Design, Synthesis, and Molecular Modeling of Asymmetric Tolterodine Derivatives as Anticancer Agents

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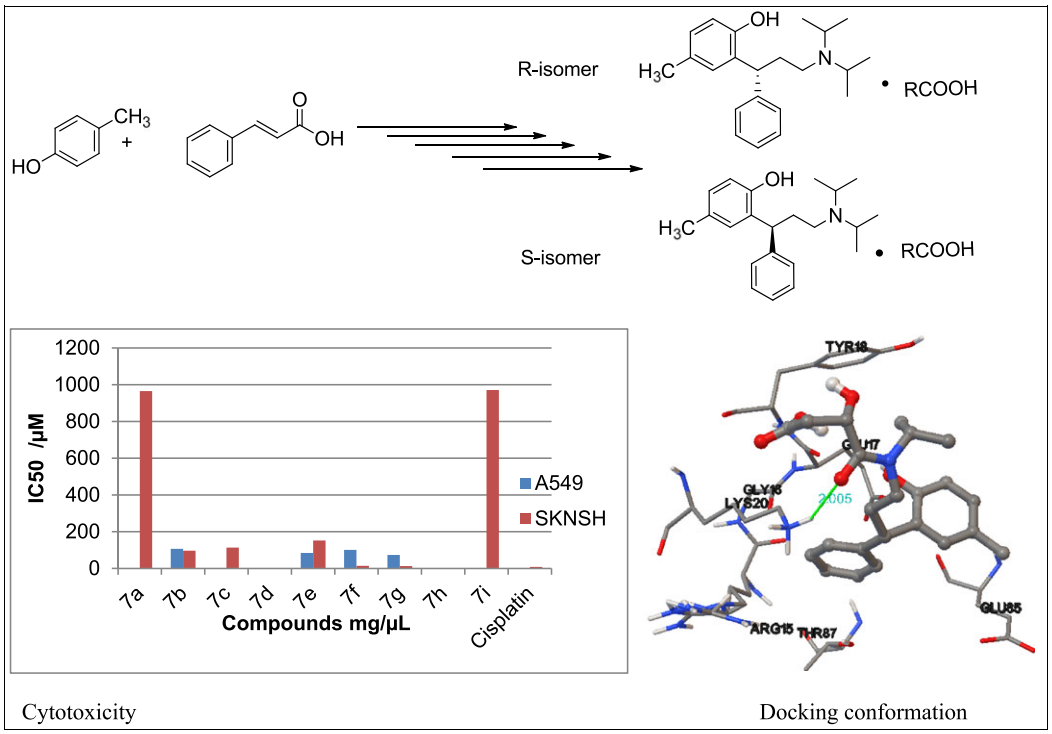
Received December 6, 2017
DOI 10.1002/jhet.3274
Published online 00 Month 2018 in Wiley Online Library (wileyonlinelibrary.com).



An efficient and short enantioselective synthesis of (S)- and (R)-tolterodine acid isomers (**7a**–**7i**) was performed a 6-methyl-4-phenylchroman-2-one intermediate from inexpensive and commercially available starting materials. A series of tolterodine acid hybrids **7** were synthesized and characterized by infrared, ^{1}H NMR, ^{13}C NMR, X-ray diffraction, and mass spectral analysis followed by anticancer activity on human cancer cell lines including A549 and SKNSH. Our results revealed the final compounds exhibited moderate to potent activity against A549 and SKNSH. Compounds **7g** and **7f** were more cytotoxic than cisplatin against all tested two human cancer cell lines, with half maximal inhibitory concentration values of 13.2, 14.3, and 8.5 µ*M*, respectively. In the present investigation, possible binding interaction of the target compounds with 3IVX protein, ligand conformations, including hydrogen bonds and the bond lengths, was analyzed. AutoDock 4.2 chemokine receptor has been investigated by molecular docking and was used to predict the affinity, activity, and binding orientation of ligand with the target protein and to analyze best conformations. Compound **7h** exhibited more binding energy (ΔG = −5.52 kcal/mol) and dissociation constant (KI = 89.8 µ*M*) with amino acids Glu 17 and Thr 87 interacting. Further studies are warranted to fully evaluate the analogues as the potential prodrugs with improved physiochemical properties.

INTRODUCTION

The most recent population-based data from the National Cancer Institute's Surveillance, Epidemiology, and End Results program demonstrate that 83% of childhood cancer patients survive at least 5 years [1]. Among patients who survive 5 years, the vast majority (i.e., greater than 90%) will not experience a recurrence and are considered cured of their cancer. Estimates place the number of pediatric cancer survivors living in the

USA at more than 420,000 at the end of 2013 [2]. Treatments for chronic obstructive pulmonary disease (COPD) include the use of bronchodilators that increase lung air flow, the two major classes being inhaled b-2 adrenergic agonists and muscarinic M3 antagonists. Long acting b-2 agonists (LABAs) and long acting muscarinic antagonists (LAMAs) have been, and continue to be, developed to enable once-daily inhalation dosing. Significant efficacy improvements can be seen from the combination of LABAs and LAMAs [3]. Chronic bronchitis and emphysema are components of COPD that damages the airways of the lungs leading to reduced air flow. COPD is a major cause of disability and is predicted to be the third largest cause of death by 2030 [4]. Moreover, triple therapy, from the optimal synergistic combination of a LABA, a LAMA, and an inhaled corticosteroid, represents an exciting new treatment paradigm for this debilitating condition [5].

A clear advantage of inhalation is the potential to target the active drug directly to the site of action and, importantly, minimize the potential for side effects associated with systemic drug exposure. Effective inhaled drugs require a number of essential attributes to be secured: very low dose and optimal material properties (to facilitate delivery), suitable solubility and pharmacokinetics, and the desired pharmacodynamic duration of action in the lung. Although the medicinal chemistry associated with inhaled drug discovery is distinct from classical oral drug design, many of the key insights and learnings may be transferable into more mainstream medicinal chemistry. The lack of selectivity of tolterodine (**1**) and oxybutynin (**3**) (Fig. 1) may be the result of the interaction of different conformations **1** of the ligands with different receptor subtypes, and so subtype selective ligands, which recognize the different localizations and functions of receptor subtypes, may provide the possibility of developing more ideal drugs. Herein we describe our work on the conformational restriction of the amine part of anticancer drugs like **7a**–**7i**.

DNA double-strand breaks (DSBs) are resolved by the nonhomologous end joining [6] and homologous recombination [7] repair pathways, which are part of the cellular DNA damage response (DDR) network. Among the many different DNA lesions, DNA DSBs are the most deleterious [8]. It has been estimated that a single unrepaired DSB is sufficient to induce apoptosis [9]. Sporadic and hereditary DDR mutations are widespread in many tumors [10], and while these mutations drive tumorigenesis, they also provide a context in which to obtain selectivity, as inhibition of a functional DDR pathway in transformed cells is selectively toxic because of decreased genetic buffering (synthetic lethality) [11]. DNA is an established target for chemotherapeutic intervention; approximately 70% of small-molecule anticancer agents target DNA [12].

Biological molecules tagged with fluorescent groups have been used for a myriad of purposes from cell imaging to automated DNA sequencing. Fluorophoric ligands have also been extensively used in drug discovery to create fluorometric assays for high throughput screening, which offer significant advantages over the radiometric variants. Therefore, innovations in this field, particularly where methods can improve existing biological assays to enable high throughput screening, are still highly desirable. Interestingly, a recent report by Mazzone *et al*. has detailed the fortuitous discovery of muscarinic antagonist activity associated with the fluorescent styryl dyes FM1-43 and FM2-10 (Fig. 1) [13]. Herein we describe our work on the conformational restriction of the amine part of antimuscarinic drugs like **1** and **3**. (Fig. 1) [14]. Hence, many studies aimed at discovery of subtype-selective or tissue-selective muscarinic receptor antagonists have been

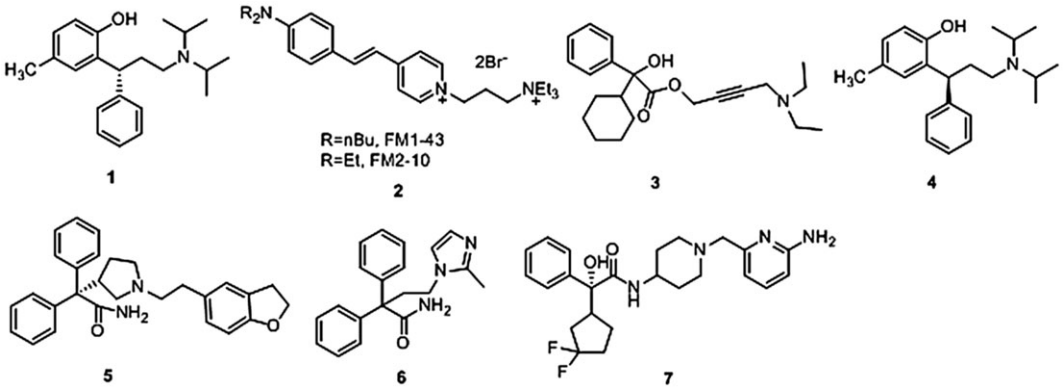


Figure 1. Biological importance of fluorescent groups with DNA sequencing (**1**–**7**).

reported, including those on tolterodine (**4**) [15], darifenacin (**5**) [16], KRP-197 (**6**) [17], and **7** [18] (Fig. 1).

(R)-Tolterodine, a potent muscarinic antagonist, is an important urological drug used for the treatment of an overactive bladder [19]. Several different approaches have been reported for the asymmetric synthesis of tolterodine so far, utilizing asymmetric hydrogenation [20], conjugate addition of arylboronic acids [21], and Corey–Bakshi–Shibata reduction [22] as a key step. In most of the syntheses, cyclic precursors such as coumarins [20] and indenones [23] were used in the key catalytic enantio selective step except for the early approaches based on chiral auxiliaries [24] or hydroformylation reaction [25]. Although some of the syntheses achieved high levels of enantio selectivity [21,22], a more efficient method with no extra steps for manipulating unnecessary functionalities is still required.

RESULTS AND DISCUSSIONS

Chemistry. To investigate the function of the acid derivatives of tolterodines, **7a**–**7i** were synthesized as shown in Figure 2. P-cresol (**1**) was converted into 6-methyl-4-phenylchroman-2-one (**3**) with good yield of 77%. Further, the compound 6-methyl-4-phenylchroman-2-one (3) (1.2 mmol) in methanol to give 2-(3-hydroxy-1-phenylpropyl)-4-methylphenol (**4**) with good yields (71%). An alcohol (**4**) (0.8 g, 1.3 mmol) in a solvent reacted with mesyl chloride (0.5 g, 1.0 mmol) to give desired product **5**. The compound 3-(5-methyl-2-((methylsulfonyl)oxy)phenyl)-3-phenylpropyl methansulfonate (**5**) (1.5 mmol) and diisopropylamine (1.2 mL, 1.2 mmol), Br_2 in CH_3COOH (3 mL) at room temperature for 5 h to give 3-(2-hydroxy-5-methylphenyl)-*N*,*N*-diisopropyl-3-phenyl propan-1-aminium bromide (**6**). Furthermore, the compound 6 (0.3 mmol) and different acids (0.2 mmol) were added in ethanol (14 mL) and the mixture is heated and then cooled for crystal formation as (R)-*N*,*N*-diisopropyl-3-(2-hydroxy-5-methylphenyl)-3-phenylpropanamine-L-hydrogentartrate **7a** (Fig. 3).

The structure of compound **7a** was confirmed based on the following analytical data as infrared (IR) signals υ cm^{-1}, 3571s cm^{-1} for OH stretching, 3045 cm^{-1} for Ar═CH stretching, 2988w cm^{-1} for CH stretching, 1697s cm^{-1} for CO stretching, 1582s and 1508w cm^{-1} aromatic –C═C vibrations, 1376 cm^{-1} for CN stretching; ^{1}H-NMR δ ppm at 1.15, 2.24 doublets for 12H, 2H's belongs to CH_3, CH_2, δ 2.17 singlet for $ArCH_3$, 4.01–4.13 singlet for CHOH protons, δ 7.05, 7.31 multiplet for aromatic hydrogens, δ 6.73, 6.80 doublet for aromatic hydrogens, 8.14 singlet for PhOH, and δ at 8.42 singlet for COOH; ^{13}C NMR (100 MHz, DMSO-d_6,) δ 17.6, 17.5 for $(CH_3)_2CH$, δ 20.5 for $ArCH_3$, δ 31.9 for CH_2CH, δ 41.0 for $(CH_3)_2CH$), δ 45.5 for CH_2N, δ 53.3 for ArCH, δ for 72.4 CHOH, δ 115.2 for ArC_2, δ 126.0 for ArC_4, δ 127.2 for ArC_{19}, δ 127.5 for $ArC_{18,20}$, δ 127.8 for ArC_3, δ 127.9 for ArC_5, δ 129.4 for ArC_6, δ 143.9 for ArC_{16}, δ 152.4 for ArC_1, δ 174.5 for $ArC_{1',4'}$. LC–MS (ESI): 475.5 $(M+1)^+$; fragmentation pattern was in accordance with the assigned

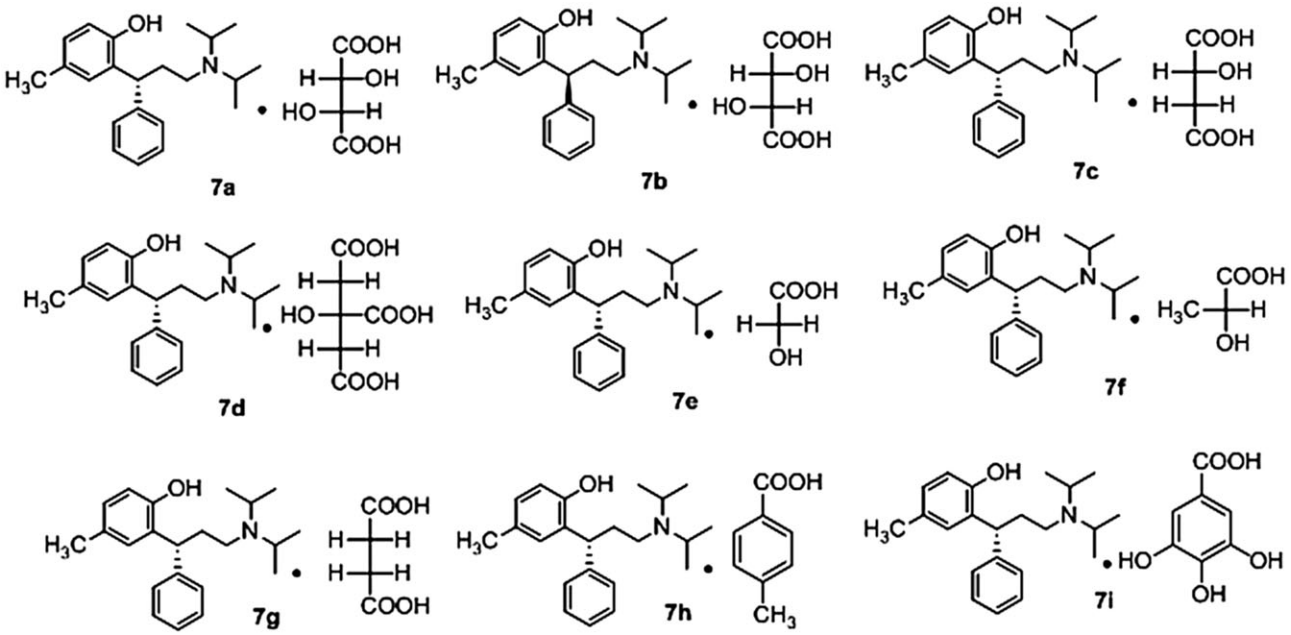


Figure 2. Synthesis of target compounds (**7a**–**7i**).

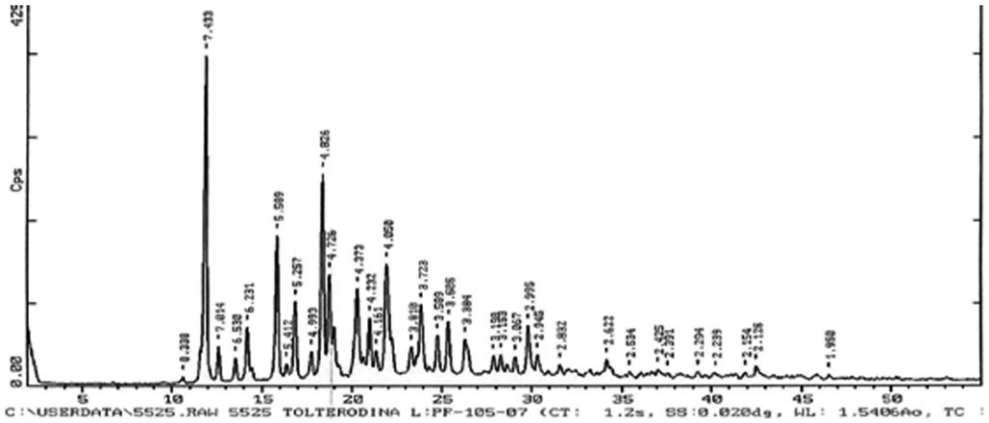
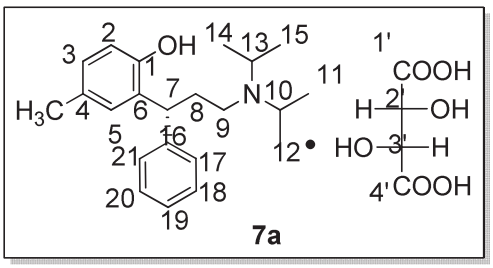


Figure 3. Cytotoxicity of title compounds (**7a–7i**).

structure and the assignment of carbon atoms of **7a** given below for further information Pl (see the Experimental section for details).



Biology. ***Cytotoxicity.*** The results were represented as percentage of cytotoxicity/viability. From the percentage of cytotoxicity, the half maximal inhibitory concentration (IC_{50}) values were calculated and presented in Table 1 and Figure 3. Treatment with the compounds reduced the viability of human cancer cell lines in a concentration-dependent manner, with IC_{50} values in the low micromolar range. Four different concentrations of compounds 7**a**–**7i** were tested on three cancer cell lines. IC_{50} values were given in micromolar concentrations (μM).

Table 1

Anticancer activity of title compounds **7a–7i**.

S. No.	Compounds	A549	SKNSH
1	**7a**	>1000	965.3
2	**7b**	106.7	96.6
3	**7c**	>1000	113.6
4	**7d**	>1000	>1000
5	**7e**	84.5	152.4
6	**7f**	101.0	14.3
7	**7g**	73.7	13.2
8	**7h**	>1000	>1000
9	**7i**	>1000	971.0
10	Cisplatin	0.92	8.5

The topless approach has been known as a popular tool in lead optimization for identifying optimal substituents. The approach involves systematic changes in electronic, steric, and hydrophobic properties of substituents and is particularly useful in cases where no structural information about the biological target is available. In our initial study, a similar strategy was used to examine the effect of the substituents (acids) on the asymmetric tolterodine acid moiety in **7g** and **7f** on the potency as summarized in Table 1 and Figure 3. For SKNSH human neuroblastoma cell line, the analogues **7g** and **7f** exhibited excellent active with IC_{50} values of 13.2 and 14.3 μM, respectively. Further, compounds **7g** and **7e** showed a moderate to good potential activity present in the cell line A549.

Docking studies. Molecular docking study was performed by using AutoDock 4.2, which was a suite of automated docking tools and was used to predict the affinity, activity, and binding orientation of ligand with the target protein and to analyze best conformations [32]; the protein with all the 12 compounds were loaded individually into auto-dock tools (ADT) and evaluate 10 finest conformations. In the present investigation, we focused mainly on the binding energy, hydrogen bonds, and distance between the protein and ligand. Binding energies were measured; these consist of Van der Waals forces, hydrogen bondings, π–π interactions, cation–π interactions, and so on. From investigation, the binding interaction of the target compounds with 3IVX protein and ligand conformations, including hydrogen bonds and the bond lengths, was analyzed in Table 2 and Figure 4. AutoDock 4.2 chemokine receptor has been investigated by molecular docking and was used to predict the affinity, activity, and binding orientation of ligand with the target protein and to analyze best conformations. Compound **7h** exhibited more binding energy ($\Delta G = -5.52$ kcal/mol) and dissociation constant (KI = 89.8 μM) with two amino acids Glu 17 and Thr 87.

Table 2

Docking conformation of title compounds (**7a**–**7i**).

Ligand	Interacting amino acids	Grid X-Y-Z coordinates	Binding energy ΔG (kcal/mol)	Dissociation constant (KI) (μ*M*)
7A	Arg 15, Trp 22	15.947, 20.382, 24.919	−2.28	21.19
7C	Lys 20	15.947, 20.382, 24.919	−3.5	2.71
7D	Arg 21, Trp 22, Trp 22, Thr 21	15.947, 20.382, 24.919	−2.76	9.56
7E	Arg 15, Glu 17	15.947, 20.382, 24.919	−3.7	1.94
7F	Arg 15, Trp 22	15.947, 20.382, 24.919	−4.01	1.16
7G	Lys 20, Thr 87	15.947, 20.382, 24.919	−3.6	2.29
7H	Glu 17, Thr 87	15.947, 20.382, 24.919	−5.52	89.8
7I	Thr 21, Trp 22, Arg 23, Arg 23	15.947, 20.382, 24.919	−3.44	3.01

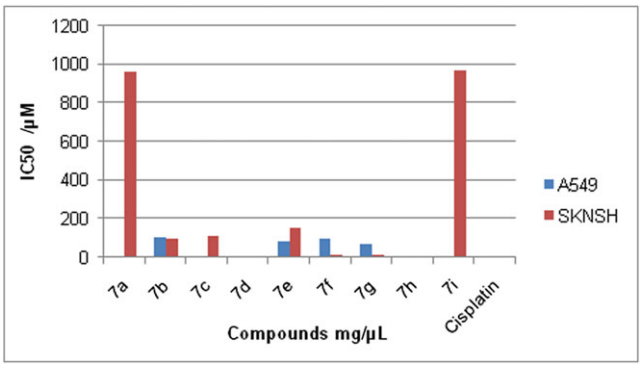


Figure 4. Docking conformations of asymmetric tolterodine acid derivatives (**7a**–**7i**). [Color figure can be viewed at wileyonlinelibrary.com]

Further, the isomer **7f**, **7e**, and **7g** shows moderate to good binding interactions with the amino acids Arg 15, Trp 22, Arg 15, Glu 17, Lys 20, and Thr 87 of ΔG = −4.01, −3.7, and −3.6 kcal/mol, respectively. The order of the molecular docking conformations is **7h** > **7f** > **7e** > **7g** > **7c** > **7i** > **7d** > **7a**.

CONCLUSIONS

In summary, this work reveals the hidden potentials in new framework design. (R)-tolterodine acid derivatives were prepared from 6-methyl-4-phenylchroman-2-one intermediate and different acids without affecting core SAR. The compounds **7a**–**7i** were screened for their *in vitro* anticancer activity against A549 and SKNSH cell lines by MTT microcultured tetrazolium assay for the determination of IC_{50} and values of the synthesized compounds along with the standard drugs (cisplatin) for comparison. The compounds **7g** showed excellent activity with IC_{50} of 13.2 μ*M* and were more potent than standard drug cisplatin with IC_{50} of 8.5 μ*M*. Further, the compound **7g** showed excellent activity with IC_{50} value 13.2 μM. Docking studies supported the concept of condensation of tolterodine with different carboxylic acid to its bioisosteric, which is crucial for establishing hydrogen bond interaction with receptor (3IVX). Furthermore, the established presence of R-conformation offered a positive ray of hope in the potential inhibition of pathway, a crucial strategy for anticancer therapies.

EXPERIMENTAL

Chemistry. ***Preparations.*** All chemicals were purchased from commercial suppliers and were used without further purification. All reactions were performed under inert nitrogen atmosphere employing dry solvents. Precoated thin-layer chromatography (TLC) silica gel plates (Kieselgel 60 F254, Merck, SRL [Sisco Research Laboratories], India) were used for monitoring reactions, and the spots are visualized under UV lamp (254 nm). Purification was performed by column chromatography using silica gel (particle size 60–120 mesh, Merck). Melting points were determined in open capillary tubes on Cintex melting point apparatus and are uncorrected. IR (KBr) spectra were recorded on a PerkinElmer 400 FTIR spectrometer (υ_{max} in cm^{-1}) or a Varian 670-IR FTIR spectrometer (attenuated total reflection) in the frequency range of 600–4000 cm^{-1}, and we observed vibrational frequencies for strong (s) and weak (w) peaks. ^1H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$/DMSO-d_6 on a Bruker DRX-300 (300, 400 MHz FT

NMR) or a Varian Mercury 500 MHz spectrometer. Proton chemical shifts are presented in δ ppm with reference to TMS. *J* values are presented in Hz. Mass spectra were recorded using Jeol SX-102 spectrometer. Determinations were carried out using an equipment VARIO EI III version H of elementary analysensysteme under the following conditions of operation.

Synthesis of 6-methyl-4-phenylchroman-2-one (3). P-cresol (**1**) (1.5 g, 1.3 mmol), is added to trans-cinnamic acid (**2**) (1.3 g, 1.2 mmol) and *p*-$MeC_6H_4SO_3H$ (0.8 mmol) in toluene, and the reaction mixture was refluxed for 4 h until complete reaction. The solvent distilled off from the reaction mixture, and it is cooled for crystal formation. The product is isolated using mobile phase hexane : ethyl acetate (8:2) and yields 77%; mp 185–187°C, IR (KBr, cm^{-1}) υ_{max}; 3020w (Ar═CH), 2920w (CH), 1766s (C═O), 1508w, 1493s, 1451w (Ar–C═C), 1288 (C–O–C); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 2.26 (s, 3H, CH_3Ph), 3.20 (dd, *J* = 15.8, 6.3 Hz, 2H, CH_2CO), 4.29 (dd, *J* = 16.9, 1H, CHPh), 6.81–7.42 (m, 8H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 20.7 ($ArCH_3$), 37.1 (CH_2CO), 40.7 (ArCH), 116.6 (ArC_2), 125.1 (ArC_4), 127.2 (ArC_{13}), 127.4 ($ArC_{11,15}$), 128.4 ($ArC_{12,14}$), 128.9 (ArC_5), 129.1 (ArC_4), 134.1 (ArC_6), 140.2 (ArC_{10}), 149.4 (ArC_1), 167.5 (CO); LC–MS (ESI): 239 $(M+1)^+$; Elemental analysis C 80.65, H 5.93; found C 81.68, H 5.99.

Synthesis of 2-(3-hydroxy-1-phenylpropyl)-4-methylphenol (4). The compound **3** (1 g, 1.2 mmol) in methanol (12 mL) in the presence of base K_2CO_3 stirred at room temperature for 7 h, the solvent was distilled, and then water is charged and the mixture is agitated. The product is isolated using TLC mobile phase hexane : ethyl acetate (8:2) and dried; mp 172–174°C, IR (KBr, cm^{-1}) υ_{max}; 3420.01s (OH), 3060w (Ar═CH), 2923s, 2885w (CH), 1654w, 1609s (Ar–C═C); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.17 (s, 3H, CH_3Ph), 2.13–2.15 (m, 2H, CH_2CH), 2.68 (s, 1H, OH), 3.52–3.74 (m, 2H, CH_2OH), 4.57 (dd, *J* = 10.2, 6.0 Hz, 1H, CHPh), 6.70–6.85 (m, 3H, ArH), 7.19–7.29 (m, 5H, ArH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 20.7 ($ArCH_3$), 36.9 (CH_2CH), 38.6 (ArCH), 60.7 (CH_2OH), 115.9 (ArC_2), 126.2 (ArC_4), 127.9 (ArC_{14}), 128.2 (ArC_3), 128.4 ($ArC_{13,15}$), 129.1 ($ArC_{12,16}$), 130.2 (ArC_5), 134.4 (ArC_6), 144.0 (ArC_{11}), 151.4 (ArC_1); LC–MS (ESI): 243 $(M+1)^+$; Elemental analysis C 79.31, H 7.49; found C 80.62, H 8.13.

Synthesis of 3-(5-methyl-2-((methylsulfonyl)oxy)phenyl)-3-phenylpropyl methansulfonate (5). An alcohol (**4**) (0.8 g, 1.3 mmol) is dissolved in MeOH (10 mL), and the mixture is cooled and reacted with mesyl chloride (0.5 g, 1.0 mmol) at room temperature for 8 h. The mixture is washed with water and an acid. The organic phase is heated, and a solvent is distilled. The desired product 5 is obtained by using column chromatography (EtOAc : hexane); mp 190–192°C, IR (KBr, cm^{-1}) υ_{max}; 3462br (OH), 3028w (Ar═CH), 2924s, 2901w (CH), 1620w, 1490s (Ar–C═C), 1268 m (C–O-S) 1196 m, 1169s (S–O); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 2.32 (s, 3H, CH_3Ph), 2.47 (dd, *J* = 6.3, 14.2 Hz, 2H, CH_2CH), 2.92, 2.99 (s, 3H, CH_3SO), 2.99 (s, 3H, CH_3SO), 4.18 (m, 2H, CH_2OH), 4.56 (dd, *J* = 5.4, 7.8 Hz, 1H, CHPh), 7.03 (dd, *J* = 7.8, 2H, ArH), 7.13 (d, 1H, *J* = 8.2, ArH), 7.20–7.34 (m, 5H, ArH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm 21.1 ($ArCH_3$), 34.2 (CH_2CH), 37.1, (CH_3SO), 37.8 (CH_3SO), 39.7 (ArCH), 67.9 (CH_2O), 121.4 (ArC_2), 126.7 (ArC_4), 127.8 (ArC_{14}), 128.4 (ArC_3), 128.5 ($ArC_{13,15}$), 128.9 ($ArC_{12,16}$), 135.5 (ArC_4), 137.1 (ArC_{11}), 144.8 (ArC_1); LC–MS (ESI): 399 $(M+1)^+$; Elemental analysis C 54.25, H 5.56, S 16.09; found C 58.24, H 8.89, S 16.68.

Synthesis of 3-(2-hydroxy-5-methylphenyl)-N,N-diisopropyl-3-phenylpropan-1-aminium bromide (6). **5** (1 g, 1.5 mmol) is dissolved in ethanol (13 mL) and heated and then added diisopropyl amine (1.2 mL, 1.2 mmol), Br_2 in CH_3COOH (3 mL) at ambient temperature for 5 h. After completion, the reaction solvents are distilled off and a mixture of solvent/water is added to wash the product. An alkaline solution in alcohol is charged and heated to reflux until complete reaction, and then solvent is distilled. The reaction mixture was washed with water, the organic phase is stirred, and an acid is charged and then is cooled for crystals formation. The product is isolated by using TLC, and crude product was obtained, using column chromatography ratio (EtOAc : hexane).

Synthesis of (R)-2-(3-(diisopropylamino)-1-phenylpropyl)-4-methylphenol tartrate (7a) or (R)-N,N-diisopropyl-3-(2-hydroxy-5-methylphenyl)-3-phenylpropanamine-L-hydrogentartrate (7a). 3-(2-hydroxy-5-methylphenyl)-*N*,*N*-diisopropyl-3-phenylpropan-1-aminium bromide (**6**) (1 g, 0.3 mmol) is dissolved in a mixture of solvents and water, tartaric acid (0.8 g, 0.2 mmol) in ethanol (14 mL) refluxed for 10 h. The mixture is heated to reflux and filtered, and then the mixture is cooled; the product is isolated and dried; the dry product was using column chromatography ratio EtOAc : hexane (2:8); mp 210–214°C, IR (KBr, cm^{-1}) υ_{max}; 3571s (OH), 3045 (Ar═CH), 2988w (CH), 2920w (CH), 1697s (CO), 1582s, 1508w, 1487w (Ar–C═C), 1376 (CN), 1265 (C–O); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.15 (d, *J* = 4.0 Hz, 12H, $(CH_3)_2$CH), 2.17 (s, 3H, CH_3Ph), 2.44 (d, *J* = 4.5 Hz, 2H, CH_2CH), 2.81 (m, 2H, $(CH_3)_2$CH), 3.50 (m, *J* = 6.4 Hz, 2H, CH_2N), 4.01 (s, 2H, CHOH), 4.13 (s, 2H, CHOH), 4.32 (t, *J* = 7.6 Hz, 1H, CHPh), 6.73 (d, *J* = 9.2 Hz, 1H, ArH), 6.80 (d, *J* = 2.0, 8.0 Hz, 1H, ArH), 7.05 (s, 1H, ArH), 7.15 (t, *J* = 6.8 Hz, 1H, ArH), 7.31 (m, 4H, ArH), 8.14 (s, 1H, PhOH), 8.42 (s, 2H, COOH); ^{13}C NMR (100 MHz, DMSO-d_6,) δ ppm 17.6, 17.5 $(CH_3)_2$CH), 20.5 ($ArCH_3$), 31.9 (CH_2CH), 41.0 $(CH_3)_2$CH), 45.5 (CH_2N), 53.3 (ArCH), 72.4 (CHOH), 115.2 (ArC_2), 126.0 (ArC_4), 127.2 (ArC_{19}),

127.5 ($ArC_{18,20}$), 127.8 (ArC_3), 127.9 (ArC_5), 129.4 (ArC_6), 143.9 (ArC_{16}), 152.4 (ArC_1), 174.5 ($ArC_{1',4'}$); LC–MS (ESI): 475.5 $(M+1)^+$; Elemental analysis C 65.48, H 7.79, N 3.25; found C 65.66, H 7.84, N 2.94.

(S)-N,N-Diisopropyl-3-(2-hydroxy-5-methylphenyl)-3-phenylpropanamine-L-hydrogen tartrate (7b). mp 220–222°C, IR (KBr, cm^{-1}) υ_{max}; 3570 m (OH), 2988w (Ar═CH), 2945w, 2707w (CH), 1697b (C═O), 1582s, 1508w, 1487w (Ar–C═C), 1375 m (CN), 1265s (C–O); ^1H NMR (400 MHz, DMSO-d_6) δ ppm 1.15 (d, J = 4.0 Hz, 12H, $(CH_3)_2CH$), 2.17 (s, 3H, CH_3Ph), 2.44 (a, 2H, CH_2CH), 2.81 (m, 2H, $(CH_3)_2CH$), 3.50 (m, J = 6.4 Hz, 2H, CH_2N), 4.04 (s, 2H, CHOH), 4.15 (s, 2H, CHOH), 4.32 (t, J = 7.6 Hz, 1H, CHPh), 6.73 (d, J = 9.2 Hz, 1H, ArH), 6.80 (d, J = 2.0, 8.0 Hz, 1H, ArH), 7.05 (s, 1H, ArH), 7.15 (t, J = 6.8 Hz, 1H, ArH), 7.31 (m, 4H, ArH), 8.16 (s, 1H, PhOH), 8.48 (s, 2H, COOH); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 17.6, 17.5 $(CH_3)_2CH$), 20.5 ($ArCH_3$), 31.9 (CH_2CH), 41.0 $(CH_3)_2CH$), 45.5 (CH_2N), 53.3 (ArCH), 72.4 (CHOH), 115.2 (ArC_2), 126.0 (ArC_4), 127.2 (ArC_{19}), 127.5 ($ArC_{18,20}$), 127.8 (ArC_3), 127.9 (ArC_5), 129.4 (ArC_6), 143.9 (ArC_{16}), 152.4 (ArC_1), 174.5 ($ArC_{1',4'}$); LC–MS (ESI): 475.5 $(M+1)^+$; Elemental analysis C 65.48, H 7.79, N 3.25; found C 65.66, H 7.84, N 2.94.

(R)-2-(3-(Diisopropylamino)-1-phenylpropyl)-4-methylphenol-(R)-2-hydroxysuccinate (7c). mp 209–211°C, IR (KBr, cm^{-1}) υ_{max}; 3434s (OH), 3046w (Ar═CH), 2924w, 2860w (CH), 1738b (C═O), 1608s, 1540w, 1481w (Ar–C═C), 1310 m (CN), 1252s (C–O); ^1H NMR (400 MHz, DMSO-d_6) δ ppm 1.15 (d, J = 4.2 Hz, 12H, $(CH_3)_2CH$), 2.17 (s, 3H, CH_3Ph), 2.26 (d, 2H, CH_2), 2.44 (d, J = 6.4 Hz, 2H, CH_2CH), 2.81 (m, 2H, $(CH_3)_2CH$), 3.50 (t, J = 6.4 Hz, 2H, CH_2N), 4.05 (s, 1H, CHOH), 4.13 (s, 1H, CHOH), 4.32 (t, J = 7.6 Hz, 1H, CHPh), 6.73 (d, J = 9.2 Hz, 1H, ArH), 6.80 (d, J = 2.0, 8.0 Hz, 1H, ArH), 7.05 (s, 1H, ArH), 7.15 (t, J = 6.8 Hz, 1H, ArH), 7.31 (m, 4H, ArH), 8.20 (s, 1H, PhOH), 8.42 (s, 2H, COOH); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 14.94 $(CH_3)_2CH$), 18.85 ($ArCH_3$), 38.28 ($CH_{2'}$), 45.77 ($ArCH_3$), 55.30 $(CH_3)_2CH$), 62.00 (CH_2N), 63.61 (ArCH), 101.62 (ArC_2), 105.56 (ArC_{19}), 114.74 ($ArC_{18,20}$), 127.94 (ArC_3), 136.02 (ArC_5), 146.37 (ArC_6), 153.26 (ArC_{16}), 158.80 (ArC_1), 165.78 ($ArC_{1',4'}$); LC–MS (ESI): 459.5 $(M+1)^+$; Elemental analysis C 66.28, H 7.23, N 3.15; found C 65.12, H 7.16, N 2.16.

(R)-2-(3-(Diisopropylamino)-1-phenylpropyl)-4-methylphenol-2-hydroxypropane-1,2,3-tricarbo xylate (7d). mp 220–222°C, IR (KBr, cm^{-1}) υ_{max}; 3433s (OH), 3052w (Ar═CH), 2966w, 2922w, 2859w (CH), 1737b (C═O), 1603s, 1531w, 1471w (Ar–C═C), 1322 m (CN), 1213s (C–O); ^1H-NMR (400 MHz, $CDCl_3$) δ ppm 1.37 (d, J = 6.8 Hz, 12H, $(CH_3)_2CH$), 2.16 (s, 3H, CH_3Ph), 2.30 (s, 4H, CH_2), 2.42 (d, J = 6.0 Hz, 2H, CH_2CH), 3.99 (t, J = 6.8 Hz, 2H, CH_2N), 5.31 (s, 1H, CHOH), 5.66 (t, J = 7.6 Hz, 1H, CHPh), 6.76–6.78 (d, J = 8.4 Hz, 2H, ArH), 7.17–7.19 (m, 4H, ArH), 7.56 (d, J = 9.2 Hz, 2H, ArH), 8.01 (s, 1H, PhOH), 8.48 (s, 1H, COOH), 8.96 (s, 2H, COOH); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 21.20 $(CH_3)_2CH$), 38.28 $(CH_{2'})_2$, 40.12 ($C_{3'}$), 45.18 ($ArCH_3$), 53.30 $(CH_3)_2CH$), 57.25 (CH_2N), 61.01 (ArCH), 114.99 (ArC_2), 125.52 (ArC_{19}), 128.08 ($ArC_{18,20}$), 135.33 (ArC_3), 148.07 (ArC_5), 152.26 (ArC_6), 156.54 (ArC_{16}), 159.38 ($ArC_{1'}$), 165.38 ($ArC_{3'}$), 172.29 ($ArC_{1',5'}$); LC–MS (ESI): 516.6 $(M+1)^+$; Elemental analysis C 70.12, H 9.17, N 4.30; found C 71.10, H 9.20, N 4.36.

(R)-2-(3-(Diisopropylamino)-1-phenylpropyl)-4-methylphenol-2-hydroxyacetate (7e). mp 198–200°C, IR (KBr, cm^{-1}) υ_{max}; 3439s (OH), 3046w (Ar═CH), 2926w, 2858w (CH), 1634b (C═O), 1602s, 1525w, 1476w (Ar–C═C), 1393 m (CN), 1117s (C–O); ^1H NMR (400 MHz, DMSO-d_6) δ ppm 2.11 (d, J = 6.8 Hz, 12H, $(CH_3)_2CH$), 2.22 (s, 3H, CH_3Ph), 2.37–2.39 (t, J = 6.8 Hz, 2H, CH_2N), 2.80 (d, J = 6.0 Hz, 2H, CH_2CH), 2.89 (s, 2H, CH_2), 4.01 (t, J = 7.6 Hz, 1H, CHPh), 5.03 (s, 1H, CHOH), 6.63–6.65 (d, J = 8.4 Hz, 2H, ArH), 7.02–7.06 (m, J = 8.4 Hz, 4H, ArH), 7.57 (d, J = 9.2 Hz, 2H, ArH), 7.91 (s, 1H, PhOH), 9.08 (s, 1H, COOH); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 17.21 $(CH_3)_2CH$), 32.45 (CH_2), 38.28 ($C_{2'}$), 45.24 ($ArCH_3$), 53.93 $(CH_3)_2CH$), 57.23 (CH_2N), 61.52 (ArCH), 114.70 (ArC_2), 117.16 (ArC_{19}), 129.59 ($ArC_{18,20}$), 144.79 (ArC_3), 145.19 (ArC_5), 153.27 (ArC_6), 155.24 (ArC_{16}), 157.51 (ArC_1), 165.26 ($ArC_{1'}$); LC–MS (ESI): 400.5 $(M+1)^+$; Elemental analysis C 67.10, H 10.15, N 3.25; found C 68.13, H 7.28, N 4.30.

(R)-2-(3-(Diisopropylamino)-1-phenylpropyl)-4-methylphenol-(R)-2-hydroxypropanoate (7f). mp 201–203°C, IR (KBr, cm^{-1}) υ_{max}; 3436s (OH), 3054w (Ar═CH), 2926w, 2859w (CH), 1633b (C═O), 1612s, 1506w (Ar–C═C), 1399w (CN), 1120s (C–O); ^1H NMR (400 MHz, DMSO-d_6) δ ppm 2.10 (d, J = 6.4 Hz, 12H, $(CH_3)_2CH$), 2.27 (s, 3H, CH_3Ph), 2.39–2.40 (t, J = 5.6 Hz, 2H, CH_2N), 2.86 (d, J = 6.4 Hz, 2H, CH_2CH), 2.99 (d, J = 7.0 Hz, 3H, $CHCH_3$), 3.01 (s, 1H, $CHCH_3$), 4.02 (t, J = 5.6 Hz, 1H, CHPh), 5.05 (s, 1H, CHOH), 6.64–6.66 (d, J = 8.8 Hz, 2H, ArH), 7.01–7.03 (d, J = 8.8 Hz, 4H, ArH), 7.32 (d, J = 8.4 Hz, 2H, ArH), 9.57 (s, 1H, PhOH), 10.22 (s, 1H, COOH); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 17.21 $(CH_3)_2CH$), 23.48 (CH_3), 32.45 (CH_2), 38.28 ($C_{2'}$), 45.24 ($ArCH_3$), 53.93 $(CH_3)_2CH$), 57.23 (CH_2N), 61.52 (ArCH), 113.34 (ArC_3), 114.70 (ArC_2), 117.16 (ArC_{19}), 129.59 ($ArC_{18,20}$), 144.79 (ArC_4), 145.19 (ArC_5), 154.28 (ArC_6), 157.51 (ArC_{16}), 165.26 (ArC_1), 174.28 ($C_{1'}$); LC–MS (ESI): 415.5 $(M+1)^+$; Elemental analysis C 66.27, H 7.20, N 3.14; found C 65.10, H 7.15, N 2.13.

(R)-2-(3-(Diisopropylamino)-1-phenylpropyl)-4-methylphenol succinate (7g). mp 210–213°C, IR (KBr, cm^{-1}) υ_{max}; 3436bs (OH), 3050w (Ar═CH), 2925w, 2857w (CH), 1634b (C═O), 1608b, 1537w, 1413w (Ar–C═C), 1314w (CN), 1116s (C–O); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.09 (d, J = 6.2 Hz, 12H, $(CH_3)_2CH$), 2.28 (s, 3H, CH_3Ph), 2.38–2.41 (t, J = 5.6 Hz, 2H, CH_2N), 2.63 (d, J = 6.4 Hz, 2H, CH_2CH), 4.04–4.06 (t, J = 5.6 Hz, 4H, CH_2CH_2), 5.08 (t, J = 5.2 Hz, 1H, CHPh), 6.63–6.67 (m, 4H, ArH), 7.08 (d, J = 8.4, 2H, ArH), 7.43 (d, J = 8.5, 2H, ArH), 9.42 (s, 1H, PhOH), 9.58 (s, 1H, COOH), 10.28 (s, 1H, COOH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.03 $(CH_3)_2CH$), 30.73 ($CH_{2',2'}$), 52.76 ($C_{2'}$), 52.97 ($ArCH_3$), 55.21 $(CH_3)_2CH$), 57.18 (CH_2N), 64.03 (ArCH), 113.88 (ArC_2), 127.62 (ArC_{19}), 136.27 ($ArC_{18,20}$), 142.80 (ArC_3), 144.20 (ArC_5), 150.33 (ArC_6), 151.95 (ArC_{16}), 158.60 (ArC_1), 164.82 ($ArC_{1',4'}$); LC–MS (ESI): 443.5 $(M+1)^+$; Elemental analysis C 67.13, H 8.12, N 3.24; found C 68.12, H 6.25, N 3.30.

(R)-2-(3-(Diisopropylamino)-1-phenylpropyl)-4-methylphenol-4-methylbenzoate (7h). mp 197–199°C, IR (KBr, cm^{-1}) υ_{max}; 3436bs (OH), 3032w (Ar═CH), 2927w, 2907w (CH), 1634m (C═O), 1614b, 1544w (ArC═C), 1389w (CN), 1243w (C–O); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.30 (d, J = 6.6 Hz, 12H, $(CH_3)_2CH$), 2.96 (s, 3H, CH_3Ph), 3.56–3.60 (t, J = 5.8 Hz, 2H, CH_2N), 4.38 (s, 3H, $ArCH_3$), 5.16 (d, J = 6.5 Hz, 2H, CH_2CH), 5.26 (t, J = 5.4 Hz, 1H, CHPh), 6.84 (d, J = 8.5 Hz, 2H, ArH), 7.26 (d, J = 8.4 Hz, 4H), 7.42–7.46 (m, 2H, ArH), 7.89 (m, 2H, ArH), 8.21 (s, 2H, ArH), 9.29 (s, 1H, PhOH), 10.22 (s, 1H, COOH); ^{13} C NMR (75 MHz, $CDCl_3$) δ 18.03 $(CH_3)_2CH$), 53.11 ($ArCH_3$), 53.39 ($ArCH_{3'}$), 56.29 $(CH_3)_2CH$), 57.63 (CH_2N), 64.42 (ArCH), 114.75 (ArC_2), 120.03 ($ArC_{5'}$) 128.05 (ArC_{19}), 136.80 ($ArC_{18,20}$), 141.82 (ArC_3), 143.12 ($ArC_{4'}$), 145.22 (ArC_5), 150.81 (ArC_6), 151.90 (ArC_{16}), 152.38 ($ArC_{2',6'}$), 156.78 (ArC_1), 158.29 ($ArC_{1'}$), 165.25 ($ArC_{1''}$); LC–MS (ESI): 461.6 $(M+1)^+$; Elemental analysis C 67.12, H 7.12, N 3.27; found C 68.10, H 6.24, N 3.32.

(R)-2-(3-(Diisopropylamino)-1-phenylpropyl)-4-methylphenol-3,4,5-trihydroxybenzoate (7i). mp 200–201°C, IR (KBr, cm^{-1}) υ_{max}; 3444b (OH), 3042w (Ar═CH), 2927w, 2860w (CH), 1634 m (C═O), 1602b, 1544w (Ar–C═C), 1393w (CN), 1113w (C–O); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.28–1.31 (d, J = 6.5 Hz, 2H, CH_2CH), 2.30 (s, 3H, CH_3Ph), 2.94 (d, J = 6.4 Hz, 12H, $(CH_3)_2CH$), 3.55–3.60 (t, J = 5.4 Hz, 1H, CHPh), 3.56–3.58 (t, J = 7.2 Hz, 2H, CH_2N), 4.38 (s, 3H, ArOH), 5.29–5.30 (d, J = 6.5 Hz, 2H, CH_2CH), 7.52–7.55 (d, J = 8.8 Hz, 4H), 8.01–7.03 (d, J = 8.8 Hz, 2H), 8.17–8.19 (dd, J = 9.5 Hz, 2H, ArH), 8.46 (s, 2H, ArH), 9.49 (s, 1H, PhOH), 9.89 (s, 1H, COOH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.71 $(CH_3)_2CH$), 41.19 ($ArCH_3$), 45.26 ($ArCH_{3'}$), 53.20 $(CH_3)_2CH$), 57.30 (CH_2N), 61.05 (ArCH), 111.75 (ArC_2), 112.23 ($ArC_{5'}$), 122.05 (ArC_{19}), 124.83 (ArC_3), 125.49 ($ArC_{4'}$), 126.88 (ArC_5), 129.82 (ArC_6), 132.56 ($ArC_{18,20}$), 147.73 (ArC_{16}), 149.71 ($ArC_{2',6'}$), 152.32 (ArC_1), 159.60 ($ArC_{1'}$), 165.42 ($ArC_{1''}$); LC–MS (ESI): 495.6 $(M+1)^+$; Elemental analysis C 67.15, H 6.10, N 3.28; found C 68.04, H 6.12, N 3.30.

Elemental analysis report of (R)-N,N-diisopropyl-3-(2-hydroxy-5-methylphenyl)-3-phenyl propanamine-L-hydro gentartrate (7a). Physical properties: MF $C_{26}H_{37}NO_7$, MW 475.6, white crystalline powder, soluble in hydrochloric acid and methanol, slightly soluble in water and ethanol, practically insoluble in toluene, insoluble in sodium hydroxide. Determinations were carried out using an equipment VARIO EI III version H of elementary analysensysteme under the following conditions of operation. Carrier gas, Helium UAP; carrier gas flow, 150 mL/min; detector, thermal conductivity; temperature of reduction reactor, 500°C; temperature of combustion reactor, 950°C; calibration compound, acetanilide (Merck).

The compounds were analyzed in a SIEMENS D5000, using K_α radiation of Cu (λ = 1.5406 Å), during 53 min with interval of $2° < 2\theta < 55°$. Diffractogram and list with characteristic peaks in 2θ values, interplanar distances, and relative intensities are provided in Table 3 and Figure 5.

Anticancer activity. Cellular viability in the presence of test compounds was determined by MTT microcultured tetrazolium assay following the reported protocol [26]. All the experiments were carried out in triplicates. Cytotoxicity assay against two different human cancer cell lines (A549 human lung adeno carcinoma epithelial cell line and SKNSH human neuroblastoma cell line) was employed in the current study.

Table 3

Elemental analysis report of target compound **7a**.

Sample identification	Analyzed quantity (mg)	% Experimental			Mean	Peak area (mean)	% Theoretical*
		First analysis	Second analysis	Third analysis			
7a	1.4580	N: 3.258	N: 3.253	N: 3.100	N: 3.203	1804.66	N: 2.945
	1.5690	C: 65.48	C: 65.55	C: 65.40	C: 65.47	27,943.66	C: 65.66
	2.3200	H: 7.796	H: 7.668	H: 7.760	H: 7.741	10,925.00	H: 7.842

*Calculated with molecular weight calculator; see 6.31 by Matthew Monroe.

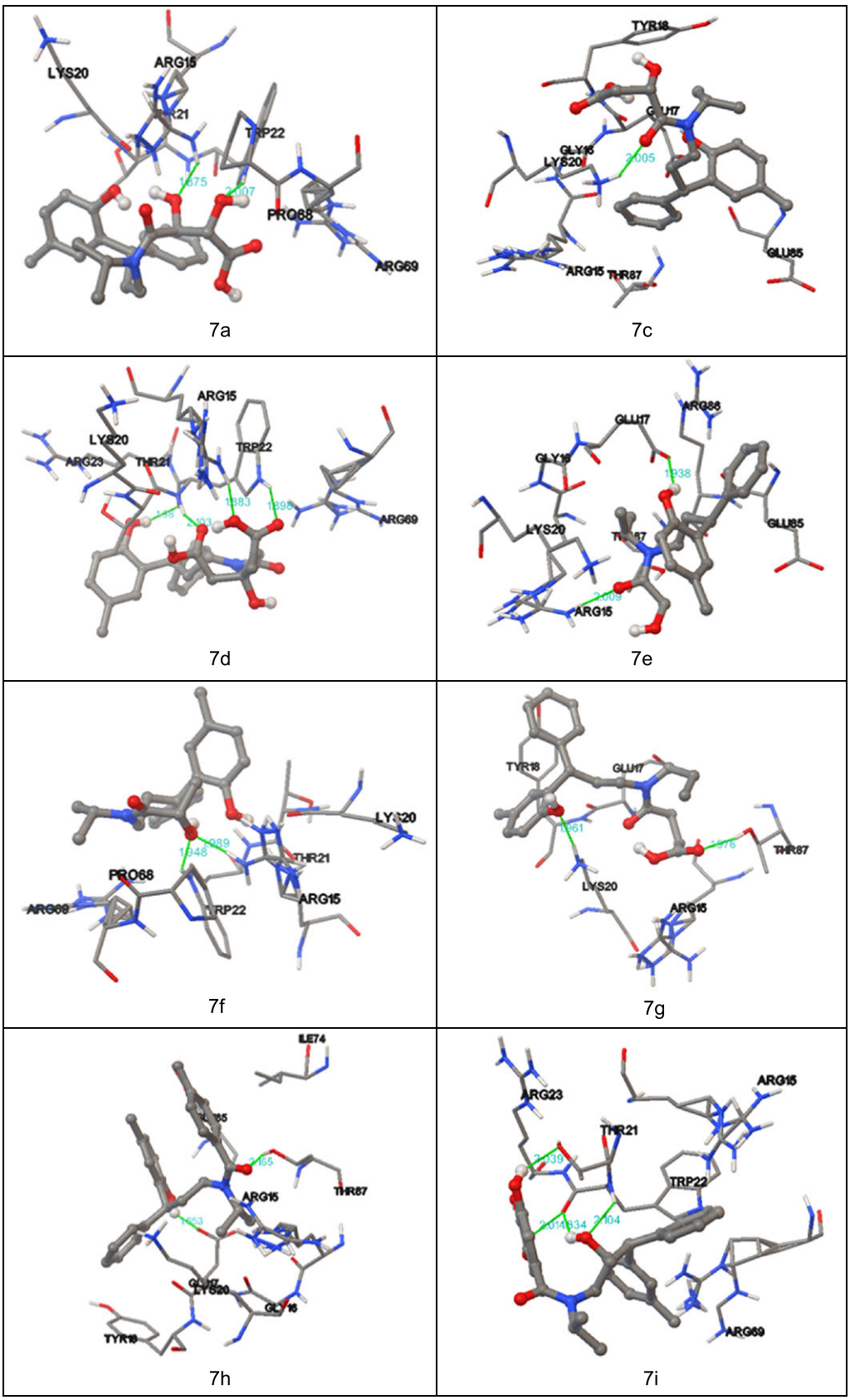
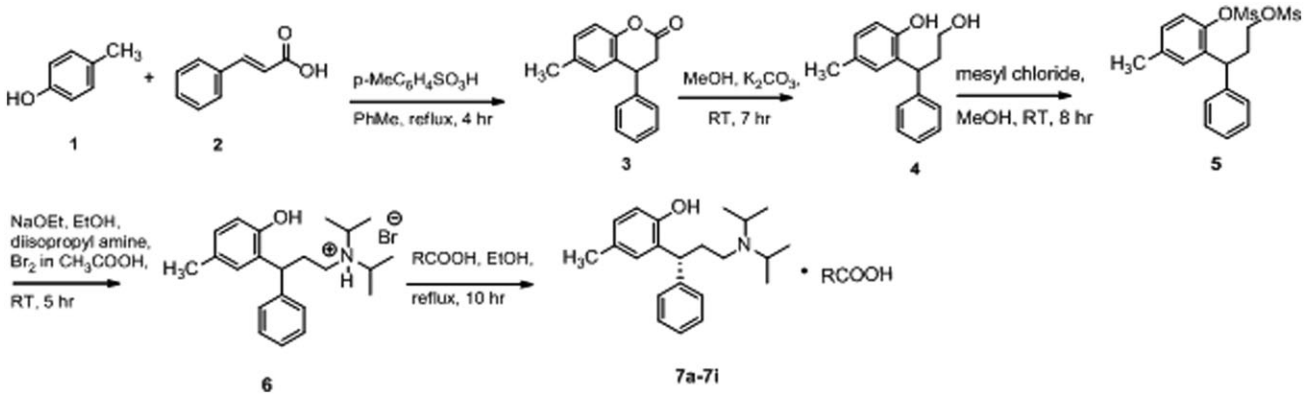


Figure 5. X-ray diffraction of target compound **7a**. [Color figure can be viewed at wileyonlinelibrary.com]

Scheme 1. Synthesis of novel derivatives of tolterodine R-isomers.



Procedure.

- Day 1: One full confluent T-25 flask was trypsinized, and 5 mL of complete media was added to trypsinized cells and centrifuged in a sterile 15 mL falcon tube at 500 rpm in the swinging bucket rotor (−400 *g*) for 5 min. Media was removed, and the cells were resuspended to 1.0 mL with complete media, and cells were counted. The cells were diluted to 75,000 cells per mL incomplete media. One hundred microliter of cells (7500 total cells) were added in each well and incubated overnight in a humid incubator with 5% CO_2 at 37°C so that the cells adhere to the surface. Different concentrations of compounds were prepared by dissolving in DMSO.
- Day 2: Different concentrations of compounds were added to the adherent cells in triplicates (1 μL per each well) and incubated for 48 h with DMSO alone as control.
- Day 4: MTT (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (sigma catalog no. M2128) was dissolved in phosphate-buffered saline at 5 mg/mL and filter sterilized and stored at 4°C. Ten microliter of MTT solution was added to each well and incubated for 2 h at 37°C in the incubator. Then the media was aspirated, and plates were dried, and 100 μL of DMSO (solvent) was added to each well. Plate was covered with tinfoil and agitated on orbital shaker for 15 min.

Molecular docking studies. The ligands were sketched in SYBYL 6.7 and saved it in Mol2 format [27]. All the sketched molecules were converted to energy minimized 3D structures by using Gasteiger–Huckel charges [28] for *in silico* protein–ligand docking using AutoDock tools. Each molecule was docked separately. Initially, the molecule was loaded, torsions were set and saved it in PDBQT format. All the heteroatoms were removed from the 3IVX, PDB (crystal structure of pantothenate synthetase in complex with 2-(2-(benzofuran-2-ylsulfonylcarbamoyl)-5-methoxy-1*H*-indol-1-yl)acetic acid) [29] to make complex receptor free of any ligand before docking [30]. The PDB was also saved in PDBQT format. All calculations for protein–ligand flexible docking were performed using the Lamarckian genetic algorithm method. A grid box with the dimensions of X, 15.137 Å; Y, 17.850 Å; and Z, −3.573 Å, with a default grid spacing of 0.375 Å was used. The best conformation was chosen with the lowest docked energy [31] after the docking search was completed. The interactions of 3IVX protein and ligand conformations, including hydrogen bonds and the bond lengths, were analyzed (Scheme 1).

Acknowledgments. One of the author (V. V. R.) is thankful to our Research Supervisor Dr. Lav Kumar and Dr. Anjali Jha for providing us required facilities and motivation for completion of the research work. We also extend our gratitude towards Department of Chemistry, GIS, GITAM University.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.