



## Original article

## Synthesis, biological evaluation, and docking studies of gigantol analogs as calmodulin inhibitors

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## ABSTRACT

Several analogs of gigantol (**1**) were synthesized to evaluate their effect on the complexes  $\text{Ca}^{2+}$ –calmodulin (CaM) and  $\text{Ca}^{2+}$ –CaM–CaM sensitive phosphodiesterase 1 (PDE1). The compounds belong to four structural groups including, 1,2-diphenylethanes (**2–11**), diphenylmethanes (**13–15**), 1,3-diphenylpropanones (**16–18**), and 1,3-diphenylpropanes (**20–22**). *In vitro* enzymatic studies showed that all compounds except **11** inhibited the complex  $\text{Ca}^{2+}$ –CaM–PDE1 with  $\text{IC}_{50}$  values ranging from 9 to 146  $\mu\text{M}$ . On the other hand, all analogs but **11**, **12** and **15** quenched the extrinsic fluorescence of the CaM biosensor *h*CaM–M124C–*mBBr* to different extent, then revealing different affinities to CaM; their affinity constants ( $K_m$ ) values were in the range of 3–80  $\mu\text{M}$ . Molecular modeling studies indicated that all these compounds bound to CaM at the same site that the classical inhibitors trifluoperazine (TFP) and chlorpromazine (CPZ). Some of these analogs could be worthy candidates for developing new anti-tumor, local anesthetics, antidepressants, antipsychotic, or smooth muscle relaxant drugs, with anti-CaM properties due to their good affinity to CaM and the straightforwardness of their synthesis. In addition they could be valuable tools for the study of  $\text{Ca}^{2+}$ –CaM functions.

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## 1. Introduction

Gigantol (**1**) is a naturally occurring 1,2-diphenylethane (bibenzyl) isolated from several medicinal orchids. In previous investigations, the anti-inflammatory, antinociceptive, phytotoxic, and spasmolytic properties of **1** and a few synthetic 1,2-diphenylethanes were demonstrated [1–5]. It was also found that for maximum spasmolytic activity, these products should have oxygenated substituents on both aromatic rings [5]. The spasmolytic 1,2-diphenylethanes or bibenzyls also inhibited the complex  $\text{Ca}^{2+}$ –calmodulin (CaM)–CaM-sensitive phosphodiesterase 1 (PDE1) and potentiated the antispasmodic action of chlorpromazine (CPZ), a classical CaM inhibitor [5]. Altogether, these results suggested that these compounds were CaM inhibitors and that their smooth muscle relaxant activity was mediated by CaM [1,5].

CaM is a major cellular  $\text{Ca}^{2+}$ –binding protein, which is ubiquitously expressed in all the eukaryotic cells [6]. This protein does not possess enzymatic activity, however modulates several enzymes

and ion channels involved in a variety of physiological events including regulation of metabolism, cytoskeleton, ion transport, protein folding, cell proliferation, cell division as well as protein phosphorylation and dephosphorylation, among others. Therefore,  $\text{Ca}^{2+}$ –CaM and  $\text{Ca}^{2+}$ –CaM–protein complexes are of significant biomedical interest as molecular targets for the development of new drugs useful for treating pathological processes related with CaM [7]. Indeed, CaM-induced target enzyme activation can be blocked by several classes of pharmacological effectors: antipsychotics, antidepressants, muscle relaxants, and local anesthetics.

In this study we describe the synthesis and the anti-CaM effect (*in vitro* and *in silico*) of twenty compounds (**2–22**) structurally related to gigantol (**1**), which on the basis of the above considerations could be a suitable lead for the discovery of new CaM antagonists. As compound **1**, analogs **2–11** contain an ethylene bridge and two aromatic rings, with oxygenated or nitrogenated substituents of diverse polarity in different positions; these modifications would be useful for ascertaining the influence of the nature and location of the substituents along the 1,2-diphenylethane core on the anti-CaM effect of gigantol (**1**). Compound **12**, includes a heteroatom in the bridge and possesses no substituents at the aromatic rings. On the other hand, to assess the contribution of the size and nature of the carbon chain between the two phenyls, analogs **13–22** were also

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analyzed. Analogs **13–15** are diphenylmethanes, **16–18** 1,3-diphenylpropenones (chalcones), and **20–22** are 1,3-diphenylpropanes. Thus, the last three set of compounds differ in the number of carbon atoms linking the two aromatic rings and their degree of oxidation. It is important to point out that diphenylmethanes, 1,3-diphenylpropanes or the chalcones have not previously tested as anti-CaM agents therefore the corresponding scaffold could be a new leads for the development of new CaM antagonists.

## 2. Results and discussion

Compounds **1–10** were obtained using the Wittig reaction, a method widely used for the synthesis of 1,2-diphenylethanes [5]. In all cases, the phosphonium salts were prepared by the reaction of triphenylphosphine with an appropriated alkyl halide; the resulting salts were dissolved in THF and deprotonated with NaH to yield a mixture of *Z*- and *E*-stilbenes, which were reduced by treatment with catalytic amounts of 10% Pd–C over H<sub>2</sub> (4 atm) to afford the 1,2-diphenylethanes [5]; the general route employed is summarized in Scheme 1. The yields ranged from 41 to 72%. Compounds **4–7**, **9**, and **10** are new chemical entities, while **3** was isolated from a *Dendrobium* species [8]. Finally, bibenzyls **2** and **8** were previously synthesized [9]. Diphenylmethanes **13** and **14** were obtained by an acid catalyzed intramolecular rearrangement of benzyl phenyl ether **26** (Scheme 2), which in turn was prepared by an *O*-alkylation of guaiacol with 3,5-dimethoxybenzyl bromide (**25**) in basic conditions [10]. Chalcones **16–19** were synthesized via a base catalyzed aldol condensation of the appropriate acetophenone (**27a–27c**) with a suitable benzaldehyde derivative (**24b**, **24c** and **24g–24h**). Finally, 1,3-diphenylpropanes **20–22** were obtained by one-step catalytic reduction of chalcones **16** [11], **18**, and **19** using H<sub>2</sub>SO<sub>4</sub> and 10% Pd/C, in ethanol at 60 °C (Scheme 3). Compounds **17–22** are reported by the first time. The structures of synthetic analogs **1–11**, **13**, **14**, and **17–22** were elucidated on the basis of 1D-, 2D-NMR, and MS studies.

The *in vitro* anti-CaM activity of compounds **1–18** and **20–22** was determined using two methodologies; the first one, enzymatic in nature, employed PDE1 as monitoring enzyme and, assesses the action of the test compounds on the complex Ca<sup>2+</sup>–CaM–PDE1 [12,13]. The second analysis measures the direct interaction and affinity of any compound to the complex Ca<sup>2+</sup>–CaM; for this assay

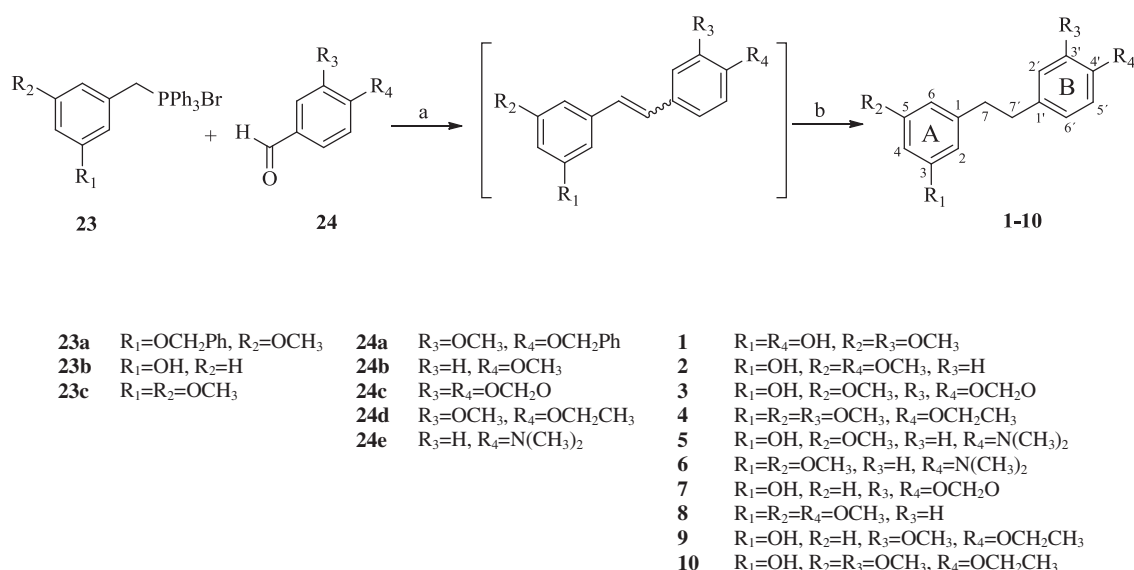
a *h*CaM M124C–*m*BBr fluorescent biosensor was used [14]. The results (Table 1 and Fig. 1) showed that all compounds but **11**, **12**, and **15** did not bind to the complex Ca<sup>2+</sup>–CaM. These three compounds have in common the absence of substituents in rings A and B of the 1,2-diphenylethane, diphenylmethane or benzylphenoxycy cores (see Scheme 1) suggesting that the substitution at both aromatic rings, regardless of the nature and number of the bridge carbons, is an important structural feature for Ca<sup>2+</sup>–CaM binding. This assumption was further confirmed throughout the docking analysis (*vide infra*, Table 1). In any case, however, the position of the phenols did not have a clear impact on the affinity to the protein.

Regarding their effect on the complex Ca<sup>2+</sup>–CaM–PDE1, **11** and **12** did not inhibit the hydrolysis of cAMP; however **15** clearly displayed an inhibitory effect of the enzymatic activity with an IC<sub>50</sub> of 15 μM. The latter results suggested that **15** could be affecting any components enzymatic assay including the snake nucleotidase or the PDE1. These results showed the pitfalls of the enzymatic assay and the robustness of direct measurements using a *h*CaM M124C–*m*BBr fluorescent biosensor.

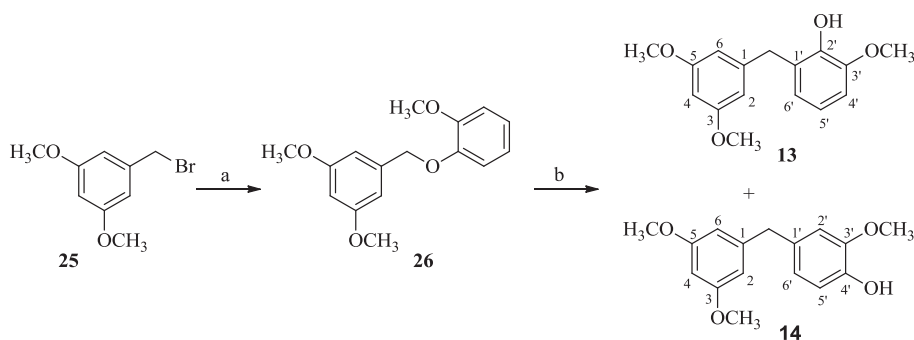
The remaining analogs tested showed different affinities to the protein with *K<sub>m</sub>* values in the range of 3–80 μM. On the other hand, compounds **11**, **12** and **8** did not affect the complex Ca<sup>2+</sup>–CaM–PDE1; the other compounds (**1–7**, **9**, **10**, **13–18**, **20–22**) however, inhibited the activation of PDE1 with IC<sub>50</sub> values ranging from 9 to 75 μM.

Among the group of 1,2-diphenylethanes, compounds **4–6** exhibited the highest affinity to the protein with *K<sub>m</sub>* of 9, 12 and 10 μM, respectively. The better affinity of compound **5** in comparison to **1** revealed that the presence of a tertiary amine in ring B increased the affinity to the protein by five times. On the other hand, comparison of the *K<sub>m</sub>* values of **1** and **10** (*K<sub>m</sub>* of 60 and 25 μM, respectively), clearly indicated that the etherification of the free phenol group at C-4 in ring B improved the affinity for the protein almost three fold. The lower *K<sub>m</sub>* value for compound **4** (*K<sub>m</sub>* = 9.88 μM), in comparison with **10** (*K<sub>m</sub>* = 25.26 μM) further supports this comportment. Comparison of the *K<sub>m</sub>* values of compounds **4**, **10**, and **9** (9, 25, and 58 μM, respectively) with those of **2**, **3**, **7**, and **8** (*K<sub>m</sub>* of 80, 45, 63, and 21 μM, respectively), strengthened the relevance of hydrophobic substituents for better affinity to Ca<sup>2+</sup>–CaM *in vitro*.

In the enzymatic study, in general the 1,2-diphenylethanes showed a similar trend of effects (Table 1) since compounds **4–6**



**Scheme 1.** Reagents and conditions for the synthesis of 1,2-diphenylethanes **1–10**: (a) NaH, THF; (b) H<sub>2</sub>, 10% Pd–C, EtOAc.



**Scheme 2.** Reagents and conditions for the synthesis of compounds **13** and **14**: (a) guaiacol,  $K_2CO_3$ , acetone; (b)  $H_3PO_4$ , toluene.

were among those that clearly inhibited the activation of PDE1 with  $IC_{50}$  between 18 and 36  $\mu M$ . The exception was compound **15** which did not bind to  $Ca^{2+}$ -CaM but showed good inhibitory effect of the enzymatic activity of the complex  $Ca^{2+}$ -CaM-PDE1. This effect could be due to a direct interaction of **15** with PDE1.

In the group of diphenylmethanes, **13** and **14** showed similar affinity to CaM ( $K_m$  values  $\sim 33 \mu M$ ) as well as similar inhibitory effect on enzymatic activity of the complex  $Ca^{2+}$ -CaM-PDE1. Compared with all compounds tested, their affinity to CaM was comparable to that of compound **10**.

The 1,3-diphenylpropanes **20–22** showed  $K_m$  values ranged between 8.7 and 54  $\mu M$ , being **20** the most affine. Comparison of the  $K_m$  values of compounds **21** and **7** indicated that the increment of the chain length between the two phenyl rings increases the affinity to  $Ca^{2+}$ -CaM. This difference could be due to the highest conformational flexibility of 1,3-diphenylpropanes in comparison to 1,2-diphenylethanes which allows better coupling of the former group to the protein, as could be inferred from the docking analysis (*vide infra*).

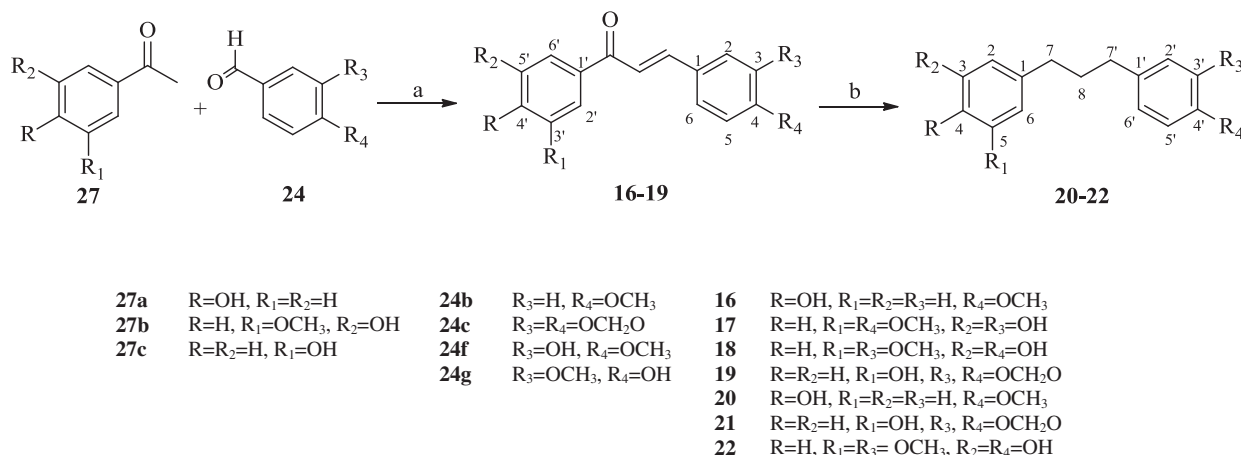
During the preparation of compounds **20** and **22**, chalcones **16–19** were obtained as intermediates and thereafter included in this study. The results summarized in Table 1 indicated that intermediates **17** and **18** displayed the highest affinity to  $Ca^{2+}$ -CaM among all compounds tested. Indeed, comparison of **1**, **18** and **22** ( $K_m = 60.8$ , 5.3 and 54.3  $\mu M$ ) revealed that the presence of a conjugated ketone group between the two phenyl rings significantly increased the affinity for CaM. However, comparison between **16** and **20** ( $K_m = 10.2$  and 8.7  $\mu M$ ) indicated that the substitution pattern in the two rings could be more important than the nature of the chain linking them. The better affinity of

compound **17** over the remaining compounds could be also explained in terms of structural energy; thus, according with the docking analysis, **17** shows one hydrogen bond interaction (*vide infra*).

Altogether, the results revealed that among similar compounds those possessing hydrophobic substituents had the best affinity for the complex  $Ca^{2+}$ -CaM and better inhibitory effect of the complex  $Ca^{2+}$ -CaM-PDE1, being the most relevant compounds 1,2-diphenylethane **4**, chalcones **17** and **18** as well as 1,3-diphenylpropane **20**. In most cases the better affinity for the protein correlated with the inhibitory effect of the enzyme activity.

## 2.1. Docking

To investigate the binding mode of gigantol (**1**) analogs to CaM (PDB code 1LIN), docking studies were performed using the program AUTODOCK 4.0.2 [15,16]. Those compounds exhibiting the highest affinity to the protein, according to the fluorescence assay, were selected; accordingly, the structures of compounds **4**, **11**, **13**, **17** and **20** were optimized with the program Gaussian 09 using the density functional theory method (DFT) at the B3LYP/3-21g level. Initially, the ligands were docked to the entire protein; then, the best conformations were docked in a smaller area (grid) in order to refine the results. The compounds analyzed bound to the pocket corresponding to sites **I**, **IV**, **IV** and **IV**, respectively (Fig. 2) as TFP, a classical CaM antagonist [17]. Fig. 2 shows the theoretical binding model of **4**, **13**, **17** and **20**, superimposed with the X-ray structure of the protein with the classical inhibitor TFP. In all cases, regardless of the binding site, one of the two phenyl groups of the scaffold sink in the hydrophobic pockets establishing contact similar to those of



**Scheme 3.** Reagents and conditions for the synthesis of 1,3-diphenylpropanones **16–19** and 1,3-diphenylpropanes **20–22**: (a) KOH, EtOH; (b) Pd-C 10%, EtOH-H<sub>2</sub>SO<sub>4</sub>.

**Table 1**Activity on the  $\text{Ca}^{2+}$ –CaM–PDE1 and properties of binding on the  $\text{Ca}^{2+}$ –CaM complex of compounds **1**–**22**.

| Compound  | Enzymatic PDE1 assay                            | Fluorescence quenching               |             |
|-----------|-------------------------------------------------|--------------------------------------|-------------|
|           | $\text{IC}_{50}$ ( $\mu\text{M}$ ) <sup>a</sup> | $K_m$ ( $\mu\text{M}$ ) <sup>b</sup> | $h^c$       |
| CPZ       | 10.3 ± 1.02                                     | 5.55 ± 0.09                          | 1.28 ± 0.02 |
| <b>1</b>  | 114.18 ± 6.17                                   | 60.82 ± 4.12                         | 1.23 ± 0.04 |
| <b>2</b>  | 9.38 ± 2.48                                     | 80.04 ± 6.17                         | 0.96 ± 0.05 |
| <b>3</b>  | 31.29 ± 5.12                                    | 45.48 ± 1.98                         | 1.59 ± 0.06 |
| <b>4</b>  | 18.44 ± 6.53                                    | 9.88 ± 0.40                          | 2.01 ± 0.12 |
| <b>5</b>  | 30.10 ± 2.90                                    | 12.03 ± 0.38                         | 1.55 ± 0.05 |
| <b>6</b>  | 36.33 ± 7.39                                    | 10.00 ± 0.19                         | 2.55 ± 0.12 |
| <b>7</b>  | 75.89 ± 9.02                                    | 63.77 ± 2.86                         | 1.52 ± 0.06 |
| <b>8</b>  | 146.35 ± 32.46                                  | 21.82 ± 1.32                         | 2.12 ± 0.14 |
| <b>9</b>  | 46.09 ± 4.01                                    | 58.08 ± 5.11                         | 1.26 ± 0.13 |
| <b>10</b> | 54.69 ± 6.22                                    | 25.26 ± 1.58                         | 1.88 ± 0.20 |
| <b>11</b> | NI                                              | NB                                   | NB          |
| <b>12</b> | NI                                              | NB                                   | NB          |
| <b>13</b> | 56.15 ± 14.89                                   | 33.79 ± 5.06                         | 1.01 ± 0.06 |
| <b>14</b> | 54.73 ± 6.47                                    | 33.07 ± 4.58                         | 1.00 ± 0.06 |
| <b>15</b> | 15.02 ± 4.19                                    | NB                                   | NB          |
| <b>16</b> | 22.53 ± 6.52                                    | 10.27 ± 0.70                         | 1.82 ± 0.17 |
| <b>17</b> | 37.68 ± 6.81                                    | 3.89 ± 0.05                          | 1.72 ± 0.03 |
| <b>18</b> | 57.27 ± 15.21                                   | 5.31 ± 0.21                          | 1.49 ± 0.05 |
| <b>20</b> | 62.68 ± 3.59                                    | 8.70 ± 0.15                          | 1.35 ± 0.03 |
| <b>21</b> | 22.94 ± 4.05                                    | 14.21 ± 0.85                         | 0.90 ± 0.03 |
| <b>22</b> | 32.54 ± 6.06                                    | 54.33 ± 6.41                         | 0.97 ± 0.06 |

NB = not bind.

NI = not inhibit.

<sup>a</sup> Concentration inhibiting by 50% the activity of the enzyme.<sup>b</sup> Apparent constant.<sup>c</sup> Hill coefficient.

TFP or W7, which are classical inhibitors of CaM [18]. In the case of compound **4**, the binding site in the docking model consists of Phe92, Leu105, Met109, Met124, Glu127, Ala128, Val136, and Met144. Compound **13** binding site comprises Phe19, Leu32, Met51, Glu54, Val55, Ile-53, and Phe68. Compound **17** binding pocket includes Phe19, Leu32, Val35, Met36, Leu39, Gln41, Ile63, Phe68, Met51, Ile52, and Val55. Finally, in the case of **20**, Phe19, Met51, Ile52, Val55, Ile63, Phe68 and Met71 were part of the pocket. Although there are not clear clues of which are the structural features responsible for the affinity of these compounds to CaM, the highest affinity of **17** to the protein might be due to differences in the contact surfaces and/or protein conformation upon interaction of the ligands, despite some common amino acid residues at the binding sites; additionally this compound show one hydrogen bond interactions between Met51/O and hydroxyl in the position R<sub>2</sub> of **17**. The results of the docking study revealed also those compounds **4**, **13**, **17** and **20** form mainly hydrophobic and/or  $\pi$ – $\pi$  interactions with the protein as does AAA. In the case of compound **11**, the docking study predicted no binding to the classical CaM binding sites **I**–**IV** (Fig. 2).

### 3. Conclusions

Several 1,2-diphenylethanes, 1,3-diphenylpropanones, diphenylmethanes, and 1,3-diphenylpropanes with CaM inhibitor properties were discovered. The simplicity and good yields of their synthesis make these compounds good candidates for the development of new CaM-inhibitors. Although there are not clear clues of what are the structural features responsible for the affinity of these compounds to CaM, some of them showed better affinity for the protein than gigantol (**1**) and CPZ [18]. The best ligand was chalcone **17** with a  $K_m$  value of 3.89  $\mu\text{M}$ . Among the 1,2-diphenylethanes and 1,3-diphenylpropanes, only derivatives **4**–**6** and **20**, respectively, possessed good affinity to the protein with  $K_m$  values approximately six fold than **1**. Compounds **4**, **13**, **17** and **21** bind to

CaM in the same region that the classical inhibitors TFP and W-7 mainly through hydrophobic, according to a docking analysis.

Once more the results of our study clearly show that different ligands bind differentially to CaM complexes ( $\text{Ca}^{2+}$ –CaM or  $\text{Ca}^{2+}$ –CaM–PDE1) which could have different implications at physiological level. Thus, in the present work compound **15** did not bind to  $\text{Ca}^{2+}$ –CaM but showed good inhibitory effect of the enzymatic activity of the complex  $\text{Ca}^{2+}$ –CaM–PDE1; however, further work is needed to demonstrate the **15** selectively bind to the complex  $\text{Ca}^{2+}$ –CaM–PDE1 or to PDE1. The relevance of differentially activate one particular complex could result in selective pharmacological activity and a decrease of side effects of any potential drug.

## 4. Experimental

### 4.1. Chemistry

#### 4.1.1. General experimental procedures

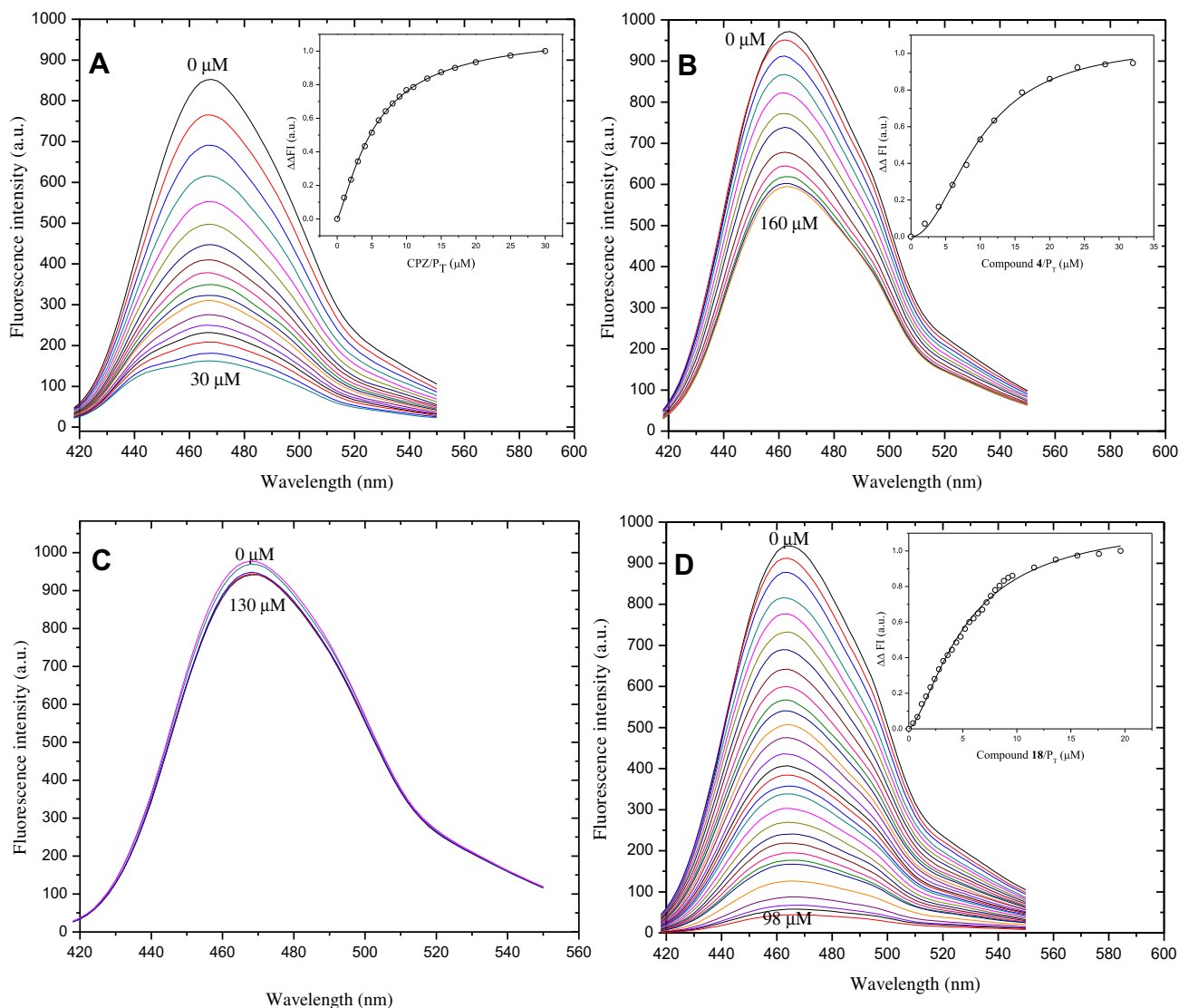
Melting points were determined on a Fisher-Johns apparatus and are uncorrected. IR spectra were obtained using KBr disks on a Perkin–Elmer FT 1605 spectrophotometer. NMR spectra were recorded in  $\text{CDCl}_3$  on a Varian Unity Plus 500 spectrometer either at 300 and 400 ( $^1\text{H}$ ) or 100 ( $^{13}\text{C}$ ) MHz, using tetramethylsilane (TMS) as an internal standard. Electron-impact mass spectra (EIMS) were registered on a JEOL SX 102 mass spectrometer. Open column chromatography was carried out on silica gel 60 (70–230 mesh, Merck, Darmstadt, Germany). Analytical and preparative TLC was performed on precoated silica gel 60 F<sub>254</sub> plates (Merck, Darmstadt, Germany).

#### 4.1.2. Synthetic intermediates

Diphenylethane (**11**), benzyl phenyl ether (**12**), diphenylmethane (**15**), 4-benzyloxy-3-methoxybenzaldehyde (**24a**), 4-methoxybenzaldehyde (**24b**), 3,4-(methylenedioxy)benzaldehyde (**23c**), 4-ethoxy-3-methoxybenzaldehyde (**24d**), 4-(dimethylamino)benzaldehyde (**24e**), 3-hydroxy-4-methoxybenzaldehyde (**24f**), 4-hydroxy-3-methoxybenzaldehyde (**24g**), 4'-hydroxyacetophenone (**27a**), 3'-hydroxy-5'-methoxyacetophenone (**27b**), 3'-hydroxyacetophenone (**27c**), and guaiacol were purchased from Sigma–Aldrich (St Louis, MO, USA). 3-Benzyloxy-5-methoxybenzyltriphenylphosphonium bromide (**23a**), 3-hydroxybenzyltriphenylphosphonium bromide (**23b**), 3,5-dimethoxybenzyltriphenylphosphonium bromide (**23c**), and 3,5-dimethoxybenzyl bromide (**25**), were prepared as previously described in Ref. [5].

#### 4.1.3. General procedure for the synthesis of 1,2-diphenylethanes **1**–**10**

The Wittig salt (**23a**–**23c**; 4.4 mmol) and NaH (4.5 mmol) were dissolved in dry tetrahydrofuran (50 mL) under a  $\text{N}_2$  atmosphere; then, the appropriated aromatic aldehyde (**24a**–**24e**; 4.4 mmol) was added to the mixture, which was stirred during 3 h at room temperature. After this period of time, water was added and the product extracted with EtOAc. The resulting organic phases were washed with brine and water, dried (anh.  $\text{Na}_2\text{SO}_4$ ) and evaporated *in vacuo* to give crude oily residues which were purified by open column chromatography (silica gel; 30 g), hexane–EtOAc to yield a mixture of *Z*- and *E*-stilbenes; the mixture were directly hydrogenated at 60 lb/in<sup>2</sup> (30 °C) in EtOAc (40 mL) over 10% palladium on carbon (0.1 g) for 3 h; the catalyst was then filtered off, and the resulting mixtures evaporated to dryness. These crude reaction mixtures were then purified by open column chromatography to give the final products **1**–**10**. In all cases, for 1 g of crude reaction mixture the chromatographic column was packed with 30 g of silica gel. The eluents consisted in mixtures of hexane– $\text{CH}_2\text{Cl}_2$  (1:1 → 0:1) and  $\text{CH}_2\text{Cl}_2$ –MeOH (1:0 → 9:1).



**Fig. 1.** Fluorescence spectra and titration curves of  $\text{Ca}^{2+}$ -hCaM-M124C-mBBr in the presence of CPZ (A), **4** (B), **11** (C), and **18** (D). Buffer was 100 mM of potassium acetate (pH 5.1) at 37 °C and 1 mM  $\text{CaCl}_2$ . Samples were excited at 381 nm, and emission spectra recorded for light scattering effects from 400 to 550 nm. The absolute changes of maximal fluorescence emission were plotted against the ration compounds/protein total and fitted to the binding equation model to obtain the  $K_m$ .

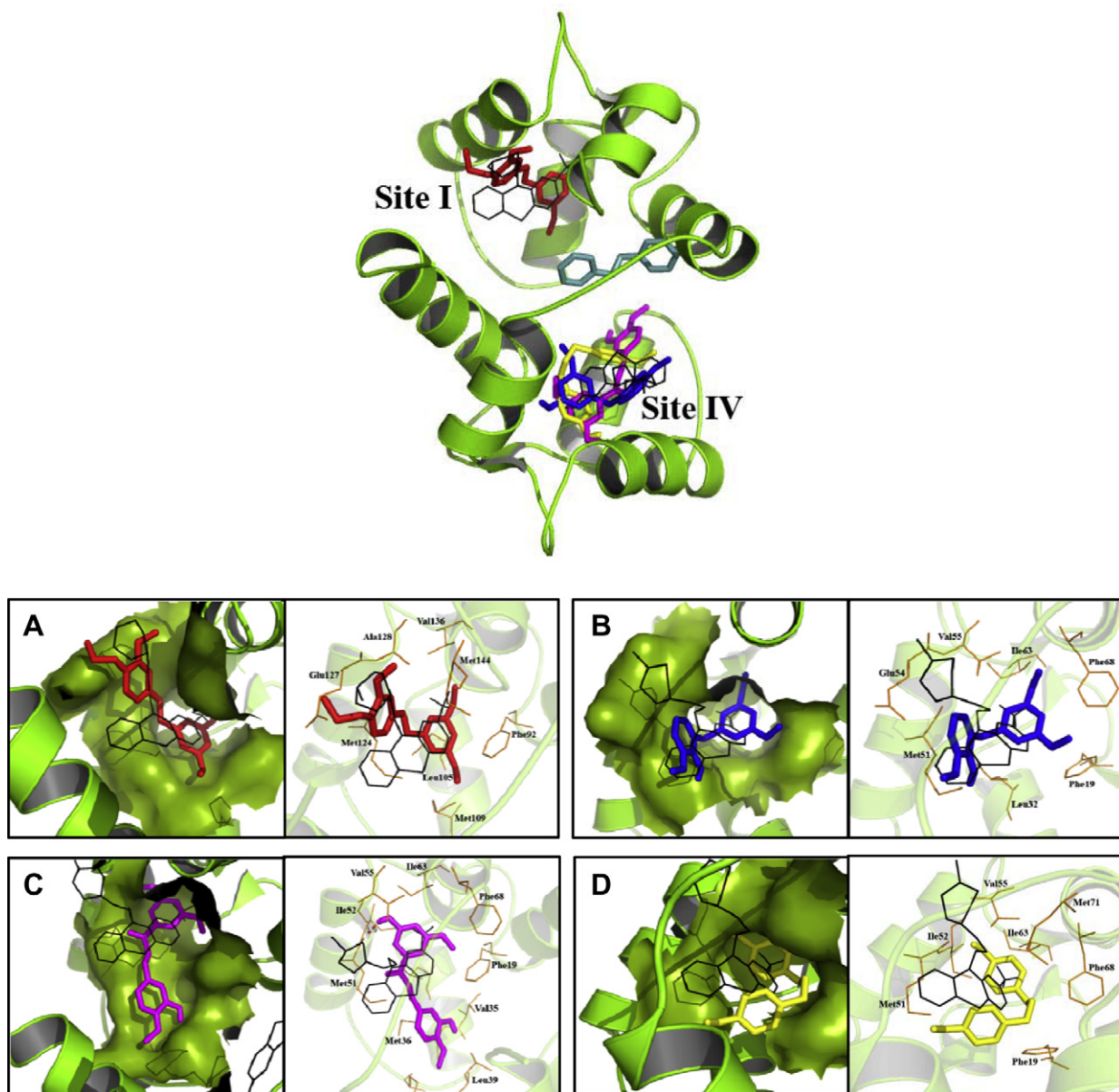
**4.1.3.1. 4-[2-(3-Hydroxy-5-methoxyphenyl)ethyl]-2-methoxyphenol (1).** The pure compound **1** (0.47 g, yield 41.3%) was obtained as a colorless oil and was identical to a reference standard isolated from *Scaphyglottis livida* (Orchidaceae) [19].

**4.1.3.2. 3-Methoxy-5-[2-(4-methoxyphenyl)ethyl]phenol (2).** Compound **2** was obtained as an amber oil (0.47 g, yield 41%); UV (MeOH)  $\lambda_{\text{max}}$  276, 208 nm; IR (KBr)  $\nu_{\text{max}}$  3401, 2933, 1597, 1453, 1244, 1148  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  2.75–2.85 (4H, m, H-7, H-7'), 3.75 (3H, s,  $\text{CH}_3\text{O}-5$ ), 3.79 (3H, s,  $\text{CH}_3\text{O}-4'$ ), 4.77 (1H, s, HO-3), 6.25 (2H, d,  $J = 1.8$  Hz, H-2, H-6), 6.32 (1H, t,  $J = 1.8$  Hz, H-4), 6.82 (2H, d,  $J = 8.8$  Hz, H-3', H-5'), 7.11 (2H, d,  $J = 8.8$  Hz, H-2', H-6');  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  36.8 (C-7'), 38.4 (C-7), 55.5 ( $\text{CH}_3\text{O}-4'$ ), 59.2 (C-4), 107.0 (C-6), 108.0 (C-2), 113.9 (C-3', C-5'), 129.5 (C-2', C-6'), 133.9 (C-1'), 144.8 (C-1), 156.8 (C-4'), 158.1 (C-3), 161.1 (C-5); EM-IE ( $m/z$ ) 258 [ $\text{M}^+$  (65)], 121 (100). HREIMS [ $\text{M}^+$  ( $m/z$ )] 258.1254 (calcd for  $\text{C}_{16}\text{H}_{18}\text{O}_3$  258.1256).

**4.1.3.3. 3-[2-(1,3-Benzodioxol-5-yl)ethyl]-5-methoxyphenol (3).** Diphenylethane **3** was obtained as yellow crystals (0.74 g, yield 61.7%); mp 115 °C; UV (MeOH)  $\lambda_{\text{max}}$  281, 212 nm; IR (KBr)  $\nu_{\text{max}}$  3306, 2927, 1592,

1484, 1243  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  2.75–2.80 (4H, m, H-7, H-7'), 3.75 (3H, s,  $\text{CH}_3\text{O}-5$ ), 5.02 (1H, s, HO-3), 5.92 (2H, s,  $-\text{OCH}_2\text{O}-$ ), 6.25 (2H, d,  $J = 2.2$  Hz, H-2, H-6), 6.32 (1H, t,  $J = 2.2$  Hz, H-4), 6.63 (1H, dd,  $J = 7.9, 1.6$  Hz, H-6'), 6.65 (1H, d,  $J = 1.6$  Hz, H-5'), 6.72 (1H, d,  $J = 7.9$  Hz, H-2');  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  37.2 (C-7'), 38.2 (C-7), 55.2 ( $\text{CH}_3\text{O}-3$ ), 99.0 (C-4), 100.7 ( $-\text{OCH}_2\text{O}-$ ), 108.1 (C-6), 106.8 (C-2), 107.9 (C-2'), 108.9 (C-5'), 121.2 (C-6'), 135.5 (C-1'), 144.4 (C-1), 145.7 (C-4'), 147.5 (C-3'), 156.5 (C-3), 160.9 (C-5); EM-IE ( $m/z$ ) 272 [ $\text{M}^+$  (44)], 135 (100). HREIMS [ $\text{M}^+$  ( $m/z$ )] 272.1045 (calcd for  $\text{C}_{16}\text{H}_{16}\text{O}_4$  272.1049).

**4.1.3.4. 4-[2-(3,5-Dimethoxyphenyl)ethyl]-1-ethoxy-2-methoxybenzene (4).** Diphenylethane **4** was obtained as a yellow powder (1.0 g, yield 72.1%); mp 82 °C; UV (MeOH)  $\lambda_{\text{max}}$  279, 208 nm; IR (KBr)  $\nu_{\text{max}}$  3072, 2935, 1598, 1469, 1136  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.45 (3H, t,  $J = 6.8$  Hz,  $\text{CH}_3\text{CH}_2\text{O}-$ ), 2.84 (4H, br s, H-7, H-7'), 3.76 (6H, s,  $\text{CH}_3\text{O}-3$ ,  $\text{CH}_3\text{O}-5$ ), 3.83 (3H, s,  $\text{CH}_3\text{O}-3'$ ), 4.07 (2H, q,  $J = 6.8$  Hz,  $\text{CH}_3\text{CH}_2\text{O}-$ ), 6.31 (1H, t,  $J = 2.4$  Hz, H-4), 6.34 (2H, d,  $J = 2.4$  Hz, H-2, H-6), 6.67 (1H, d,  $J = 2.0$  Hz, H-2'), 6.71 (1H, dd,  $J = 8.0, 2.0$  Hz, H-6'), 6.79 (1H, d,  $J = 8.0$  Hz, H-5');  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  14.8 ( $\text{CH}_3\text{CH}_2\text{O}-$ ), 37.3 (C-7), 38.4 (C-7'), 55.2 ( $\text{CH}_3\text{O}-3$ ,  $\text{CH}_3\text{O}-5$ ), 55.8



**Fig. 2.** Binding model of compounds with the complex  $\text{Ca}^{2+}$ -CaM. CaM is represented in green cartoon. The TFP is depicted in black lines, compound **4** in red sticks, **11** in cyan sticks, **13** in blue sticks, **17** in magenta sticks and **20** in yellow stick. Hydrophobic pockets are shown in surface and interacting amino acids within the CaM-ligands complex of the compounds **4**(A), **13**(B), **17**(C) and **20**(D). This figure was generated using the program PyMOL. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

( $\text{CH}_3\text{O}-3'$ ), 64.3 ( $\text{CH}_3\text{CH}_2\text{O}-$ ), 97.8 (C-4), 106.5 (C-2, C-6), 112.0 (C-2'), 112.7 (C-5'), 120.2 (C-6'), 134.3 (C-1'), 144.2 (C-1), 146.4 (C-4'), 149.0 (C-3'), 160.7 (C-3, C-5); EM-IE ( $m/z$ ) 316 [ $\text{M}^+$  (36)], 165 (100), 137 (70). HREIMS [ $\text{M}]^+(m/z)$  316.1670 (calcd for  $\text{C}_{19}\text{H}_{24}\text{O}_4$  316.1675).

**4.1.3.5. 3-[2-[4-(Dimethylamino)phenyl]ethyl]-5-methoxyphenol (5).** Compound **5** was obtained as a yellow powder (0.57 g, yield 48.2%); mp 146 °C; UV (MeOH)  $\lambda_{\text{max}}$  253, 208 nm; IR (KBr)  $\nu_{\text{max}}$  3081, 2933, 1599, 1454, 1149  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  2.77 (4H, br s, H-7, H-7'), 2.93 (6H, s,  $(\text{CH}_3)_2\text{N}-$ ), 3.75 (3H, s,  $\text{CH}_3\text{O}-5$ ), 5.70 (1H, br s, HO-3), 6.20 (1H, br s, H-4), 6.26 (1H, t,  $J = 2.4$  Hz, H-2), 6.32 (1H, br s, H-6), 6.82 (2H, d,  $J = 8.4$  Hz, H-3', H-5'), 7.09 (2H, d,  $J = 8.4$  Hz, H-2', H-6');  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  36.6 (C-7'), 38.1 (C-7), 41.6 ( $(\text{CH}_3)_2\text{N}-$ ), 55.2 ( $\text{CH}_3\text{O}-5$ ), 98.9 (C-4), 106.6 (C-6), 108.0 (C-2), 114.2 (C-3', C-5'), 129.1 (C-2', C-6'), 131.9 (C-1'), 144.6 (C-1), 148.3 (C-4'), 156.8 (C-3),

160.8 (C-5); EM-IE ( $m/z$ ) 271 [ $\text{M}^+$  (27)], 134 (100), 118 (6). HREIMS [ $\text{M}]^+(m/z)$  271.1244 (calcd for  $\text{C}_{17}\text{H}_{21}\text{NO}_2$  271.1572).

**4.1.3.6. N-[4-[2-(3,5-Dimethoxyphenyl)ethyl]phenyl]-N,N-dimethylamine (6).** Compound **6** was obtained as a white powder (0.77 g, yield 61.8%); mp 41 °C; UV (MeOH)  $\lambda_{\text{max}}$  254, 207 nm; IR (KBr)  $\nu_{\text{max}}$  3430, 2927, 1596, 1522, 1346, 1159  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  2.29 (4H, s, H-7, H-7'), 2.98 (6H, s,  $\text{N}(\text{CH}_3)_2$ ), 3.76 (6H, s,  $\text{CH}_3\text{O}-3$ ,  $\text{CH}_3\text{O}-5$ ), 6.28 (1H, m, H-4), 6.64 (2H, m, H-2, H-6), 6.73 (2H, d,  $J = 8.1$  Hz, H-3', H-5'), 7.12 (2H, d,  $J = 8.4$  Hz, H-2', H-6');  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  37.4 (C-7'), 38.0 (C-7), 41.3 ( $\text{N}(\text{CH}_3)_2$ ), 55.8 ( $\text{CH}_3\text{O}-3$ ,  $\text{CH}_3\text{O}-5$ ), 97.6 (C-4), 106.5 (C-2, C-6), 112.8 (C-3', C-5'), 128.6 (C-2', C-6'), 131.6 (C-1'), 145.1 (C-1), 148.3 (C-4'), 161.5 (C-3, C-5); EM-IE ( $m/z$ ) 285 [ $\text{M}^+$  (69)], 134 (100), 118 (21). HREIMS [ $\text{M}]^+(m/z)$  285.1244 (calcd for  $\text{C}_{18}\text{H}_{23}\text{NO}_2$  285.1729).

**4.1.3.7. 3-[2-(1,3-Benzodioxol-5-yl)ethyl]phenol (7).** This title compound was prepared following the same general procedure except that two equivalent of NaH (9.0 mmol) was used. Compound **7** was obtained as a yellow powder (0.58 g, yield 54.8%); mp 85 °C; UV (MeOH)  $\lambda_{\max}$  281, 209 nm; IR (KBr)  $\nu_{\max}$  3367, 2858, 1483, 1244, 1041  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  2.82 (4H, s, H-7, H-7'), 4.63 (1H, s, HO-3), 5.92 (2H, s,  $-\text{OCH}_2\text{O}-$ ), 6.61 (5H, m, H-2, H-2', H-3', H-4, H-6'), 6.75 (1H, m, H-6), 7.14 (1H, m, H-5);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  37.4 (C-7'), 38.0 (C-7), 100.7 ( $-\text{OCH}_2\text{O}-$ ), 108.1 (C-5'), 108.9 (C-2'), 112.8 (C-4), 115.3 (C-2), 120.9 (C-6), 121.2 (C-6'), 129.5 (C-5), 135.5 (C-1'), 143.5 (C-1), 145.6 (C-4'), 147.5 (C-3'), 155.5 (C-3); EM-IE ( $m/z$ ) 242 [ $\text{M}^+$  (64)], 135 (100), 105 (8), 77 (22). HREIMS [ $\text{M}^+$  ( $m/z$ )] 242.0941 (calcd for  $\text{C}_{15}\text{H}_{14}\text{O}_3$  242.09243).

**4.1.3.8. 1,3-Dimethoxy-5-[2-(4-methoxyphenyl)ethyl]benzene (8).** Compound **8** was obtained as an amber oil (0.53 g, yield 44.5%); UV (MeOH)  $\lambda_{\max}$  277, 208 nm; IR (KBr)  $\nu_{\max}$  2998, 2935, 1596, 1463, 1246, 1153  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  2.80–2.87 (4H, m, H-7, H-7'), 3.76 (6H, s,  $\text{CH}_3\text{O}-3$ ,  $\text{CH}_3\text{O}-5$ ), 3.78 (3H, s,  $\text{CH}_3\text{O}-4'$ ), 6.30–6.34 (3H, m, H-2, H-4, H-6), 6.82 (2H, d,  $J = 8.7$  Hz, H-3', H-5'), 7.10 (2H, d,  $J = 8.7$  Hz, H-2', H-6');  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  36.7 (C-7), 38.4 (C-7'), 55.21 ( $\text{CH}_3\text{O}-3$ ,  $\text{CH}_3\text{O}-5$ ), 55.23 ( $\text{CH}_3\text{O}-4'$ ), 97.9 (C-4), 106.5 (C-2, C-6), 113.7 (C-3', C-5'), 129.3 (C-2', C-6'), 133.8 (C-1), 144.2 (C-1'), 157.8 (C-4'), 160.7 (C-3, C-5); EM-IE ( $m/z$ ) 272 [ $\text{M}^+$  (24)], 151 (6), 121 (100), 91 (14), 77 (20). HREIMS [ $\text{M}^+$  ( $m/z$ )] 272.1410 (calcd for  $\text{C}_{17}\text{H}_{20}\text{O}_3$  272.1412).

**4.1.3.9. 3-[2-(4-Ethoxy-3-methoxyphenyl)ethyl]phenol (9).** This title compound was prepared following the same general procedure except that two equivalent of NaH (9.0 mmol) was used. Compound **9** was obtained as yellow crystals (0.67 g, yield 56.3%); mp 54 °C; UV (MeOH)  $\lambda_{\max}$  279, 207 nm; IR (KBr)  $\nu_{\max}$  3536, 2944, 1590, 1516, 1362, 1229  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.45 (3H, t,  $J = 7.0$  Hz,  $\text{CH}_3\text{CH}_2\text{O}-$ ), 2.84 (4H, s, H-7, H-7'), 3.84 (3H, s,  $\text{CH}_3\text{O}-3$ ), 4.07 (2H, q,  $J = 7.0$  Hz,  $\text{CH}_3\text{CH}_2\text{O}-$ ), 4.82 (1H, br s, HO-3), 6.64–6.66 (2H, m, H-2', H-4), 6.66–6.72 (2H, m, H-2, H-6), 6.75 (1H, d,  $J = 7.5$  Hz, H-6'), 6.79 (1H, d,  $J = 7.5$  Hz, H-5'), 7.14 (1H, td,  $J = 7.5, 1.2$  Hz, H-5);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  14.8 ( $\text{CH}_3\text{CH}_2\text{O}-$ ), 37.3 (C-7), 37.9 (C-7'), 55.8 ( $\text{CH}_3\text{O}-$ ), 64.3 ( $\text{CH}_3\text{CH}_2\text{O}-$ ), 112.0 (C-2'), 112.8 (C-4), 115.4 (C-2, C-5'), 120.2 (C-6), 120.9 (C-6'), 129.4 (C-5), 134.3 (C-1'), 143.7 (C-1), 146.4 (C-4'), 148.9 (C-3'), 155.5 (C-3); EM-IE ( $m/z$ ) 272 [ $\text{M}^+$  (37)], 165 (96), 137 (100), 107 (15), 77 (10). HREIMS [ $\text{M}^+$  ( $m/z$ )] 272.1414 (calcd for  $\text{C}_{17}\text{H}_{20}\text{O}_3$  272.1412).

**4.1.3.10. 3-[2-(4-Ethoxy-3-methoxyphenyl)ethyl]-5-methoxyphenol (10).** Compound **10** was obtained as an amber oil (0.81 g, yield 60.8%); UV (MeOH)  $\lambda_{\max}$  280, 208 nm; IR (KBr)  $\nu_{\max}$  3451, 2935, 1600, 1456, 1145  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.45 (3H, t,  $J = 7.1$  Hz,  $\text{CH}_3\text{CH}_2\text{O}-$ ), 2.82 (4H, br s, H-7, H-7'), 3.75 (3H, s,  $\text{CH}_3\text{O}-3'$ ), 3.85 (3H, s,  $\text{CH}_3\text{O}-3$ ), 4.07 (2H, q,  $J = 7.1$  Hz,  $\text{CH}_3\text{CH}_2\text{O}-$ ), 4.82 (1H, s, HO-3), 6.25 (2H, d,  $J = 1.8$  Hz, H-2, H-6), 6.32 (1H, t,  $J = 1.8$  Hz, H-4), 6.66 (1H, d,  $J = 2.0$  Hz, H-2'), 6.69 (1H, dd,  $J = 8.1, 2.0$  Hz, H-6'), 6.79 (1H, d,  $J = 8.1$  Hz, H-5');  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  15.1 ( $\text{CH}_3\text{CH}_2\text{O}-$ ), 37.4 (C-7'), 38.4 (C-7), 55.5 ( $\text{CH}_3\text{O}-3$ ), 56.2 ( $\text{CH}_3\text{O}-3'$ ), 64.7 ( $\text{CH}_3\text{CH}_2\text{O}-$ ), 99.3 (C-4), 107.1 (C-6), 108.2 (C-2), 112.6 (C-2'), 113.3 (C-5'), 120.6 (C-6'), 134.6 (C-1'), 144.8 (C-1), 146.8 (C-4'), 149.4 (C-3'), 156.8 (C-3), 161.2 (C-5); EM-IE ( $m/z$ ) 302 [ $\text{M}^+$  (38)], 165 (90), 137 (100), 122 (19), 94 (32), 77 (30). HREIMS [ $\text{M}^+$  ( $m/z$ )] 302.1512 (calcd for  $\text{C}_{18}\text{H}_{22}\text{O}_4$  302.1518).

#### 4.1.4. Synthesis of diphenylmethanes **13** and **14**

**4.1.4.1. Synthesis of intermediate 1,3-dimethoxy-5-[2-(2-methoxyphenoxy)methyl]benzene (26).** To a solution of 8.5 g (36.8 mmol) of 3,5-dimethoxybenzyl bromide (**25**) in acetone (65 mL), 5.0 g (40.3 mmol) of guaiacol, 5.5 g (39.9 mmol) of  $\text{K}_2\text{CO}_3$ , and 1.0 g

(6.1 mmol) of KI were added. The mixture was stirred and heated to reflux for 10 h; afterward, the solvent was removed *in vacuo*. To the previous resulting mixture 30 mL of water were added at room temperature and extracted with EtOAc ( $2 \times 25$  mL). The combined organic layers were consecutively washed with an aqueous solution of NaOH (5%, 20 mL), and brine ( $2 \times 25$  mL), and finally dried over anhydrous  $\text{Na}_2\text{SO}_4$ . After elimination of the solvent *in vacuo*, the crude reaction mixture was purified by flash column chromatography on silica gel (hexane-EtOAc, 9:1; for 1.0 g of crude reaction, 30 g of silica gel were packed in the column) to give compound **26** (6.72 g, yield 66.7%). UV (MeOH)  $\lambda_{\max}$  275, 207 nm; IR (KBr)  $\nu_{\max}$  2939, 2837, 1598, 1505, 1456, 1251  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  3.78 (6H, s,  $\text{CH}_3\text{O}-3$ ,  $\text{CH}_3\text{O}-5$ ), 3.89 (3H, s,  $\text{CH}_3\text{O}-2'$ ), 5.10 (2H, s,  $-\text{CH}_2\text{O}-$ ), 6.38 (1H, t,  $J = 2.4$  Hz, H-4), 6.60 (2H, d,  $J = 2.4$  Hz, H-2, H-6), 6.81–6.95 (4H, m, ArH);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  55.3 ( $\text{CH}_3\text{O}-3$ ,  $\text{CH}_3\text{O}-5$ ), 55.9 ( $\text{CH}_3\text{O}-2'$ ), 71.0 ( $-\text{CH}_2\text{O}-$ ), 99.8 (C-4), 104.9 (C-2, C-6), 111.9 (C-3'), 114.3 (C-6'), 120.8 (C-4'), 121.5 (C-5'), 139.8 (C-1), 148.1 (C-2'), 149.7 (C-1'), 160.9 (C-3, C-5); EM-IE ( $m/z$ ) 274 [ $\text{M}^+$  (27)], 151 (100), 77 (43). HREIMS [ $\text{M}^+$  ( $m/z$ )] 274.1204 (calcd for  $\text{C}_{16}\text{H}_{18}\text{O}_4$  274.1205).

**4.1.4.2. 2-(3,5-Dimethoxybenzyl)-6-methoxyphenol (13) and 4-(3,5-dimethoxybenzyl)-2-methoxyphenol (14).** 1.5 g (5.5 mmol) of **26**, 1.7 mL (29.1 mmol) of  $\text{H}_3\text{PO}_4$ , and 18 mL of toluene were mixed at room temperature. The mixture was stirred and heated under reflux for 6.5 h. The resulting solution was allowed to cool to room temperature; then, 15 mL of water and  $\text{Na}_2\text{CO}_3$ , to pH 12, were added to the reaction mixture, from which the crude products were extracted with EtOAc ( $2 \times 15$  mL). The combined organic layers were washed with brine (10 mL), and dried ( $\text{Na}_2\text{SO}_4$ ). After solvent removal, the residue was purified by flash chromatography (hexane-EtOAc, 9:1; for 1.0 g of crude reaction, 30 g of silica gel were packed in the column) to give products diphenylmethane **13** (0.4 g, yield 26.8%) as yellow powder mp 80 °C, and **14** as a colorless oil (36 mg, yield 2.4%). **13**: UV (MeOH)  $\lambda_{\max}$  273, 226 nm; IR (KBr)  $\nu_{\max}$  3406, 2957, 1590, 1468, 1208, 1159  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  3.74 (3H, s,  $\text{CH}_3\text{O}-3'$ ), 3.75 (6H, s,  $\text{CH}_3\text{O}-3$ ,  $\text{CH}_3\text{O}-5$ ), 3.95 (2H, s,  $-\text{CH}_2-$ ), 5.52 (1H, br s, HO-2'), 6.31 (1H, t,  $J = 2.3$  Hz, H-4), 6.35 (2H, d,  $J = 2.3$  Hz, H-6), 6.66 (1H, dd,  $J = 8.0, 1.7$  Hz, H-6'), 6.84 (1H, dd,  $J = 8.0, 1.7$  Hz, H-4'), 6.94 (1H, t,  $J = 8.0$  Hz, H-5');  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  35.7 ( $-\text{CH}_2-$ ), 55.2 ( $\text{CH}_3\text{O}-3$ ,  $\text{CH}_3\text{O}-5$ ), 61.3 ( $\text{CH}_3\text{O}-3'$ ), 97.9 (C-4), 107.0 (C-2, C-6), 113.9 (C-4'), 122.4 (C-5'), 124.9 (C-6'), 133.5 (C-1'), 142.9 (C-1), 145.4 (C-2'), 148.9 (C-3'), 160.8 (C-3, C-5); EM-IE ( $m/z$ ) 274 [ $\text{M}^+$  (100)], 151 (72.5), 115 (41). HREIMS [ $\text{M}^+$  ( $m/z$ )] 274.1206 (calcd for  $\text{C}_{16}\text{H}_{18}\text{O}_4$  274.1205). **14**: UV (MeOH)  $\lambda_{\max}$  280, 219 nm; IR (KBr)  $\nu_{\max}$  3437, 2935, 1594, 1511, 1460, 1152  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  3.75 (6H, s,  $\text{CH}_3\text{O}-3$ ,  $\text{CH}_3\text{O}-5$ ), 3.88 (3H, s,  $\text{CH}_3\text{O}-3'$ ), 3.94 (2H, s,  $-\text{CH}_2-$ ), 5.72 (1H, s, HO-4'), 6.30 (1H, t,  $J = 2.4$  Hz, H-4), 6.43 (2H, d,  $J = 2.4$  Hz, H-2, H-6), 6.72, (1H, dd,  $J = 6.8, 2.4$  Hz, H-6'), 6.74–6.80 (2H, m, H-2', H-5');  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  35.6 ( $-\text{CH}_2-$ ), 55.2 ( $\text{CH}_3\text{O}-3$ ,  $\text{CH}_3\text{O}-5$ ), 56.0 ( $\text{CH}_3\text{O}-3'$ ), 97.8 (C-4), 107.0 (C-2, C-6), 108.7 (C-2'), 119.4 (C-5'), 122.7 (C-6') 126.6 (C-1'), 143.2 (C-1), 143.4 (C-4'), 146.4 (C-3'), 160.6 (C-3, C-5); EM-IE ( $m/z$ ) 274 [ $\text{M}^+$  (29)], 151 (100), 115 (20.5). HREIMS [ $\text{M}^+$  ( $m/z$ )] 274.1200 (calcd for  $\text{C}_{16}\text{H}_{18}\text{O}_4$  274.1205).

#### 4.1.5. General procedure for the synthesis of 1,3-diphenylpropenones **16–19**

Equimolar portions of acetophenones **27a–27c** (10 mmol) and aromatic aldehydes **24b**, **24c** or **24g** (10 mmol) were dissolved in ethanol (15 mL). 5 mL of a 40% aqueous KOH solution was then slowly added drop wise. The mixture was stirred and heated to reflux for 10 h. The mixture of reaction was poured into ice-water and acidified with cold diluted HCl (2N). The precipitate was

filtered and purified by flash column chromatography on silica gel (hexane-EtOAc, 9:1) to give the final products **16–19**. For 1.0 g of crude reaction, 30 g of silica gel were packed in the column.

**4.1.5.1. (2E)-1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (16).** Compound **16** (1.96 g, yield 77.4%) was obtained as yellow powder; mp 190 °C; UV (MeOH)  $\lambda_{\max}$  344, 236 nm; IR (KBr)  $\nu_{\max}$  3123, 2839, 1645, 1563, 1429, 1166  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  3.89 (3H, s,  $\text{CH}_3\text{O}-4$ ), 4.89 (1H, s,  $\text{HO}-4'$ ), 6.96–6.98 (4H, m, H-3, H-3', H-5, H-5'), 7.45 (1H, d,  $J = 15.6$  Hz,  $=\text{CH}-\alpha$ ), 7.63 (2H, d,  $J = 8.7$  Hz, H-2, H-6), 7.81 (1H, d,  $J = 15.9$  Hz,  $=\text{CH}-\beta$ ), 8.03 (2H, d,  $J = 8.7$  Hz, H-2', H-6');  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  55.4 ( $\text{CH}_3\text{O}-4$ ), 114.4 (C-3, C-5), 115.4 (C-3', C-5'), 119.5 (C- $\alpha$ ), 127.7 (C-1), 130.1 (C-1'), 130.7 (C-2, C-6), 131.0 (C-2', C-6'), 144.1 (C- $\beta$ ), 159.7 (C-4), 161.6 (C-4'), 188.9 (C=O); EM-IE ( $m/z$ ) 254 [ $\text{M}^+$  (100)], 239 (21), 161 (18), 121 (20). HREIMS [ $\text{M}^+$ ]( $m/z$ ) 254.0950 (calcd for  $\text{C}_{16}\text{H}_{14}\text{O}_3$  254.0943).

**4.1.5.2. (2E)-3-(3-hydroxy-4-methoxyphenyl)-1-(3-hydroxy-5-methoxyphenyl)prop-2-en-1-one (17).** Chalcone **17** (2.16 g, yield 72.0%) was obtained as yellow powder; mp 161 °C; UV (MeOH)  $\lambda_{\max}$  356, 207 nm; IR (KBr)  $\nu_{\max}$  3476, 3263, 2840, 1646, 1562, 1266  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  3.82 (3H, s,  $\text{CH}_3\text{O}-4$ ), 3.91 (3H, s,  $\text{CH}_3\text{O}-5'$ ), 6.60 (1H, t,  $J = 2.1$  Hz, H-4'), 6.98 (1H, d,  $J = 8.3$  Hz, H-5), 7.01–7.03 (2H, m, H-2', H-6'), 7.19 (1H, dd,  $J = 8.3, 2.1$  Hz, H-6), 7.21 (1H, d,  $J = 2.1$  Hz, H-2), 7.41 (1H, d,  $J = 15.7$  Hz,  $=\text{CH}-\alpha$ ), 7.67 (1H, d,  $J = 15.7$  Hz,  $=\text{CH}-\beta$ );  $^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  54.5 ( $\text{CH}_3\text{O}-5'$ ), 54.9 ( $\text{CH}_3\text{O}-4$ ), 104.6 (C-6'), 105.5 (C-4'), 107.6 (C-2'), 111.1 (C-5), 113.7 (C-2), 119.4 (C- $\alpha$ ), 122.14 (C-6), 127.9 (C-1), 140.7 (C-1'), 145.4 (C- $\beta$ ), 146.6 (C-3), 150.5 (C-4), 158.7 (C-3'), 161.2 (C-5'), 191.0 (C=O); EM-IE ( $m/z$ ) 300 [ $\text{M}^+$  (100)], 285 (34), 177 (35), 142 (41). HREIMS [ $\text{M}^+$ ]( $m/z$ ) 300.0980 (calcd for  $\text{C}_{17}\text{H}_{16}\text{O}_5$  300.0998).

**4.1.5.3. (2E)-1-(3-hydroxy-5-methoxyphenyl)-3-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one (18).** Chalcone **18** (1.91 g, yield 63.7%) was obtained as yellow powder; mp 161–162 °C; UV (MeOH)  $\lambda_{\max}$  360, 207 nm; IR (KBr)  $\nu_{\max}$  3407, 2943, 1648, 1565, 1428, 1149  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  3.83 (3H, s,  $\text{CH}_3\text{O}-3$ ), 3.93 (3H, s,  $\text{CH}_3\text{O}-5'$ ), 6.60 (1H, t,  $J = 2.0$  Hz, H-4'), 6.84 (1H, d,  $J = 8.4$  Hz, H-6), 7.04 (1H, t,  $J = 2.0$  Hz, H-2'), 7.06 (1H, t,  $J = 2.0$  Hz, H-6'), 7.22 (1H, dd,  $J = 8.4, 2.0$  Hz, H-5), 7.33 (1H, d,  $J = 2.0$  Hz, H-2), 7.46 (1H, d,  $J = 15.6$  Hz,  $=\text{CH}-\alpha$ ), 7.71 (1H, d,  $J = 15.6$  Hz,  $=\text{CH}-\beta$ );  $^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  54.5 ( $\text{CH}_3\text{O}-5'$ ), 55.1 ( $\text{CH}_3\text{O}-3$ ), 104.7 (C-2'), 105.4 (C-4'), 107.6 (C-6'), 110.8 (C-2), 115.2 (C-5), 118.8 (C- $\alpha$ ), 123.5 (C-6), 126.8 (C-1), 140.3 (C-1'), 145.9 (C- $\beta$ ), 148.0 (C-4), 149.7 (C-3), 158.6 (C-3'), 161.1 (C-5'), 191.2 (C=O); EM-IE ( $m/z$ ) 300 [ $\text{M}^+$  (100)], 177 (28), 145 (23). HREIMS [ $\text{M}^+$ ]( $m/z$ ) 300.0980 (calcd for  $\text{C}_{17}\text{H}_{16}\text{O}_5$  300.0998).

**4.1.5.4. (2E)-3-(1,3-benzodioxol-5-yl)-1-(3-hydroxyphenyl)prop-2-en-1-one (19).** To a solution of (1.36 g, 10 mmol) 4'-hydroxyacetophenone (**27c**), (1.5 g, 10 mmol) of 3,4-(methylenedioxy) benzaldehyde (**24c**) in ethanol (15 mL), an aqueous solution of KOH (40%, 5 mL) was added slowly. The mixture was stirred and heated to reflux for 10 h, and then the suspension was poured into ice-water. The precipitate was filtered and dried to yield (E)-3'-hydroxy-3,4-methylenedioxydiphenylpropenone (**19**) (1.7 g, 63.7%) as an amorphous yellow powder; UV (MeOH)  $\lambda_{\max}$  361, 206 nm; IR (KBr)  $\nu_{\max}$  3406, 2945, 1644, 1563  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  5.18 (1H, br s,  $\text{HO}-3'$ ), 6.07 (2H, s,  $-\text{OCH}_2\text{O}-$ ), 6.89 (1H, d,  $J = 7.9$  Hz, H-5), 6.92 (1H, dd,  $J = 7.3, 3.8$  Hz, H-4'), 7.09 (1H, dd,  $J = 8.2, 1.6$  Hz, H-6'), 7.18 (1H, dd,  $J = 7.9, 1.8$  Hz, H-6), 7.27 (1H, d,  $J = 2.0$  Hz, H-2), 7.37 (1H, dd,  $J = 5.6, 4.9$  Hz, H-2'), 7.45 (1H, d,  $J = 15.6$  Hz,  $=\text{CH}-\alpha$ ), 7.59 (1H, t,  $J = 7.8$  Hz, H-5'), 7.88 (1H, d,  $J = 15.8$  Hz,  $=\text{CH}-\beta$ ). HREIMS [ $\text{M}^+$ ]( $m/z$ ) 268.2639 (calcd for  $\text{C}_{16}\text{H}_{12}\text{O}_4$  268.2640).

#### 4.1.6. Synthesis of 1,3-diphenylpropanes **20–22**

**4.1.6.1. 4-[3-(4-Methoxyphenyl)propyl]phenol (20).** Chalcone **16** (3.0 g) was dissolved in a solution of  $\text{H}_2\text{SO}_4$  (2.0 mL) in EtOH (100 mL) and hydrogenated at 60 lb/in<sup>2</sup> (60 °C) over 10% palladium on carbon (0.3 g) for 4 h; the catalyst was filtered and the solvent removed *in vacuo*. The resulting solution was then tried with 50 mL of water and the crude products were extracted with EtOAc (2  $\times$  25 mL). The combined organic layers were washed with brine (20 mL), and dried ( $\text{Na}_2\text{SO}_4$ ). After solvent removal, the residue was purified by flash chromatography to give diphenylpropane **20**: (2.67 g, yield 92.4%) as yellow powder; mp 48 °C; UV (MeOH)  $\lambda_{\max}$  277, 226 nm; IR (KBr)  $\nu_{\max}$  3418, 2941, 1608, 1512, 1353, 1216  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.80–1.90 (2H, m, H-8), 2.50–2.60 (4H, m, H-7, H-7'), 3.78 (3H, s,  $\text{CH}_3\text{O}-4'$ ), 4.41 (1H, br s,  $\text{HO}-4$ ), 6.73 (2H, d,  $J = 8.8$  Hz, H-3, H-5), 6.82 (2H, d,  $J = 8.8$  Hz, H-3', H-5'), 7.02 (2H, d,  $J = 8.8$  Hz, H-2, H-6), 7.08 (2H, d,  $J = 8.8$  Hz, H-2', H-6');  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  33.4 (C-8), 34.8 (C-7, C-7'), 55.3 ( $\text{CH}_3\text{O}-4'$ ), 113.7 (C-3', C-5'), 115.1 (C-3, C-5), 129.3 (C-2', C-6'), 129.4 (C-2, C-6), 134.4 (C-1'), 134.5 (C-1), 153.5 (C-4), 157.5 (C-4'); EM-IE ( $m/z$ ) 242 [ $\text{M}^+$  (71)], 135 (18), 121 (100), 107 (24). HREIMS [ $\text{M}^+$ ]( $m/z$ ) 242.1301 (calcd for  $\text{C}_{16}\text{H}_{18}\text{O}_2$  242.1307).

**4.1.6.2. 4-[3-(1,3-Benzodioxol-5-yl)propyl]phenol (21).** Compound **19** (1.5 g, 5.6 mmol) was dissolved in a solution of  $\text{H}_2\text{SO}_4$  (1.0 mL) in EtOH (50 mL), and hydrogenated at 60 lb/in<sup>2</sup> (60 °C) over 10% palladium on carbon (0.15 g) for 4 h. The catalyst was filtered off and the filtrate evaporated. The resulting solution was then tried with 25 mL of water and the crude products were extracted with EtOAc (2  $\times$  25 mL). The combined organic layers were washed with brine (20 mL), and dried ( $\text{Na}_2\text{SO}_4$ ). After solvent removal, the residue was purified by flash chromatography to give diphenylpropane **21** (1.48 g, yield 58%) as yellow powder; mp 84 °C; UV (MeOH)  $\lambda_{\max}$  281, 209 nm; IR (KBr)  $\nu_{\max}$  3477, 3368, 2928, 1586, 1500, 1483, 1244  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.26 (2H, m, H-8), 2.82 (4H, s, H-7, H-7'), 4.63 (1H, br s,  $\text{HO}-3$ ), 5.92 (2H, s,  $-\text{OCH}_2\text{O}-$ ), 6.61 (1H, dd,  $J = 8.0, 1.6$  Hz, H-6'), 6.64–6.68 (3H, m, H-2, H-2', H-4), 6.72 (1H, d,  $J = 8.0$  Hz, H-5'), 6.75 (1H, d,  $J = 7.6$  Hz, H-6), 7.14 (1H, dt,  $J = 7.6, 2.4$  Hz, H-5);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  37.4 (C-8), 37.9 (C-7, C-7'), 100.7 ( $-\text{OCH}_2\text{O}-$ ), 108.1 (C-5'), 112.5 (C-2'), 112.8 (C-4), 115.3 (C-2), 120.9 (C-6), 121.1 (C-6'), 129.5 (C-5), 135.5 (C-1'), 143.6 (C-1), 145.6 (C-4'), 147.5 (C-3'), 155.4 (C-3); EM-IE ( $m/z$ ) 256 [ $\text{M}^+$  (60)], 149 (100), 119 (16). HREIMS [ $\text{M}^+$ ]( $m/z$ ) 256.1090 (calcd for  $\text{C}_{16}\text{H}_{16}\text{O}_3$  256.1099).

**4.1.6.3. 4-[3-(3-Hydroxy-5-methoxyphenyl)propyl]-2-methoxyphenol (22).** Compound **22** (crystalline solid, mp 77 °C, 0.25 g, yield 54.2%) was prepared from chalcone **18** (0.48 g) following the method described for **21**. UV (MeOH)  $\lambda_{\max}$  279, 207 nm; IR (KBr)  $\nu_{\max}$  3355, 3039, 2931, 1611, 1591, 1513, 1455, 1196, 813  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.89 (2H, q,  $J = 7.6$  Hz, H-8), 2.55 (2H, t,  $J = 7.6$  Hz, H-7), 2.56 (2H, t,  $J = 7.6$  Hz, H-7'), 3.76 (3H, s,  $\text{CH}_3\text{O}-3'$ ), 3.86 (3H, s,  $\text{CH}_3\text{O}-5$ ), 5.49 (2H, br s,  $\text{HO}-3$ ,  $\text{HO}-4'$ ), 6.24 (2H, m, H-2, H-4), 6.32 (1H, d,  $J = 3.0$  Hz, H-2'), 6.67 (2H, m, H-6, H-6'), 6.83 (1H, d,  $J = 8.0$  Hz, H-5'),  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  32.8 (C-8), 35.0 (C-7, C-7'), 35.4 (C-7, C-7'), 55.2 ( $\text{CH}_3\text{O}-3'$ ), 55.9 ( $\text{CH}_3\text{O}-5$ ), 98.2 (C-4), 106.8 (C-6), 107.9 (C-2), 111.0 (C-2'), 114.2 (C-5'), 120.9 (C-6'), 134.2 (C-1'), 143.6 (C-1), 145.1 (C-4'), 146.3 (C-3'), 156.6 (C-3), 160.8 (C-5); EM-IE ( $m/z$ ) 288 [ $\text{M}^+$  (9)], 150 (4), 138 (100), 122 (4), 77 (7), 65 (4). HREIMS [ $\text{M}^+$ ]( $m/z$ ) 288.3378 (calcd for  $\text{C}_{17}\text{H}_{20}\text{O}_4$  288.3380).

## 4.2. Biological study

### 4.2.1. Phosphodiesterase activity assay

Phosphodiesterase activity was measured according to the method described by Rivero and coworkers with some modifications [12].

Briefly, CaM (0.08  $\mu$ g) was incubated with 0.015 units of PDE1 from bovine brain during 30 min in 40  $\mu$ L of assay solution containing 0.063 units of 5'-nucleotidase (*Crotalus atrox* venom from Sigma), 45 mM Tris–HCl, 5.6 mM magnesium acetate, 45 mM imidazole, 2.5 mM calcium chloride and 10 mM BSA, pH 7.0. Tested compounds were then added to the assay medium at 0.5, 1, 2, 3, 4, 7, 13, 20, 32, 50, and 65 mM in MeCN–water (1:1), and the samples incubated during 30 min; thereafter 10  $\mu$ L of 10.8 mM cAMP were added to start the assay. After 15 min, the assay was stopped by the addition of 190  $\mu$ L of malachite green solution. The amount of inorganic phosphate released was measured at 700 nm, correlated with the activity of the PDE1. All the results are expressed as the mean of at least six experiments  $\pm$  SEM. The IC<sub>50</sub> (concentration inhibiting by 50% the activity of the enzyme) values were determined by non-linear regression analysis by fitting to hyperbolic inhibition.

#### 4.2.2. Steady-state fluorescence (biosensor CaM)

All measurements were conducted with an ISS-PC1 spectrofluorometer (ISS, Champaign, IL), with sample stirring at 37 °C. The protein hCaM M124C–mBBr (5  $\mu$ M) [14] was incubated in a phosphate buffer (100 mM; pH 5.1) and 10 mM CaCl<sub>2</sub>. Fluorescence emission spectra were acquired with excitation and emission slit widths of 4 and 8 nm, respectively. The excitation wavelength was 381 nm and emission wavelengths were measured from 400 to 550 nm. The fractional degree of saturated hCaM M124C–mBBr with ligand ( $y$ ), was calculated by changes in fluorescence upon ligand binding according to  $y = (F - F_0)/(F_{\infty} - F_0)$ , where  $F_{\infty}$  represents the fluorescence intensity at saturation of the ligand,  $y$  was plotted as a function of the inhibitor concentration, and the apparent constants ( $K_m$ ) concentration that produces an effect that is 50% of maximum effect ( $E_{\max}$ ) were obtained by fitting to equation [20].

$$y = \frac{E_{\max} L^h}{K_m + L^h}$$

where  $y$  represents the fractional degree of fluorescence intensity at 470 nm,  $K_m$  is the apparent constant for the ligands,  $E_{\max}$  is the maximal value of effect,  $L$  is the concentrations of the ligand and  $h$  is the Hill coefficient. The data were analyzed using the program Origin 8.0 (OriginLab, Northampton, MA).

#### 4.3. Docking

To generate more accurate and physically realistic models of the protein CaM (PDB code 1LIN), after several iterations of rebuilding and refinement, we perform a final all-atom refinement of CaM with the idealization application of the Rosetta3.1 release [21]. This application rebuilds molecules using ideal bond lengths, bond angles, and torsion angles. The starting conformation of the ligands was optimized using the program Gaussian 09, revision A.02 (Gaussian Inc., Wallingford, CT) at DTF B3LYP/3-21g level of theory. Blind docking was carried out using AutoDock4 version 4.2 software (<http://autodock.scripps.edu/>) [15,16] using the default parameters the Lamarckian genetic algorithm with local search, number of individuals in population (150), maximum number of energy evaluations (2.5 million), maximum number of generations (27 000), rate of gene mutation (0.02), rate of crossover (0.8) and 100 runs for docking. The initial grid box size was 60 Å  $\times$  60 Å  $\times$  60 Å in the  $x$ ,  $y$  and  $z$  dimensions. The refined docking analysis was performed in a smaller grid box, with 30 Å  $\times$  30 Å  $\times$  30 Å dimensions, centered in the ligand. All calculations were made using a parallel distributed memory supercomputer (Kanbalam, Dirección General de Cómputo y de Tecnologías de Información y Comunicación, UNAM) which contains 1368 processors AMD Opteron, around 3 terabyte of memory and 160 terabyte of storage (<http://www.super.unam.mx/>).

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#### Appendix. Supplementary data

Supplementary data (NMR spectra) associated with this article can be found, in the online version at doi:10.1016/j.ejmech.2011.03.057.

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