}\text{H}_{17}\text{NO}_4$ ) C, H, N.

#### 5,6,7,8-Tetramethoxy-4-methylquinolin-2(1H)-one (1q)

This compound was prepared as a brown solid in 70% yield by following Method B: mp 158–160 °C. IR (KBr) 1653, 1597, 1556, 1483, 1393, 1378, 1259, 1170, 1099, 1073, 1012, 952  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  9.65 (brs, 1 H), 6.27 (s, 1 H), 3.93 (s, 3 H), 3.85 (s, 3 H), 3.81 (s, 3 H), 3.80 (s, 3 H), 2.52 (s, 3 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  161.6, 148.9, 147.8, 147.6, 141.8, 134.6, 129.2, 121.3, 110.8, 61.2, 23.1; EIMS ( $m/z$ , relative intensity) 279 ( $\text{M}^+$ , 88), 264 (100), 249 (6), 236 (45), 221 (37), 206 (20), 178 (29), 135 (6), 79 (4); HRMS calcd for  $\text{C}_{14}\text{H}_{17}\text{NO}_5$  279.1107, found 279.1108. Anal. ( $\text{C}_{14}\text{H}_{17}\text{NO}_5$ ) C, H, N.

#### 5,6,7,8-Tetramethoxy-1,4-dimethylquinolin-2(1H)-one (1r)

Yellowish solid, mp 66–68 °C. IR (KBr) 1655, 1578, 1551, 1458, 1416, 1400, 1387, 1372, 1260, 1089, 1043, 1021  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  6.35 (s, 1 H), 3.99 (s, 3 H), 3.85 (s, 3 H), 3.83 (s, 3 H), 3.76 (s, 3 H), 3.68 (s, 3 H), 2.53 (s, 3 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  163.1, 149.8, 148.0, 146.3, 142.0, 138.5, 132.5, 121.6, 113.6, 61.8, 61.4, 61.3, 61.1, 35.2, 23.7; EIMS ( $m/z$ , relative intensity) 293 ( $\text{M}^+$ , 100), 278 (90), 263 (21), 248 (40), 220 (38), 192 (28), 164 (7), 131 (4), 123 (11), 93 (10), 84 (15); HRMS calcd for  $\text{C}_{15}\text{H}_{19}\text{NO}_5$  293.1263, found 293.1265. Anal. ( $\text{C}_{14}\text{H}_{17}\text{NO}_4$ ) C, H, N.

### 3-(Benzo[d][1,3]dioxol-4-yl(methyl)amino)-3-oxopropanoic Acid (**2**)

A mixture of diketo ester **8** (2.0 g, 7.5 mmol) and 5% aqueous sodium hydroxide solution (15 mL) was heated at 60 °C for 2 h. The solution was concentrated to about one-quarter of the original volume and cooled in an ice bath. The mixture was acidified to pH 3.5 by the addition of 2 N HCl. The mixture was extracted with chloroform, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated to dryness under vacuum to give acid **2** (1.5 g, 85%) as colorless solid: mp 115–117 °C. IR (KBr) 2914, 1737, 1663, 1614, 1486, 1459, 1386, 1362, 1253, 1196, 1054, 1028, 794 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 6.83 (m, 2 H), 6.64 (dd, *J* = 7.8, 1.8 Hz, 1 H), 6.00 (s, 2 H), 3.26 (s, 3 H), 3.21 (s, 2 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) δ 170.0, 167.8, 149.1, 142.4, 123.1, 123.0, 119.7, 109.2, 101.9, 36.3, 36.2; EIMS (*m/z*, relative intensity) 237 (M<sup>+</sup>, 35), 220 (3), 193 (10), 151 (100), 122 (23), 93 (49), 69 (17), 55 (26); HRMS calcd for C<sub>11</sub>H<sub>11</sub>NO<sub>5</sub> 237.0637, found 237.0644.

### General Procedure for CBz Group Deprotection

A mixture of CBz-protected amine (5.0 mmol) and 10% Pd-C catalyst (300 mg) in EtOAc (30 mL) was hydrogenated at 20 psi for 6 h. The suspension was filtered and the filtrate was evaporated. The residue was dissolved in CHCl<sub>3</sub> (30 mL) and the solution was extracted with dilute HCl (3 × 50 mL). The combined aqueous extracts were then made basic with concd NH<sub>4</sub>OH, and the aqueous mixture was again extracted with CHCl<sub>3</sub>. The combined extracts were dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated to provide amines in high yield, which were pure enough to use for the next step without further purification. A little portion of each compound was taken out and purified by silica gel column chromatography for analysis.

### Benzo[d][1,3]dioxol-4-amine (**3a**)

Yield 92%. Colorless oil. IR (KBr) 3360, 2887, 1654, 1499, 1467, 1358, 1255, 1212, 1212, 1050, 925 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 6.70 (m, 1 H), 6.37 (m, 2 H), 5.9 (s, 2 H), 3.37 (brs, 2 H); EIMS (*m/z*, relative intensity) 137 (M<sup>+</sup>, 84), 108 (8), 91 (8), 79 (80), 52 (100); HRMS calcd for C<sub>7</sub>H<sub>7</sub>NO<sub>2</sub> 137.0477, found 137.0478.

### *N*-Methylbenzo[d][1,3]dioxol-4-amine (**3b**)

Yield 95%. Colorless oil. IR (neat) 3401, 2972, 1646, 1474, 1454, 1353, 1275, 1258, 1092, 1052, 926 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 6.73 (t, *J* = 8.1 Hz, 1 H), 6.29 (m, 2 H), 5.87 (s, 2 H), 3.58 (brs, 1 H), 2.86 (s, 3 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) δ 146.9, 134.0, 133.6, 122.3, 105.7, 100.4, 98.9, 30.6; positive ion ESIMS (*m/z*, relative intensity) 152 (MH<sup>+</sup>, 100), 136 (35), 133 (8), 105 (4); HRMS calcd for C<sub>8</sub>H<sub>9</sub>NO<sub>2</sub> 152.0711, found 152.0712.

### 2,3-Dimethoxyaniline (**3g**)

Yield 96%. Colorless oil. IR (neat) 3367, 2938, 1613, 1499, 1478, 1321, 1264, 1228, 1132, 1087, 1052, 1003, 775 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 6.81 (t, *J* = 8.4 Hz, 1 H), 6.25 (m, 2 H), 3.84 (m, 2 H), 3.80 (s, 3 H), 3.79 (s, 3 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) δ 152.8, 140.5, 135.7, 124.0, 108.6, 102.0, 59.5, 55.5; positive ion ESIMS (*m/z*, relative intensity) 153 (M<sup>+</sup>, 100), 138 (100), 110 (6), 95 (25), 67 (10); HRMS calcd for C<sub>8</sub>H<sub>11</sub>NO<sub>2</sub> 153.0790, found 153.0786.

### 2,3-Dimethoxy-*N*-methylaniline (**3h**)

Yield 93%. Colorless oil. IR (neat) 3417, 1935, 1602, 1514, 1482, 1304, 1263, 1218, 1161, 1110, 1079, 1050, 1003 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 6.93 (t, *J* = 8.1 Hz, 1 H), 6.31 (m, 2 H), 4.30 (brs, 1 H), 3.84 (s, 3 H), 3.77 (s, 3 H), 2.82 (s, 3 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) δ 152.2, 143.4, 135.1, 124.3, 124.1, 108.7, 103.7, 101.0, 59.7, 55.6, 30.4; positive ion ESIMS

( $m/z$ , relative intensity) 168 ( $MH^+$ , 100), 166 (13), 152 (32), 137 (8); HRMS calcd for  $C_9H_{13}NO_2 + H^+$  168.1024, found 168.1025.

### 2,4-Dimethoxyaniline (3i)

Yield 94%. Colorless solid: mp 34–36 °C (lit<sup>32</sup> 33–35 °C). IR (neat) 3436, 2939, 2833, 1597, 1515, 1465, 1285, 1240, 1206, 1154, 1035, 919  $cm^{-1}$ ;  $^1H$  NMR ( $CDCl_3$ , 300 MHz)  $\delta$  6.80 (d,  $J = 8.1$  Hz, 1 H), 6.63 (d,  $J = 2.7$  Hz, 1 H), 6.52 (dd,  $J = 8.1, 2.7$  Hz, 1 H), 3.96 (s, 3 H), 3.91 (s, 3 H);  $^{13}C$  NMR ( $CDCl_3$ , 75 MHz)  $\delta$  152.6, 147.9, 129.5, 114.8, 103.8, 98.9, 55.3, 55.0; EIMS ( $m/z$ , relative intensity) 153 (90), 138 (100), 110 (51), 95 (41), 79 (8), 67 (18), 52 (26); HRMS calcd for  $C_8H_{11}NO_2$  153.0790, found 153.0788.

### 2,4-Dimethoxy-*N*-methylaniline (3j)

Colorless oil; IR (neat) 3419, 2935, 2831, 1596, 1520, 1454, 1438, 1286, 1259, 1206, 1153, 1034, 918  $cm^{-1}$ ;  $^1H$  NMR (300 MHz,  $CDCl_3$ )  $\delta$  6.50 (m, 3 H), 3.80 (s, 3 H), 3.76 (s, 3 H), 2.83 (s, 3 H);  $^{13}C$  NMR (75 MHz,  $CDCl_3$ )  $\delta$  151.4, 147.6, 133.5, 109.0, 103.3, 98.3, 55.2, 54.9, 30.6; EIMS ( $m/z$ , relative intensity) 167 (75), 152(100), 124 (27), 109 (28), 93 (7), 80 (6), 66 (7), 52 (9); HRMS calcd for  $C_9H_{13}NO_2$  167.0946, found 167.0947.

### 2,3,4-Trimethoxyaniline (3m)

Yield 90%. Colorless oil: IR (neat) 3442, 2937, 1496, 1478, 1424, 1266, 1204, 1128, 1085, 1041, 1012  $cm^{-1}$ ;  $^1H$  NMR ( $CDCl_3$ , 300 MHz)  $\delta$  6.33 (d,  $J = 8.1$  Hz, 1 H), 6.35 (d,  $J = 8.1$  Hz, 1 H), 3.79 (s, 3 H), 3.78 (s, 3 H), 3.69 (s, 3 H);  $^{13}C$  NMR ( $CDCl_3$ , 75 MHz)  $\delta$  145.7, 142.8, 141.1, 134.2, 109.3, 108.1, 60.6, 60.1, 56.5; EIMS ( $m/z$ , relative intensity) 183 ( $M^+$ , 100), 168 (87), 153 (15), 140 (12), 125 (43), 110 (17), 108 (12), 82 (16), 54 (31); HRMS calcd for  $C_9H_{13}NO_3$  183.0895, found 183.0897.

### 2,3,4-Trimethoxy-*N*-methylaniline (3n)

Colorless oil; IR (neat) 3411, 2936, 2830, 1611, 1506, 1486, 1268, 1228, 1203, 1158, 1094, 1026, 937  $cm^{-1}$ ;  $^1H$  NMR (300 MHz,  $CDCl_3$ )  $\delta$  6.55 (d,  $J = 8.7$  Hz, 1 H), 6.24 (d,  $J = 8.7$  Hz, 1 H), 3.84 (s, 3 H), 3.82 (s, 3 H), 3.75 (s, 3 H), 2.76 (s, 3 H);  $^{13}C$  NMR (75 MHz,  $CDCl_3$ )  $\delta$  144.8, 142.5, 140.6, 137.8, 108.0, 104.0, 60.6, 60.1, 56.4, 30.7; EIMS ( $m/z$ , relative intensity) 197 (91), 182 (100), 167 (29), 152 (10), 139 (20), 136 (6), 124 (16), 110 (5), 96 (15), 68 (22), 53 (8); HRMS calcd for  $C_{10}H_{15}NO_3$  197.1052, found 197.1050.

### 2,4,5-Trimethoxyaniline (3o)

Yield 94%. Colorless solid: mp 84–86 °C. IR (KBr) 3392, 1527, 1468, 1413, 1233, 1204, 1188, 1044, 1027, 824  $cm^{-1}$ ;  $^1H$  NMR ( $CDCl_3$ , 300 MHz)  $\delta$  6.49 (s, 1 H), 6.35 (s, 1 H), 3.78 (s, 3 H), 3.77 (s, 3 H), 3.75 (s, 3 H);  $^{13}C$  NMR ( $CDCl_3$ , 75 MHz)  $\delta$  143.7, 141.4, 140.7, 128.4, 101.8, 100.0, 57.2, 56.5; EIMS ( $m/z$ , relative intensity) 183 ( $M^+$ , 74), 168 (100), 140 (39), 125 (34), 108 (16), 94 (9), 80 (5), 44 (16); HRMS calcd for  $C_9H_{13}NO_3$  183.0895, found 183.0894.

### 2,4,5-Trimethoxy-*N*-methylaniline (3p)

Yield 91%. Colorless solid: mp 58–60 °C. IR (KBr) 3435, 2942, 1529, 1457, 1449, 1397, 1223, 1210, 1199, 1035, 824  $cm^{-1}$ ;  $^1H$  NMR ( $CDCl_3$ , 300 MHz)  $\delta$  6.51 (s, 1 H), 6.27 (s, 1 H), 3.82 (s, 3 H), 3.78 (s, 3 H), 3.76 (s, 3 H), 2.82 (s, 3 H);  $^{13}C$  NMR ( $CDCl_3$ , 75 MHz)  $\delta$  144.0, 140.5, 139.7, 133.9, 99.9, 97.4, 57.6, 56.8, 56.2, 30.9; EIMS ( $m/z$ , relative intensity) 197 ( $M^+$ , 74), 182 (100), 166 (8), 154 (31), 139 (35), 123 (31), 109 (14), 82 (12), 69 (11), 53 (12); HRMS calcd for  $C_{10}H_{15}NO_3$  197.1052, found 197.1051.

**2,3,4,5-Tetramethoxyaniline (3q)**

Colorless solid: mp 98–100 °C. (lit<sup>20</sup> 99–100 °C). IR (KBr) 3360, 2936, 1617, 1499, 1469, 1227, 1124, 1079, 1044, 1007 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 6.05 (s, 1 H), 3.90 (s, 3 H), 3.76 (s, 3 H), 3.75 (s, 3 H), 3.73 (s, 3 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) δ 149.7, 147.3, 135.7, 134.6, 94.9, 61.3, 61.0, 60.4, 56.0; EIMS (*m/z*, relative intensity) 213 (60), 198 (100), 183 (13), 168 (10), 155 (17), 140 (22), 112 (11), 84 (10), 68 (5), 53 (6); HRMS calcd for C<sub>10</sub>H<sub>15</sub>NO<sub>4</sub> 213.1001, found 213.0994.

**General Procedure to Synthesize Protected Amines from Acids**

A mixture of diphenylphosphoryl azide (12 mmol), the required acid (11 mmol) and triethylamine (12 mmol) in benzene (20 mL) was heated under reflux for 2–3 h. After the evolution of N<sub>2</sub> had ceased, benzyl alcohol (11 mmol) was added and the mixture was heated at reflux for another 2–5 h. The reaction was monitored by TLC. After cooling, the mixture was washed successively with cold 1 N HCl, aq NaHCO<sub>3</sub> solution, and brine, and then dried (Na<sub>2</sub>SO<sub>4</sub>). The organic extract was evaporated under reduced pressure and the residue was chromatographed on silica gel, eluting with ethyl ether-hexane (1:1).

**Benzyl Benzo[d][1,3]dioxol-4-ylcarbamate (6a)**

Yield 75%. Colorless oil. IR (KBr) 3400, 2900, 1735, 1616, 1537, 1450, 1374, 1220, 1101, 1013, 795 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 7.33 (m, 7 H), 7.17 (m, 1 H), 6.79 (m, 1 H), 6.57 (d, *J* = 8.4 Hz, 1 H), 5.90 (s, 2 H), 5.19 (s, 2 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) δ 153.0, 147.3, 135.8, 129.6, 128.5, 128.3, 128.2, 128.0, 125.2, 121.9, 121.5, 120.0, 113.3, 104.1, 101.1, 67.1; positive ion ESIMS (*m/z*, relative intensity) 272 (MH<sup>+</sup>, 20), 264 (6), 102 (100); HRMS calcd for C<sub>15</sub>H<sub>14</sub>NO<sub>5</sub> 272.0923, found 272.0924.

**Benzyl 2,3-Dimethoxyphenylcarbamate (6g)**

Yield 92%. Colorless oil. IR (KBr) 3422, 2940, 1736, 1607, 1531, 1481, 1459, 1421, 1299, 1232, 1203, 1102, 1043 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 7.75 (brs, 1 H), 7.33 (m, 5 H), 6.93 (t, *J* = 8.1 Hz, 1 H), 6.53 (m, 1 H), 5.11 (s, 2 H), 3.73 (s, 6 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) δ 152.9, 151.8, 136.7, 135.8, 131.8, 128.3, 128.1, 123.9, 110.6, 106.4, 66.7, 60.3, 55.4; EIMS (*m/z*, relative intensity) 287 (M<sup>+</sup>, 19), 243 (19), 228 (52), 213 (3), 179 (11), 164 (7), 136 (8), 91 (100), 77 (8), 65 (21); HRMS calcd for C<sub>16</sub>H<sub>17</sub>NO<sub>4</sub> 287.1158, found 287.1160.

**Benzyl 2,4-Dimethoxyphenylcarbamate (6i)**

Yield 95%. Colorless semisolid. IR (KBr) 3400, 2900, 1728, 1618, 1531, 1492, 1417, 1208, 1158, 1037 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 7.98 (brs, 1 H), 7.35 (m, 5 H), 7.06 (s, 1 H), 6.46 (m, 2 H), 5.17 (s, 2 H), 3.78 (s, 3 H), 3.76 (s, 3 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) δ 155.8, 153.3, 148.9, 136.2, 128.5, 128.2, 120.9, 119.8, 119.0, 103.7, 98.6, 66.7, 55.5, 55.4; EIMS (*m/z*, relative intensity) 287 (M<sup>+</sup>, 19), 243 (9), 179 (30), 152 (100), 136 (22), 124 (57), 109 (17), 91 (88), 79 (16), 65 (23); HRMS calcd for C<sub>16</sub>H<sub>17</sub>NO<sub>4</sub> 287.1158, found 287.1156.

**Benzyl 2,3,4-Trimethoxyphenylcarbamate (6m)**

Yield 73%. Colorless solid: mp 53–55 °C. IR (KBr) 3400, 2900, 1731, 1599, 1525, 1495, 1459, 1412, 1211, 1102, 1023 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 7.75 (d, *J* = 9.0 Hz, 1 H), 7.35 (m, 5 H), 6.61 (d, *J* = 9.0 Hz, 1 H), 5.17 (s, 2 H), 3.87 (s, 3 H), 3.83 (s, 3 H), 3.80 (s, 3 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) δ 153.3, 149.0, 142.4, 141.7, 136.0, 128.5, 128.3, 125.1, 112.9, 107.0, 66.8, 61.0, 60.8, 56.1; EIMS (*m/z*, relative intensity) 317 (M<sup>+</sup>, 20), 273 (8), 258 (10), 182 (65), 167 (29), 123 (27), 91 (100), 77 (2), 65 (19); HRMS calcd for C<sub>17</sub>H<sub>19</sub>NO<sub>5</sub> 317.1263, found 317.1261.

**Benzyl 2,4,5-Trimethoxyphenylcarbamate (6o)**

Yield 90%. Colorless solid: mp 99–100 °C. IR (KBr) 3400, 2900, 1728, 1614, 1531, 1457, 1456, 1407, 1202, 1137, 1036  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  7.81 (brs, 1 H), 7.35 (m, 5 H), 7.11 (s, 1 H), 6.50 (s, 1 H), 5.16 (s, 2 H), 3.82 (s, 6 H), 3.78 (s, 3 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  153.3, 144.5, 143.0, 141.5, 136.1, 128.5, 128.28, 120.5, 103.9, 97.8, 66.8, 56.59, 56.50, 56.3; EIMS ( $m/z$ , relative intensity) 317 ( $\text{M}^+$ , 44), 258 (6), 209 (8), 182 (22), 123 (33), 91 (100), 65 (14); HRMS calcd for  $\text{C}_{17}\text{H}_{19}\text{NO}_5$  317.1263, found 317.1265.

**General Procedure for N-Methylation**

A mixture of NaH (12 mmol, 60% dispersion in oil, washed twice with *n*-hexane) and mono-protected amine (8 mmol) in DMF (10 mL) was stirred at room temperature for 30 min. A solution of  $\text{CH}_3\text{I}$  (24 mmol) was added and the resulting mixture was stirred at room temperature for 2 h. Ice water (20 g) was added to this mixture and the resulting mixture was extracted with  $\text{CHCl}_3$  ( $3 \times 100$  mL). The combined extracts were washed with brine (50 mL), dried ( $\text{Na}_2\text{SO}_4$ ), and evaporated. The residue was purified by column chromatography on silica gel, eluting with a 9:1 mixture of petroleum ether and ethyl acetate. Evaporation of the solvents gave methylated carbamate in very high yield.

**Benzyl Benzo[d][1,3]dioxol-4-yl(methyl)carbamate (7a)**

Yield 94%. Colorless oil. IR (KBr) 1712, 1633, 1492, 1459, 1388, 1345, 1301, 1252, 1152, 1102, 1059, 795  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  7.26 (m, 5 H), 6.75 (m, 3 H), 5.86 (brs, 2 H), 5.16 (brs, 2 H), 3.26 (s, 3 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  155.0, 148.4, 141.9, 136.4, 128.2, 127.7, 125.3, 121.3, 120.1, 106.8, 101.0, 67.2, 36.7; positive ion ESIMS ( $m/z$ , relative intensity) 308 ( $\text{MH}^+$ , 35), 285 (100), 242 (68); HRMS calcd for  $\text{C}_{16}\text{H}_{15}\text{NO}_4 + \text{Na}^+$  308.0899, found 308.0906.

**Benzyl 2,3-Dimethoxyphenyl(methyl)carbamate (7g)**

Yield 89%. Colorless solid: mp 92–94 °C (lit<sup>33</sup> mp 92–93 °C). IR (KBr) 1707, 1587, 1488, 1479, 1389, 1316, 1265, 1157, 1075, 794  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  7.22 (m, 5 H), 6.99 (t,  $J = 8.4$  Hz, 1 H), 6.81 (m, 2 H), 5.12 (s, 2 H), 3.83 (s, 3 H), 3.73 (s, 3 H), 3.20 (s, 3 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  155.7, 153.3, 145.2, 136.7, 128.2, 127.6, 127.5, 123.5, 120.6, 111.4, 67.0, 60.6, 55.8, 37.5; positive ion ESIMS ( $m/z$ , relative intensity) 324 ( $\text{MNa}^+$ , 49), 283 (18), 279 (35), 278 (8), 277 (60), 244 (7), 243 (10); HRMS calcd for  $\text{C}_{17}\text{H}_{19}\text{NO}_4 + \text{Na}^+$  324.1212, found 324.1208.

**Benzyl 2,4-Dimethoxyphenyl(methyl)carbamate (7i)**

Yield 90%. Colorless oil. IR (KBr) 1705, 1609, 1587, 1513, 1455, 1389, 1352, 1209, 1159, 1033  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  7.22 (m, 6 H), 6.40 (m, 2 H), 5.11 (s, 2 H), 3.77 (s, 3 H), 3.66 (s, 3 H), 3.14 (s, 3 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  159.7, 155.8, 155.7, 137.2, 128.9, 128.1, 127.9, 127.4, 127.2, 125.0, 103.9, 99.2, 66.7, 55.4, 55.3, 37.5; EIMS ( $m/z$ , relative intensity) 301 ( $\text{M}^+$ , 22), 166 (33), 151 (42), 91 (100), 65 (11); HRMS calcd for  $\text{C}_{16}\text{H}_{19}\text{NO}_4$  301.1314, found 301.1315.

**Benzyl Methyl(2,3,4-trimethoxyphenyl)carbamate (7m)**

Yield 92%. Colorless oil. IR (KBr) 1708, 1598, 1497, 1471, 1418, 1390, 1352, 1212, 1155, 1094  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  7.35 (m, 5 H), 6.86 (d,  $J = 8.7$  Hz, 1 H), 6.61 (d,  $J = 8.7$  Hz, 1 H), 5.11 (s, 2 H), 3.84 (s, 6 H), 3.79 (s, 3 H), 3.21 (s, 3 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  155.9, 152.8, 149.7, 142.4, 136.6, 129.4, 128.0, 127.3, 122.7, 106.3, 66.8, 60.7, 55.8, 37.9; EIMS ( $m/z$ , relative intensity) 331 ( $\text{M}^+$ , 9), 196 (100), 181 (45), 166 (10), 147 (12), 91 (82), 77 (2), 65 (13); HRMS calcd for  $\text{C}_{18}\text{H}_{21}\text{NO}_5$  331.1420, found 331.1424.

### Benzyl Methyl(2,4,5-trimethoxyphenyl)carbamate (7o)

Yield 85%. Colorless solid: mp 98–99 °C. IR (KBr) 1704, 1608, 1515, 1455, 1386, 1330, 1206, 1148, 1036 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 7.76 (m, 5 H), 7.14 (s, 1 H), 6.98 (s, 1 H), 5.56 (s, 2 H), 4.33 (s, 3 H), 4.23 (s, 3 H), 4.16 (s, 3 H), 3.64 (s, 3 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) δ 155.9, 148.9, 148.4, 142.3, 136.8, 128.1, 127.9, 127.6, 127.3, 127.0, 123.1, 112.4, 97.6, 66.5, 56.1, 55.9, 37.2; EIMS (*m/z*, relative intensity) 331 (M<sup>+</sup>, 73), 196 (45), 181 (42), 166 (22), 137 (10), 91 (100), 65 (15); HRMS calcd for C<sub>18</sub>H<sub>21</sub>NO<sub>5</sub> 331.1420, found 331.1421.

### Ethyl 3-(Benzo[d][1,3]dioxol-4-yl(methyl)amino)-3-oxopropanoate (8)

A mixture of amine **3b** (1.51 g, 10.0 mmol) and excess diethyl malonate (15 mL) was heated at 110 °C for 3 h while argon gas was bubbled through the solution. The excess diethyl malonate and ethanol produced during the reaction were removed under reduced pressure. The crude product was purified by silica gel column chromatography, eluting with 30% ethyl acetate in hexanes, to afford carbamate **8** (2.1 g, 82%) as a colorless oil. IR (neat) 3459, 2971, 1741, 1672, 1631, 1460, 1375, 1253, 1096, 1055, 928, 795 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 6.75 (m, 2 H), 6.62 (dd, *J* = 7.8, 1.8 Hz, 1 H), 5.92 (s, 2 H), 4.00 (q, *J* = 7.2 Hz, 2 H), 3.19 (s, 2 H), 3.15 (s, 3 H), 1.11 (t, *J* = 8.4 Hz, 3 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) δ 167.2, 165.9, 148.6, 142.5, 125.0, 122.2, 120.6, 108.2, 101.4, 60.9, 41.1, 35.8, 13.8; EIMS (*m/z*, relative intensity) 265 (M<sup>+</sup>, 35), 220 (10), 178 (9), 151 (100), 120 (5), 93 (12), 77 (3), 65 (20); HRMS calcd for C<sub>13</sub>H<sub>15</sub>NO<sub>5</sub> + Na<sup>+</sup> 288.0848, found 288.0847.

### 6-Hydroxy-9-methyl-[1,3]dioxolo[4,5-*h*]quinolin-8(9*H*)-one (9)

The acid **2** (500 mg, 2.1 mmol) was added to polyphosphoric acid (5 g) at 95–100 °C and the mixture was allowed to stir for 3 h at the same temperature and then poured into ice-water. After polyphosphoric acid had dissolved, a white solid product appeared in the flask. The solid hydroxyquinolone **9** (400 mg, 88%) was collected by filtration, washed with ether and dried under vacuum. The solid was used for the next step without further purification: mp 310 °C (dec, lit<sup>18</sup> mp 310–315 °C). <sup>1</sup>H NMR (CF<sub>3</sub>CO<sub>2</sub>D, 300 MHz) δ 8.06 (d, *J* = 8.4 Hz, 1 H), 7.29 (d, *J* = 8.4 Hz, 1 H), 6.72 (s, 1 H), 6.27 (s, 2 H), 4.28 (s, 3 H); EIMS (*m/z*, relative intensity) 219 (M<sup>+</sup>, 100), 190 (27), 149 (22), 120 (15), 93 (29), 69 (27), 46 (8); HRMS calcd for C<sub>11</sub>H<sub>9</sub>NO<sub>4</sub> 219.0532, found 219.038.

### General Procedure for the Synthesis of Amides (10a-g)

Diketene (10.0 mmol) was added to a magnetically stirred solution of the substituted anilines (**3a-g**, 11.0 mmol) in benzene (10 mL) and the mixture was heated at 100 °C for 90–120 min. The reaction mixtures were monitored by TLC. After completion of the reactions, the solutions were evaporated under reduced pressure and the residues were chromatographed on silica gel, eluting with ethyl ether-hexane (1:1).

### *N*-(Benzo[d][1,3]dioxol-4-yl)-3-oxobutanamide (10a)

Yield 68%. Colorless solid: mp 64–66 °C. IR (KBr) 3300, 2800, 1716, 1668, 1636, 1542, 1419, 1451, 1358, 1254, 1178, 1158, 1068, 1022, 934, 794 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 9.10 (brs, 1 H), 7.54 (d, *J* = 8.4 Hz, 1 H), 6.77 (m, 1 H), 6.59 (d, *J* = 7.8 Hz, 1 H), 5.93 (s, 2 H), 3.57 (s, 2 H), 2.29 (s, 3 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) δ 205.1, 163.3, 147.5, 137.3, 121.8, 121.0, 114.9, 105.1, 101.3, 49.2, 31.2; EIMS (*m/z*, relative intensity) 221 (M<sup>+</sup>, 14), 163 (33), 137 (100), 109 (8), 79 (17), 52 (13); HRMS calcd for C<sub>11</sub>H<sub>11</sub>NO<sub>4</sub> 221.0688, found 221.0690.

### *N*-(Benzo[d][1,3]dioxol-4-yl)-*N*-methyl-3-oxobutanamide (10b)

Yield 70%. Colorless oil. IR (neat) 1721, 1662, 1630, 1604, 1487, 1459, 1425, 1377, 1358, 1253, 1194, 1090, 1055, 1032, 927 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 6.78 (m, 2 H), 6.63

(m, 1 H), 5.96 (s, 2 H), 3.30 (s, 2 H), 3.21 (s, 3 H), 2.11 (s, 3 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  202.1, 166.9, 148.8, 142.6, 125.3, 122.4, 120.6, 108.5, 101.5, 49.6, 35.9, 30.0; EIMS ( $m/z$ , relative intensity) 235 ( $\text{M}^+$ , 31), 178 (4), 151 (100), 122 (12), 93 (23), 65 (10); HRMS calcd for  $\text{C}_{12}\text{H}_{13}\text{NO}_4$  235.0845, found 235.0846.

### 3-Oxo-*N*-phenylbutanamide (10c)

Yield 80%, colorless solid: mp 82–84 °C (lit34 mp 84–86 °C). IR (KBr) 3300, 1716, 1661, 1599, 1544, 1444, 1410, 1314, 1231, 1157, 1029, 755  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  9.16 (brs, 1 H), 7.50 (m, 2 H), 7.26 (m, 2 H), 7.06 (m, 1 H), 3.52 (s, 2 H), 2.25 (s, 3 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  204.9, 163.7, 137.4, 128.8, 124.4, 120.0, 49.8, 31.0; EIMS ( $m/z$ , relative intensity) 177 ( $\text{M}^+$ , 8), 119 (18), 93 (100), 66 (28), 58 (12), 43 (11); HRMS calcd for  $\text{C}_{10}\text{H}_{11}\text{NO}_2$  177.0790, found 177.0794.

### *N*-Methyl-3-oxo-*N*-phenylbutanamide (10d)

Yield 85%, colorless oil. IR (neat) 1721, 1655, 1595, 1496, 1427, 1379, 1358, 1343, 1302, 1162, 1123 776  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  7.37 (m, 3 H), 7.13 (m, 2 H), 3.24 (s, 5 H), 2.04 (s, 3 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  202.2, 166.5, 143.4, 129.8, 129.5, 128.1, 127.1, 49.8, 37.2, 30.2; positive ion ESIMS ( $m/z$ , relative intensity) 382 ( $2\text{M}^+$ , 100), 192 ( $\text{MH}^+$ , 63), 122 (30), 93 (100), 66 (28), 58 (12), 43 (11); HRMS calcd for  $\text{C}_{11}\text{H}_{13}\text{NO}_2 + \text{H}^+$  192.1024, found 192.1023.

### *N*-(2-Methoxyphenyl)-3-oxobutanamide (10e)

Yield 70%, yellow solid: mp 82–84 °C (lit35 83–84 °C). IR (KBr) 3300, 1714, 1677, 1600, 1537, 1488, 1461, 1435, 1336, 1253, 1159, 750  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  9.20 (brs, 1 H), 8.27 (d,  $J = 8.1$  Hz, 1 H), 7.01 (m, 1 H), 6.96 (m, 1 H), 6.84 (m, 1 H), 3.83 (s, 3 H), 3.54 (s, 2 H), 2.25 (s, 3 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  204.3, 163.2, 148.1, 127.1, 123.9, 120.7, 119.9, 109.9, 55.6, 50.6, 30.8; EIMS ( $m/z$ , relative intensity) 207 ( $\text{M}^+$ , 47), 149 (41), 108 (100), 92 (7), 80 (35), 43 (52); HRMS calcd for  $\text{C}_{12}\text{H}_{13}\text{NO}_3$  207.0895, found 207.0891.

### *N*-(2-Methoxyphenyl)-*N*-methyl-3-oxobutanamide (10f)

Yield 75%, colorless oil. IR (neat) 1720, 1655, 1510, 1433, 1464, 1357, 1379, 1279, 1240, 1108, 795  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  7.42 (m, 1 H), 7.26 (m, 1 H), 7.02 (m, 2 H), 3.93 (s, 3 H), 3.31 (s, 3 H), 3.29 (s, 2 H), 2.42 (s, 3 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  202.3, 167.4, 154.6, 131.5, 129.7, 128.8, 121.0, 111.8, 55.2, 49.6, 36.0, 29.7; EIMS ( $m/z$ , relative intensity) 221 ( $\text{M}^+$ , 10), 190 (21), 122 (100), 94 (27), 65 (15), 43 (46); HRMS calcd for  $\text{C}_{12}\text{H}_{15}\text{NO}_3$  221.1052, found 221.1050.

### *N*-(2,3-Dimethoxyphenyl)-3-oxobutanamide (10g)

Yield 67%, colorless oil. IR (neat) 3300, 1712, 1681, 1603, 1539, 1478, 1463, 1422, 1297, 1260, 1090, 1002, 794  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  9.47 (brs, 1 H), 7.92 (d,  $J = 8.1$  Hz, 1 H), 6.99 (dd,  $J = 8.1, 16.5$  Hz, 1 H), 6.63 (d,  $J = 8.1$  Hz, 1 H), 3.88 (s, 3 H), 3.83 (s, 3 H), 3.59 (s, 2 H), 2.29 (s, 3 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  204.4, 163.4, 152.0, 137.8, 131.9, 124.0, 112.6, 107.7, 60.7, 55.7, 50.3, 31.0; EIMS ( $m/z$ , relative intensity) 237 ( $\text{M}^+$ , 68), 206 (8), 153 (51), 138 (100), 95 (18), 43 (99); HRMS calcd for  $\text{C}_{12}\text{H}_{15}\text{NO}_4$  237.1001, found 237.1009.

### *N*-(2,3-Dimethoxyphenyl)-*N*-methyl-3-oxobutanamide (10h)

Yield 70%, colorless oil. IR (neat) 1721, 1656, 1587, 1478, 1429, 1378, 1356, 1317, 1267, 1234, 1148, 1096, 1051, 1003, 794  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  6.98 (m, 1 H), 6.85 (m, 1 H), 6.63 (d,  $J = 8.1$  Hz, 1 H), 3.79 (s, 3 H), 3.74 (s, 5 H), 3.15 (s, 3 H), 2.20 (s, 3



H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  202.2, 167.1, 153.3, 136.6, 128.0, 124.1, 120.6, 112.2, 60.7, 55.7, 49.1, 36.5, 29.8; positive ion ESIMS ( $m/z$ , relative intensity) 274 ( $\text{MNa}^+$ , 68), 252 ( $\text{MH}^+$ ), 180 (100); HRMS calcd for  $\text{C}_{13}\text{H}_{17}\text{NO}_4 + \text{Na}^+$  274.1055, found 274.1054.

#### ***N*-(2,4-Dimethoxyphenyl)-3-oxobutanamide (10i)**

Yield 80%, colorless solid: mp 92–94 °C. IR (KBr) 3300, 1713, 1667, 1615, 1603, 1535, 1459, 1416, 1302, 1262, 1282, 1208, 1158, 1128, 1030, 750  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  9.00 (brs, 1 H), 8.15 (d,  $J = 8.7$  Hz, 1 H), 6.44 (d,  $J = 8.7$  Hz, 1 H), 6.40 (brs, 1 H), 3.84 (s, 3 H), 3.75 (s, 3 H), 3.54 (s, 3 H), 2.29 (s, 3 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  204.6, 162.8, 156.6, 149.7, 120.9, 120.8, 103.5, 98.6, 55.8, 55.5, 50.6, 31.0; EIMS ( $m/z$ , relative intensity) 237 ( $\text{M}^+$ , 67), 179 (55), 153 (40), 138 (100), 110 (12), 93 (5), 52 (4); HRMS calcd for  $\text{C}_{12}\text{H}_{15}\text{NO}_4$  237.1001, found 237.1001.

#### ***N*-(2,4-Dimethoxyphenyl)-*N*-methyl-3-oxobutanamide (10j)**

Yield 80%, colorless oil. IR (neat) 1720, 1655, 1609, 1586, 1513, 1462, 1421, 1379, 1317, 1283, 1210, 1162, 1113, 1031, 795  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  6.99 (d,  $J = 1.5$  Hz, 1 H), 6.42 (m, 2 H), 3.74 (s, 3 H), 3.72 (s, 3 H), 3.09 (s, 5 H), 2.05 (s, 3 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  202.4, 167.8, 160.5, 155.5, 129.1, 124.7, 104.4, 99.3, 55.4, 55.2, 49.6, 36.1, 29.7; EIMS ( $m/z$ , relative intensity) 251 ( $\text{M}^+$ , 5), 220 (3), 167 (65), 152 (100), 136 (7), 124 (23), 109 (12), 98 (22), 56 (9); HRMS calcd for  $\text{C}_{13}\text{H}_{17}\text{NO}_4$  251.1158, found 251.1161.

#### ***N*-(2,5-Dimethoxyphenyl)-3-oxobutanamide (10k)**

Yield 94%, colorless solid: mp 70–72 °C (lit<sup>36</sup> mp 67–68 °C). IR (KBr) 3300, 2800, 1715, 1676, 1600, 1537, 1486, 1465, 1430, 1361, 1279, 1221, 1159, 1046, 795  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  9.20 (brs, 1 H), 7.97 (d,  $J = 3.0$  Hz, 1 H), 6.72 (d,  $J = 9.0$  Hz, 1 H), 6.50 (dd,  $J = 9.0, 3.0$  Hz, 1 H), 3.76 (s, 3 H), 3.68 (s, 3 H), 3.51 (s, 2 H), 2.22 (s, 3 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  204.2, 163.3, 153.4, 142.3, 127.8, 110.7, 108.4, 106.3, 56.1, 55.5, 50.4, 30.7; positive ion ESIMS ( $m/z$ , relative intensity) 260 ( $\text{MNa}^+$ , 100); HRMS calcd for  $\text{C}_{12}\text{H}_{15}\text{NO}_4 + \text{Na}^+$  260.0899, found 260.0900.

#### ***N*-(2,5-Dimethoxyphenyl)-*N*-methyl-3-oxobutanamide (10l)**

Yield 80%, colorless solid: mp 69–71 °C (lit<sup>20</sup> mp 68.5–69 °C). IR (KBr) 1720, 1658, 1611, 1508, 1464, 1424, 1376, 1310, 1274, 1219, 1181, 1107, 1041, 728  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  6.81 (m, 2 H), 6.67 (m, 1 H), 3.70 (s, 3 H), 3.69 (s, 3 H), 3.12 (m, 5 H), 2.08 (s, 3 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  202.3, 167.3, 153.6, 148.7, 132.0, 114.6, 114.2, 112.5, 55.6, 49.5, 35.9, 29.7; positive ion ESIMS ( $m/z$ , relative intensity) 274 ( $\text{MNa}^+$ , 100), HRMS calcd for  $\text{C}_{13}\text{H}_{17}\text{NO}_4 + \text{Na}^+$  274.1055, found 274.1056.

#### **3-Oxo-*N*-(2,3,4-trimethoxyphenyl)butanamide (10m)**

Yield 85%, colorless solid: mp 86–88 °C. IR (KBr) 3300, 2800, 1711, 1674, 1601, 1535, 1494, 1462, 1412, 1360, 1272, 1164, 1095, 1060, 1010, 795  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  9.28 (brs, 1 H), 7.86 (d,  $J = 9.0$  Hz, 1 H), 6.53 (d,  $J = 9.0$  Hz, 1 H), 3.86 (s, 3 H), 3.80 (s, 3 H), 3.77 (s, 3 H), 3.51 (s, 2 H), 2.21 (s, 3 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  204.5, 163.1, 149.6, 143.1, 141.5, 125.0, 114.7, 106.6, 60.9, 60.6, 55.8, 49.8, 30.8; EIMS ( $m/z$ , relative intensity) 267 ( $\text{M}^+$ , 20), 209 (100), 183 (83), 168 (49), 153 (7), 140 (11), 123 (19), 108 (12), 95 (4), 80 (12), 69 (8); HRMS calcd for  $\text{C}_{13}\text{H}_{17}\text{NO}_5$  267.1107, found 267.1111.

#### ***N*-Methyl-3-oxo-*N*-(2,3,4-trimethoxyphenyl)butanamide (10n)**

Yield 78%, colorless oil. IR (neat) 1712, 1633, 1492, 1459, 1388, 1345, 1301, 1252, 1152, 1102, 1059, 795  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  6.69 (d,  $J = 9.0$  Hz, 1 H), 6.51 (d,  $J =$

9.0 Hz, 1 H), 3.73 (s, 3 H), 3.70 (s, 3 H), 3.69 (s, 3 H), 3.04 (m, 5 H), 1.96 (s, 3 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  202.0, 167.3, 153.6, 149.0, 142.2, 129.2, 122.6, 106.6, 60.8, 60.5, 55.6, 49.1, 36.5, 29.6; positive ion ESIMS ( $m/z$ , relative intensity) 282 ( $\text{MH}^+$ , 100), 251 (3), 198 (21), 183 (2); HRMS calcd for  $\text{C}_{14}\text{H}_{19}\text{NO}_5 + \text{H}$  282.1343, found 282.1341.

### 3-Oxo-*N*-(2,4,5-trimethoxyphenyl)butanamide (10o)

Yield 67%. Colorless solid: mp 90–92 °C (lit<sup>37</sup> mp 92–93 °C). IR (KBr) 3300, 2800, 1714, 1668, 1534, 1465, 1405, 1342, 1219, 1203, 1031, 750  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  9.02 (brs, 1 H), 7.97 (s, 1 H), 6.46 (s, 1 H), 3.78 (s, 3 H), 3.76 (s, 3 H), 3.73 (s, 3 H), 3.49 (s, 2 H), 2.23 (s, 3 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  204.2, 162.9, 145.0, 142.3, 120.2, 105.4, 97.3, 56.5, 56.2, 50.3, 30.8; EIMS ( $m/z$ , relative intensity) 267 ( $\text{M}^+$ , 64), 252 (5), 209 (44), 194 (32), 168 (100), 140 (28), 108 (13), 69 (15); HRMS calcd for  $\text{C}_{13}\text{H}_{17}\text{NO}_5$  267.1107, found 267.1106.

### *N*-Methyl-3-oxo-*N*-(2,4,5-trimethoxyphenyl)butanamide (10p)

Yield 81%. Colorless oil. IR (neat) 1731, 1599, 1525, 1495, 1459, 1412, 1270, 1240, 1211, 1102, 1089, 1023, 795  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  6.61 (s, 1 H), 6.46 (s, 1 H), 3.82 (s, 3 H), 3.71 (s, 6 H), 3.13 (s, 2 H), 3.08 (s, 3 H), 2.04 (s, 3 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  202.5, 167.7, 149.5, 148.8, 142.8, 123.0, 112.2, 97.3, 56.4, 56.0, 55.8, 49.5, 36.0, 29.8; EIMS ( $m/z$ , relative intensity) 281 ( $\text{M}^+$ , 37), 250 (10), 209 (12), 197 (33), 182 (100), 166 (12), 154 (17), 123 (10), 98 (11), 69 (26); HRMS calcd for  $\text{C}_{14}\text{H}_{19}\text{NO}_5$  281.1263, found 281.1261.

### 3-Oxo-*N*-(2,3,4,5-tetramethoxyphenyl)butanamide (10q)

Yield 77%. Colorless oil. IR (neat) 3500, 2800, 1710, 1682, 1604, 1532, 1486, 1464, 1410, 1364, 1219, 1129, 1098, 1006  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  9.4 (brs, 1 H), 7.73 (s, 1 H), 3.86 (s, 3 H), 3.85 (s, 6 H), 3.82 (s, 3 H), 3.77 (s, 3 H), 3.54 (s, 2 H), 2.04 (s, 3 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz)  $\delta$  204.5, 163.2, 149.0, 146.3, 138.6, 136.1, 126.9, 99.2, 61.2, 61.1, 61.0, 51.0, 49.9, 31.0; EIMS ( $m/z$ , relative intensity) 297 ( $\text{M}^+$ , 47), 282 (10), 239 (22), 198 (100), 183 (21), 155 (27), 140 (17), 127 (5), 69 (16); HRMS calcd for  $\text{C}_{14}\text{H}_{19}\text{NO}_6$  297.1212, found 297.1210.

## Expression and Purification of Human QR2 Gene

Human QR2 was purified from 3 liters of *E. coli* BL21(DE3) grown in Luria Bertani medium supplement with 100  $\mu\text{g}/\text{mL}$  ampicillin (Fisher Scientific, Pittsburg, PA) following our previously reported procedure.<sup>6</sup> Freshly prepared and non-frozen QR2 was used to set up crystallization experiments. For kinetic assays and inhibitor studies, QR2, previously stored at  $-80$  °C, was thawed on ice and then adjusted to the desired concentration by diluting in storage buffer without glycerol.

## Steady-State Kinetic Assays and $\text{IC}_{50}$ Value Determination

The activity of QR2 under steady-state conditions was evaluated on SpectraMax Plus 384 UV/Vis spectrophotometer thermostated at 23 °C by monitoring the increase in absorbance of the product formazan at 610 nm which is produced from MTT. The assays were run in 96 well plates at a final assay volume of 200  $\mu\text{L}$ . Each assay mixture contained 12 nM QR2, 17.5  $\mu\text{M}$  NMeH, and 200  $\mu\text{M}$  MTT in a reaction buffer containing 100 mM NaCl, 0.1% Triton-X100 (Fisher Biotech 93004), and 0.6 mg/mL BSA (Sigma A3059). The reactions were initiated by the addition of QR2. The initial slopes of the reaction ( $\Delta\text{Abs}/\Delta\text{time}$ ) were used to calculate the initial rates using a molar extinction coefficient of 11,300  $\text{mM}^{-1} \text{cm}^{-1}$  for MTT.

$\text{IC}_{50}$  values were determined at 23 °C in 96 well plates using an assay volume of 200  $\mu\text{L}$  and the assay conditions described above. Inhibitor concentrations ranged from 0.4  $\mu\text{M}$  to 500

$\mu\text{M}$ , and 11 different inhibitor concentrations were chosen for each  $\text{IC}_{50}$  value determination. Assays at each inhibitor concentration were performed in triplicate, and the average rate values and standard deviation at each rate were used to determine the  $\text{IC}_{50}$  values. The resulting data were used to calculate the percent inhibition values (%I) versus the non-inhibited control which consisted of reaction mixture with no inhibitor. The percent inhibition data were then plotted as a function of inhibitor concentration ([I]) and the data were fit via non-linear regression to the following equation:  $\%I = \%I_{\text{max}}/(1+(\text{IC}_{50}/[\text{I}]))$  describing a simple binding isotherm. Data were fit using the Enzyme Kinetics Module of program SigmaPlot from SPSS Scientific.  $\text{IC}_{50}$  values are reported along with standard errors.

### QR2 Crystallization and X-ray Structure Determination

Crystals of the QR2 apoenzyme were grown according to our previously reported protocols.<sup>6</sup> The resulting crystals were soaked overnight with inhibitor in a solution that consisted of 1  $\mu\text{L}$  of a 10 mM inhibitor solution (dissolved in 100% DMSO) that was mixed into 9  $\mu\text{L}$  of the mother liquor solution that contained 1.339 M ammonium sulfate, 0.1 M bis-Tris, pH 6.7, 0.1 M NaCl, 5 mM DTT and 12  $\mu\text{M}$  FAD. Crystals were then retrieved with a nylon loop which was then used to swipe the crystals through the well solution supplemented with 30% glycerol. The crystals were immediately flash-frozen by plunging into liquid nitrogen. Crystals were stored in liquid nitrogen in Dewar flasks until X-ray data collection.

X-Ray diffraction data were collected at the SER-CAT beamline 22-BM located at the Advanced Photon Source (APS) at Argonne National Laboratories. Flash-frozen crystals were mounted on a goniostat under a stream of dry  $\text{N}_2$  at 100 K. X-ray data were collected on either a MAR 225 mm or MAR315 CCD detector. Individual data sets were processed and scaled in the  $\text{P}2_12_12_1$  space group using HKL2000. Initial phases for the QR2-compound complex's were determined via molecular replacement using the program Phaser in the CCP4 suite. Model building and structural refinement were performed using the programs WinCoot and Refmac from the CCP4 suite. The atomic coordinates and structure factors have been deposited in the Protein Data bank ([www.pdb.org](http://www.pdb.org))<sup>38</sup> under PDB codes XXXX for the QR2-compound **1** complex and XXXX for the **11** complex.

### Aromatase Assay

Aromatase activity was assayed as previously reported, with the necessary modifications to assay in a 384-well plate. Briefly, the test compound (3.5  $\mu\text{L}$ ) was preincubated with 30  $\mu\text{L}$  of NADPH regenerating system (2.6 mM  $\text{NADP}^+$ , 7.6 mM glucose 6-phosphate, 0.8 U/ml glucose-6-phosphate dehydrogenase, 13.9 mM  $\text{MgCl}_2$ , and 1 mg/mL albumin in 50 mM potassium phosphate buffer, pH 7.4) for 10 min at 37 °C. The enzyme and substrate mixture (33  $\mu\text{L}$  of 80  $\mu\text{L}/\text{mL}$  CYP19 enzyme, BD Biosciences), 0.4  $\mu\text{M}$  dibenzylfluorescein, 4 mg/mL albumin in 50 mM potassium phosphate, pH 7.4, and the plate were incubated for 30 min at 37 °C before quenching with 25  $\mu\text{L}$  of 2 N NaOH. After termination of the reaction and shaking for 5 min, the plate was further incubated for 2 h at 37 °C. This enhances the ratio of signal to background. Fluorescence was measured at 485 nm (excitation) and 530 nm (emission).  $\text{IC}_{50}$  values and dose-response curves were based on three independent experiments performed in duplicate using five concentrations of test substance (Table 1).

### Cytotoxicity Testing

LNCaP (prostate), MCF7 (breast), and Hepa 1c1c7 (mouse liver) cell lines were acquired from ATCC (Manassas, VA). The LU lung cancer cell line was provided by the Department of Surgical Oncology, University of Illinois Chicago, Chicago, IL. Evaluation of cytotoxic potential was performed as described previously.<sup>39-41</sup> In brief, using 96-well plates, cells were seeded at a density of  $5.0 \times 10^4$  cells/mL, and treated with a 10  $\mu\text{L}$  solution of each compound in 10% DMSO to yield final concentrations ranging from 0–20  $\mu\text{g}/\text{mL}$ . A zero day

control plate was incubated for 30 min at 37 °C. All other plates were incubated for 72 h. After incubation, cells were fixed by the addition of 50 µL cold 50% aqueous trichloroacetic acid, incubated at 4 °C for 30 min, washed with tap water, and air-dried. Cells were then stained with 100 µL of 0.4% sulforhodamine B in 1% acetic acid for 30 min at 23 °C. After washing with 1% acetic acid (4x) to remove excess dye and air-drying, bound dye was solubilized by the addition of 200 µL 10 mM Tris base (pH 10). Finally, the plates were shaken for 5 min, and absorption at 515 nm was determined with a Beckman Coulter ELx800 plate reader. All determinations were performed in triplicate, and the 50% inhibitory (IC<sub>50</sub>) values of active samples were assessed by linear regression analyses of dose-response data.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgements

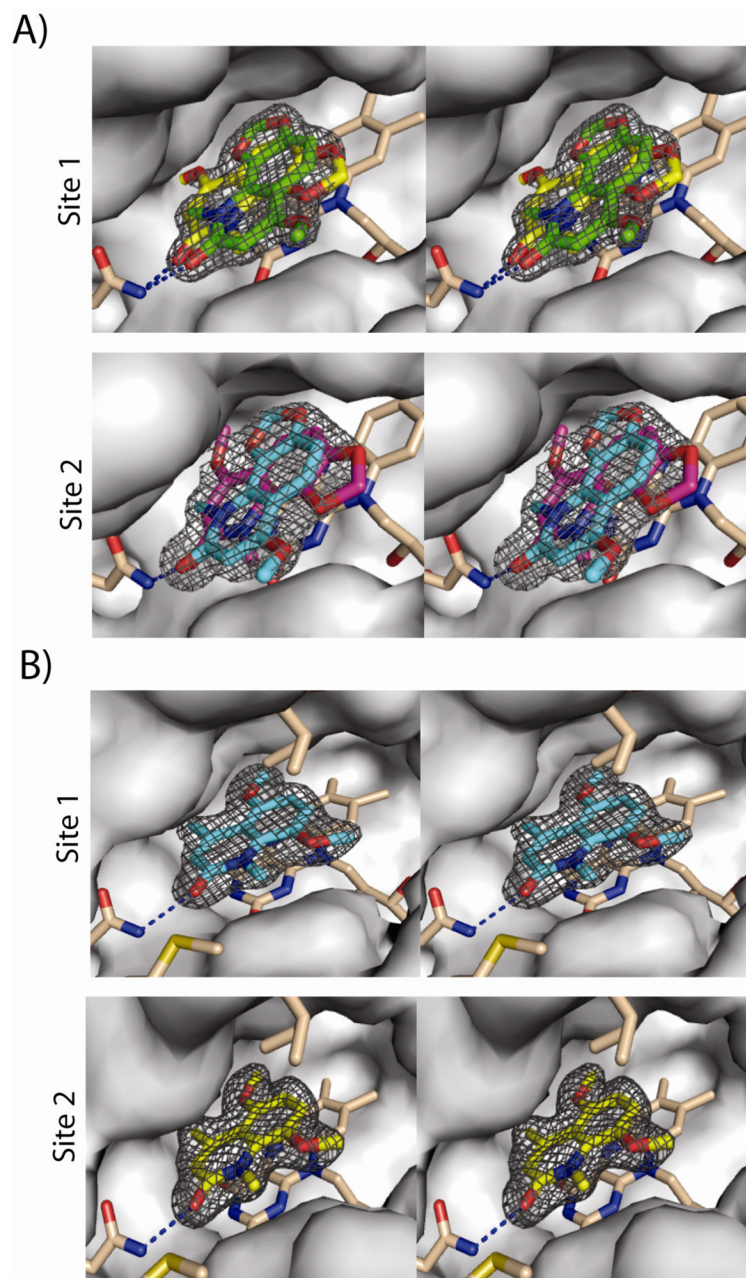
This work was supported by program project grant P01 CA48112 awarded by the National Cancer Institute. Some of this research was conducted in a facility constructed with the financial support of a Research facilities Improvement program grant C06-14499 from the National Institutes of Health.

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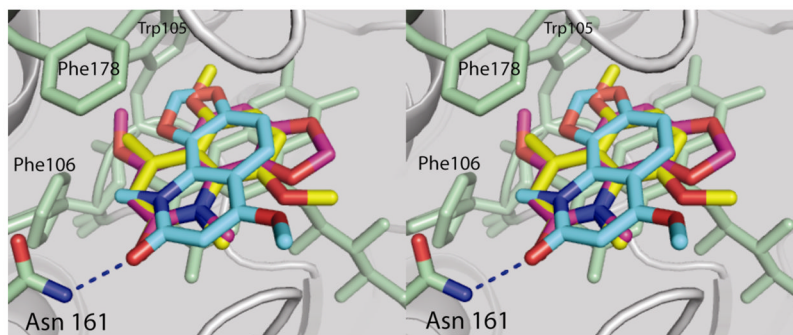
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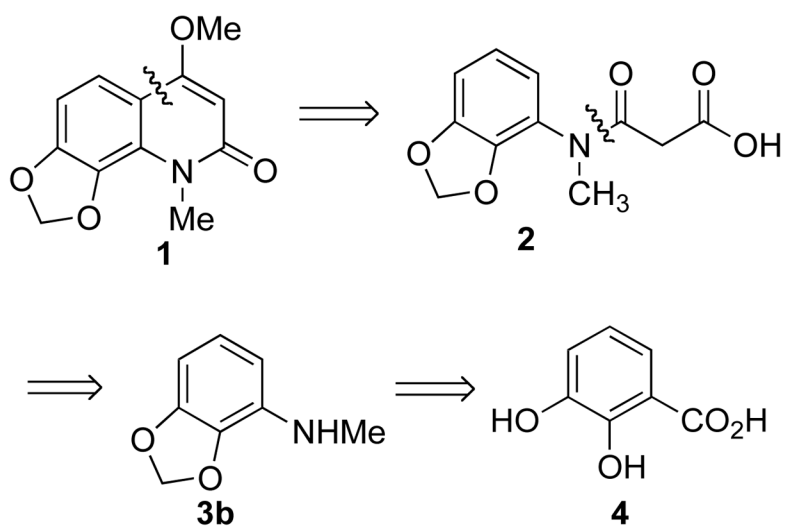
**Figure 1.** X-ray structure and electron density surrounding inhibitors in the active site of human QR2. In each stereoview, QR2 inhibitor complexes depict all FAD and enzyme carbons in grey and beige, oxygen atoms are red and all nitrogen atoms are blue. Hydrogen bonds between Asn161 and the inhibitors are represented as blue dashed lines. **(A)** QR2 in complex with compound **1**. The  $2F_o - F_c$  electron density surrounding the two orientations of compound **1** is contoured at 1s in black. Site 1, compound **1** binds in two orientations in both Site 1 (green and yellow) of chain A (top panel) and Site 2 (blue and magenta) of Chain B (lower panel). The two binding orientations are rotated by  $\sim 180^\circ$  degrees from each other. Each pose was refined at 50% occupancy. The different orientations in the two separate sites are depicted in different colors for clarity. Ile128 and Met154 have been removed for clarity. **(B)** QR2 in complex with

compound **11** binds in one orientation in both Site 1 (blue) of chain A (top panel) and Site 2 (yellow) of Chain B (lower panel). The  $2F_o - F_c$  electron density surrounding compound **11** is contoured at 1 $\sigma$  in black.

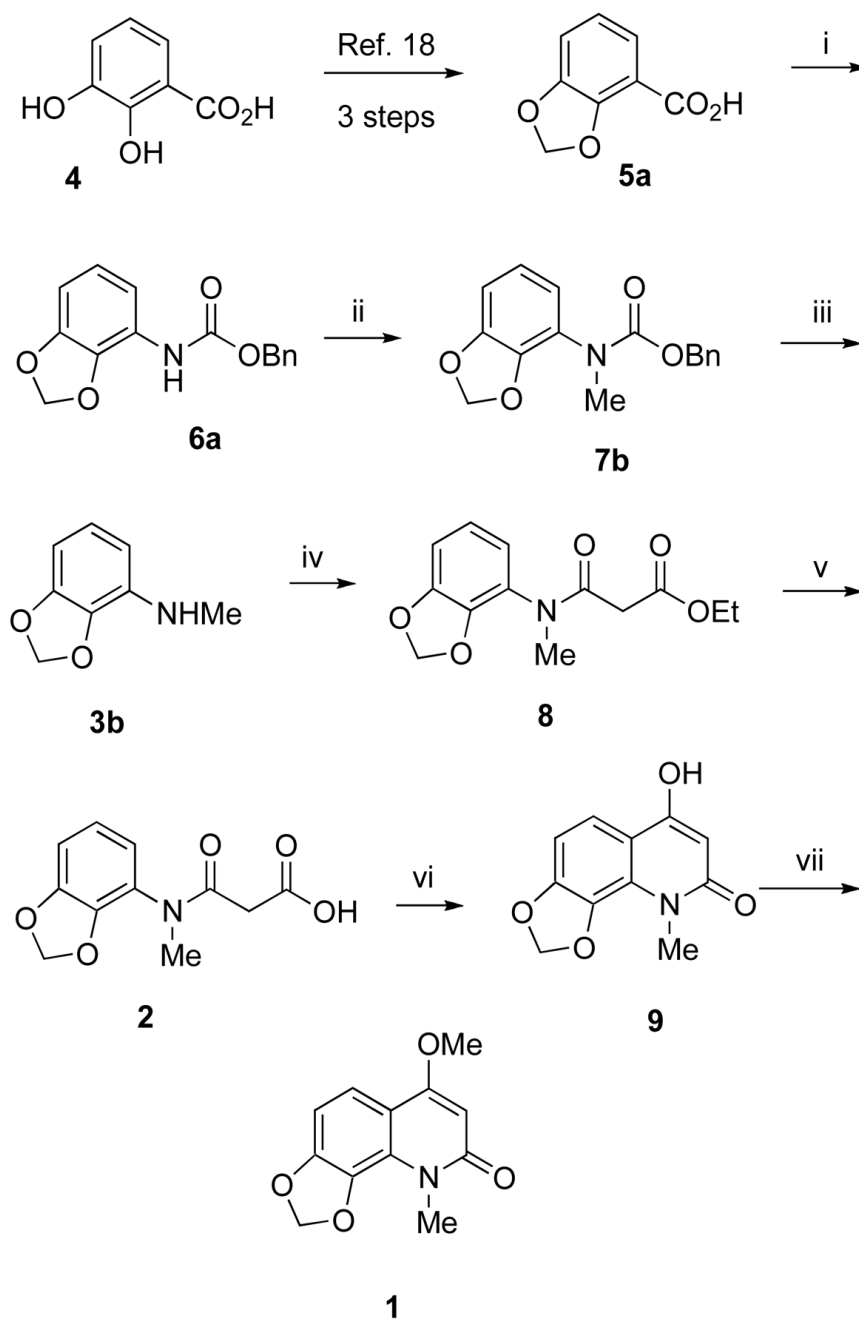




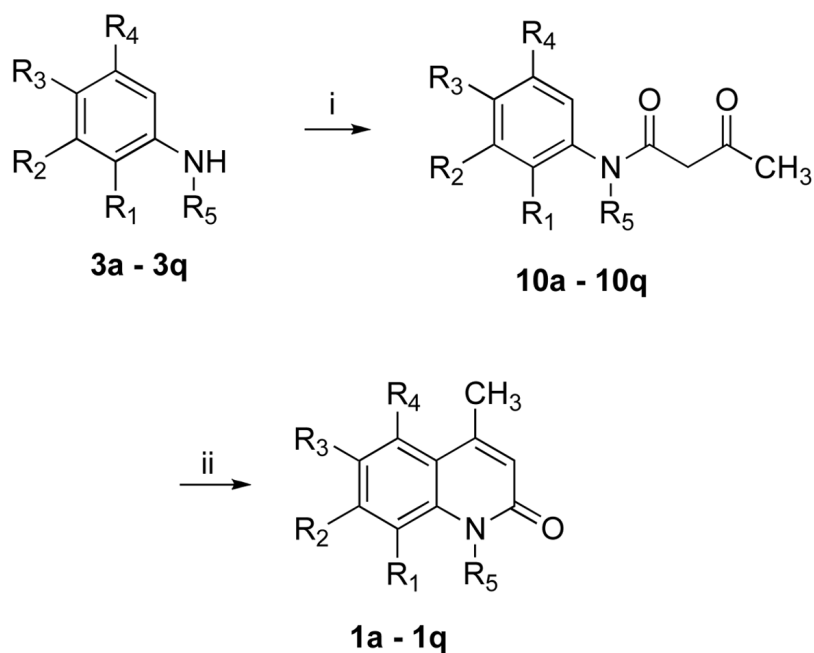
**Figure 2.** Stereoview of a superposition of compound **1** and compound **11** bound within the active sites of human QR2. The two orientations of compound **1** bound within the active sites of chain A (blue) and chain B (magenta) are shown with compound **11** (yellow).



**Scheme 1.**  
Retrosynthetic Analysis of Compound 1

**Scheme 2a.**

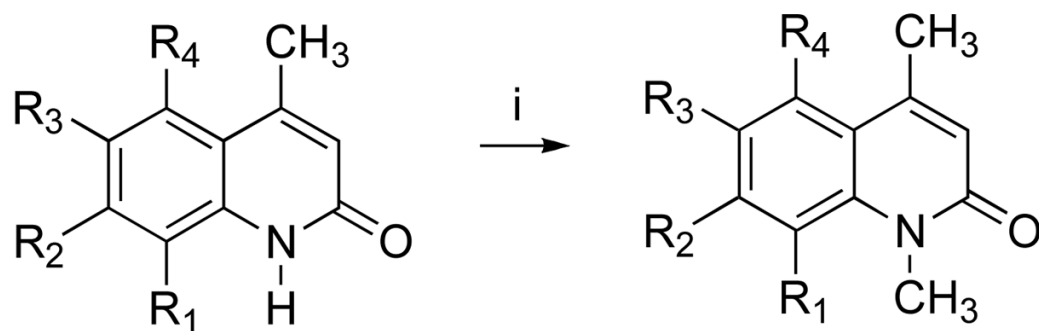
<sup>a</sup>Reagents and conditions: (i)  $(\text{PhO})_2\text{PON}_3$ ,  $\text{Et}_3\text{N}$ ,  $\text{PhCH}_2\text{OH}$ ,  $\text{PhH}$ , 3 h; (ii)  $\text{NaH}$ ,  $\text{MeI}$ , rt, 2 h; (iii)  $\text{Pd/C}$ ,  $\text{H}_2$ ,  $\text{EtOAc}$ , 30 psi, 1 h; (iv) diethyl malonate, 100–110 °C, 3 h; (v) (a)  $\text{NaOH}$ , 60 °C, 2 h, (b)  $\text{HCl}$ ; (vi)  $\text{PPA}$ , 95–100 °C 3 h; (vii)  $\text{TMSCH}_2\text{N}_2$ ,  $\text{MeOH-Et}_2\text{O}$ , rt, 24 h



- a**  $R_1, R_2 = -OCH_2O-$ ,  $R_3, R_4, R_5 = H$   
**b**  $R_1, R_2 = -OCH_2O-$ ,  $R_3, R_4 = H, R_5 = CH_3$   
**c**  $R_1, R_2, R_3, R_4, R_5 = H$   
**d**  $R_1, R_2, R_3, R_4 = H, R_5 = CH_3$   
**e**  $R_1 = OCH_3, R_2, R_3, R_4, R_5 = H,$   
**f**  $R_1 = OCH_3, R_2, R_3, R_4 = H, R_5 = CH_3$   
**g**  $R_1, R_2 = OCH_3, R_3, R_4, R_5 = H$   
**h**  $R_1, R_2 = OCH_3, R_3, R_4 = H, R_5 = CH_3$   
**i**  $R_1, R_3 = OCH_3, R_2, R_4, R_5 = H$   
**j**  $R_1, R_3 = OCH_3, R_2, R_4 = H, R_5 = CH_3$   
**k**  $R_1, R_4 = OCH_3, R_2, R_3, R_5 = H$   
**l**  $R_1, R_4 = OCH_3, R_2, R_3 = H, R_5 = CH_3$   
**m**  $R_1, R_2, R_3 = OCH_3, R_4, R_5 = H$   
**n**  $R_1, R_2, R_3 = OCH_3, R_4 = H, R_5 = CH_3$   
**o**  $R_1, R_3, R_4 = OCH_3, R_2, R_5 = H$   
**p**  $R_1, R_3, R_4 = OCH_3, R_2 = H, R_5 = CH_3$   
**q**  $R_1, R_2, R_3, R_4 = OCH_3, R_5 = H$

**Scheme 3a.**

<sup>a</sup>Reagents and conditions: (i) diketene, PhH, 95–100 °C, 3h, or CF<sub>3</sub>CO<sub>2</sub>H, rt; (ii) PPA, 95–100 °C, 3 h.



**1i**  $R_1, R_3 = \text{OCH}_3, R_2, R_4 = \text{H}$

**1o**  $R_1, R_3, R_4 = \text{OCH}_3, R_2 = \text{H}$

**1q**  $R_1, R_2, R_3, R_4 = \text{OCH}_3$

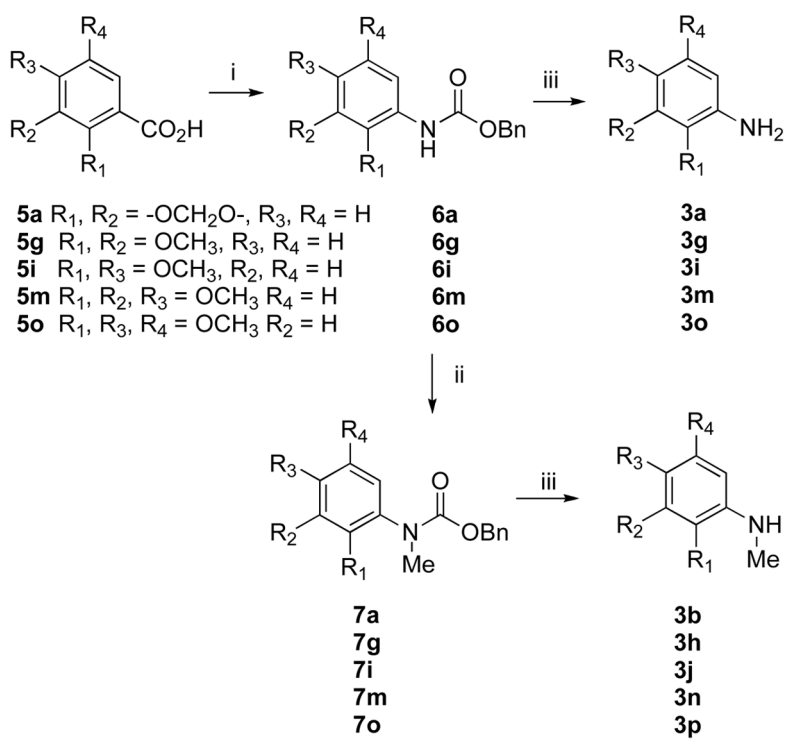
**1j**

**1p**

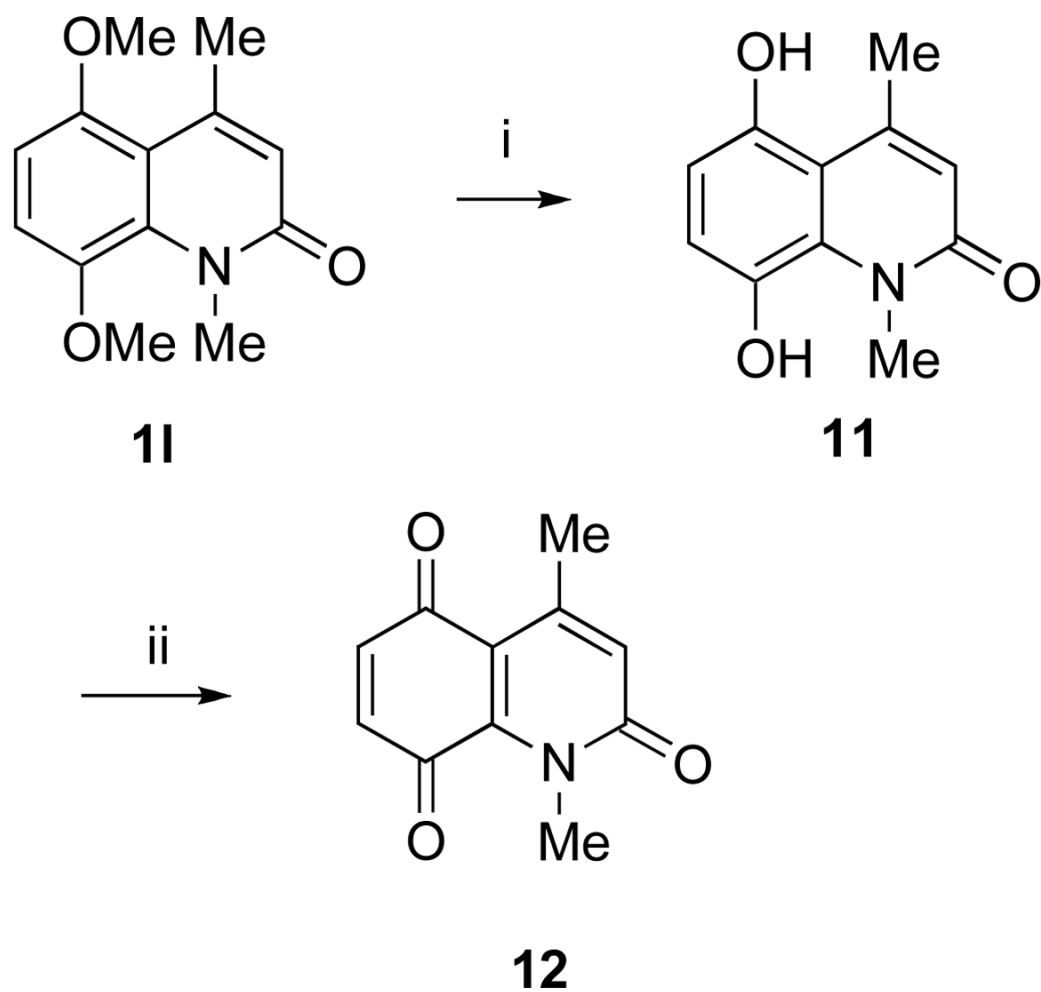
**1r**

**Scheme 4a.**

<sup>a</sup>Reagents and conditions: (i) NaH, MeI, THF, 23 °C, 12 h

**Scheme 5a.**

<sup>a</sup>Reagents and conditions: (i)  $(PhO)_2PON_3$ ,  $Et_3N$ ,  $PhCH_2OH$ ,  $PhH$ , 3 h; (ii)  $NaH$ ,  $MeI$ ,  $rt$ , 2 h; (iii)  $Pd/C$ ,  $H_2$ ,  $EtOAc$ , 30 psi, 1 h.

**Scheme 6a.**

<sup>a</sup>Reagents and conditions: (i) HBr, heat, 48 h (ii) K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>, H<sub>2</sub>SO<sub>4</sub>, acetone, 0 °C, 10 min.

**Table 1**  
Inhibition of QR2 and Aromatase by Compound 1 and Its Analogues

Quinolimonones	QR2 IC <sub>50</sub> (μM)	Aromatase IC <sub>50</sub> (μM)	Cytotoxicity IC <sub>50</sub> (μM)			
			Hepa 1c1c7	MCF7	LNCaP	LU
<b>1</b>	54.1 ± 6.7	3.92 ± 0.67	>85.81	>85.81	>85.81	>85.81
<b>1a</b>	10.8 ± 2.1	>98.49	>98.49	>98.49	>98.49	>98.49
<b>1b</b>	6.2 ± 0.8	1.25 ± 0.03	>92.14	>92.14	>92.14	>92.14
<b>1c</b>	>500	>125.7	>125.7	>125.7	>125.7	>125.7
<b>1d</b>	18.2 ± 3.1	>115.6	>115.6	>115.6	>115.6	>115.6
<b>1e</b>	24.1 ± 3.4	>105.8	>105.8	>105.8	>105.8	>105.8
<b>1f</b>	5.8 ± 0.9	2.02 ± 0.1	>98.48	>98.48	>98.48	>98.48
<b>1g</b>	29.3 ± 3.4	31.5 ± 3.1	>91.29	>91.29	>91.29	>91.29
<b>1h</b>	9.3 ± 2.3	5.76 ± 0.07	>85.80	>85.80	>85.80	>85.80
<b>1i</b>	8.8 ± 1.1	>91.29	>91.29	>91.29	>91.29	>91.29
<b>1j</b>	1.9 ± 0.2	0.96 ± 0.02	>85.80	>85.80	>85.80	>85.80
<b>1k</b>	10.8 ± 1.5	26.8 ± 13.77	>91.22	>91.22	>91.22	>91.22
<b>1l</b>	4.1 ± 0.6	0.96 ± 0.01	>85.74	>85.74	>85.74	>85.74
<b>1m</b>	10.0 ± 1.4	18.47 ± 3.7	>80.29	>80.29	>80.29	>80.29
<b>1n</b>	7.0 ± 1.1	2.72 ± 0.02	>76.01	>76.01	>76.01	>76.01
<b>1o</b>	6.0 ± 0.6	14.8 ± 0.65	34.52	42.95	45.76	35.28
<b>1p</b>	10.8 ± 1.3	0.10 ± 0.01	70.73	>76.01	>76.01	68.47
<b>1q</b>	>500	5.68 ± 0.24	>71.66	>71.66	>71.66	>71.66
<b>1r</b>	>500	0.76 ± 0.02	>68.23	>68.23	>68.23	>68.23
<b>1l</b>	>500	>90.4	NT <sup>a</sup>	NT	NT	NT
<b>12</b>	>500	>98.4	54.14	60.53	58.07	49.22
<b>Resveratrol<sup>6</sup></b>	0.96 +/- 0.13	NT	NT	NT	NT	NT
<b>Melatonin<sup>6</sup></b>	11.3 +/- 2.1	NT	N	NT	NT	NT
<b>Aminoglutethimide</b>		0.27	NT	NT	NT	NT

<sup>a</sup>Not tested.



**Table 2**  
Data Collection and Refinement Statistics for QR2-Inhibitor Complexes

QR2-Inhibitor Complex	<i>I</i>	<i>II</i>
<b>Data Collection</b>		
Space Group	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>
Unit Cell Dimensions a, b, c (Å)	56.73, 84.23, 106.31	56.34, 83.88, 106.69
Resolution (Å)	66.08 – 1.85(1.92 – 1.85)	65.49 – 1.98(2.04 – 1.98)
No. Reflections Observed	241,450	197,663
No. Unique Reflections	44,812	35,682
R <sub>merge</sub> (%)	9.3(62.9)*	8.4(29.1)*
I/σI	17.8(2.0)*	21.1(4.5)*
% Completeness	99.9(99.4)*	99.3(95.2)*
<b>Refinement</b>		
Resolution Range	66.08 – 1.84	65.49 – 1.98
No. Reflections in Working Set	42,486	33,839
No. Reflections in Test Set	2,256	1,788
R <sub>work</sub> (%)	18.0 (28.5)*	18.1 (22.2)*
R <sub>free</sub> (%)	23.1 (35.7)*	22.7 (33.0)*
Average B-factor (Å <sup>2</sup> )	22.2	26.2

\* Highest resolution data shell is given in parentheses