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Selective biocatalytic aminolysis of (±)-epichlorohydrin: Synthesis and ICAM-1 inhibitory activity of (*S*)-(+)-3-arylamino-1-chloropropan-2-ols

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ABSTRACT

Regio- and enantioselective synthesis of (*S*)-(+)-3-arylamino-1-chloropropan-2-ols has been achieved by the epoxide ring opening of (±)-epichlorohydrin with different aromatic amines in the presence of *Candida rugosa* lipase. Activities of seven model (*S*)-(+)-3-arylamino-1-chloropropan-2-ols, out of 10 compounds synthesized, have been evaluated for the inhibition of tumor necrosis factor-α TNF-α) induced expression of intercellular adhesion molecule-1 (ICAM-1), which is one of the factors responsible for the modulation of inflammation in biological systems; (*S*)-(+)-1-chloro-3-(2′-chlorophenylamino)-propan-2-ol has been found to exhibit highest activity, that is, 86% inhibition of TNF-α induced expression of ICAM-1 at a concentration of 40 μg/ml.

1. Introduction

Inflammation is caused by soluble antigen, live organisms and chemical or mechanical stress upon tissue, which serves to destroy and/or dilute the injurious materials, and remove the injured tissues. It is characterized pathologically by an increased supply of blood to the affected area, increased capillary permeability caused by retraction of the endothelial cells and infiltration of phagocytic, monocytic and polymorphonuclear cells into the site of tissue insult. The accumulation and subsequent activation of leukocytes is one of the central events in the pathogenesis of all forms of inflammation. The migration of the leukocytes to the site of inflammation is regulated in part by the expression of cell adhesion molecules, such as ICAM-1 and E-selectin.[1] These cell-adhesion molecules are induced on endothelial cells by various pro-inflammatory cytokines like IL-1α, and TNF-α and also by bacterial LPS.[2] The increased expression of cell adhesion molecules on the endothelial cells alters the adhesive property of the vasculature leading to indiscriminate infiltration of the leukocytes across the blood vessels and hence causes inflammation.[3] A critically regulated expression of these adhesion molecules is therefore highly essential for maintaining the body fluidity. A promising therapeutic approach for various inflammatory responses is to inhibit the cytokine induced expression of cell adhesion molecules.[4] Although, various natural products, synthetic drugs, antibodies and peptides have been demonstrated to inhibit the expression of these molecules, they have their own limitations for usage because of side effects.[5]

β-Amino alcohols are versatile intermediates in the synthesis of a vast range of biologically active natural and synthetic products,[6] unnatural amino acids,[7] and chiral auxiliaries for asymmetric synthesis.[8] β-Amino alcohols, like 3-arylamino-1-chloropropan-2-ols have been found as useful intermediates for various drugs. Some of the β-amino alcohols are important antihypertensive drugs too. However compounds of this class have never been tested for inhibition of cell adhesion molecules implicated in inflammation.[9,10]

The nucleophilic opening of epoxide ring in epichlorohydrin with amines constitutes a well recognized route for the synthesis of β-amino alcohols.[11] The classical approach,[12] involving heating of epoxides with amines, works less well with poorly nucleophilic amines. Moreover, the lack of appreciable regioselectivity and the need for an excess of amines in the classical methods of β-amino alcohol synthesis have led to the necessity for activation of the epoxides so as to increase their susceptibility to nucleophilic attack by amines.[13–15] However, many of these methods often involve the use of expensive and stoichiometric amounts of reagents, where

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poor regioselectivity especially with metal amides derived from primary amines, extended reaction times and undesirable side reactions such as rearrangement of the oxiranes to allyl alcohols under basic conditions[16] or polymerization in strongly acidic conditions often result in low yields of the desired products.

Chirality is an important element of biological systems because most of the biomolecules are chiral and therefore they recognize the symmetry present in the foreign molecules. It is therefore important to develop enantioselective synthetic methodologies for the synthesis of drugs, drug like molecules or their precursors and intermediates. In view of the biological importance of β-amino alcohols and that the chirality plays an important role in biological systems, development of simple, efficient and environmentally benign processes for the synthesis of enantiomerically pure β-amino alcohols is highly required. In continuation of our work on lipase catalysis in organic synthesis,[17–19] we report herein efficient synthesis of (*S*)-(+)-3-arylamino-1-chloropropan-2-ols by highly regio- and enantioselective ring opening of (±)-epichlorohydrin in the presence of *Candida rugosa* lipase (CRL) in diisopropyl ether. Lipase-catalyzed reactions offer an attractive route for industrial exploitation due to their ability to work in both aqueous & organic solvents, low cost and high stability. Most of the chiral aminoalcohols synthesized have been examined for their ICAM-1 expression inhibitory activity.

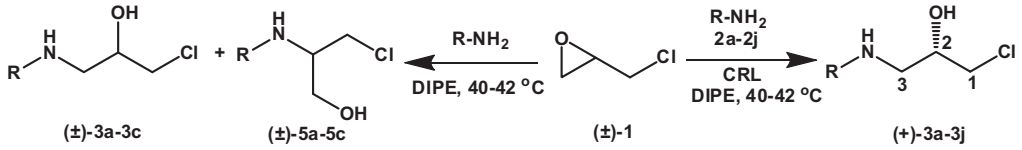
2. Results and discussion

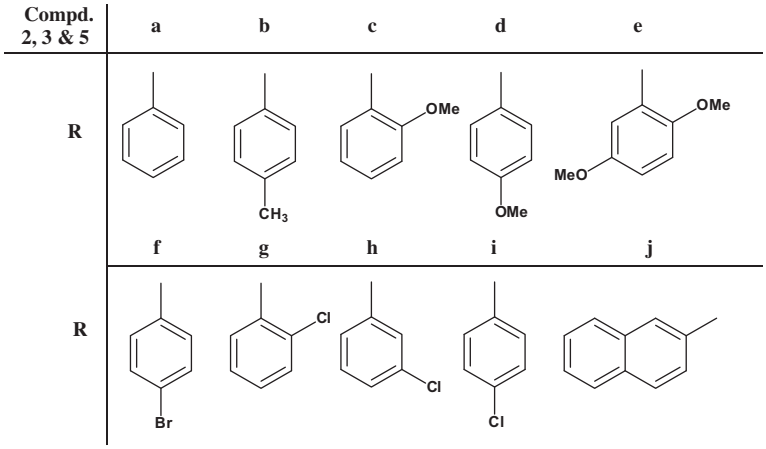
2.1. Chemistry

Four different lipases, that is, Novozyme 435 (*Candida antarctica* lipase immobilized on polyacrylate), Amano *PS* (*Pseudomonas* species lipase), Porcine pancreatic lipase (PPL) and *C. rugosa* lipase (CRL) were screened for regio- and enantioselective epoxide ring opening of (±)-epichlorohydrin with different aromatic amines **2a**–**2j** in different organic solvents. It was observed that Novozyme 435, Amano PS and PPL do not catalyze the reaction or if there is any reaction, it was non-selective leading to the formation of two products due to epoxide ring opening in epichlorohydrin from either of the two sides. *C. rugosa* lipase (CRL) in diisopropyl ether catalyzed epoxide ring opening of (±)-epichlorohydrin with appreciable rate and almost exclusive selectivity leading to the formation of only one product on TLC.

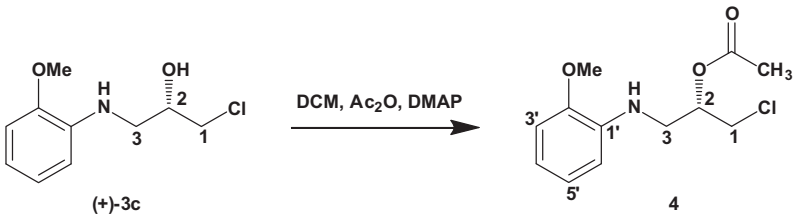
In a typical reaction, equimolar mixture of racemic epichlorohydrin **1** and the aromatic amine **2a**–**2j** was incubated with *Candida rugosa* lipase in diisopropyl ether at 40–42 °C in an incubator shaker and the reaction was monitored by analytical TLC. After 50% consumption of the starting amine in 8–12 h, reaction became too slow to complete even in one week of continued incubation. Thus, the reaction was stopped by filtering off the enzyme after about 45–50% conversion of the starting amine to a single product in 8–12 h and solvent removed under reduced pressure to obtain the crude product, which was passed through a silica gel column to get the pure products **3a**–**3j** in 72–90% yields (based on the amount of the particular enantiomer in the racemic epichlorohydrin) on elution with 10–15% ethyl acetate in petroleum ether (Scheme 1). The unreacted starting epichlorohydrin was not recovered from the column.

The products of the CRL-catalyzed reaction of (±)-epichlorohydrin **1** with aromatic amines **2a**–**2j** were unambiguously identified as 3-arylamino-1-chloropropan-2-ols **3a**–**3j** on the basis of their spectral (IR, ^{1}H NMR, ^{13}C NMR and HRMS) analysis and comparison of the spectral data with that of the known compounds **3a**–**3d** and **3f**–**3j** with those reported in the literature.[20–27] It is worth mentioning here that the compounds reported in the literature are racemic mixtures. The secondary nature of hydroxyl group in aminoalcohols **3a**–**3j** was further confirmed by acetylation of one of the compounds, 1-chloro-3-(2′-methoxyphenylamino)-propan-2-ol (**3c**) and comparison of the chemical shift value of the C-2H in the ^{1}H NMR spectrum of alcohol **3c** with the chemical shift value of the C-2H in the ^{1}H NMR spectrum of the resultant acetate **4** (Scheme 2). In the ^{1}H NMR spectrum of **3c**, the C-2H resonated at δ 3.88, however the same proton in the corresponding acetate **4** resonated at δ 5.13. The chemical shift values of other protons in the ^{1}H NMR spectra of the alcohol **3c** and the acetate **4** remained almost the same. The shift of δ 1.25 ppm in the chemical shift value of the C-2H of acetate with respect to the alcohol indicates that the hydroxyl group in **3c** is at the C-2 and thus secondary in nature. This was further confirmed by the observation of a similar





Scheme 1.



Scheme 2.

downfield shift in the chemical shift value of the C-2 of the acetate **4** with respect to that of the alcohol **3c** in their ^{13}C NMR spectra. The formation of the mono *O*-acetylated compound **4** over *N*-acetylated compound or the diacetate during acetylation of compound **3c** may be because of the secondary nature of the amino function, which is also attached to the 2-methoxyphenyl ring that exert inductive and resonance effects together with steric effects, thereby reduces the nucleophilicity of –NH group present in the compound.

This indicated that CRL-catalyzed epoxide ring opening of (±)-epichlorohydrin with different aromatic amines is completely regioselective due to the attack of the amine nucleophile only on the less hindered C-atom of the epoxide resulting in the formation of 3-arylamino-1-chloropropan-2-ols **3a**–**3j** (Scheme 1). An attempt on epoxide ring opening of (±)-epichlorohydrin with aniline (**2a**), 4-methylaniline (**2b**) and 2-methoxyaniline (**2c**) in diisopropyl ether at 40–42 °C in the absence of lipase led to the formation of two products, that is, 3-arylamino-1-chloropropan-2-ols **3a**–**3c** in 45–68% yields and 2-arylamino-3-chloropropanols **5a**–**5c** in 17 to 30% yields in 35–40 h due to the attack of the nucleophile at both the two C-ends of the epoxide (Scheme 1). The major product formed in the chemical reaction was due to the attack of the nucleophile on less hindered C-atom of the epoxide as expected, but the reaction was very slow and non-selective.

Further, evaluation of optical rotation values of 3-arylamino-1-chloropropan-2-ols **3a**–**3j** revealed that all the ten compounds obtained by CRL-catalyzed epoxide ring opening of the (±)-epichlorohydrin with different aromatic amines **2a**–**2j** are optically active and dextrorotatory. Thus the lipase-catalyzed epoxide ring opening of (±)-epichlorohydrin is enantioselective also, together with regioselective (Table 1). The enantiomeric excess (*ee*) values of (+)-3-arylamino-1-chloropropan-2-ol **3a**–**3j** obtained by CRL-catalyzed epoxide ring opening of (±)-epichlorohydrin (**1**) were determined by calculations using specific rotation values of optically pure (*S*)-(+)-β-amino alcohols **3a**–**3j** prepared by the epoxide ring opening of optically pure (*S*)-(+)-epichlorohydrin with the amines **2a**–**2j** and found to be in the range of 55–92% (Table 1). 3-Arylamino-1-chloropropan-2-ols **3a**–**3c** and 2-arylamino-3-chloropropanols **5a**–**5c** obtained by epoxide ring opening of (±)-epichlorohydrin **1** with amines **2a–2c** in diisopropyl ether in the absence of lipase CRL did not show any optical activity. Further, there was no reaction between (±)-epichlorohydrin and 4-nitroaniline, 1,2-diaminobenzene or aliphatic amines when incubated with the enzyme under identical conditions. This may be due to the weaker nucleophilicity of these amines.

2.2. Antiinflammatory activity

Ten 3-(arylamino)-1-chloro-propan-2-ols **3a–3j** have been synthesized, out of which seven β-aminoalcohols, that is, **3a**, **3b**, **3e**, **3f**, **3g**, **3h** and **3i** were evaluated for their anti-inflammatory activity using primary human umbilical vein endothelial cells. The inhibitory activities of the aminoalcohols **3a**, **3b** and **3e–3i** was evaluated on the TNF-α tumor necrosis factor-α induced expression of cell adhesion molecules, viz. ICAM-1 (intracellular adhesion molecule-1) and E-selectin that play key roles in controlling various inflammatory diseases. The results of anti-inflammatory activities of β-amino alcohols are summarized in Table 2.

1-Chloro-3-(phenylamino)propan-2-ol (**3a**) shows an IC_{50} value of 39.8 μg/ml with 38% of ICAM-1 inhibition at the maximum tolerable dose of 30 μg/ml. Methyl group substitution at the *para* position in the parent structure 1-chloro-3-(phenylamino)propan-2-ol (**3a**), that is, compound **3b** shows an IC_{50} value of 28.84 μg/ml with 81.5% ICAM-1 inhibition at the maximum tolerant dose of 40 μg/ml indicating that methyl substitution at *p*-position results in the increase in inhibitory activity of the parent compound. 1-Chloro-3-(4-bromophenylamino)propan-2-ol (**3f**) shows an IC_{50} value of 31.62 μg/ml with 76% ICAM-1 inhibition at the maximum tolerant dose of 40 μg/ml indicating that this substitution too results in increased inhibitory activity of the parent compound 1-chloro-3-(phenylamino)propan-2-ol (**3a**). 1-Chloro-3-(2′,5′-dimethoxyphenylamino)propan-2-ol (**3e**) shows no significant inhibition of ICAM-1 activity at the maximum tolerant dose of 30 μg/ml indicating that the disubstitution results in significant loss of inhibitory activity of the parent compound.

Table 1
Regio- and enantioselective lipase-catalyzed epoxide ring opening of (±)-epichlorohydrin (**1**) with aromatic amines **2a**–**2j** in diisopropyl ether (DIPE) in the presence of *Candida rugosa* lipase at 40–42 °C

Amine **2a**–**2j**	Product: (*S*)-(+)-3a–3j	Reaction time (h)	Yield[a] (%)	$[\alpha]_D^{23}$ of product	$[\alpha]_D^{23}$ of product obtained from (*S*)-(+)-epichlorohydrin	*ee* (%)
2a	(*S*)-(+)-3a	12	72	+5.6	+7.5	75
2b	(*S*)-(+)-3b	8	80	+16.7	+23.0	73
2c	(*S*)-(+)-3c	11	88	+13.7	+15.0	92
2d	(*S*)-(+)-3d	11	86	+10.5	+11.7	89
2e	(*S*)-(+)-3e	12	84	+3.7	+5.0	75
2f	(*S*)-(+)-3f	12	86	+20.0	+22.3	90
2g	(*S*)-(+)-3g	12	90	+3.0	+5.5	55
2h	(*S*)-(+)-3h	12	70	+5.0	+6.2	80
2i	(*S*)-(+)-3i	12	76	+11.2	+13.7	82
2j	(*S*)-(+)-3j	8	88	+20.0	+25.6	76

[a] Yields were calculated by assuming corresponding single enantiomer as 100% in the starting (±)-epichlorohydrin (**1**).

Table 2
TNF-α induced ICAM-1 inhibitory activity of 3-arylamino-1-chloropropan-2-ols **3a**, **3b** and **3e**–**3i**

Compound	Maximal tolerable dose (MTD, μg/ml)	% Inhibition at MTD	IC_{50} (μg/ml)[a]
(*S*)-(+)-1-Chloro-3-phenylaminopropan-2-ol (**3a**)	30	38	39.8
(*S*)-(+)-1-Chloro-3-(4′-methylphenylamino)-prop-an-2-ol (**3b**)	40	81	28.8
(*S*)-(+)-1-Chloro-3-(2′,5′-dimethoxyphenylamino)-propan-2-ol (**3e**)	30	40	—
(*S*)-(+)-1-Chloro-3-(4′-bromophenylamino)-prop-an-2-ol (**3f**)	40	76	31.6
(*S*)-(+)-1-Chloro-3-(2′-chlorophenylamino)-propan-2-ol (**3g**)	40	86	33.1
(*S*)-(+)-1-Chloro-3-(3′-chlorophenylamino)-propan-2-ol (**3h**)	30	24	—
(*S*)-(+)-1-Chloro-3-(4′-chlorophenylamino)-propan-2-ol (**3i**)	30	60	28.8

[a] '—' Denotes no inhibition.

1-Chloro-3-(2′-chlorophenylamino)propan-2-ol (**3g**) shows an IC_{50} of 33.1 μg/ml with 86% ICAM-1 inhibition at the maximum tolerant dose of 40 μg/ml indicating that the *ortho* chloro substituents increases inhibitory activity of the parent compound substantially. 1-Chloro-3-(3′-chlorophenylamino)propan-2-ol (**3h**) shows no significant inhibition of ICAM-1 activity at the maximum tolerant dose of 30 μg/ml, while 1-chloro-3-(4′-chlorophenylamino)propan-2-ol (**3i**) shows an IC_{50} of 28.8 μg/ml with 60% ICAM-1 inhibition at the maximum tolerant dose of 30 μg/ml indicating that the chloro substituents at the *ortho* or the *para* position enhances the inhibitory activity of the parent compound (Table 2).

3. Conclusion

It has been successfully demonstrated that lipase from *C. rugosa* can be used for regio- and enantioselective synthesis of (*S*)-(+)-3-arylamino-1-chloropropan-2-ols directly from (±)-epichlorohydrin, which is otherwise not possible by classical chemical methods. This method may find general utility towards the synthesis of enantiomerically enriched/pure β-aminoalcohols from racemic epichlorohydrin. It has also been discovered for the first time that (*S*)-(+)-3-arylamino-1-chloropropan-2-ols inhibit the TNF-α induced ICAM-1 expression. One of the compounds, (*S*)-(+)-1-chloro-3-(2′-chlorophenylamino)-propan-2-ol exhibited 86% inhibition of TNF-α induced expression of ICAM-1 at 40 μM concentration which can be used for further development of anti-inflammatory agents.

4. Materials and methods

4.1. Chemistry

Reactions were monitored by TLC on precoated Merck silica gel $60F_{254}$ aluminium plates. Flash column chromatography was carried out using silica gel (100–200 mesh) for purification of the compounds. The products on TLC were detected under UV light. The ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance spectrometer at 300 MHz and 75.5 MHz, respectively. Chemical shift values were reported as δ ppm relative to TMS used as internal standard. Coupling constant (*J* values) are given in Hz. The IR spectra were recorded on a Perkin–Elmer model 2000 FT–IR instrument. The optical rotation values were measured on Rudolph Research Analytical IV polarimeter. The FAB-HRMS spectra were recorded on a JEOL JMS-AX505W high-resolution mass spectrometer in positive mode using the matrix HEDS (*bis*hydroxyethyldisulphide) doped with sodium acetate. The EIMS spectra were recorded on a micromass KC 455 TOF mass spectrometer in positive ion mode. The organic solvents THF, dioxane and diisopropyl ether were used after distillation over sodium pieces. Novozyme-435 and Amano PS were obtained as gifts from Novozymes A/S (Copenhagen, Denmark) and Rasayan Inc. (San Diego, USA), respectively; *C. rugosa* lipase (CRL) and Porcine pancreatic lipase (PPL) were purchased from Sigma Chemical Co. (USA). These lipases were used after storing in vacuo over P_2O_5 for 24 h.

4.1.1. General procedure for CRL-catalyzed epoxide ring opening of (±)-epichlorohydrin (1)

To a solution of the aromatic amine (**2a–2j**, 10.0 mmol) and (±)-epichlorohydrin (**1**, 10.0 mmol) in diisopropyl ether (50 ml), *C. rugosa* lipase (half of the amount of amine w/w) was added and the resulting suspension was stirred at 40–42 °C for 8–12 h. The progress of the reaction was monitored periodically by TLC and at 45–50% completion, the reaction was quenched by filtering off the enzyme. The solvent was removed under reduced pressure, and the residue thus obtained was purified by column chromatography on silica gel using petroleum ether–ethyl acetate (93:7 to 82:18) to obtain the pure products, (*S*)-(+)-3-arylamino-1-chloropropan-2-ols **3a–3j** in 70–90% yields. Compounds **3a–3j** were unambiguously identified on the basis of their spectral (IR, ^{1}H NMR, ^{13}C NMR and HRMS/EIMS) analysis and comparison of the physical and the spectral data of the known compounds **3a**–**3d** and **3f**–**3j** with those reported in the literature.[20–27]

4.1.1.1. (*S*)-(+)-1-Chloro-3-(2′,5′-dimethoxyphenylamino)-propan-2-ol (3e). It was obtained as a light brown oil (500 mg) in 84% yield. R_f: 0.50 (ethyl acetate/petroleum ether, 1:4); $[\alpha]_D^{23}$ +3.7 (0.2, $CHCl_3$); IR (nujol): 3413, 2940, 2834, 1614, 1522, 1462, 1219, 1169, 1048 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$: δ 3.22 and 3.35 (2H, 2dd, 1H each, J = 6.9 & 13.4 Hz and 4.6 & 13.4 Hz, C-3H$_\alpha$ & C-3H$_\beta$), 3.64 (2H, m, C-1H), 3.74 and 3.79 (6H, 2s, 3H each, 2× OCH_3), 4.07 (1H, m, C-2H), 6.18 (1H, dd, J = 2.7 & 8.5 Hz, C-4′H), 6.26 (1H, d, J = 2.7 Hz, C-6′H) and 6.66 (1H, d, J = 8.6 Hz, C-3′H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 46.70 (C-1), 47.52 (C-3), 55.46 and 55.93 (2× OCH_3), 69.75 (C-2), 98.46 (C-6′), 99.59 (C-4′), 110.15 (C-3′), 138.65 (C-1′), 141.67 (C-2′), 154.66 (C-5′); ESIMS *m/z* calculated for $C_{11}H_{16}NO_3Cl$ $[M+1]^+$ 246.71, observed $[M+1]^+$ 246.72.

4.1.2. Synthesis of 2-acetoxy-1-chloro-3-(2′-methoxyphenylamino)-propane (4)

To a solution of (*S*)-(+)-1-chloro-3-(2′-methoxyphenylamino)-propan-2-ol (**3c**, 2.0 mmol) and catalytic amount of dimethylaminopyridine (DMAP) in dichloromethane (10 ml), acetic anhydride (2.2 mmol) was added and the reaction mixture was stirred at room temperature for 3 h until TLC examination indicated completion of the reaction. The reaction mixture was washed with a saturated solution of sodium bicarbonate (3 × 5 ml) and brine solution (3 × 5 ml), dried over sodium sulfate and evaporated under reduced pressure to afford the crude product which was purified by column chromatography over silica gel using petroleum ether and ethyl acetate as eluent to afford 2-acetoxy-1-chloro-3-(2′-methoxyphenylamino)-propane (**4**) as a brownish oil in 92% yield (470 mg); R_f, 0.60 in 20% ethyl acetate in petroleum ether.

IR (nujol): 3419 (NH), 1742 (–O–C=O), 1602, 1515, 1224, 1043, 740 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.02 (3H, s, $OCOCH_3$), 3.39 and 3.66 (2H, d, J = 5.7 Hz, C-3H), 3.66 (2H, m, C-1H), 3.75 (3H, s, OCH_3), 4.38 (1H, m, NH), 5.13 (1H, m, C-2H), 6.65 and 6.77 (4H, m, C-3′H, C-4′H, C-5′H and C-6′H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.82 ($OCOCH_3$), 43.57 and 44.04 (C-1 and C-3), 55.38 (OCH_3), 71.48 (C-2), 109.64 (C-6′), 110.04 (C-3′), 117.19 (C-4′), 121.28 (C-5′), 137.21 (C-1′), 146.80 (C-2′), 170.24 ($OCOCH_3$); ESIMS m/z calculated for $C_{12}H_{15}NO_3Cl$ $[M+1]^+$ 257.72, observed $[M+1]^+$ 257.70.

4.1.3. General procedure for non-enzymatic epoxide ring opening of (±)-epichlorohydrin (1)

The aromatic amine (**2a**–**2c**, 10.0 mmol) was dissolved in diisopropyl ether (50 ml) and (±)-epichlorohydrin (**1**, 10.0 mmol) was added into it. The resulting solution was stirred at 45 °C and progress of the reaction was monitored periodically by TLC. The TLC examination of the reaction mixture revealed the formation of two products and reaction remained incomplete even after 40 h of stirring. The solvent was removed under reduced pressure, and the residue thus obtained was purified by column chromatography on silica gel using petroleum ether/ethyl acetate (93:7 to 82:18) to obtain the pure products, 3-arylamino-1-chloropropan-2-ols **3a–3c** in 45–68% yields and 2-arylamino-3-chloropropanols **5a**–**5**c in 17–30% yields. Compounds **3a–3c** and **5a–5c** were unambiguously identified on the basis of their spectral (IR, ^1H NMR, ^{13}C NMR and HRMS/EIMS) analysis and comparison of their physical and spectral data with those reported in the literature. Further, the physical characteristics and spectral data of **3a**–**3c** were found identical with the corresponding data of (*S*)-(+)-3-arylamino-1-chloropropan-2-ols **3a–3c** synthesized by the CRL-catalyzed epoxide ring opening (±)-epichlorohydrin, except that their optical rotations were zero.

4.1.3.1. 3-Chloro-2-phenylaminopropanol (5a). It was obtained as a brownish oil (330 mg) in 18% yield. R_f: 0.38 in 20% ethyl acetate in petroleum ether; $[\alpha]_D^{23}$ 0.0 (*c* 0.2, $CHCl_3$); IR (nujol): 3338 (NH), 3062, 1599, 1504, 1228, 852 and 751 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.32 (2H, dd, J = 7.8 and 15.6 Hz, C-3H), 3.60 (2H, m, C-1H), 3.93 (1H, br s, C-2H), 5.32 (1H, d, J = 4.8 Hz, OH), 5.46 (1H, d, J = 4.8 Hz, NH), 6.60 (1H, m, C-4′H), 6.75 (2H, dd, J = 1.8 and 8.7 Hz, C-2′H and C-6′H) and 7.15 (2H, m, C-3′H and C-5′H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 48.12 and 55.08 (C-1 and C-3), 67.64 (C-2), 112.07 (C-2′ and C-6′), 115.69 (C-4′), 129.08 (C-3′ and C-5′) and 147.61 (C-1′); ESIMS m/z calculated for $C_9H_{12}NOCl$ $[M+1]^+$ 186.66, observed $[M+1]^+$ 186.64.

4.1.3.2. 3-Chloro-2-(4′-methylphenylamino)propanol (5b). It was obtained as a brownish oil (590 mg) in 30% yield. R_f: 0.36 in 20% ethyl acetate in petroleum ether; $[\alpha]_D^{23}$ 0.0 (*c* 0.2, $CHCl_3$); IR (nujol): 3583 (NH), 3342 (OH), 1616, 1519, 1231, 805, 752 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.25 (3H, s, CH_3), 3.37 (2H, dd, J = 8.1 and 14.5 Hz, C-3H), 3.62 (2H, m, C-1H), 4.10 (1H, br s, C-2H), 6.76 (2H, d, J = 8.1 Hz, C-2′H and C-6′H), 7.05 (2H, d, J = 7.8 Hz, C-3′H and C-5′H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 27.93 (CH_3), 54.46 and 54.81 (C-1 and C-3), 65.99 (C-2), 121.20 (C-4′), 122.98 (C-2′ and C-6′), 137.68 (C-3′ and C-5′), 154.01 (C-1′); ESIMS m/z calculated for $C_{10}H_{14}NOCl$ $[M+1]^+$ 200.68, observed $[M+1]^+$ 200.58.

4.1.3.3. 3-Chloro-2-(2′-methoxyphenylamino)propanol (5c). It was obtained as colourless oil (360 mg) in 17% yield. R_f: 0.32 in 20% ethyl acetate in petroleum ether; $[\alpha]_D^{23}$ 0.0 (*c* 0.2, $CHCl_3$); IR (nujol): 3849 (NH), 3400 (OH), 1593, 1494, 1240, 807, 753 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.16 (2H, m, C-3H), 3.55 (2H, m, C-1H), 3.68 (1H, m, C-2H), 3.78 (3H, s, OCH_3), 5.04 (1H, m, OH), 6.87 (1H, t, J = 6.9 Hz, C-6′H), 6.87 (1H, t, J = 5.7 Hz, C-5′H), 6.98 (2H, m, C-3′H and C-4′H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 48.63 and 55.33 (C-1, C-3 and OCH_3), 68.58 (C-2), 112.40 (C-6′), 120.59 (C-3′), 123.65 (C-4′), 124.09 (C-5′), 138.52 (C-1′), 154.09 (C-2′); ESIMS m/z calculated for $C_{10}H_{14}NO_2Cl$ $[M+1]^+$ 216.68 observed $[M+1]^+$ 216.64.

4.2. Antiinflammatory activity

Anti-ICAM-1 and E-selectin were purchased from Pharmingen, USA. M-199 media, L-glutamine, endothelial cell growth factor, trypsin, Pucks saline, HEPES, *o*-phenylenediamine and anti-mouse IgG-HRP were purchased from Sigma Chemical Co., USA. Fetal calf serum was purchased from Biological Industries, Israel.

4.2.1. Cells and cell culture

Primary endothelial cells were isolated from human umbilical cord using mild trypsinization.[28] The cells were grown in M 199 medium supplemented with 15% heat inactivated fetal calf serum, 2 mM L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, 0.25 μg/ml amphotericin B, endothelial cell growth factor (50 μg/ml) and heparin (5 U/ml). At confluence, the cells were subcultured using 0.05% trypsin–0.01 M EDTA solution and were used between passages three to four. The viability of cells was determined by trypan blue exclusion test. The purity of endothelial cells was determined by E-selectin expression.

4.2.2. Modified ELISA for measurement of ICAM-1 and E-selectin

Cell-ELISA was used for measuring the expression of ICAM-1 and E-selectin on the surface of the endothelial cells.[29,30] Endothelial cells were then incubated with or without the test compound at desired concentrations for the required period, followed by treatment with TNF-α (10 ng/ml) or LPS (1 μg/ml) for 16 h for ICAM-1 expression and 4 h for E-selectin expression. The cells were fixed with 1.0% glutaraldehyde using non-fat dry milk (3.0% in PBS blocked non-specific binding of the antibody). Following incubation overnight at 4 °C with ICAM-1 and E-selectin mAb, diluted in blocking buffer, the cells were washed with PBS and incubated with peroxidase-conjugated goat anti-mouse secondary Ab. The cells were again washed with PBS and exposed to the peroxidase substrate (*o*-phenylenediamine dihydrochloride 40 mg/100 ml in citrate phosphate buffer, pH 4.5). The colour development reaction was stopped by the addition of 2 N sulphuric acid and absorbance at 490 nm was measured using an automated microplate reader (Spectramax 190, Molecular Devices, USA).

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