Organic Process Research & Development

Communication

pubs.acs.org/OPRD

An Efficient Synthesis of 1-(2-Methoxyphenoxy)-2,3-epoxypropane: Key Intermediate of β-Adrenoblockers[§]

Lokeswara Rao Madivada,[†] Raghupathi Reddy Anumala,[†] Goverdhan Gilla,[†] Mukkanti Kagga,[‡] and Rakeshwar Bandichhor*,[†]

[†]Innovation Plaza, IPD, R&D, Dr. Reddy's Laboratories Ltd., Survey Nos. 42, 45,46, and 54, Bachupally, Qutubullapur - 500073, Andhra Pradesh, India

[‡]Institute of Science and Technology, Center for Environmental Science, JNT University, Kukatpally, Hyderabad - 500 072, Andhra Pradesh, India

Supporting Information

ABSTRACT: An efficient process for the preparation of 1-(2-methoxyphenoxy)-2,3-epoxypropane, a key intermediate for the synthesis of ranolazine is described.

INTRODUCTION

β-Adrenoblockers block norepinephrine and epinephrine from binding to the β-receptors present in the nerves.[1] These β-adrenoblockers are used in the management of heart rhythm disorders, high blood pressure, heart failure, angina, tremor, pheochromocytoma, and migraines. 1-(2-Methoxyphenoxy)-2,3-epoxypropane **4** is widely used as a key intermediate for the synthesis of β-adrenoblocker moprolol, anxiolytic mephenoxalone, antianginal ranolazine,[2−5] and skeletal muscle relaxant methocarbamol [6] (see Figure 1).

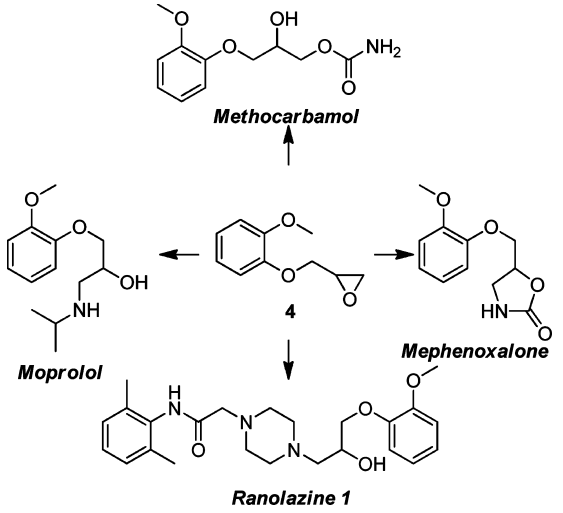
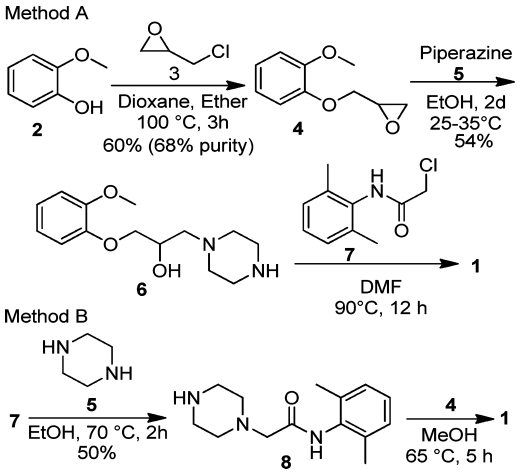


Figure 1. Structure of β-blockers and methacarbamol.

In general, synthetic approaches to the active pharmaceutical ingredients (APIs) ought to be safer, simple, and cost-effective. From the process development standpoint, in-depth optimization efforts tend to offer several advantages (e.g., robustness, use of nontoxic solvents, less energy intensive, etc.) in the manufacturing of the drug substances.

In order to exemplify an application of epoxypropane (**4**) in the synthesis of ranolazine (**1**), we embarked our studies on the synthesis of epoxypropane **4**. The first reported synthetic route[5] for **1** as shown in Scheme 1 (Method A) where the starting material 2-methoxyphenol (**2**) was allowed to react with epichlorohydrin (**3**), affording aryloxy epoxypropane (**4**). Thereafter, reaction of **4** with piperazine (**5**) gave rise to aryloxypiperazine intermediate (**6**). Subsequently, condensation of 2-chloro-*N*-(2,6-dimethylphenyl)acetamide (**7**) with **6** afforded ranolazine (**1**).

Scheme 1. Precedented synthetic approach (methods A and B)



Additionally, there is a different approach, Scheme 1 (Method B), that involves the reaction of 2-chloro-*N*-(2,6-dimethylphenyl)acetamide (**7**) with piperazine to obtain aniline piperazine intermediate **8**, which upon condensation with **4** afforded **1**.

Despite the proven potential of the procedures disclosed in Scheme 1, there are certain disadvantages in the transformation of **2** to **4**: (a) moderate yields (~60%) and purity (~60%), (b) formation of significant amount of byproducts, (c) use of carcinogenic and expensive solvent, e.g., dioxane (Class II) and

Received: March 6, 2012

   dx.doi.org/10.1021/op300056k | *Org. Process Res. Dev.* XXXX, XXX, XXX−XXX

ethers (Class III); (d) purification of crude material by using fractional distillation at higher temperature.[7−9] Moreover, there is no literature precedence that details the impurity profiles and their root cause.

To study the impurity profile of **4**, reactions were conducted by following the reported procedures.[5,6] There were three major impurities (other than starting materials) observed as shown in Figure 2, and the experimental results are depicted in

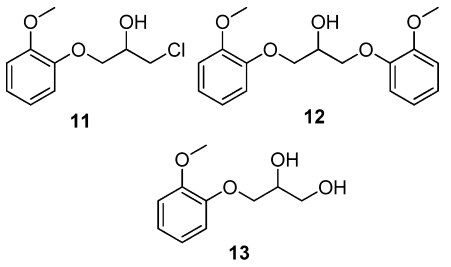


Figure 2. Structures of impurities identified in epoxypropane **4**.

Table 1. Experimental results obtained by repeating the reported procedures for the synthesis of 4

			purity by HPLC (%)			
entry	reference no.	quantity of **2** (g)	**4**	**11**	**12**	**13**
1	7	25	50.48	0.79	43.37	2.74
2	8	100	58.36	1.31	23.18	5.3

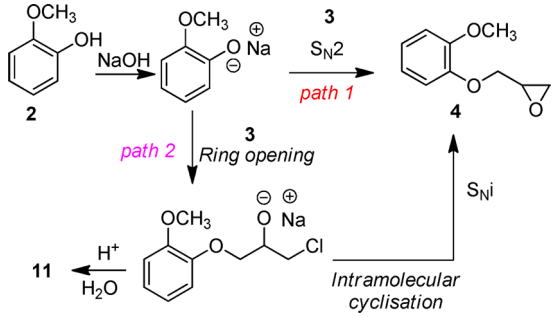
Table 1. On the basis of LC/MS analysis these impurities were identified as chloro compound **11**, dimer compound **12**, and dihydroxy compound **13**. These impurities were synthesized and confirmed by spectral data and HPLC analysis.[10]

RESULTS AND DISCUSSION

Synthesis of **4** involves *O*-alkylation of 2-methoxyphenol **2** with epichlorohydrin **3** in the presence of base. Generally, the *O*-alkylation reaction with epichlorohydrin proceeds via two mechanisms: (1) the substitution of chlorine with phenoxide and (2) the epoxide ring-opening with phenoxide followed by intramolecular epoxidation to afford the epoxypropane **4** as shown in Scheme 2.

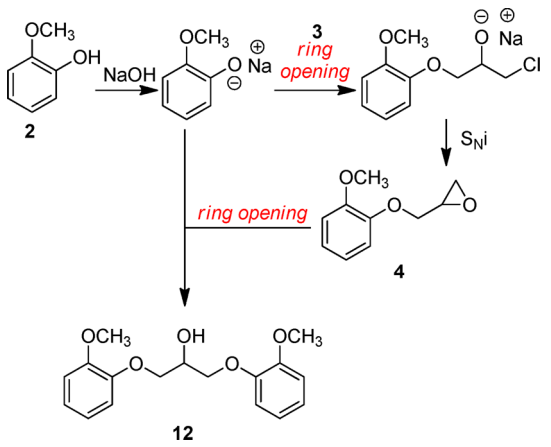
Route Cause for the Formation of Impurities. Formation of chloro compound **11** during the transformation of **2** to **4** indicates that the reaction proceeds through chloro compound **11** as an in situ intermediate as shown in path 2

Scheme 2. Fate of epichlorohydrin 3 in the presence of phenol 2 and base



(Scheme 2). Preparation of **4** involves the usage of sodium hydroxide as base that triggers the formation of **13** via **11**. Another major impurity that forms during the synthesis of **4** is dimer impurity **12** due to the reaction of residual quantity of 2-methoxyphenoxide with **4** in the presence of base as shown in Scheme 3.

Scheme 3. Formation of dimer 12



Removal of the impurities was found to be expensive due to the loss of the desired material and energy consumption that makes the process less efficient with the commercial scale. Reported processes[5,6] involve product distillation to remove the impurities which leads to the low yields. In lieu of this, controlling the formation of the impurities is considered to be a strategy superior to the purification of the impurities. A design of experiment (DoE) tool has also been employed to obtain the best possible conditions for achieving the improved quality. However, the DoE did not offer significant improvement in the quality; the attempted studies are in the Supporting Information.

Optimization of Solvent during the Synthesis of 4. As a part of optimization efforts, we have studied key parameters such as solvent, base mol ratio, and epichlorohydrin **3** mol ratio. Solvent as a reaction medium plays a major role in the formation of impurities. Different solvents were screened to understand the formation of dimer impurity **12**. It was found that the formation of dimer **12** is greater in organic solvent as compared with that in water (entry 3) as shown in Table 2. Since the product is insoluble in water, usage of water as a solvent is more advantageous than the organic solvents for isolation. Usage of organic solvent leads to an increase in the cycle time and makes the process expensive with impeded efficiency as it involves distillation to isolate the highly soluble product.

Optimization of Epichlorohydrin 3 Mol Ratio during the Synthesis of 4. Preparation of **4** involves the reaction of 2-methoxyphenol **2** with epichlorohydrin **3**. As a part of optimization strategy, the reactions were conducted by using 1.0, 2.0, 3.0, and 5.0 mol ratio of epichlorohydrin, and the dimer impurity **12** was found to be increasing with the fewer equiv of **3**. This indicates that 2-methoxyphenoxide which remained in the reaction mass would have reacted with **4**, leading to an increase in dimer impurity **12**. Improved results were observed with 3.0 and 5.0 mol ratio of epichlorohydrin **3** as shown in Table 3 (entries 3 and 4).

dx.doi.org/10.1021/op300056k | Org. Process Res. Dev. XXXX, XXX, XXX−XXX

Table 2. Optimization solvent for the synthesis of 4

			analysis by HPLC (%) of crude material						
entry	solvent	conversion of **2**	**4**	**11**	**12**	**13**	temperature (°C)	epichlorohydrin (equiv)	NaOH (equiv)
1	water and 1,4-dioxane	96	72.6	24.0	–	–	25−30	3	1.5
2	toluene and water	94	58.4	1.3	23.2	0.1	25−30	3	1.5
3	**water**	**96**	**84.0**	**7.8**	**3.7**	**1.5**	25−30	3	1.5
4	toluene	76	62.0	3.8	26.5	1.2	25−30	3	1.5
5	dimethylformamide	96	81.5	0.3	13.7	0.04	25−30	3	1.5
6	dichloromethane	93	70.4	4.1	12.4	3.7	25−30	3	1.5
7	neat	63	54.8	0.0	19.6	0.3	25−30	3	1.5

Table 3. Optimization of epichlorohydrin 3 mol ratio for the synthesis of 4

			analysis by HPLC (%) of crude material					
entry	epichlorohydrin **3** (mol ratio)	conversion of **2** (%)	**4**	**11**	**12**	**13**	temperature (°C)	NaOH (equiv)
1	1.0	84	45.3	0.1	34.7	0.4	25−30	1.5
2	2.0	94	82.5	1.3	10.0	1.8	25−30	1.5
3	3.0	99	85.5	2.5	5.3	1.9	25−30	1.5
4	5.0	99	85.4	9.6	2.2	1.1	25−30	1.5

Table 4. Optimization of sodium hydroxide mol ratio for the synthesis of 4

			purity by HPLC (%)					
entry	sodium hydroxide (mol ratio)	conversion of **2** (%)	**4**	**11**	**12**	**13**	temperature (°C)	epichlorohydrin (equiv)
1	0.5	99	39.5	55.8	1.2	1.8	25−30	3
2	1.0	93	76.2	18.8	2.3	0.9	25−30	3
3	1.25	99	84.4	6.0	4.3	1.6	25−30	3
4	**1.50**	**99**	**85.5**	**2.5**	**5.3**	**1.9**	25−30	3
5	2.0	94	81.3	0.8	9.1	2.6	25−30	3
6	3.0	94	67.9	0.3	20.0	2.9	25−30	3

Table 5. Optimization of sodium hydroxide mode of addition for the synthesis of 4

			purity by HPLC (%)						
entry	sodium hydroxide mode of addition	conversion of **2** (%)	**4**	**11**	**12**	**13**	temperature (°C)	epichlorohydrin (equiv)	NaOH (equiv)
1	**two lots (0.5 mol ratio in first lot and 1.0 mol ratio in second lot)**	**99**	**95.9**	**0.5**	**0.6**	**0.2**	25−30	3	1.5
2	single lot (1.5 mol ratio)	99	85.5	2.5	5.3	1.9	25−30	3	1.5

Table 6. Optimization of reaction mass maintenance temperature for the synthesis of 4

			purity by HPLC (%)					
entry	maintenance temperature (°C)	conversion of **2** (%)	**4**	**11**	**12**	**13**	NaOH (equiv)	epichlorohydrin (equiv)
1	10−15	87.1	87.87	2.1	7.9	0.1	1.5	3
2	25−35	99.2	95.87	0.5	0.6	0.2	1.5	3
3	50−55	97.5	91.17	4.02	1.9	0.4	1.5	3

Optimization of Sodium Hydroxide Mol Ratio during the Synthesis of 4. The sodium hydroxide mol ratio plays a pivotal role in the synthesis of **4**. Reactions were conducted by using different mol ratios of sodium hydroxide (0.5, 1.0, 1.25, 1.5, 2.0 and 3.0). Synthesis of **4** involves two steps. The first step is opening of the epoxide ring with phenoxide and the second step is the intramolecular cyclization of chlorine with alkoxide as shown in Scheme 3. Experimental results obtained by using different mol ratios of sodium hydroxide as shown in Table 4, indicate that (1) by decreasing the sodium hydroxide mol ratio, a higher level of chloro impurity **11** is observed, (2) by increasing the sodium hydroxide mol ratio, a higher level of dimer impurity **12** is found. Optimum results were obtained with 1.5 mol ratio of sodium hydroxide (entry 4) along with the ∼5% of **12** and ∼2% of **11**.

To further minimize the formation of impurities, sodium hydroxide was added in two lots. The first lot contained 0.5 mol ratio that was used for the reaction of 2-methoxyphenol **2** and epichlorohydrin **3** to get the chloro compound **11**. After completion of first half of the reaction, the aqueous layer containing the unreacted 2-methoxyphenoxide was removed from the reaction mixture by layer separation to avoid the formation of dimer impurity **12**. To the chloro compound **11** was added 1.0 mol ratio of sodium hydroxide for the cyclization reaction to obtain epoxypropane **4**. Formation of dimer impurity was controlled significantly (entry 1 of Table 5) by

dx.doi.org/10.1021/op300056k | Org. Process Res. Dev. XXXX, XXX, XXX−XXX

Table 7. Optimization of epichlorohydrin addition time for the synthesis of 4

entry	addition time of 3 equiv epichlorohydrin	conversion of 2 (%)	purity by HPLC (%) 4	11	12	13	NaOH (equiv)	temperature (°C)
1	15−20 min	95.7	93.6	3.7	1.5	0.3	1.5	25−30
2	45−60 min	96.5	98.2	0.2	0.3	0.3	1.5	25−30
3	2−3 h	94.9	97.7	0.4	0.3	0.1	1.5	25−30

removing the aqueous layer before proceeding to the second reaction.

After completion of the cyclization reaction, the upper layer containing compound **4** was separated and washed with 10% aq sodium hydroxide solution to further improve the quality of **4**.

Optimization of Reaction Maintenance Temperature during the Synthesis of 4. Temperature always plays significant role in the reaction progress; therefore, as a part of our optimization strategy the synthesis of **4** was performed at different temperatures. Experimental results revealed that compound **4** was obtained with best yield and purity at 25−30 °C, compared with lower and higher temperatures such as 10−15 °C and 50−55 °C, respectively, as shown in Table 6.

Synthesis of **4** involves addition of epichlorohydrin to the reaction mixture containing 2-methoxy phenol **2** and sodium hydroxide in water. Addition and stirring impact of epichlorohydrin **3** in the synthesis of **4** were studied. No major changes were observed by adding epichlorohydrin to the reaction mixture for longer time (45−60 min or 2−3 h) and less purity was obtained by adding epichlorohydrin **3** in 15−20 min as shown in Table 7. Quality of epichlorohydrin was assessed by using GC.[11] The purities were measured against peak area ratios.

Excess epichlorohydrin (as per reaction mechanism 1 equiv is sufficient, but 3 equiv were used in the process) used in the process was recovered (93−95%) from the reaction mass by distillation at below 90 °C under reduced pressure (650−700 mmHg). Since epichlorohydrin along with the product was separated from the aqueous layer, it was thus observed that the trace amount of **3** (instead of a significant amount) was found to be present in aqueous waste (LoD: 0.5%).[11] The quality of the recovered epichlorohydrin was found to be unchanged and was reused for the synthesis of **4**.[11] Experiments were conducted by including all of the optimized parameters, and consistent yield and quality were obtained as shown in Table 8.

Table 8. Experimental results by incorporating all optimal conditions for the synthesis of 4

entry	quantity of 2 (g)	conversion of 2 (%)	purity by HPLC (%) 4	2	3	11	12	13
1	100	95	97.2	0.2	0.2	0.1	0.4	0.3
2	100	94	98.3	0.2	0.3	0.1	0.3	0.4
3	100	94	98.2	0.2	0.1	0.1	0.3	0.3

CONCLUSION

We have developed a high-yielding and efficient synthesis of the key starting material **4** of ranolazine, **1**, that has the following advantages over the reported processes; (1) avoids the usage of expensive organic solvent, e.g., dioxane and ethers by incorporating water, (2) avoids energy-intensive high-vacuum distillation, (3) includes a simple workup procedure, (4) is amenable to the control of all the possible impurities at below 0.5% and (5) unreacted epichlorohydrin **3** that was recovered and reused.

EXPERIMENTAL SECTION

Solvents and reagents were obtained from commercial sources and used without further purification. The ^{1}H and ^{13}C spectra were measured in DMSO-d_6 using 200 or 400 MHz on a Varian Gemini and Varian Mercury plus 2000 FT NMR spectrometer; the chemical shifts were reported in δ (ppm). IR spectra were recorded in the solid state as a KBr dispersion using a Perkin-Elmer 1650 FT IR spectrometer. The mass spectrum (70 eV) was recorded on an HP 5989 A LC/MS spectrometer. The melting points were determined by using the capillary method on Polmon (model MP-96) melting point apparatus. The solvents and reagents were used without further purification.

Preparation of 1-(2-Methoxyphenoxy)-2,3-epoxypropane 4. To a stirring solution of 2-methoxy phenol **2** (10 kg, 80.55 mol) and water (40 L) at about 30 °C was added sodium hydroxide (1.61 kg, 40.25 mol) and water (10 L). After stirring for 30−45 min, epichlorohydrin **3** (22.35 kg, 241.62 mol) was added and stirred for 10−12 h at 25−35 °C. Layers were separated, and water (40 L) was added to the organic layer (bottom layer) containing product. Sodium hydroxide solution (3.22 kg, 80.5 mol) and water (10 L) were added at 27 °C and stirred for 5−6 h at 27 °C. The bottom product layer was separated and washed with sodium hydroxide solution (3.0 kg 75 mol) and water (30 L). Excess epichlorohydrin (**3**) was recovered by distillation of the product layer at below 90 °C under vacuum (650−700 mmHg) to give 13.65 kg (94%) of title compound with 98.3% purity by HPLC, 0.2% of 2-methoxy phenol **2**, 0.1% of epichlorohydrin **3**, 0.1% of chlorohydrin **11**, 0.3% of dimer **12** and 0.3% of dihydroxy **13**. ^{1}H NMR (400 MHz, $CDCl_3$, δ) 6.8−7.0 (m, 4H), 4.3 (dd, J = 5.6 Hz, 5.4 Hz, 1H), 3.8 (dd, J = 5.6 Hz, 5.3 Hz, 1H), 3.7 (s, 3H), 3.2−3.4 (m, 1H), 2.8 (dd, J = 5.6 Hz, 5.4 Hz, 1H), 2.7 (dd, J = 5.6 Hz, 5.3 Hz, 1H); IR (KBr, cm^{-1}) 2935 (C−H, aliphatic), 1594 and 1509 (C═C, aromatic), 1258 and 1231 (C−O−C, aralkyl ether), 1125 and 1025 (C−O−C, epoxide); MS (m/z) 181 (M^+ + H).

Preparation of 1-Chloro-3-(2-methoxyphenoxy)-propan-2-ol (11). Mixture of epoxypropane **4** (100 g, 0.555 mol) and conc HCl (400 mL, 3.835 mol) was stirred at 25−35 °C for 8−10 h. Toluene (200 mL) and water (200 mL) were added to the reaction mixture and stirred for 10−15 min. Layers were separated, and aqueous layer was extracted with toluene (100 mL). Total organic layer was distilled completely at below 70 °C under vacuum (650−700 mmHg). The obtained crude was distilled further under vacuum (650−700 mmHg) at vapor temperature 145−150 °C (bath temperature 215−235 °C) to afford the 98.4 g (89%) of titled compound. ^{1}H NMR (400 MHz, $CDCl_3$, δ) 6.8−7.2 (m, 4H), 5.5 (s, 1H), 4.1−4.2 (m, 1H), 4.05 (dd, J = 4.8, 4.8 Hz, 2H), 3.76 (s, 3H), 3.7 (dd, J = 4.8, 4.8 Hz, 2H); IR (KBr, cm^{-1}) 3459 (O−H), 2938 (C−H, aliphatic), 1594 and 1508 (C═C, aromatic), 1250 and 1225 (C−O−C, epoxide); MS (m/z) 217.2 (M^+ + H).

dx.doi.org/10.1021/op300056k | Org. Process Res. Dev. XXXX, XXX, XXX−XXX

Preparation of 1,3-Bis(2-methoxyphenoxy)propan-2-ol 12. To a stirring solution of 2-methoxy phenol **2** (50 g, 0.402 mol) and 1, 4-dioxane (130 mL) at 25−35 °C were added sodium hydroxide (24.1 g, 0.604 mol), water (40 mL), and epichlorohydrin **3** (18.6 g, 0.50 mol). After the reaction mass stirred at 100 °C for 5−6 h, toluene (100 mL) and water (100 mL) were added at 25−35 °C. Layers were separated; aqueous layer was extracted with toluene (100 mL). Total organic layer was distilled completely at below 75 °C under vacuum (650−700 mmHg) followed by distilling the product at vapor temperature 210 °C (bath temperature 310−311 °C) under vacuum (650−700 mmHg) to give the 60 g (49%) of the title compound. ^{1}H NMR (400 MHz, $CDCl_3$, δ) 6.8−7.0 (m, 8H), 5.3 (d, J = 5.2 Hz, 1H), 4.15−4.2 (m, 1H), 4.05 (dd, J = 5.6 Hz, 4H), 3.74 (s, 6H); IR (KBr, cm^{-1}) 2922 (C−H, aliphatic), 1593 and 1508 (C═C, aromatic), 1252 and 1223 (C−O−C, aralkyl ether); MS (m/z) 305 (M^+ + H).

Preparation of 3-(2-Methoxyphenoxy)propane-1,2-diol 13. Fine powder of sodium hydroxide (0.03 g, 0.001 mol) and glycidol (12.1 g, 0.163 mol) were charged into 2-methoxy phenol **2** (20 g, 0.161 mol) at 90−95 °C, and the resultant reaction mixture stirred for 10−12 h. Acetic acid (1.0 mL) was added to the reaction mixture at 0−5 °C and stirred for 10−15 min. Dichloromethane (20 mL) and water (10 mL) were added to the reaction mixture and stirred for 30 min. Layers were separated, aqueous layer was extracted with dichloromethane (20 mL). Total organic layer was distilled at below 40 °C under vacuum. To the resultant crude was added *n*-hexane (50 mL) at 27 °C and stirred for 60 min. Precipitated solids were collected by filtration and washed with *n*-hexane (20 mL). The obtained compound was purified in isopropyl alcohol to afford 13.4 g (56%) of the titled compound. ^{1}H NMR (400 MHz, $CDCl_3$, δ) 6.8−7.2 (m, 4H), 4.9 (d, J = 5.2 Hz, 1H), 4.6 (d, J = 5.6 Hz, 1H), 3.95 (m, 1H), 3.75 (s, 3H), 3.45 (dd, J = 5.2 Hz, 2H); IR (KBr, cm^{-1}) 3274 (O−H), 2928 (C−H, aliphatic), 1595 and 1508 (C═C, aromatic), 1257 and 1227 (C−O−C, aralkyl ether); MS (m/z) 220.9 (M^+ + Na).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Telephone: +91 4044346000. Fax: +91 4044346285. E-mail: rakeshwarb@drreddys.com.

Notes

[§]DRL-IPD Communication number: IPDO-IPM-00301

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the management of Dr. Reddy's Laboratories Ltd. for supporting this work. Co-operation from the colleagues of API-R&D, IPDO, Dr. Reddy's Laboratories Ltd. is highly appreciated.

■ REFERENCES

(1) (a) Frishman, W. H.; Cheng-Lai, A.; Nawarskas, J. Current Cardiovascular Drugs. *Curr. Sci. Group* **2005**, 152. (b) Arcangelo, V. P.; Peterson, A. M.Peterson, A.ds *Pharmacotherapeutics for Advanced Practice: A Practical Approach*; Lippincott Williams & Wilkins: Philadelphia, PA, 2006; p 205.

(2) Allely, M. C.; Alps, B. J. *Br. J. Pharmacol.* **1988**, *93*, 375−382.

(3) Hale, S. L.; Kloner, R. A. *J. Cardiovasc. Pharmacol. Ther.* **2006**, *11*, 249−255.

(4) Fraser, H.; Belardinelli, L.; Wang, L.; Light, P. E.; McVeigh, J. J.; Clanachan, A. S. *J. Mol. Cell. Cardiol.* **2006**, *41*, 1031−1038.

(5) Jerling, M. *Clin. Pharmacokinet.* **2006**, *45*, 469−491.

(6) Bredikhin, A. A.; Strunskaya, I. E.; Zakharychev, V. D.; Krivolapov, B. D.; Litvinov, A. I.; Bredikhina, A. Z. *Tetrahedron: Asymmetry* **2005**, *16*, 3361−3366.

(7) (a) Kluge, A. F.; Clark, R. D.; Strosberg, A. M.; Pascal, J. G.; Whiting, R. US Patent 4,567,264, 1986.

(8) (a) Reddy, M. S.; Eswaraiah, S.; Satyanarayana, R. Indian Pat. Appl. 2942/CHE/2007, 2007. (b) Rahul, S.; Venkateswaran, S. C.; Lalit, W. WO/2008/047,388, A2, 2008.

(9) Kopka, K.; Wagner, S.; Riemann, B.; Law, M. P.; Puke, C.; Luthra, S. K.; Pike, V. W.; Wichter, T.; Schmitz, W.; Schober, O.; Schafers, M. *Bioorg. Med. Chem.* **2003**, *11*, 3513−3527.

(10) HPLC method: symmetry shield RP-18, 250 mm × 4.6 mm, 5 μm; flow: 1 mL/min; eluent A: water, pH adjusted to 5.0 with dil H_3PO_4; B: acetonitrile and water in the ratio of 800:200 (v/v); gradient: 0 min: 70% A, 30% B; 5 min: 70% A, 30% B; 35 min: 30% A, 70% B; 55 min: 30% A, 70% B; 57 min: 70% A, 30% B; 65 min: 70% A, 30% B, UV detection at 223 nm.

(11) GC method: column: DB-624; length: 30 m; ID: 0.32 mm; film thickness: 1.8 m; detector: 280 °C (FID); injector: 270 °C; split ratio: 1.5; flow: 2 mL/min; load: 0.2 L; diluent: dichloromethane; column oven temperature: initially held at 50 °C for 0 min, then increased to 250 °C at a rate of 15 °C/min and held at 250 °C for 20 min. Limit of detection (LoD) epichlorohydrin in wastewater is 0.5%.