Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech



Design, synthesis and biological evaluation of new ionone derivatives as potential neuroprotective agents in cerebral ischemia

Ajay Kumar Srivastava^a, Preeti Dohare^b, Madhur Ray^b, Gautam Panda^{a,*}

^a Medicinal & Process Chemistry Division, Central Drug Research Institute, CSIR, Chattar Manzil Palace, Lucknow 226001, UP, India ^b Pharmacology Division, Central Drug Research Institute, CSIR, Lucknow 226001, UP, India

ARTICLE INFO

ABSTRACT

A new series of ionone derived allylic alcohols have been evaluated for anti-ischemic activity. Out of them, **12f** and **13b** decreased infarct volume to $23.98 \pm 4.7 \text{ mm}^3$ and $93.98 \pm 24.8 \text{ mm}^3$ as compared to ischemic group.

 $\ensuremath{\textcircled{}^{\odot}}$ 2010 Elsevier Masson SAS. All rights reserved.

175

Article history: Received 15 October 2009 Received in revised form 15 January 2010 Accepted 18 January 2010 Available online 28 January 2010

Keywords: Ionone Allylic alcohol Cerebral ischemia Stroke

1. Introduction

Stroke being the major cause for cerebral ischemia, is the third largest cause of death and disability in adults [1]. To-date there exists only one FDA-approved treatment for ischemic stroke: i.e., the serine protease tissue-type plasminogen activator (t-PA, a thrombolytic agent). Recombinant human t-PA treatment has benefited ischemic stroke patients if given within 3-4 h of symptoms onset. After the focal ischemic stroke, brain tissues undergo necrotic cell death due to the significant blood flow reduction. During this process the nearby tissues surrounded to the necrotic region known as "ischemic penumbra" remains metabolically active and provides an opportunity for post-stroke therapy. There are several mechanisms proposed for the neuroprotective therapy which focus on lowering the activation of various toxic pathways and activate the endogenous neuroprotective mechanisms [2–5]. Recently it has been established that the neurons in the ischemic penumbra undergo apoptosis after several hours or days and thus can be treated by antiapoptotic agents even after the onset of the stroke [6]. Most of the reported compounds for the protection of cerebral ischemia act as NMDA receptor antagonists, antiinflammatory agents [2–4], antiapoptotic agents [5] or antioxidants [7–9].

Recently some of the antioxidants like vinpocetine **1**, its analog **2** [7], and curcumin **3** [10] have been reported as potential neuropreventive agents. Fabio et al. have reported GV150526A analogs **4** as potent glycine binding site antagonists in animal models of cerebral ischemia [11]. Suh et al have also identified biological activity and mechanism of quinolone analog **5** as anti-ischemic agent [12]. MK 801; **6**, {(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate} is an orally active potent NMDA receptor antagonist [13,15]. Spiromorpholone-benzopyran derivatives **7a–b** have also been found to act as anti-ischemic activators of mitochondrial ATP-sensitive potassium channel [16].

In search of new and potent anti-ischemic agents, recently we have reported synthesis and biological evaluation of amino-acid based benzodiazepines **8**, **9** as new class of anti-ischemic agents (Fig. 1) [17–19]. However this series of compounds did not exhibit very good potency *in vivo*. After careful searching of the literature, we selected ionones **10**, **11** as starting materials due to their profound biological activities and wide occurrence in nature (Fig. 2) [20–22].

Recently ionone derivatives have been reported as nuclear hormone receptor ligands for the stimulation and/or improvement of mammalian and particularly human skin [23]. Neuroprotection by attenuating nitrosative [20] and oxidative stress [25,26] by curcuma oil containing the same class of compounds is also reported. Inspired by these findings we sought to incorporate the



^{*} Corresponding author. Tel.: +91 522 2364635; fax: +91 522 2623405.

E-mail addresses: gautam_panda@cdri.res.in, gautam.panda@gmail.com (G. Panda).

^{0223-5234/\$ –} see front matter @ 2010 Elsevier Masson SAS. All rights reserved. doi:10.1016/j.ejmech.2010.01.039



Fig. 1. Recently reported anti-ischemic agents.

aromatic rings with various substituents in the parent α and β -ionones and to study them for the treatment of cerebral ischemia. Herein, we report a new series of ionone derivatives with anti-apoptotic property as neuroprotective agents for the treatment of stroke.

2. Results and discussion

2.1. Chemistry

The compounds **12a–m** and **13a–g** were synthesized from reaction of appropriate Grignard reagent with α -ionone **10** and β -ionone **11** respectively (Scheme 1) [27]. Substituted bromobenzenes were synthesized by reacting *p*-bromophenol with various alkyl halides in presence of K₂CO₃ in acetone which was converted into corresponding Grignard reagents on reacting with activated Mg in dry THF. The synthesized allyl alcohols were tested for their biological activity. In order to study the structure–activity relationship (SAR), *C* Log *P* values of the synthesized compounds were also calculated using chem. draw^(R) programme. The structures of the prepared compounds and their biological activities are



Fig. 2. Structures of some active unsaturated ketones.



Scheme 1. Syntheses of the compounds.

listed in Table 1. Curcumin; **3**, a well known neuropreventive agent [10] which is structurally similar to the synthesized compounds was used as parent compound for biological studies. MK 801 **6**, a potent NMDA receptor antagonist was used as the reference compound for *in vitro* and *in vivo* biological evaluations.

2.2. Biological studies

2.2.1. In vitro testing of compounds

The forebrain neurons were isolated according to the method of Dohare et al. [24]. Three groups were assigned to the isolated neuron: (1) no treatment (2) hydrogen peroxide treated and (3) pretreated with test compound followed by H₂O₂ treatment. For H_2O_2 treatment 1 \times 10⁶ cells/100 µL were treated with equal volume of H₂O₂ (concentration of 300 µM) for 1 h at 37 °C. For pretreated group 1×10^6 neuronal cells/100 µL were pretreated with compound (100 μ M) for 20 min at 37 °C followed by H₂O₂ treatment. For no treatment group, 1×10^6 neuronal cells/100 μ L were maintained for 1 h and 20 min at 37 °C. No treatment, H₂O₂ and/or compound treated neurons were re-suspended at a concentration of 1 \times 10⁶ cells/100 μ L in staining solution containing binding buffer, FITC-labeled Annexin V and Propidium Iodide (PI). Fluorescence was determined according to the manufacturer's protocol (Calbiochem, Apoptosis Detection kit). Acquisition and analysis were performed with the Cell Quest software package (Becton Dickinson, UK, Ltd.) [17].

Representative dot plots of viable and apoptotic cell populations of control, ischemic, MK 801 treated, curcumin treated and compound treated groups (Fig. 3). The lower left hand quadrant represents viable cells, lower right hand quadrant represents early apoptosis, Upper right hand quadrant represents late apoptotic/ necrotic and upper left hand quadrant represents necrotic cells respectively. Apoptotic population was decreased in **12f** and **13b** treated group in comparison to Ischemic/Reperfusion (I/R) group.

2.2.1.1. In vitro testing results. The isolated neurons were labeled with Annexin V-FITC and PI. Control neurons (LL) showed no FITC/ PI positive staining (1.15%/1.18%). The number of FITC positive neurons (LR) was increased to 57.77% in ischemic neurons. **12f** and **13b** significantly decreased the number of FITC positive neurons to 4.31% and 4.9%, respectively. The number of viable neurons was also increased by the treatment. In no treatment ischemic group, viability was 30.23% (cells falling in the 4th quadrant, lower left). Among the compounds tested, **13b** and **12f** increased the neurons viability significantly to 91.9% and 82.3%. **13a** and **12a** showed the neuron viability of 75.25 and 61.7% respectively. Moreover **13d**, **13c**, **12c** showed 69.25, 66.4, 60.9% viability respectively. On the other hand **12i**, **12j**, **12k**, **12l**, **12m** and **12h** showed the apoptotic

Table 1				
Svnthesize	d compounds and	their in	vitro	activity.

Entry	Treatment	Ionone	R	C Log P	Upper left	Upper right	Lower left	Lower right
					(Necrosis)	(late apoptotic)	(Normal viable)	(Early apoptotic)
1	Control	-	-	-	1.02 ± 0.40	2.22 ± 0.70	93.66 ± 2.19	3.09 ± 1.40
2	Ischemia	-	-	-	0.72 ± 0.37	11.26 ± 2.36	$\textbf{30.23} \pm \textbf{4.21}$	57.77 ± 5.40
3	Reference (MK-801)	-	-	-	1.96 ± 0.90	1.56 ± 3.90	82.45 ± 2.40	14.9 ± 5.60
4	Curcumin	-	-	-	1.89 ± 0.67	$\textbf{7.30} \pm \textbf{2.80}$	79.90 ± 6.50	24.15 ± 4.65
	(Parent compound)							
5	12a	α-ionone	C ₆ H ₄ -3-OCH ₃	5.699	$\textbf{3.89} \pm \textbf{3.30}$	9.40 ± 0.96	61.70 ± 17.30	25.02 ± 19.70
6	12b	α-ionone	C_6H_4 -4-OCH ₃	5.699	1.14 ± 0.54	19.72 ± 6.38	$\textbf{37.75} \pm \textbf{12.90}$	41.37 ± 17.20
7	12c	α-ionone	C ₆ H ₄ -4-OC ₅ H ₁₁	7.815	0.61 ± 0.41	12.70 ± 10.39	60.91 ± 7.60	25.77 ± 3.18
8	12d	α-ionone	C ₆ H ₄ -4-SCH ₃	6.339	0.58 ± 0.21	15.43 ± 0.90	$\textbf{33.43} \pm \textbf{14.27}$	50.55 ± 14.70
9	12e	α-ionone	C_6H_4 -2-OCH ₃	5.699	0.42 ± 0.15	15.20 ± 2.70	44.4 ± 1.60	39.80 ± 3.80
10	12f	α-ionone	C ₆ H ₄ -4-OC ₃ H ₇	6.757	$\textbf{3.00} \pm \textbf{2.90}$	$\textbf{0.70} \pm \textbf{0.50}$	91.90 ± 4.07	4.31 ± 0.60
11	12g	α-ionone	C ₆ H ₄ -4-OC ₂ H ₅	6.228	1.32 ± 0.80	16.30 ± 4.20	50.80 ± 12.70	31.40 ± 14.90
12	12h	α-ionone	C_6H_4 -4- CF_3	6.663	0.057 ± 0.03	22.23 ± 1.69	5.09 ± 2.80	72.60 ± 1.50
13	12i	α-ionone	C ₆ H ₄ -4-C(CH ₃) ₃	7.606	0.05 ± 0.02	30.40 ± 4.93	7.36 ± 4.20	62.10 ± 5.40
14	12j	α-ionone	C ₆ H ₅	5.78	0.05 ± 0.03	30.47 ± 4.09	6.50 ± 3.80	62.90 ± 4.70
15	12k	α-ionone	C ₆ H ₃ -2,5-CH ₃	6.728	0.045 ± 0.02	$\textbf{38.30} \pm \textbf{3.60}$	6.40 ± 3.60	55.10 ± 7.20
16	121	α-ionone	C ₆ H ₃ -3,4-OCH ₃	5.438	0.065 ± 0.04	$\textbf{38.50} \pm \textbf{9.04}$	3.90 ± 2.26	57.36 ± 10.90
17	12m	α-ionone	2-thienyl	5.426	0.025 ± 0.009	42.09 ± 6.60	6.80 ± 4.60	50.90 ± 3.30
18	13a	β-ionone	C_6H_4 -4-OCH ₃	5.759	$\textbf{3.42} \pm \textbf{2.81}$	9.06 ± 1.38	75.25 ± 1.16	12.20 ± 5.30
19	13b	β-ionone	C ₆ H ₄ -3-OCH ₃	5.759	$\textbf{7.39} \pm \textbf{4.10}$	5.35 ± 1.42	82.30 ± 1.70	4.95 ± 4.50
20	13c	β-ionone	C ₆ H ₄ -4-SCH ₃	6.399	0.21 ± 0.09	2.5 ± 0.9	66.40 ± 6.80	$\textbf{30.89} \pm \textbf{9.80}$
21	13d	β-ionone	C ₆ H ₄ -4-OC ₅ H ₁₁	7.875	$\textbf{0.26} \pm \textbf{0.21}$	4.02 ± 1.2	69.25 ± 4.90	26.48 ± 7.80
22	13e	β-ionone	C ₆ H ₄ -4-OC ₃ H ₇	6.817	0.52 ± 0.19	16.60 ± 1.80	29.00 ± 15.50	53.84 ± 16.30
23	13f	β-ionone	C ₆ H ₄ -4-OC ₂ H ₅	6.288	0.93 ± 0.40	15.62 ± 2.19	$\textbf{33.10} \pm \textbf{16.4}$	50.34 ± 18.90
24	13g	β-ionone	C ₆ H ₄ -2-OCH ₃	5.759	0.32 ± 0.05	14.50 ± 1.40	19.00 ± 8.90	66.10 ± 7.50

population of neuron of 62.1%, 62.9%, 55.1%, 57.36%, 50.9% and 72.6% respectively and the viability was greatly decreased.

2.2.2. In vivo testing

Animals: Initially all rats were evaluated for neurological deficit according to Wang et al. [28]. Rats showing any deficit were excluded from the study. Male Sprague–Dawley rats 21 weighing 210–250 g were housed and maintained on a 12-h light/12-h dark cycle with access to food and water. The experiments were approved by the Animal Ethics committee of the Institute following strict guidelines on the care and use of experimental animals.

2.2.2.1. Focal embolic model. Blood was drawn from the femoral vein of a donor rat. Embolic cerebral ischemia was induced as reported with slight modifications [29]. Briefly, the animal was anaesthetized with chloral hydrate (400 mg/kg) intraperitoneally. The right CCA and ECA were clamped temporarily using microvascular clips. A PE 50 catheter was advanced rostrally up to 17 mm in the ICA until its tip was 1–2 mm away from the origin of the MCA. The clot of 3 μ L volume was then slowly injected into the MCA. The clips on the right CCA and the right ECA were removed and the incision was closed. The animal was allowed to recover from anesthesia. The sham operated rats underwent same surgical procedure but no clot was injected into them. The rectal temperature in the animals was kept at 37 °C throughout the surgical procedure with the help of heating lamps. No significant change in rectal temperatures was observed in all groups.

2.2.2.2. Experimental protocol. The following groups were assigned using various test agents *in vivo* and *in vitro* neuronal study:

- 1. Sham operated control
- 2. I/R + no treatment
- 3. I/R + pre-treatment with MK 801 (Reference compound) (1 mg/Kg Body weight, i.v.) [30].
- 4. I/R + pre-treatment with Curcumin (Parent Compound) (300 mg/Kg Body weight, i.p.) [29]

- 5. I/R + pre-treatment with **13b** (100 mg/Kg Body weight, i.p.) (Figs. 4 and 5)
- 6. I/R + pre-treatment with **12f**. (100 mg/Kg Body weight, i.p.) (Figs. 4 and 5)

The dose and route of reference compound MK 801 and curcumin were administered as reported earlier. The test agents used in this study were **13b** and **12f**. Dose of 100 mg/Kg was given 30 min prior to MCAO *via* i.p. route.

2.2.2.3. Neurological evaluation. The observation was made up to 24 h for signs of neurological deficits. An observer who was unaware of the identity of the groups assessed neurological deficits after 24 h of clot implant (before sacrifice) and scored as described by the Wang et al. [28] as follows: Score 0 for no observable neurological deficit (normal); score 1 for failure to extend left forepaw on lifting the whole body by tail (mild); score 2 for circling to the contralateral side (moderate); score 3 for leaning to the contralateral side at rest or no spontaneous motor activity (severe).

2.2.2.3.1. Determination of cerebral infarct. Staining of frozen tissue slices was done with 2,3,5-triphenyltetrazolium chloride (TTC) [10] which is colored red by electron transport in active mitochondria and it defines a region in which mitochondrial function is severely compromised by not staining that region thus differentiation can be done by color. Red color depicts the viable tissue while the pale or white colored region depicts the dead or infarcted tissue. 24 h after reflow all the rats were given overdose of chloral hydrate for decapitation. Brain was quickly removed and placed in an ice-cold saline. Seven coronal slices of 2-mm thickness were cut and treated with 2% 2,3,5-triphenyltetrazolium chloride (TTC) for 15 min at 37 °C. The stained brain sections were fixed with 4% formalin and analyzed. The deep red and white area of each brain slice was determined using image analyzer software [26] for infarct area. The volume of infarction was obtained from the product of average slice thickness (2 mm) and sum of infarction area in all the seven slices.

The edema volume in each brain was determined using the same image analyzer software. For the determination of edema



Fig. 3. Extent of apoptosis and necrosis in neurons by flow cytometry (Cell Quest Analysis system).

volume, the contralateral brain volume was subtracted from ipsilateral brain volume. Volume of contralateral and ipsilateral brain slice was calculated from the product of the average slice thickness (2 mm) and the sum of areas in all seven-brain slices. Edema correlated significantly with the neurological score.

2.2.2.4. Result

2.2.2.4.1. Pre-treatment of compound. The parent compound Curcumin was given at the dose of 300 mg/kg and both compounds were given through i.p. route doses of 100 mg/Kg body weight each, while MK 801, the reference compound was given 1 mg/Kg body weight, through the i.v. route as its effective dose *via* i.p. is little higher [13–15,31,32].

This observation shows the compound offer protection by attenuating neurological deficit.

Pre-ischemic treatment with MK 801 (1 mg/Kg body weight; i.v.) and curcumin (300 mg/Kg body weight, i.p.) decreased the infarct volumes to 78.670 \pm 10.10 mm³ (P < 0.001) and 89.8700 \pm 12.320 mm³ (P < 0.001) as compared to the ischemic group to 244.390 \pm 6.03 mm³ as shown in Fig. 6a. Pre-ischemic treatment with **12f** and **13b** decreased the infarct volumes to 23.98 \pm 4.7 mm³ (P < 0.001) and 93.98 \pm 24.8 mm³ (P < 0.001) as compared to the ischemic group to 244.390 \pm 6.03 mm³ as shown in Fig. 6a. Edema volume in the ischemic animals (n - 5) was 96.89 \pm 12.35 mm³. Pre-treatment with MK 801 and curcumin

decreased the edema volume to $28.790 \pm 5.90 \text{ mm}^3$ (P < 0.001) and $41.76 \pm 10.6 \text{ mm}^3$ (P < 0.001). Pre-treatment with **12f** and **13b** decreased the edema volume to $13.67 \pm 8.64 \text{ mm}^3$ (P < 0.001) and $32.23 \pm 7.34 \text{ mm}^3$ (P < 0.001) (Fig. 6). Pre-ischemic treatment with **12f** and **13b** significantly reduced the edema and infarct volume.

3. Conclusion

In conclusion we have synthesized a new series of ionone derived allylic alcohols with potent anti-ischemic activity. Two compounds of the series showed significant activity in vivo. The structure-activity relationship shows that the oxygen on aromatic part is necessary as most of the compounds lacking this were found to be inactive. The increase in chain length of alkoxy substituent on benzene ring shows enhancement in activity up to three carbons. Further increment shows loss in activity. Introduction of heteroaryl at the place of aromatic moiety does not show any significant change. The potential of the use of compound for treating cerebrovascular disorder was evaluated in animal models. The delayed death in the cortex penumbra region and the thalamus is clinically well identified and thalamic damage is frequently associated with aphasia and severe memory impairment in humans. Blocking of the apoptotic damage by the compound may offer a therapeutic window for pharmacological intervention after cerebral ischemia, which is well observed in our experiments. Collectively, our data



suggest that combined strategies targeting apoptosis as well as necrosis will be necessary to protect ischemic damage in the ischemic and non-ischemic brain structures after a cerebral ischemic insult. Further pharmacological evaluations and dose-



Fig. 4. Pre-ischemic treatment with MK 801, curcumin, compound **12f** and **13b** improved the neurological scores to 1.98 (S.E.M. \pm 0.34); 2.45 (S.E.M. \pm 0.78); 2.9 (S.E.M. \pm 0.50) and 3.27 (S.E.M. \pm 0.70) as compared to 5.20 (S.E.M. \pm 0.60); *P* < 0.05; (Fig. 2) of ischemic group. Mean neurological disability scores paralleled the changes in infarct volume in case of untreated and treated group.

response studies *via* different route of administration are currently underway and will be reported in due course.

4. Experimental section

4.1. General

All chemicals, reagents, and solvents for the synthesis of the compounds were of analytical grade, purchased from commercial sources and used without further purification, unless otherwise specified. Tetrahydrofuran (THF) was dried by distillation over sodium/benzophenone ketal and stored over molecular sieves (3 Å). All reactions were monitored by thin layer chromatography over silica gel coated TLC plates. The spots on TLC were visualized by warming ceric sulphate (2% CeSO₄ in 2 N H₂SO₄) sprayed plates in hot plate or in an oven at about 100 °C. Silica gel (60–120 mesh) was used for column chromatography. Melting points were determined in capillary tubes on an electrically heated apparatus and were uncorrected. IR spectra were recorded on Perkin Elmer 881 or FT IR 820/PC instrument and values are expressed in cm⁻¹. FABMS were recorded on a JEOL/SX-102 spectrometer and ESMS were recorded using a Micromass LC-MS system. ¹H and ¹³C NMR were recorded on Brucker Advance DPX 200 MHz using TMS as an internal standard (chemical shifts are expressed as δ values, I in



Fig. 5. Upper Panel showing the representative TTC stained Brain sections of sham, ischemic, **12f** and **13b** treated group of rats (n - 5). The pale or white colored area shows the dead tissue and red area depicts the viable tissue. There is a significant decrease in the infarct volume in compound treated group of rats. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

hertz). Elementary analysis was carried out on Carlo ERBA-1108 analyzer. The purity of the synthesized compounds was determined by LichroCART Chiradex column ($250 \times 4 \text{ mm}, 5 \mu \text{m}$) column using water and methanol as eluent at 25 °C and in all cases the extent of purity was >95%.

4.2. General procedure for the preparation of compounds

To a suspension of activated Mg in dry THF catalytic amount of iodine was added and stirred for 15 min. To this a solution of bromo compound in THF was added and stirred until the temperature of vessel rises indicating the formation of Grignard reagent. The



Fig. 6. Bar chart showing the edema volume in sham, ischemic, MK 801, curcumin, **12f** and **13b** treated group of rats (n - 5). There is a significant decrease in the edema volume in compound treated group of rats. (P < 0.001).

solution was further stirred for 30 min and cooled to 0 °C before adding the solution of ionone in THF. The reaction mixture was stirred for 15 min and quenched by adding saturated NH₄Cl solution. The aqueous layer was extracted three times by ethyl acetate. The combined organic layer was dried by sodium sulphate and crude was purified by using column chromatography on silica gel.

4.2.1. 2-(3-Methoxy-phenyl)-4-(2,6,6-trimethyl-cyclohex-2-enyl)but-3-en-2-ol (**12a**)

Yield 85%; IR: (neat, cm⁻¹) 3321, 3021, 2961, 2919, 2862, 2361, 1421, 1216, 761; ¹H NMR (200 MHz, CDCl₃): δ 7.28–7.20 (m, 1H, Ar<u>H</u>), 7.05–7.01 (m, 2H, Ar<u>H</u>), 6.80–6.75 (m, 1H, Ar<u>H</u>), 5.75 (d, J = 15.4 Hz, 1H), 5.57–5.40 (m, 2H), 3.79 (s, 3H, OC<u>H</u>₃), 2.14 (d, J = 9.2 Hz, 1H), 1.99 (bs, 1H, O<u>H</u>), 1.63 (s, 3H, C<u>H</u>₃), 1.46–1.39 (m, 2H, C<u>H</u>₂), 1.23–1.14 (m, 2H, C<u>H</u>₂), 0.89 (s, 3H, C<u>H</u>₃), 0.83 (s, 3H, C<u>H</u>₃). MASS (FAB): m/z (%): 283 (100, [M⁺ - H₂O]); Anal. Calcd for C₂₀H₂₈O₂: C 79.96%; H 9.39%; found: C 79.82%, H 9.26%.

4.2.2. 2-(4-Methoxy-phenyl)-4-(2,6,6-trimethyl-cyclohex-2-enyl)but-3-en-2-ol (**12b**)

Yield 87%; IR; (neat, cm⁻¹) 3392, 3020, 2961, 2910, 2856, 2361, 1461, 1216, 762; ¹H NMR (200 MHz, CDCl₃): δ 7.32 (d, J = 8.7 Hz, 2H, ArH), 6.79 (d, J = 8.7 Hz, 2H, ArH), 5.71(d, J = 15.4 Hz, 1H), 5.50–5.38 (m, 2H), 3.76 (s, 3H, OCH₃), 2.11 (d, J = 9.2 Hz, 1H), 1.98 (bs, 1H, OH), 1.58 (s, 6H, CH₃), 1.49–1.34 (m, 2H, CH₂), 1.19–1.13 (m, 2H, CH₂), 0.89 (s, 3H, CH₃), 0.83 (s, 3H, CH₃). ¹³C NMR (50 MHz, CDCl₃): δ 158.78, 139.97, 139.88, 139.41, 134.4, 129.46, 129.38, 126.83, 121.45, 113.79, 96.587, 74.53, 55.40, 54.40, 32.62, 32.15, 30.49, 30.36, 28.10, 27.55, 23.47. MASS (FAB): m/z (%): 283 (100, [M⁺ – H₂O]). Anal. Calcd for C₂₀H₂₈O₂: C 79.96%; H 9.39%; found: C 79.67%, H 9.19%.

4.2.3. 2-(4-Pentyloxy-phenyl)-4-(2,6,6-trimethyl-cyclohex-2-enyl)but-3-en-2-ol (**12c**)

Yield 78%; IR; (neat, cm⁻¹) 3431, 3022, 2962, 2920, 2862, 2361, 1441, 1216, 762; ¹H NMR (200 MHz, CDCl₃): δ 7.36 (d, *J* = 8.7 Hz, 2H, ArH), 6.85 (d, *J* = 8.7, 2H, ArH), 5.75 (d, *J* = 15.4 Hz, 1H), 5.54–5.39 (m, 2H), 3.94 (t, *J* = 6.5 Hz, 2H, OCH₂), 2.13 (d, *J* = 9.3 Hz, 1H), 1.99 (bs, 1H, OH), 1.82–1.74 (m, 2H), 1.62 (s, 3H, CH₃), 1.58 (s, 3H, CH₃), 1.49–1.40 (m, 6H), 1.19–1.12 (m, 2H, CH₂), 0.96–0.92 (m, 3H, CH₃), 0.89 (s, 3H, CH₃), 0.83 (s, 3H, CH₃). MASS (FAB): *m/z* (%): 339 [100, (M⁺ – H₂O)]. Anal. Calcd for C₂₄H₃₆O₂: C 80.85%; H 10.18%; found: C 80.64%, H 10.02%.

4.2.4. 2-(4-Methylsulfanyl-phenyl)-4-(2,6,6-trimethyl-cyclohex-2-enyl)-but-3-en-2-ol (**12d**)

Yield 92%; IR; (neat, cm⁻¹) 3430, 3020, 2962, 2920, 2862, 2361, 1441, 1216, 762; ¹H NMR (200 MHz, CDCl₃): δ 7.34 (d, *J* = 8.7 Hz, 2H, ArH), 7.18 (d, *J* = 8.7 Hz, 2H, ArH), 5.71 (d, *J* = 15.4 Hz, 1H), 5.52–5.38 (m, 2H), 2.46 (s, 3H, SCH₃), 2.10 (d, *J* = 9.3 Hz, 1H), 1.99 (bs, 1H, OH), 1.59 (s, 3H, CH₃), 1.56 (s, 3H, CH₃), 1.45–1.38 (m, 2H, CH₂), 1.19–1.12 (m, 2H, CH₂), 0.89 (s, 3H, CH₃), 0.81 (s, 3H, CH₃). ¹³C NMR (50 MHz, CDCl₃): δ 144.69, 138.97, 137.11, 134.24, 129.95, 129.85, 126.92, 126.18, 121.57, 96.58, 74.63, 54.39, 32.62, 32.11, 30.49, 30.35, 28.11, 27.52, 23.45, 16.35. MASS (FAB): *m*/*z* (%): 316 (75, [M⁺]), 299 (100, [M⁺ − H₂O]). Anal. Calcd for C₂₀H₂₈OS: C 75.90%; H 8.92%; found: C 75.84%, H 8.72%.

4.2.5. 2-(2-Methoxy-phenyl)-4-(2,6,6-trimethyl-cyclohex-2-enyl)but-3-en-2-ol (**12e**)

Yield 93%; IR; (neat, cm⁻¹) 3592, 3430, 3010, 2959, 2915, 2862, 2358, 1451, 1216, 762; ¹H NMR (200 MHz, CDCl₃): δ 7.32–7.18 (m, 2H, Ar<u>H</u>), 6.95–6.83 (m, 2H, Ar<u>H</u>), 5.71 (d, *J* = 15.4 Hz, 1H), 5.33–5.25 (m, 2H), 3.84 (s, 3H, OC<u>H₃</u>), 2.08 (d, *J* = 9.5 Hz, 1H), 1.95 (bs, 1H, O<u>H</u>), 1.63 (s, 3H, C<u>H₃</u>), 1.55 (s, 3H, C<u>H₃</u>), 1.54–1.43 (m, 2H, C<u>H₂</u>), 1.17–1.12 (m, 2H, C<u>H₂</u>), 0.86 (s, 3H, C<u>H₃</u>), 0.72 (s, 3H, C<u>H₃</u>). ¹³C NMR (50 MHz,

CDCl₃): δ 157.22, 139.55, 134.97, 134.73, 134.62, 128.64, 128.52, 126.88, 121.22, 121.15, 121.06, 111.19, 96.58, 74.78, 55.50, 54.246, 32.57, 32.34, 32.27, 28.40, 27.60, 23.45, 23.30. MASS (FAB): *m/z* (%): 283 (100, [M⁺ – H₂O]). Anal. Calcd for C₂₀H₂₈O₂: C 79.96%; H 9.39%; found: C 79.64%, H 9.19%.

4.2.6. 2-(4-Propoxy-phenyl)-4-(2,6,6-trimethyl-cyclohex-2-enyl)but-3-en-2-ol (**12f**)

Yield 85%; IR; (neat, cm⁻¹) 3523, 3320, 2965, 2920, 2761, 2361, 1441, 1210, 760; ¹H NMR (300 MHz, CDCl₃): δ 7.30 (d, *J* = 8.7 Hz, 2H, Ar<u>H</u>), 6.78 (d, *J* = 8.7 Hz, 2H, Ar<u>H</u>), 5.72 (d, *J* = 15.4 Hz, 1H), 5.50–5.37 (m, 2H), 3.89 (t, *J* = 6.5 Hz, 2H, OC<u>H</u>₂CH₂CH₃), 2.03 (d, *J* = 9.3 Hz, 1H), 1.99 (bs, 1H, O<u>H</u>), 1.80 (m, 2H, OCH₂C<u>H</u>₂CH₃), 1.57 (s, 6H, C<u>H</u>₃), 1.45–1.38 (m, 2H, C<u>H</u>₂), 1.21–1.12 (m, 2H, C<u>H</u>₂), 1.04 (t, *J* = 7.3 Hz, 3H, OCH₂C<u>H</u>₂C<u>H</u>₃), 0.89 (s, 3H, C<u>H</u>₃), 0.81 (s, 3H, C<u>H</u>₃). ¹³C NMR (50 MHz, CDCl₃): δ 158.36, 139.43, 134.40, 129.44, 126.76, 121.45, 114.36, 96.58, 74.55, 69.69, 54.39, 32.63, 32.14, 30.51, 28.10, 27.55, 23.46, 23.07, 11.05. MASS (FAB): *m/z* (%): 311 (100, [M⁺ – H₂O]). Anal. Calcd for C₂₂H₃₂O₂: C 80.44%; H 9.82%; found: C 80.34%, H 9.63%.

4.2.7. 2-(4-Ethoxy-phenyl)-4-(2,6,6-trimethyl-cyclohex-2-enyl)but-3-en-2-ol (**12g**)

Yield 74%; IR: (neat, cm⁻¹) 3631, 3420, 2896, 2921, 2865, 2361, 1431, 1206, 761; ¹H NMR (300 MHz, CDCl₃): δ 7.30 (d, *J* = 8.7 Hz, 2H, Ar<u>H</u>), 6.77 (d, *J* = 8.7 Hz, 2H, Ar<u>H</u>), 5.70 (d, *J* = 15.4 Hz, 1H), 5.49–5.37 (m, 2H), 3.98 (q, *J* = 6.9 Hz, 2H, OCH₂CH₃), 2.10 (d, *J* = 9.3 Hz, 1H), 1.99 (bs, 1H, O<u>H</u>), 1.57 (s, 6H, C<u>H</u>₃), 1.39 (t, *J* = 6.9 Hz, 3H, OCH₂CH₃) 1.45–1.38 (m, 2H, CH₂), 1.21–1.12 (m, 2H, CH₂), 0.89 (s, 3H, C<u>H</u>₃), 0.81 (s, 3H, C<u>H</u>₃). ¹³C NMR (50 MHz, CDCl₃): δ 158.74, 158.15, 147.88, 139.81, 139.73, 139.47, 135.21, 134.43, 133.50, 133.40, 129.45, 129.31, 126.82, 121.43, 114.34, 114.04, 96.59, 74.52, 63.56, 55.19, 54.41, 32.91, 32.63, 32.33, 32.17, 30.46, 30.32, 28.09, 27.56, 23.48, 15.34, 14.67. MASS (FAB): *m*/*z* (%): 297 (100, [M⁺ – H₂O]). Anal. Calcd for C₂₁H₃₀O₂: C 80.21%; H 9.62%; found: C 80.02%, H 9.38%.

4.2.8. 2-(4-Trifluoromethyl-phenyl)-4-(2,6,6-trimethyl-cyclohex-2-enyl)-but-3-en-2-ol (**12h**)

Yield 77%; IR; (neat, cm⁻¹) 3404, 3020, 2961, 2922, 2862, 2361, 1618, 1449, 1327, 1216, 1166, 1127, 1074, 759; ¹H NMR (300 MHz, CDCl₃): δ 7.60 (s, 4H, Ar<u>H</u>), 5.77 (d, *J* = 15.4 Hz, 1H), 5.60–5.50 (m, 1H), 5.44(s, 1H), 2.16 (d, *J* = 9.3 Hz, 1H), 2.01 (bs, 1H, O<u>H</u>), 1.67 (s, 3H, C<u>H₃), 1.60 (s, 3H, C<u>H₃), 1.45–1.40 (m, 2H, CH₂), 1.24–1.18 (m, 2H, C<u>H₂), 0.95 (s, 3H, C<u>H₃), 0.91 (s, 3H, C<u>H₃), 1.³C NMR (50 MHz, CDCl₃): δ 150.05, 136.52, 132.34, 129.38, 124.32, 123.84, 120.13, 73.12, 52.69, 30.92, 30.29, 28.81, 26.50, 25.68, 21.73. MASS (ESI MS): *m/z* (%): 321 (100, [M⁺ - H₂O]). Anal. Calcd for C₂₀H₂₅F₃O: C 70.98%; H 7.45%; found: C 70.78%, H 7.29%.</u></u></u></u></u>

4.2.9. 2-(4-tert-Butyl-phenyl)-4-(2,6,6-trimethyl-cyclohex-2-enyl)-but-3-en-2-ol (**12i**)

Yield 84%; IR; (neat, cm⁻¹) 3689, 3412, 3021, 2352, 1415, 1208, 761; ¹H NMR (300 MHz, CDCl₃): δ 7.46–7.37 (m, 4H, Ar<u>H</u>), 5.82 (d, *J* = 15.4 Hz, 2H), 5.62–5.53 (m, 1H), 5.45 (s, 1H), 2.20 (d, *J* = 9.4 Hz, 1H), 2.04 (bs, 1H, O<u>H</u>), 1.67 (s, 3H, C<u>H₃</u>), 1.65 (s, 3H) 1.50–1.41 (m, 2H, C<u>H₂</u>), 1.36 (s, 9H), 1.26–1.15 (m, 2H, C<u>H₂</u>), 0.95 (s, 3H, C<u>H₃</u>), 0.89 (s, 3H, C<u>H₃</u>). MASS (ESI MS): *m*/*z* (%): 309 (100, [M⁺ – H₂O]). Anal. Calcd for C₂₃H₃₄O: C 84.60%; H 10.50%; found: C 84.54%, H 10.37%.

4.2.10. 2-Phenyl-4-(2,6,6-trimethyl-cyclohex-2-enyl)-but-3-en-2-ol (**12j**)

Yield 81%; IR; (neat, cm⁻¹) 3691, 3408, 3020, 2358, 1435, 1216, 761; ¹H NMR (300 MHz, CDCl₃): δ 7.51–7.48 (m, 2H, Ar<u>H</u>), 7.38–7.33 (m, 2H, ArH), 7.28–7.26 (m, 1H, ArH), 5.80 (d, *J* = 15.4 Hz, 1H), 5.57–

5.48 (m, 1H), 5.43 (s, 1H), 2.16 (d, J = 9.3 Hz, 1H), 2.02 (bs, 1H, O<u>H</u>), 1.67 (s, 3H, C<u>H</u>₃), 1.61 (s, 3H, C<u>H</u>₃), 1.47–1.43 (m, 2H, C<u>H</u>₂), 1.22–1.17 (m, 2H, C<u>H</u>₂), 0.92 (s, 3H, C<u>H</u>₃), 0.85 (s, 3H, C<u>H</u>₃). MASS (ESI MS): m/z (%): 253 (100, [M⁺ - H₂O]). Anal. Calcd for C₁₉H₂₆O: C 84.39%; H 9.69%; found: C 84.04%, H 9.37%.

4.2.11. 2-(2,5-Dimethyl-phenyl)-4-(2,6,6-trimethyl-cyclohex-2-enyl)-but-3-en-2-ol (**12k**)

Yield 91%; IR; (neat, cm⁻¹) 3423, 3021, 2972, 2359, 1655, 1216, 1045, 761, 671; ¹H NMR (300 MHz, CDCl₃): δ 7.38 (s, 1H, ArH), 7.08–7.03 (m, 2H, ArH), 5.84 (d, *J* = 15.6 Hz, 1H), 5.43–5.40 (m, 2H), 2.45 (s, 3H, CH₃), 2.35 (s, 3H, CH₃), 2.18 (d, *J* = 12.8 Hz, 1H), 2.00 (bs, 1H, OH), 1.82–1.80 (m, 1H), 1.73 (s, 3H, CH₃), 1.62 (s, 3H, CH₃), 1.50–1.38 (m, 2H, CH₂), 1.21–1.12 (m, 1H, CH₂), 0.92 (s, 3H, CH₃), 0.83 (s, 3H, CH₃). ¹³C NMR (75 MHz, CDCl₃): δ 142.65, 137.56, 133.47, 132.68, 131.65, 130.89, 128.62, 126.47, 125.27, 119.90, 73.95, 52.72, 31.07, 30.40, 28.24, 26.48, 25.79, 21.79, 20.13, 19.93. MASS (ESI MS): *m/z* (%): 281 (100, [M⁺ – H₂O]). Anal. Calcd for C₂₁H₃₀O C 84.51%; H 10.13%; found: C 84.45%, H 10.03%.

4.2.12. 2-(3,4-Dimethoxy-phenyl)-4-(2,6,6-trimethyl-cyclohex-2-enyl)-but-3-en-2-ol (**12l**)

Yield 83%; IR; (neat, cm⁻¹) 3670, 3220, 2962, 2872, 2859, 2361, 1421, 1216, 762; ¹H NMR (300 MHz, CDCl₃): δ 7.04–6.99 (m, 1H, ArH), 6.90–6.82 (m, 2H, ArH), 5.75 (d, *J* = 15.4 Hz, 1H), 5.57–5.48 (m, 1H), 5.42 (s, 1H), 3.90 (s, 3H, OCH₃), 3.88 (s, 3H, OCH₃), 2.16(d, *J* = 9.3 Hz, 1H), 2.00 (bs, 1H, OH), 1.65 (s, 3H, CH₃), 1.61 (s, 3H, CH₃), 1.47–1.40 (m, 2H, CH₂), 1.22–1.17 (m, 2H, CH₂), 0.93 (s, 3H, CH₃), 0.86 (s, 3H, CH₃). MASS (ESI MS): *m/z* (%): 313 (100, [M⁺ – H₂O]). Anal. Calcd for C₂₁H₃₀O₃: C 76.33%; H 9.15%; found: C 76.34%, H 9.10%.

4.2.13. 2-Thiophen-3-yl-4-(2,6,6-trimethyl-cyclohex-2-enyl)-but-3-en-2-ol (**12m**)

Yield 67%; IR; (neat, cm⁻¹); 3777, 3620, 3430, 3020, 2966, 2359, 1664, 1426, 1216, 1044, 761; ¹H NMR (300 MHz, CDCl₃): δ 7.23 (d, *J* = 4.9 Hz, 1H, ArH), 7.08–6.96 (m, 2H, ArH), 6.28 (d, *J* = 15.4 Hz, 1H), 5.90–5.81 (m, 1H), 5.44 (s, 1H), 2.18 (d, *J* = 9.3 Hz, 1H), 2.02 (bs, 1H, OH), 1.75 (s, 3H, CH₃), 1.65 (s, 3H, CH₃), 1.48–1.43 (m, 2H, CH₂), 1.23–1.13 (m, 2H, CH₂), 0.95 (s, 3H, CH₃), 0.87 (s, 3H, CH₃). ¹³C NMR (50 MHz, CDCl₃): δ 142.02, 139.06, 134.03, 130.02, 125.80, 123.20, 119.93, 71.73, 53.42, 52.50, 31.18, 30.49, 29.48, 28.42, 26.34, 25.78, 21.79. MASS (ESI MS): *m/z* (%): 259 (100, [M⁺ – H₂O]). Anal. Calcd for C₁₇H₂₄OS: C 73.86%; H 8.75%; found: C 73.80%, H 8.47%.

4.2.14. 2-(4-Methoxy-phenyl)-4-(2,6,6-trimethyl-cyclohex-1-enyl)but-3-en-2-ol (**13a**)

Yield 87%; IR; (neat, cm⁻¹) 3691, 3468, 3120, 2351, 1435, 1216, 761; ¹H NMR (200 MHz, CDCl₃): δ 7.40 (d, *J* = 8.7 Hz, 2H, Ar<u>H</u>), 6.87 (d, *J* = 8.7 Hz, 2H, Ar<u>H</u>), 6.43–6.20 (m, 1H), 5.76–5.71 (m, 1H), 3.81 (s, 3H, OC<u>H₃</u>), 2.16–2.13 (m, 2H), 1.98 (bs, 1H, O<u>H</u>), 1.85–1.84 (m, 2H), 1.67–1.44 (m, 2H), 1.34 (s, 6H, C<u>H₃</u>), 0.98 (s, 6H, C<u>H₃</u>). MASS (FAB): *m*/*z* (%): 282 (100, [M⁺ – H₂O]). Anal. Calcd for C₂₀H₂₈O₂: C 79.96%; H 9.39%; found: C 79.74%, H 9.37%.

4.2.15. 2-(3-Methoxy-phenyl)-4-(2,6,6-trimethyl-cyclohex-1-enyl)but-3-en-2-ol (**13b**)

Yield 87%; IR; (neat, cm⁻¹) 3581, 3388, 3023, 2358, 1395, 1216, 761, 671; ¹H NMR (200 MHz, CDCl₃): δ 7.26–7.21 (m, 1H, Ar<u>H</u>), 7.09–7.05 (m, 2H, Ar<u>H</u>), 6.81–6.77 (m, 1H, Ar<u>H</u>), 6.13 (d, *J* = 16.1 Hz, 1H), 5.73 (d, *J* = 16.1 Hz, 1H), 3.80 (s, 3H, OC<u>H</u>₃), 1.99–1.93 (m, 3H, C<u>H</u>₂), and O<u>H</u>), 1.68 (s, 3H, C<u>H</u>₃), 1.65 (s, 3H, C<u>H</u>₃), 1.60–1.56 (m, 2H, C<u>H</u>₂), 1.47–1.41 (m, 2H, C<u>H</u>₂), 0.99 (s, 6H, C<u>H</u>₃), ¹³C NMR (50 MHz, CDC<u>I</u>₃): δ 159.9, 149.4, 140.5, 137.1, 129.6, 128.9, 126.4, 118.1, 112.8, 111.4, 75.3, 55.6, 39.8, 34.5, 33.1, 30.3, 29.2, 21.8, 19.7. MASS (FAB): *m/z* (%): 283

[100, (M⁺ – H₂O)]. Anal. Calcd for $C_{20}H_{28}O_2$ C 79.96%; H 9.39%; found: C 79.84%, H 9.27%.

4.2.16. 2-(4-Methylsulfanyl-phenyl)-4-(2,6,6-trimethyl-cyclohex-1-enyl)-but-3-en-2-ol (**13c**)

Vield 68%; IR; (neat, cm⁻¹) 3690, 3412, 3020, 2358, 1651, 1435, 1116, 761; ¹H NMR (200 MHz, CDCl₃): δ 7.43–7.36 (m, 2H, Ar<u>H</u>), 7.21 (d, *J* = 7.9 Hz, 2H, Ar<u>H</u>), 6.11 (d, *J* = 16.1 Hz, 1H,), 5.70 (d, *J* = 16.1 Hz, 1H), 2.47 (s, 3H, CH₃), 2.16 (bs, 1H, O<u>H</u>) 1.96–1.93 (m, 2H, CH₂), 1.65 (s, 3H, CH₃), 1.64 (s, 3H, CH₃), 1.60–1.55 (m, 2H, CH₂), 1.46–1.33 (m, 2H, CH₂), 0.99 (s, 6H, CH₃). Mass (FAB): *m*/*z* (%): 298 [100, (M⁺ – H₂O)]. Anal. Calcd for C₂₀H₂₈OS: C 75.90%; H 8.92%; found: C 75.68%, H 9.67%.

4.2.17. 2-(4-Pentyloxy-phenyl)-4-(2,6,6-trimethyl-cyclohex-1-enyl)-but-3-en-2-ol (**13d**)

Yield 79%; IR; (neat, cm⁻¹) 3691, 3345, 3400, 3028, 2345, 1415, 1214, 761; ¹H NMR (300 MHz, CDCl₃): δ 7.36 (d, *J* = 8.7 Hz, 2H, ArH), 6.85 (d, *J* = 8.7 Hz, 2H, ArH), 6.85 -6.20 (m, 1H), 5.75 -5.54 (m, 1H), 3.94 (t, *J* = 6.5 Hz, 2H, OCH₂CH₂CH₂CH₂CH₂CH₃), 2.16 -2.13 (m, 3H), 1.99 (bs, 1H, OH), 1.80 - 1.74 (m, 2H), 1.68 - 1.61 (m, OCH₂CH₂CH₂CH₂CH₃), 1.56 -1.51 (m, 2H, CH₂), 0.96 - 0.92 (m, 3H, CH₃), 1.43 (s, 3H, OCH₂CH₂CH₂CH₂CH₃), 1.34 (s, 3H, CH₃), 0.99 (s, 3H, CH₃), 0.93 (s, 3H, CH₃). MASS (FAB): *m/z* (%): 339 [100, (M⁺ - H₂O)]. Anal. Calcd for C₂₄H₃₆O₂: C 80.85%; H 10.18%; found: C 80.68%, H 9.97%.

4.2.18. 2-(4-Propoxy-phenyl)-4-(2,6,6-trimethyl-cyclohex-1-enyl)but-3-en-2-ol (**13e**)

Yield 74%; IR; (neat, cm⁻¹) 3408, 3320, 2358, 1435, 1216, 761; ¹H NMR (200 MHz, CDCl₃): δ 7.36 (d, J = 8.7 Hz, 2H, ArH), 6.80 (d, J = 8.7 Hz, 2H, ArH), 6.06 (d, J = 15.4 Hz, 1H), 5.69 (d, J = 15.4 Hz, 1H), 3. 89 (t, J = 6.5 Hz, 2H, OCH₂CH₂CH₃), 1.97–1.94 (m, 3H, CH₂ and OH), 1.82–1.75 (m, 2H, OCH₂CH₂CH₃), 1.57(s, 6H, CH₃), 1.45–1.38 (m, 2H, CH₂), 1.21–1.12 (m, 2H, CH₂), 1.04 (t, 3H, J = 7.35 Hz, OCH₂CH₂CH₃), 0.89 (s, 3H, CH₃), 0.81(s, 3H, CH₃). ¹³C NMR (50 MHz, CDCl₃): δ 158.36, 130.90, 126.97, 114.67, 96.563, 69.72, 41.16, 29.48, 28.43, 26.52, 23.05, 22.24, 15.93, 11.04. MASS (FAB): m/z (%): 311 (100, [M⁺ - H₂O]). Anal. Calcd for C₂₂H₃₂O₂: C 80.44%; H 9.82%; found: C 80.04%, H 9.67%.

4.2.19. 2-(4-Ethoxy-phenyl)-4-(2,6,6-trimethyl-cyclohex-1-enyl)but-3-en-2-ol (**13***f*)

Yield 78%; IR; (neat, cm⁻¹) 3691, 3514, 2920, 2298, 1435, 1212, 762; ¹H NMR (200 MHz, CDCl₃): δ 7.32 (d, *J* = 8.7 Hz, 2H, Ar<u>H</u>), 6.82 (d, *J* = 8.7 Hz, 2H, Ar<u>H</u>), 6.43–6.20 (m, 1H), 5.76–5.71 (m, 1H), 3.98 (q, 2H, *J* = 6.9 Hz, OCH₂CH₃), 2.14–2.11 (m, 2H), 1.98 (bs, 1H, O<u>H</u>), 1.71–1.38 (m, 4H), 1.51 (t, *J* = 6.9 Hz, 3H, OCH₂C<u>H₃</u>), 1.38 (s, 6H, C<u>H₃</u>), 0.98 (s, 6H, C<u>H₃</u>). MASS (FAB): *m*/*z* (%): 297 (100, [M⁺ – H₂O]). Anal. Calcd for C₂₁H₃₀O₂: C 80.21%; H 9.62%; found: C 79.34%, H 9.37%.

4.2.20. 2-(2-Methoxy-phenyl)-4-(2,6,6-trimethyl-cyclohex-1-enyl)-but-3-en-2-ol (**13**g)

Yield 88%; IR; (neat, cm⁻¹) 3691, 3408, 3120, 2358, 1395, 1216, 761; ¹H NMR (200 MHz, CDCl₃): δ 7.35–7.17 (m, 2H, Ar<u>H</u>), 6.96–6.84 (m, 2H, Ar<u>H</u>), 5.82 (d, *J* = 15.9 Hz, 1H), 5.67 (d, *J* = 15.9 Hz, 1H), 3.86 (s, 3H, OC<u>H</u>₃), 1.95–1.89 (m, 2H, C<u>H</u>₂), 1.68 (s, 3H, CH₃), 1.61 (s, 3H, C<u>H</u>₃), 1.58–1.51 (m, 2H, C<u>H</u>₂), 1.43–1.28 (m, 2H, C<u>H</u>₂), 0.94 (s, 3H, C<u>H</u>₃), 0.91 (s, 3H, C<u>H</u>₃). ¹³C NMR (50 MHz, CDCl₃): δ 157.17, 144.91, 136.09, 135.48, 134.80, 131.21, 129.58, 128.24, 127.89,126.92, 126.36, 122.38, 121.06, 120.63, 118.80, 111.32, 96.58, 55.67, 41.17, 37.56, 35.30, 29.35, 28.46, 25.95, 25.51, 24.23, 23.33, 22.22, 17.51. MASS

(FAB): m/z (%): 283 (100, [M⁺ – H₂O]). Anal. Calcd for C₂₀H₂₈O₂: C 79.96; H 9.39; found: C 79.54%, H 9.47%.

Acknowledgments

The authors wish to acknowledge Mr. A. L. Vishwakarma and Mr. Ramesh Chandra for their technical assistance. Ajay and Preeti thank Council of Scientific and Industrial Research (CSIR) for providing fellowship. This manuscript bear CDRI communication number 7721.

Appendix. Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2010.01.039.

References

- G.W. Petty, R.D. Brown Jr., J.P. Whisnant, J.D. Sicks, W.M. O'Fallon, D.O. Wiebers, Stroke 31 (2000) 1062–1068.
- [2] C. Wakade, M.M. Khan, L.M. De Sevilla, Q.G. Zhang, V.B. Mahesh, D.W. Brann, Endocrinology 149 (2008) 367–379.
- [3] K.T. Lu, R.Y. Chiou, L.G. Chen, M.H. Chen, W.T. Tseng, H.T. Hsieh, Y.L. Yang, J. Agric. Food Chem. 54 (2006) 3126–3131.
- [4] M. Strokin, O. Chechneva, K.G. Reymann, G. Reiser, Neuroscience 140 (2006) 547–553.
- [5] P. Maher, T. Akaishi, K. Abe, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 16568– 16573.
- [6] B.R.S. Broughton, D.C. Reutens, C.G. Sobey, Stroke 40 (2009) e331-e339.
- [7] A. Nemes, L. Czibula, C. Szántay Jr., A. Gere, B. Kiss, J. Laszy, I. Gyertyán, Z. Szombathelyi, C. Szántay, J. Med. Chem. 51 (2008) 479–486.
- [8] J. Folbergrova, Q. Zhao, K. Katsure, B.K. Siesjo, Proc. Natl. Acad. Sci. U.S.A. 92 (1995) 5057–5061.
- [9] M. Habeck, Drug Discov. Today 7 (2002) 157-159.
- [10] J. Jiang, W. Wang, Y.J. Sun, M. Hu, F. Li, D.Y. Zhu, Eur. J. Pharmacol. 561 (2007) 54–62.
- [11] R.D. Fabio, N. Conti, E.D. Magistris, A. Feriani, S. Provera, F.M. Sabbatini, A. Reggiani, L. Rovatti, R.J. Barnaby, J. Med. Chem. 42 (1999) 3486–3493.
- [12] C.-H. Park, J. Lee, H.Y. Jung, M.J. Kim, S.H. Lim, H.T. Yeo, E.C. Choi, E.J. Yoon, K.W. Kim, J.H. Cha, S.-H. Kim, D.-J. Chang, D.-Y. Kwon, F. Li, Y.-G. Suh, Bioorg. Med. Chem. 15 (2007) 6517–6526.
- [13] E. Cam, B. Yulug, E. Ozan, J. Neuropsych. Clin. N. 20 (3) (2008) 367.
- [14] E.H.F. Wong, J.A. Kemp, T. Priestley, A.R. Knight, G.N. Woodruff, L.L. Iversen, Proc. Natl. Acad. Sci. U.S.A. 83 (1986) 7104–7108.
- [15] S. Miyamoto, J.N. Leipzig, J.A. Lieberman, G.E. Duncan, Neuropsychopharmacology 22 (2000) 400–412.
- [16] M.C. Breschi, V. Calderone, M. Digiacomo, M. Manganaro, A. Martelli, F. Minutolo, S. Rapposelli, L. Testai, F. Tonelli, A. Balsamo, J. Med. Chem. 51 (2008) 6945–6954.
- [17] J.K. Mishra, P. Garg, P. Dohare, A. Kumar, M.I. Siddiqui, M. Ray, G. Panda, Bioorg. Med. Chem. Lett. 17 (2007) 1326–1331.
- [18] J.K. Mishra, G. Panda, J. Comb. Chem. 9 (2007) 321-338.
- [19] J.K. Mishra, G. Panda, Tetrahedron Lett. 11 (2005) 1881-1887.
- [20] J.-R. Liu, X.-R. Sun, H.-W. Dong, C.-H. Sun, W.-G. Sun, B.-Q. Chen, Y.-Q. Song, B.-
- F. Yang, Int. J. Cancer 122 (2008) 2689–2698.
 [21] H. Hatcher, R. Planalp, J. Cho, F.M. Torti, S.V. Torti, Cell. Mol. Life Sci. 65 (2008) 1631–1652.
- [22] A.S. Strimpakos, R.A. Sharma, Antioxd. Redox Signal. 10 (2008) 511–545.
- [23] D.M. Anthony, B.K. Ann, B.D. Lynn, B.A. Sun, C.S. Louis, S.C. Elizabeth, US Patent, 2004, No. WO2004037213 (A2).
- [24] P. Dohare, S. Varma, M. Ray, Nitric Oxide 19 (2008) 1-11.
- [25] P. Rathore, P. Dohare, S. Varma, A. Ray, U. Sharma, N.R. Jaganathanan, M. Ray, Neurochem. Res. 33 (2008) 1672–1682.
- [26] S.K. Sandur, M.K. Pandey, B. Sung, K.S. Ahn, A. Murakami, G. Sethi, P. Limtrakul, V. Badmaev, B.B. Aggarwal, Carcinogenesis 28 (2007) 1765–1773.
- [27] W. Oroshnik, G. Karmas, R.A. Mallory, J. Am. Chem. Soc. 76 (1954) 2325–2329.
- [28] C.X. Wang, Y. Yang, T. Yang, A. Shuaib, Brain Res. Protoc. 7 (2001) 115–120.
- [29] P. Dohare, P. Garg, V. Jain, C. Nath, M. Ray, Behav. Brain Res. 193 (2008) 289– 297.
- [30] I. Margaill, S. Parmentier, J. Callebert, M. Allix, R.G. Boulu, M. Plotkine, J. Cereb. Blood Flow Metab. 16 (1996) 107–113.
- [31] A.C. Foster, R. Gill, G.N. Woodruff, J. Neurosci. 12 (1988) 4745-4754.
- [32] J. Li, M.C. Henman, T. Tatlisumak, G.G. Shaw, K.M. Doyle, Brain Res. 1055 (2005) 180–185.