Asian Journal of Chemistry; Vol. 32, No. 9 (2020), 2180-2186

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2020.22722

Synthesis, Characterization and Biological Evaluation of Some Novel Regioisomers of Ropivacaine Analogues: An Anaesthetic Drug

S.P.N. SHARAVANAN[1], C.S. VENKATESAN[2], D. BHASKARA SURESH RAJU[2] and S. KABILAN[1,*]

[1]Department of Chemistry, Faculty of Science, Annamalai University, Annamalai Nagar-608002, India
[2]Gland Pharma Ltd, Research and Development, D.P. Pally, Hyderabad-500043, India

*Corresponding author: E-mail: profdrskabilanau@gmail.com

Received: 14 March 2020; *Accepted*: 26 May 2020; *Published online*: 20 August 2020; AJC-20010

A series of ropivacaine analogues were synthesized by altering the methyl group substituent in the aromatic ring. The synthesized compounds **(2a-f and 3a-f)** were characterized by using IR, NMR and HRMS analysis and then compounds **3a-3f** were screened *in vitro* anticancer (MTT assay) activity against breast cancer cell line (MCF-7), antibacterial and antioxidant (DPPH scavenging assay) studies. The biological studies revealed that the compounds **3c** and **3f** have shown a good inhibitory activity against MCF-7 cell line. Compounds **3e**, **3b**, **3c** and **3f** showed moderate antioxidant activity. There was no inhibition observed against both Gram-positive and Gram-negative bacteria.

Keywords: Ropivacaine, Regioisomers, Biological activity.

INTRODUCTION

Ropivacaine analogues forming -C-N- bond with alkyl group regioisomers [1-4] of *S*-ropivacaine [5,6] analogues by altering the methyl group in a sequence-specific manner under physiological conditions are of potential clinical and biological interest [7-10]. In particular, local or regional anaesthetic drugs are given to the patient for pain relief during and after surgery [11]. The first optically pure anaesthetic drug is ropivacaine, even though both *R*- and *S*-isomers are showing anaesthetic property, however, *R*-isomer showing side effects on cardiovascular [6]. Compare to *R*-isomers, *S*-enantiomer of ropivacaine shows better anaesthetic property along with few side effects compared to the *R*-isomer [6]. Ropivacaine analogues also exhibit the anti-inflammatory, antioxidant and antimicrobial properties [12,13]. Schmidt and Rosenkranz [14] reported the results of Murphy *et al*. [15] who showed that 0.5% tetracaine was toxic to *Pseudomonas*.

Ropivacaine (**I**), bupivacaine (**II**) and mepivacaine (**III**) have the same chemical structure except for the alkyl group present in piperidyl ring, $-C_3H_7$ in ropivacaine, $-C_4H_9$ in bupivacaine and $-CH_3$ in mepivacaine (Fig. 1). A small difference in the length of alkyl chain is known to affect the antimicrobial activity of hydrophobic inhibitors. Similarly small changes by altering the position of methyl group present in the aromatic ring of ropivacaine showing excellent antioxidant and anticancer activity.

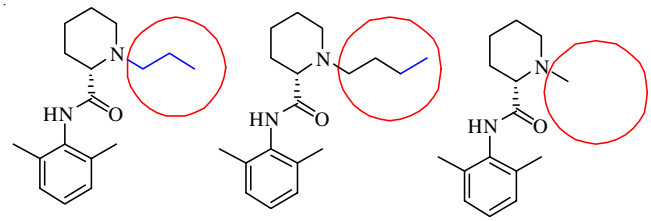


Fig. 1. Chemical structures of ropivacaine and bupivacaine

The present study is first towards the identification, synthesis and characterization, and *in vitro* biological studies like antibacterial, antioxidant and anticancer activity studies of ropivacaine analogues which were not reported till date. Analogues profiling study will be of enormous significance for the synthetic chemists to understand the source of the possible analogues throughout the synthesis of ropivacaine. As part of our continuous efforts to develop potent bioactive molecules, we hereby report the synthesis of regioisomers of

S-ropivacaine analogues by altering the methyl group present in the aromatic ring by without disturbing the chirality and piperidyl ring substitution. The ropivacaine analogues were screened for their cytotoxicity against an MCF-7 cell line, through MTT assay method, antioxidant and antimicrobial evaluation of regioisomers.

EXPERIMENTAL

Melting points were recorded in open capillary melting point apparatus and are uncorrected. IR spectra were recorded using a Shimadzu FTIR Spectrophotometer (Model no.: IR AFFINITY-1S WL) while ^{1}H NMR spectra were recorded using a Bruker-Avance 400 (400 MHZ) at IICT, Hyderabad, India performed at room temperature, using TMS as an internal standard. ^{13}CNMR spectra were recorded on the same instrument at 100 MHz and are referenced using the central line of solvent signal ($CDCl_3$ (triplet at 77 ppm), DMSO-d_6 (septet at 39.5 ppm). LC-HRMS spectra were recorded using an instrument from Agilent QTOF 6530 model. Chromatographic purifications were performed using silica gel 100-200 and thin-layer chromatography (TLC) was performed on precoated silica gel sheets from Merck (Kiesel 60GF_{254}, 0.2 mm thickness). All reagents and solvents were commercially obtained from Sigma-Aldrich, Avra and Spectrochem and used directly without further purification.

MTT assay (cell growth inhibition assay): Cell viability was evaluated by the MTT assay with three independent experiments with six concentrations of compounds in triplicates. Cells were trypsinized and preform the trypan blue assay to know viable cells in the cell suspension. Cells were counted by haemocytometer and seeded at a density of 5.0×10^3 cells / well in 100 µL media in 96 well plate culture medium and incubated overnight at 37 ºC. After incubation, take off the old media and added fresh media 100 µL with different concentrations of the test compound in represented wells in 96 plates. After 48 h, discarded the drug solution and added the fresh media with MTT solution (0.5 mg/mL) was added to each well and plates were incubated at 37 ºC for 3 h. At the end of incubation time, precipitates are formed as a result of the reduction of the MTT salt to chromophore formazan crystals by the cells with metabolically active mitochondria. The optical density of solubilized crystals in DMSO was measured at 570 nm on a microplate reader [16]. The percentage of growth inhibition was calculated using the following formula:

$$\text{Cytotoxicity } (\%) = \frac{\text{Test optical density}}{\text{Control optical density}} \times 100$$

Antibacterial activity: The bacterial strains were obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. Bacterial strains were used Gram-negative *Escherichia coli* and Gram-positive *Bacillus Subtilis* bacteria. Bacterial Media (nutrient agar media) were made with 13 g of nutrient agar was mixed with distilled water and then sterilized in an autoclave at 15 lb pressure for 15 min. The sterilized media were poured into petri dishes. The solidified plates were pored with 5 mm dia cork pore. The plates with wells were used for antibacterial studies.

Antioxidant activity: The antioxidant activity of synthesized compounds **3a-f** were determined through sequestration capacity of free radical DPPH (2,2-diphenyl-1-picrylhydrazyl). To a 5 mL ethanol (80% v/v) containing 0.1 mM DPPH was added compound **3a-f** with the increasing concentrations 5, 10, 25, 50, 75, 100 µg/mL and incubated for 30 min. After incubation, the sample were recorded using spectrophotometer at wavelength 517 nm. Ascorbic acid (µg/mL) has been used as a standard reference. The DPPH radical scavenging activity was calculated from the absorption according to the following equation:

$$\text{Radical scavenging activity } (\%) = \frac{OD_{control} - OD_{sample}}{OD_{control}} \times 100$$

Molecular docking study: The critical interactions between aldehyde dehydrogenase 1 (ALDH1A1) with ropivacaine analogues have been carried out using Schrodinger suite 9.3.5. The 3D coordinates of crystallographic structure of ALDH1A1 protein complex44 (PDB ID: 5L2N) was downloaded from protein Data Bank (www.rscb.com). The protein prepared using P*rotein Preparation Wizard* [17]. The prepared protein minimized using force field OPLS-2005 (Optimized Potential for Liquid Simulations) [18] up to root mean square deviation (RMSD) value of 0.3 Å. Using ligprep module, all the ligands are prepared. With Glide docking using extra precision (XP) mode the prepared protein and ligands are docked to find the best fit molecules in the active site of ALDH1A1.

General procedure for the synthesis of compounds 2a-f: To a stirred solution of (*S*)-piperidine-2-carboxylic acid (0.5 g, 3.87 mmol) in toluene (10 mL) and cool to 0-5 ºC. To this, dropwise addition of 4 M HCl in 1,4-dioxane (1.4 mL), stirred for 10 min and slowly raise the temperature to room temperature and again stirred for 1 h. Then the reaction mixture was cooled to 0-5 ºC and slowly dropwise addition of $POCl_3$ (1.21 g, 7.83 mmol), stirred for 10 min and 2,4-dimethylamine (1.41 g, 11.64 mmol) was added. After addition the reaction mixture stirred at same temperature for about 10 min and left the reaction mixture at room temperature for about overnight. The completion of the reaction was monitored by TLC (mobile phase conditions: 10% methanol:MDC). The reaction mixture was concentrated completely gave a crude compound, which was diluted with water (10 mL) and pH adjusted to 11.0-11.5 by using aqueous 2 N NaOH solution. The aqueous layer was extracted with ethyl acetate (3 × 25 mL) and dried over anhydrous sodium sulphate. The organic layer was concentrated to give solid compound. The crude compound was further purified by column chromatography using 100-200 silica gel and methanol and dichloromethane as an eluent.

***N*-(2,4-dimethylphenyl)piperidine-2-carboxamide (2a):** Yield: 0.28 g: ^{1}H NMR ($CDCl_3$,400 MHz): δ_H 3.41 (1H, dd, H_2), 2.02-2.06 (1H, m, H_{3a}), 1.79-1.82 (1H, m, H_{3b}), 1.47-1.62 (4H, m, H_4 & H_5), 3.06-3.11 (1H, m H_{6a}), 2.74-2.80 (1H, m, $6H_b$), 6.98 (1H, s, H_9), 7.01(1H, s, H_{11}), 7.7 (1H, d, H_{12}), 2.23 (3H, s, H_{13}), 2.28 (3H, s, H_{14}), 8.78 (1H, bs, -*NH*CO). ^{13}C NMR (DMSO-d_6, 100 MHz): δ_C 171.81 (C1), 134.27 (C10), 133.01 (C7), 130.97 (C9), 128.62 (C8), 127.11 (C11), 122.26 (C12), 60.34 (C2), 45.45 (C6), 29.67 (C3), 25.69 (C5), 23.63

(C4), 20.80 (C14), 17.66 (C13). Mass (ESI) calcd. for $C_{14}H_{20}N_2O$: 232.32, found; 233.1 $[M+H]^+$. IR (KBr, ν_{max}, cm^{-1}): 3294.42, 3257.77 (NH), 2926.01 (CH), 1683.86 (C=O), 1533.41& 1448.54 (C=C).

***N*-(2,3-Dimethylphenyl)piperidine-2-carboxamide (2b):** ^1H NMR ($CDCl_3$,400 MHz): δ_H 3.47 (1H, dd, H_2), 2.05 (1H, dd, H_{3a}), 1.81 (1H, t, H_{3b}),1.48-1.62 (4H, m, H_4 & H_5), 3.10 (1H, dd, H_{6a}), 2.79 (1H, t, $6H_b$), 6.97 (1H, d, H_{10}), 7.08 (1H, t, H_{11}), 7.63 (1H, d, H_{12}), 2.29 (3H, s, H_{13}), 2.15 (3H, s, H_{14}), 8.91 (1H, bs, -*NH*CO). ^{13}C NMR (DMSO-d_6, 100 MHz): δ_C 171.84 (C1), 137.18 (C9), 135.29 (C7), 128.30 (C8), 126.79 (C10), 125.81 (C11), 121.01 (C12), 60.29 (C2), 45.39 (C6), 29.47 (C3), 25.56 (C5), 23.57 (C4), 20.55 (C14), 13.58 (C13). Mass (ESI) calcd. for $C_{14}H_{20}N_2O$: 232.32, found; 233.2 $[M+H]^+$. IR (neat, ν_{max}, cm^{-1}): 329.49 (NH), 2924.09, 2852.72 (CH), 1672.28 (C=O), 1529.55, 1469.76 (C=C)

N-(*o*-Tolyl)piperidine-2-carboxamide (2c): ^1H NMR ($CDCl_3$, 400 MHz): δ_H 3.42 (1H, dd, H_2), 2.02-2.06 (1H, m, H_{3a}), 1.81 (1H, t, H_{3b}), 1.45-1.63 (4H, m, H_4 & H_5), 3.06-3.11 (1H, m, H_{6a}), 2.75-2.81 (1H, m, $6H_b$), 7.02 (1H, t, H_{10}), 7.16-7.22 (2H, m, H_{11} & H_{12}), 7.98 (1H, d, H_9), 2.27 (3H, s, H_{13}), 8.91 (1H, bs, -*NH*CO). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ_C 171.90 (C1), 135.73 (C7), 130.30 (C9), 128.22 (C8), 126.70 (C10), 124.54 (C11), 121.89 (C12), 60.38 (C2), 45.45 (C6), 29.63 (C3), 25.79 (C5), 22.65 (C4), 17.70 (C13). Mass (ESI) calcd. for $C_{13}H_{18}N_2O$: 218.30, found; 219.2 $[M+H]^+$. IR (KBr, ν_{max}, cm^{-1}): 3356.14, 3298.28 (NH), 2924.09, 2852.72 (CH), 1691.57 (C=O), 1525.69 & 1450.47 (C=C).

***N*-(3,5-Dimethylphenyl)piperidine-2-carboxamide (2d):** ^1H NMR ($CDCl_3$, 400 MHz): δ_H 3.44 (1H, dd, H_2), 2.00-2.03 (1H, m, H_{3a}), 1.80-1.81 (1H, t, H_{3b}),1.43-1.59 (4H, m, H_4 & H_5), 3.06 (1H, m, H_{6a}), 2.74 (1H, t, $6H_b$), 6.73 (1H, s, H_{10}), 7.22 (2H, s, H_8 & H_{12}), 2.29 (6H, s, H_{13} & H_{14}), 8.81 (1H, bs, -*NH*CO). ^{13}C NMR (DMSO-d_6, 100 MHz): δ_C 171.88 (C1), 138.62 (C9 & C11), 137.64 (C7), 125.76 (C10), 117.23 (C8 & C12), 60.41 (C2), 45.48 (C6), 29.55 (C3), 25.75 (C5), 23.70 (C4), 21.32 (C13 & C14). Mass (ESI) calcd. for $C_{14}H_{20}N_2O$: 232.32, found; 233.2 $[M+H]^+$. IR (KBr, ν_{max}, cm^{-1}): 3458.37, 3302.13 (NH), 2922.16, 2852.72 (CH), 1672.28 (C=O), 1462.04(C=C)

***N*-(3,4-Dimethylphenyl)piperidine-2-carboxamide (2e):** ^1H NMR ($CDCl_3$, 400 MHz): δ_H 3.33 (1H, dd, H_2), 2.02-2.05 (1H, m, H_{3a}), 1.81-1.82 (1H, m, H_{3b}), 1.43-1.61 (4H, m, H_4 & H_5), 3.05 (1H, m, H_{6a}), 2.75 (1H, t, $6H_b$), 7.06 (1H, d, H_{11}), 7.30 (1H, d, H_{12}), 7.38 (1H, s, H_8), 2.21 & 2.24 (6H, s, H_{14} & H_{13}), 8.75 (1H, bs, -*NH*CO).^{13}C NMR (DMSO-d_6, 100 MHz): δ_C 171.86 (C1), 137.13 (C9), 135.57 (C7), 132.27 (C10), 129.90 (C11), 120.81 (C8), 116.95 (C12), 60.49 (C2), 45.60 (C6), 29.71 (C3), 25.89 (C5), 23.82 (C4), 19.84 (C13), 19.14 (C14). Mass (ESI) calcd. for $C_{14}H_{20}N_2O$: 232.32, found; 233.2 $[M+H]^+$. IR (KBr, ν_{max}, cm^{-1}): 3346.50, 3300.20 (NH), 2926.01, 2854.65 (CH), 1683.86 (C=O), 1519.91, 1450.47 (C=C).

***N*-Mesitylpiperidine-2-carboxamide (2f):** ^1H NMR ($CDCl_3$, 400 MHz): δ_H 3.51 (1H, dd, H_2), 2.06-2.09 (1H, m, H_{3a}), 1.80-1.84 (1H, m, H_{3b}), 1.47-1.66 (4H, m, H_4 & H_5), 3.15 (1H, m, H_{6a}), 2.94 (1H, t, $6H_b$), 6.86 (2H, s, H_9 & H_{11}), 2.25 (3H, s, H_{13}), 2.11 (6H, s, H_{14} & H_{15}), 8.31 (1H, bs, -*NH*CO). ^{13}C NMR (DMSO-d_6, 100 MHz): δ_C 172.0 (C1), 136.68 (C10), 134.89 (C8 & C12), 130.82 (C7), 128.82 (C9 & C11), 116.97 (C12), 60.24 (C2), 45.43 (C6), 29.99 (C3), 25.31 (C5), 23.64 (C4), 20.87 (C15), 18.33 (C13 & C14). Mass (ESI) calcd. for $C_{15}H_{22}N_2O$: 246.35, found; 247.2 $[M+H]^+$. IR (neat, ν_{max}, cm^{-1}): 3251.98 (NH), 2924.09, 2854.65 (CH), 1660.71 (C=O), 1502.55, 1442.75 (C=C).

General procedure for the synthesis of compounds 3a-f: To a stirred solution of *N*-(2,4-dimethylphenyl)piperidine-2-carboxamide (**2a**) (0.25 g, 1.07 mmol) in 4 mL DMF at room temperature, K_2CO_3 (0.232 g, 1.61mmol), 1-chloropropane (0.10g, 1.30 mmol) and catalytic amount of KI were added. The resultant reaction mixture was stirred at 90-100 ºC for about 2 h. The reaction was monitored by TLC. After completion of reaction, the reaction mass was cooled to room temperature and added ice cold water and extract with ethyl acetate, separate the organic layer and dried over anhydrous sodium sulphate. Concentrate the organic layer at < 35 ºC to give crude compound. The obtained crude compound was purified by column chromatography using neutral aluminum oxide, ethyl acetate/ petroleum ether as an eluent to collect the pure fractions and concentrated to give pure compound (yield: 252 mg, 85%).

(*S*)-*N*-(2,4-Dimethylphenyl)-1-propylpiperidine-2-carboxamide (3a): ^1H NMR ($CDCl_3$,400 MHz): δ_H 3.14 (1H, dd, H_2), 1.41-1.72 (7H, m, H_{3b}, H_4, H_5 & H_8), 2.84 (1H, dd, H_{6a}), 2.59-2.66 (1H, m, H_{6b}), 2.03-2.23 (3H, m, H_{3a}, H_7), 0.89 (3H, t, H_9), 8.66 (1H, bs, -*NH*CO), 7.81 (1H, d, H_{15}), 6.99 (1H, s, H_{12}), 7.01 (1H, d, H_{14}), 2.23 & 2.29 (6H, s, H_{16} & H_{17}). ^{13}C NMR (DMSO-d_6, 100 MHz): δ_C 172.84 (C1), 134.02 (C13), 133.29 (C10), 131.01 (C12), 128.12 (C11), 127.24 (C14), 121.85 (C15), 68.35 (C2), 58.78 (C7), 50.99 (C6), 29.84 (C3), 24.56 (C5), 23.29 (C4), 20.79 (C17), 20.56 (C8), 17.64 (C16), 11.70 (C9). LC-HRMS (ESI) calcd. for $C_{17}H_{26}N_2O$: 274.2045, found; 275.2242 $[M+H]^+$. IR (neat, ν_{max}, cm^{-1}): 3348.42 (NH), 2922.16 (CH), 1685.79 (C=O), 1479.40 & 1456.26 (C=C).

(*S*)-*N*-(2,3-Dimethylphenyl)-1-propylpiperidine-2-carboxamide(3b): ^1H NMR ($CDCl_3$,400 MHz): δ_H 3.16 (1H, dd, H_2), 1.48-1.76 (7H, m, H_{3b}, H_4, H_5 & H_8), 2.85 (1H, dd, H_{6a}), 2.61-2.68 (1H, m, H_{6b}), 2.04-2.11 & 2.21-2.28 (3H, m, H_7, H_{3a}), 0.90 (3H, t, H_9), 8.72 (1H, bs, -*NH*CO), 7.71 (1H, d, H_{15}), 7.11 (1H, t, H_{14}), 6.97 (1H, d, H_{13}), 2.17 & 2.31(6H, s, H_{16} & H_{17}). ^{13}C NMR (DMSO-d_6, 100 MHz): δ_C 172.97 (C1), 137.18 (C10), 135.62 (C12), 127.61 (C11), 126.48 (C14), 125.94 (C13), 120.39 (C15), 68.40 (C2), 58.81 (C7), 51.04 (C6), 29.85 (C3), 24.60 (C5), 23.30 (C4), 20.60 (C17), 20.55 (C8), 13.43 (C16), 11.70 (C9). LC-HRMS (ESI) calcd. for $C_{17}H_{26}N_2O$: 274.2045, found; 275.2153 $[M+H]^+$ IR (neat, ν_{max}, cm^{-1}): 3342.64 (NH), 2929.87, 2858.51 (CH), 1693.50 (C=O), 1521.84 & 1469.76 (C=C).

(*S*)-1-propyl-N-(*o*-tolyl)piperidine-2-carboxamide (3c): ^1H NMR ($CDCl_3$,400 MHz): δ_H 3.13-3.18 (1H, m, H_2), 1.46-1.76 (7H, m, H_{3b}, H_4, H_5 & H_8), 2.86 (1H, dd, H_{6a}), 2.59-2.67 (1H, m, H_{6b}), 2.03-2.26 (3H, m, H_{3a}, H_7), 0.90 (3H, t, H_9), 8.79 (1H, s, -*NH*CO), 8.02 (1H, d, H_{15}), 7.17-7.24 (2H, m, H_{12} & H_{14}), 7.04 (1H, t, H_{13}), 2.28 (3H, s, H_{16}). ^{13}C NMR (DMSO-d_6, 100 MHz): δ_C 172.84 (C1), 136.03 (C10), 130.35 (C12), 127.67 (C11), 126.82 (C13), 124.28 (C14), 121.51 (C15), 68.31 (C2), 58.75 (C7), 50.93 (C6), 29.64 (C3), 24.48 (C5),

23.26 (C4), 20.59 (C8), 17.69 (C16), 11.71(C9). LC-HRMS (ESI) calcd. for $C_{16}H_{24}N_2O$: 260.1889, found; 261.2074 $[M+H]^+$. IR (neat, ν_{max}, cm^{-1}): 3334.92 (NH), 2931.80 (CH), 1695.43 (C=O), 1521.84 & 1452.40 (C=C).

(*S*)-*N*-(3,5-dimethylphenyl)-1-propylpiperidine-2-carboxamide (3d): ^{1}H NMR ($CDCl_3$,400 MHz): δ_H 3.13 (1H, m, H_2), 1.47-1.76 (7H, m, H_{3b}, H_4, H_5 & H_8), 2.78 (1H, dd, H_{6a}), 2.50-2.57 (1H, m, H_{6b}), 2.00-2.22 (3H, m, H3a, H7), 0.92 (3H, t, H9), 8.64 (1H, s, -NHCO), 7.21 (2H, s, C11 & H15), 6.74 (1H, s, H13), 2.30 (6H, s, H16 & H17). ^{13}C NMR (DMSO-d_6, 100 MHz): δ_C 172.91 (C1), 138.73 & 137.91 (C12, C14 & C10), 125.55 (C13), 116.85 (C11 & C15), 68.56 (C2), 58.73 (C7), 51.03 (C6), 29.99 (C3), 24.72 (C5), 23.35 (C4), 21.35 (C16 & C17), 20.20 (C8) 11.73 (C9). LC-HRMS (ESI) calcd. for $C_{17}H_{26}N_2O$: 274.2045, found; 275.2214 $[M+H]^+$. IR (neat, ν_{max}, cm^{-1}): 3329.14 (NH), 2922.16 (CH), 1693.50 (C=O), 1598.99 & 1456.26 (C=C).

(*S*)-*N*-(3,4-Dimethylphenyl)-1-propylpiperidine-2-carboxamide (3e): ^{1}H NMR ($CDCl_3$,400 MHz): δ_H 3.13 (1H, m, H_2), 1.48-1.76 (7H, m, H_{3b}, H_4, H_5 & H_8), 2.77 (1H, dd, H_{6a}), 2.50-2.57 (1H, m, H_{6b}), 2.00-2.19 (3H, m, H_{3a}, H_7), 0.91 (3H, t, H9), 8.62 (1H, bs, -*NH*CO), 7.29 (1H, d, H_{15}), 7.38 (1H, s, H_{11}), 7.07 (1H, d, H_{14}), 2.25 & 2.22 (6H, s, H_{16} & H_{17}). ^{13}C NMR (DMSO-d_6, 100 MHz): δ_C 172.80 (C1), 137.22 (C12), 135.81 (C10), 132.07 (C13), 129.94 (C14), 120.47 (C11), 116.61 (C15), 68.59 (C2), 58.74 (C7), 51.07 (C6), 30.05 (C3), 24.76 (C5), 23.39 (C4), 20.21 (C8), 19.86 & 19.11 (C16 & C17), 11.74 (C9). LC-HRMS (ESI) calcd. for $C_{17}H_{26}N_2O$: 274.2045, found; 275.2213 $[M+H]^+$. IR (neat, ν_{max}, cm^{-1}): 3327.21 (NH), 2931.80 (CH), 1685.79 (C=O), 1521.84 (C=C).

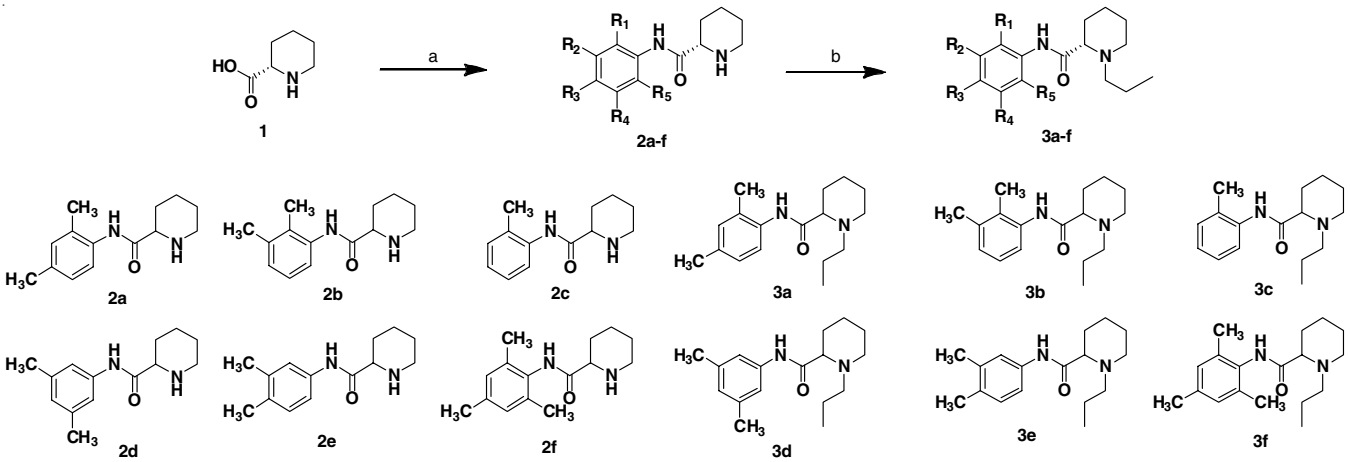
(S)-*N*-Mesityl-1-propylpiperidine-2-carboxamide (3f): ^{1}H NMR ($CDCl_3$,400 MHz): δ_H 3.21 (1H, m, H_2), 1.48-1.79 (7H, m, H_{3b}, H_4, H_5 & H_8), 2.85 (1H, dd, H_{6a}), 2.75-2.82 (1H, m, H_{6b}), 1.99-2.20 (3H, m, H_{3a}, H_7), 0.90 (3H, t, H_9), 8.06 (1H, s, -*NH*CO), 6.89 (2H, s, H_{13} & H_{15}), 2.20 (6H, s, H_{17} & H_{19}), 2.28 (3H, s, H_{18}). ^{13}C NMR (DMSO-d_6, 100 MHz): δ_C 173.19 (C1), 136.70 (C14), 135.08 (C12 & C16), 130.96 (C11), 129.03 (C13 & C15), 68.70 (C2), 59.43 (C7), 51.68 (C6), 30.84 (C3), 24.96 (C5), 23.54 (C4), 20.84 (C18), 20.65 (C8), 18.58 (C17 & C19), 11.58 (C9). LC-HRMS (ESI) calcd. for $C_{18}H_{28}N_2O$: 288.2202, found; 289.2391 $[M+H]^+$. IR (neat, ν_{max}, cm^{-1}): 3223.05 (NH), 2931.80 (CH), 1653.00 (C=O), 1519.91 (C=C).

RESULTS AND DISCUSSION

In present study, a series of ropivacaine analogues is synthesized and characterized. Instead of substituted 2,6-dimethyl groups in the aromatic ring of ropivacaine, methyl groups were introduced in other alternative positions in the aromatic ring keeping heterocyclic *N*-alkyl group remains unchanged. The synthesized analogues were characterized by IR, mass and NMR spectral analysis.

Linear synthesis of ropivacaine analogues starting from the key synthetic intermediate (*S*)-piperidine-2-carboxylic acid (**Scheme-I**) is well documented and involves chlorination in the presence of phosphoryl chloride. The formed acid chloride is subjected to condensation with substituted aniline (by altering methyl group position in the aromatic ring) in the presence of base potassium carbonate to give **2a-f.** The isolated product further condensed with 1-chloro propane gave analogues of ropivacaine **3a-f**.

Characterization: In IR spectrum of compound **3a**, amide carbonyl appeared as a sharp band at 1685.79 cm^{-1} and –NH stretching appeared at 3348.42 cm^{-1}. LC-HRMS spectrum of compound **3a** showed a molecular ion peak at *m/z* 275.2242 $[M+H]^+$ in the positive mode, which complies with the exact molecular weight 274.2045. From the ^{1}H NMR spectrum, small broad singlet at δ 8.66 ppm is due to the exchangeable proton -NH. The peak appeared at δ 7.81ppm (1H, doublet), δ 6.99 ppm (1H, singlet), δ 7.02 (1H, doublet) attribute to aromatic proton H-15, H-12 and H-14, respectively. The peak appeared at δ 2.23 and 2.29 ppm corresponds to the two methyl groups present in the aromatic ring and the peak at 0.89 ppm (3H, triplet) belongs to H-9 proton. The methine proton (H-2) which is attached to the carbonyl group appeared at δ 3.14 ppm (1H, doublet of doublet). The enantiotopic -CH_2 proton H-3 splitted into two peaks *i.e*. H-3a and H-3b. The peak appeared at 2.03-2.23 ppm (3H, multiplet) correspond to H-3a and H-7. The peak appeared at δ 1.41-1.72 ppm (7H, multiplet) correspond to H-



Scheme-I: Synthetic scheme for the preparation of ropivacaine; **Reaction conditions**: a. i) 4M HCl in dioxane, toluene, 0-5 °C to room temperature, 1 h; ii) $POCl_3$, arylamine, 0-5 °C to room temperature, O/N. b. 1-Chloro propane, K_2CO_3, KI (Cat), DMF, 90-100 °C, 2 h

3b, H-4, H-5 and H-8. The peak at 2.84 ppm (1H, doublet of doublet) attributes to H_{6a} and the peak at 2.59-2.66 ppm (1H, multiplet) attribute to H_{6b}. In DEPT-135° NMR spectrum showed seven peaks above the plane and six peaks below the plane confirming the presence of $3CH_3$, $6CH_2$ and 4CH groups in the molecule.

Antioxidant activity: The antioxidant activity of compounds **(3a-f)** was studied using free radical DPPH scavenging assay as described by Brand-Williams *et al*. [19]. The concentration required to capture 50% of the free radical DPPH (IC_{50}) was calculated. Ascorbic acid (µg/mL) used as a reference standard. Ropivacaine and compound **3f** showed the highest activity, while other compounds are showed the least activity. Besides all the compounds show lesser activity compared to standard ascorbic acid, which showed the IC_{50} 27.20. The antioxidant activity results are given in Table-1.

TABLE-1
DPPH ANTIOXIDANT STUDIES OF COMPOUNDS **3a-f**

Compound	IC_{50}
RVH	42.12
3a	102.2
3b	88.23
3c	82.68
3d	94.06
3e	50.81
3f	83.19
Ascorbic acid (STD)	27.42

***in vitro* Cytotoxic studies on MCF-7 cancer cell lines:** *in vitro* Antitumor evaluation of the synthesized compounds was carried out, with compounds **3a-f** being screened for anticancer activity using MCF-7 tumour cells, with ropivacaine as the standard drug. The anticancer studies were studied of the synthesised compounds using MTT assay, and the results show that compounds **3c**, **3f** and **RV** exhibited excellent anticancer activity, while compounds **3e**, **3d** and **3a** showed a moderate activity. From the above anticancer activity results, the methyl substitution at *ortho*-amine plays an important role in anticancer activity. When both *ortho*-positions were blocked by methyl group (ropivacaine) exhibited good anticancer activity, even though compounds **3f** and **3c** are very similar in structure to ropivacaine, but have slightly lesser activity compared to ropivacaine, which may be due to additional methyl group attached at *para*-position and reduction of one methyl group at the *ortho*-position. Besides if the compound consist of one methyl group at *ortho*-position while other methyl group is present at an alternative place, the activity would be less. From this, it is clear evidence that both *ortho*-substitutions are significant for good antioxidant activity. This may be due to the two methyl group adjacent to the amine restricted the rotation. The activity results are given in Table-2.

Antibacterial studies: All the synthesized compounds were screened for their antibacterial efficacy against a Gram-positive and Gram-negative pathogenic bacteria including resistant strains *E. coli* (Gram-negative) and *Bacillus subtilis* (Gram-positive). All the compounds shows no inhibition against both Gram-positive and Gram-negative bacteria.

TABLE-2
IC_{50} VALUES (EXPRESSED IN μg^{-1}) FOR COMPOUNDS **3a-f**, AGAINST MCF-7 CELLS

Compound	IC_{50} (µg)
RV	22.34
3c	38.35
3f	30.29
3e	51.75
3d	57.05
3b	75.43
3a	57.37

Pharmacokinetic properties prediction: Physio-chemical properties and the pharmacokinetic properties (ADME) of the synthesized compounds were assessed to evaluate the drug-likeness of the compounds. The *Qikprop 3.5* module of Schrödinger software was used to calculate physically significant descriptors and pharmaceutically relevant properties for all the hit compounds. The drug-likeness nature of synthesized compounds were calculated based on Lipinski's rule of five [20]. Based on Lipinski's rule of five, basic requirement of drug should possess molecular weight (mol_MW) of less than 500 amu, Hydrogen bond donor < 5 (Accpt HB) and hydrogen bond acceptor < 10 (Donor HB). Molecular descriptors of the compound like SASA (total solvent accessible surface area), FOSA (hydrophobic component of SASA), FISA (hydrophilic component of SASA), PISA (π component of SASA), WPSA (weakly polar component of the SASA), volume (total solvent-accessible volume in cubic angstroms), QP log S (predicted aqueous solubility, log S), QPlog BB (predicted brain/blood partition coefficient) are likely to satisfy their admissible range for a drug. SASA, PISA, WPSA descriptors for a drug should be well within 300.0-1000.0, 0.0-450.0 and 0.0-175.0 range, respectively. The descriptors FISA and FOSA describing the hydrophilic component and hydrophobic component of SASA should be at 7.0-330.0 and 0.0-750.0 range. The predicted brain/blood partition coefficient should be between -3.0 to 1.2 [21,22]. All the synthesized compounds obey the Lipinski rule of five and molecular descriptors without any penalty. The corresponding values of molecular descriptors are well within the range (Table-3).

Molecular docking study: Molecular docking study was carried out to find the binding affinity and interactions between the compounds (**2a-f** & **3a-f**) and the ALDH1A1 protein (PDB ID: 5L2N). The binding score of compounds **2a-f** and **3a-f** was varies from -4.322 to -8.077 (Table-4). Compounds **2d**, **2e**, **2a** and **3f** have hydrogen bond interaction with Gly458. Compounds **2c** and **2b** has hydrogen bond interaction with phe466 and compounds **3a**, **3b**, **3c** has hydrogen bond interaction with Cys302. Out of 12 compounds, compounds **2d**, **2e**, **2f** and **3e** showed good binding score with protein 5L2N. The interaction between the protein (PDB Id: 5L2N) and compounds are given in Figs. 2 and 3.

Conclusion

A series of ropivacaine analogues **(3a-f)** were synthesized by altering the methyl group present in the aromatic ring. It was further characterized by using advanced spectroscopic and

TABLE-3
ADME PROPERTIES OF ROPIVACAINE ANALOGUES

Compd.	MW	Dipole	SASA	FOSA	FISA	PISA	WPSA	Volume	Qp log Po/w	Qp log S	QP log BB	Donor HB	Accpt HB
2a	232.3	4.49	492.5	326.9	59.1	106.4	0	843.3	1.947	-2.257	0.328	2	4
2b	232.3	5.58	495.4	326.4	49.1	119.8	0	843.6	2.031	-2.305	0.41	2	4
2c	218.3	5.91	484.8	279.1	44.0	161.6	0	809.5	1.902	-2.206	0.405	2	4
2d	232.3	5.99	519.6	366.3	60.5	92.8	0	871.6	2.08	-2.71	0.282	2	4
2e	232.3	4.84	524.3	354.7	57.6	111.9	0	871.5	2.117	-2.789	0.299	2	4
2f	246.3	4.37	527.3	421.6	35.8	69.8	0	905.5	2.444	-2.828	0.518	2	4
3a	274.4	5.37	561.6	432.1	25.8	103.6	0	999.1	3.092	-3.033	0.488	1	4.5
3b	274.4	5.88	557.6	407.5	28.2	121.7	0	998.6	3.097	-2.962	0.47	1	4.5
3c	260.4	4.68	511.8	330.1	42.9	138.8	0	923.7	2.559	-2.168	0.364	1	4.5
3d	274.4	4.89	592.7	473.6	35.4	83.5	0	1029.7	3.184	-3.58	0.379	1	4.5
3e	274.4	4.47	546.6	430.9	42.7	72.9	0	986.4	2.868	-2.771	0.35	1	4.5
3f	288.4	4.93	601.6	506.0	22.6	72.9	0	1062.2	3.467	-3.738	0.505	1	4.5

TABLE-4
XP GLIDE RESULTS OF COMPOUND **2a-f** AND **3a-f** WITH PROTEIN 5L2N

Compounds	XP GScore	XP PhobEn	Glide evdw	Glide ecoul	Glide energy	XP H Bond	Interacting residues
2d.sdf	-7.515	-1.47	-30.658	-2.055	-32.714	-0.67	Gly458
3e.sdf	-8.077	-1.778	-32.783	-2.653	-35.436	0	–
2e.sdf	-7.314	-1.624	-29.555	-2.206	-31.761	-0.432	Gly458
2a.sdf	-6.569	-1.825	-26.941	-1.159	-28.1	0	Gly458
2c.sdf	-6.51	-1.518	-25.962	-3.621	-29.582	0	Phe466, Trp178
2f.sdf	-7.037	-1.739	-31.13	-0.344	-31.474	0	–
2b.sdf	-6.827	-1.837	-28.793	-1.994	-30.787	0	Phe466
3a.sdf	-6.879	-0.832	-33.407	-3.244	-36.65	0	Cys302, Tyr297
3f.sdf	-6.563	-1.35	-30.697	1.585	-29.112	-0.027	Gly458, Phe171
3d.sdf	-6.503	-1.35	-28.517	-0.716	-29.234	0	–
3c.sdf	-5.734	-1.503	-33.883	-2.63	-36.513	-0.307	Trp178, Cys302
3b.sdf	-4.322	-0.76	-32.221	-2.975	-35.195	0	Cys302, Tyr297

Glide evdw = van der Waals interaction energies, Glide ecoul = Coulomb interaction energies.

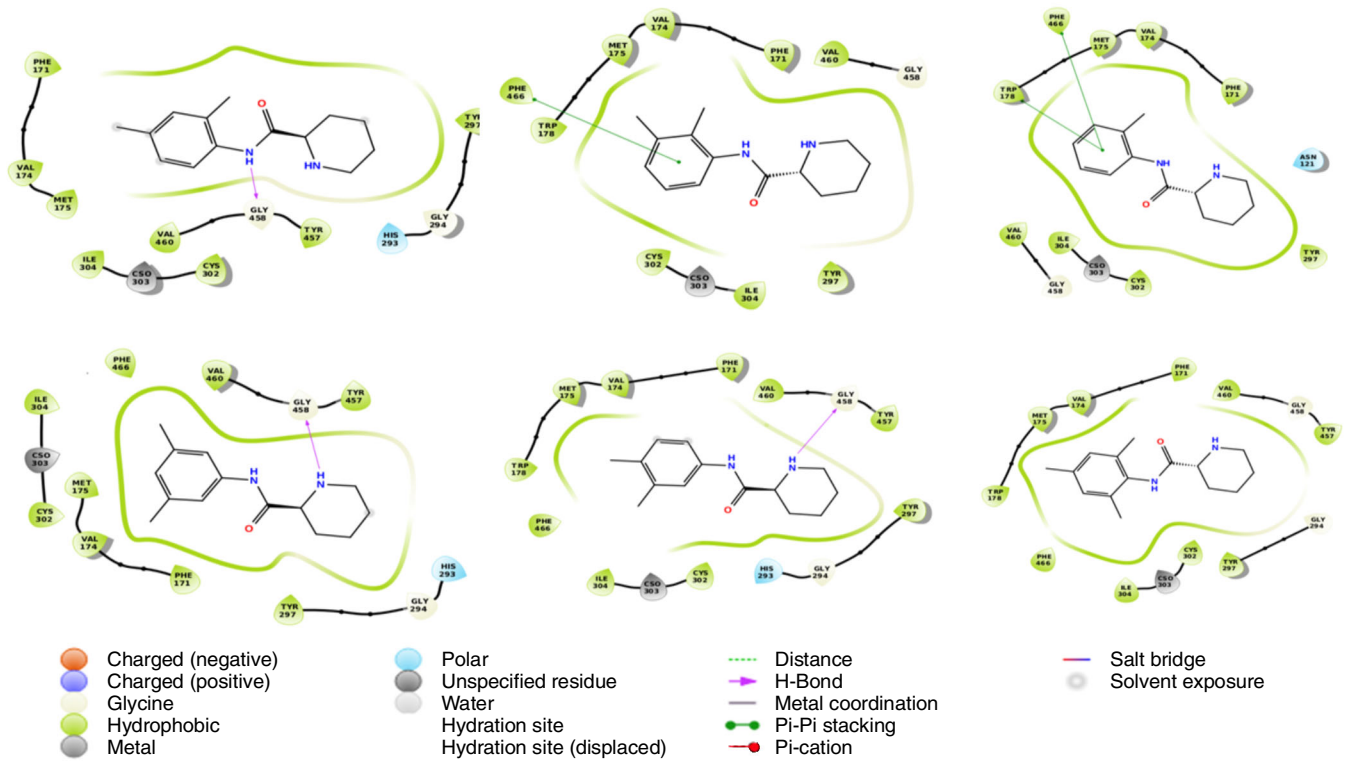


Fig. 2. 2D ligand interaction with protein 5L2N of compound **2a-f**

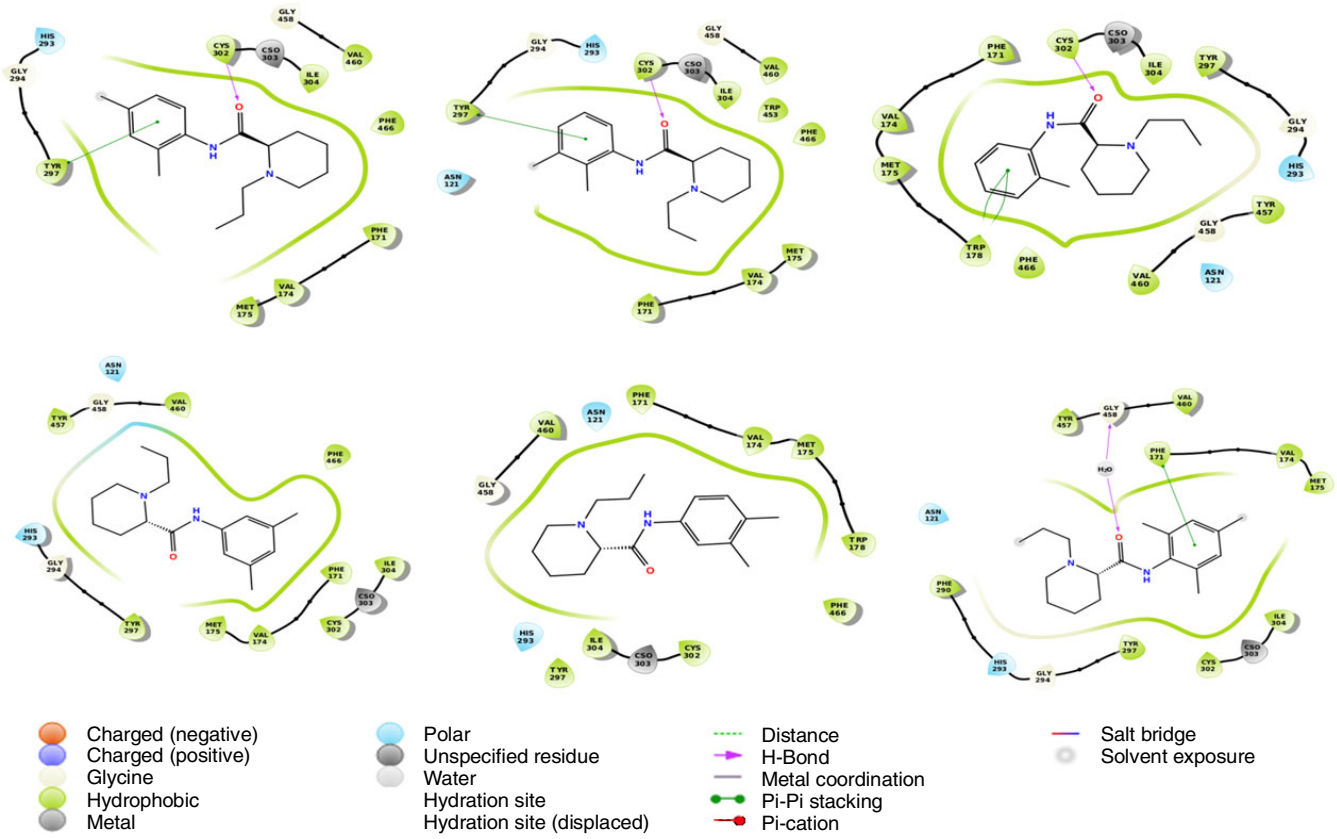


Fig. 3. 2D ligand interaction with protein 5L2N of compound **3a-f**

spectrometric techniques like IR, NMR (^{1}H, ^{13}C and DEPT analysis) and HRMS. From the biological activity studies, it is concluded that methyl group present in the aromatic ring play an important role, where compounds **3f** and **3c** showed a moderate antioxidant and anticancer activity. Besides that, none of the synthesized compounds (**3a-f**) showed antibacterial activity.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. Y.A. Ruetsch, T. Böni and A. Borgeat, *Curr. Top. Med. Chem.*, **1**, 175 (2001); https://doi.org/10.2174/1568026013395335
2. W. Li, L. Ding, H.-M. Liu and Q. You, *Med. Chem. Res.*, **27**, 954 (2018); https://doi.org/10.1007/s00044-017-2118-0
3. B.F. Tullar, *J. Med. Chem.*, **14**, 891 (1971); https://doi.org/10.1021/jm00291a033
4. M.D. Karkas, *Chem. Soc. Rev.*, **47**, 5786 (2018); https://doi.org/10.1039/c7cs00619e
5. J.H. Mcclure, *Br. J. Anaesth.*, **76**, 300 (1996); https://doi.org/10.1093/bja/76.2.300
6. S.R. Verner, Requesting a quote *S*-(-)-1-Propyl-2′,6′-pipecoloxylidide Hydrochloride Monohydrate, Process for its Preparation and Pharmaceutical Preparation Containing It, European Patent, EP0239710 A1 (1990).
7. B. Akerman, I.B. Hellberg and C. Trossvik, *Acta Anaesthesiol. Scand.*, **32**, 571 (1988); https://doi.org/10.1111/j.1399-6576.1988.tb02788.x
8. A. De Iuliis, L. Zanatta, E. Vincenti and L. Galzigna, *Il Farmaco*, **56**, 153 (2001); https://doi.org/10.1016/S0014-827X(01)01043-6
9. M.B. Dalvi, R.S. Kenny and G.R. Kawle, Process for Preparation of (DL)-Norepinephrine Acid Addition Salt, A Key Intermediate of (*R*)-(-)-Norepinephrine, World Patent WO 2014/009964 A1 (2014).
10. E.H. Howard, C.F. Cain, C.W. Kang and J.R. Del Valle, *J. Org. Chem.*, **85**, 1680 (2020); https://doi.org/10.1021/acs.joc.9b02382
11. O. Yosef-Brauner, N. Adi, T. Ben Shahar, E. Yehezkel and E. Carmeli, *J. Clin. Diagn. Res.*, **9**, 1 (2015); https://doi.org/10.1111/crj.12091
12. O.N. Aydin, M. Eyigor and N. Aydin, *Eur. J. Anaesthesiol.*, **18**, 687 (2001); https://doi.org/10.1097/00003643-200110000-00008
13. C. Raedler, C. Lass-Florl, F. Puhringer, C. Kolbitsch, W. Lingnau and A. Benzer, *Br. J. Anaesth.*, **83**, 657 (1999); https://doi.org/10.1093/bja/83.4.657
14. R.M. Schmidt and H.S. Rosenkranz, *J. Infect. Dis.*, **121**, 597 (1970); https://doi.org/10.1093/infdis/121.6.597
15. J.T. Murphy, H.F. Allen and A.B. Mangiaracine, *Arch. Ophthal.*, **53**, 63 (1955); https://doi.org/10.1001/archopht.1955.00930010065006
16. A. Venkanna, B. Siva, B. Poornima, P.R. Rao Vadaparthi, K.R. Prasad, K.A. Reddy, G.B.P. Reddy and K.S. Babu, *Fitoterapia*, **95**, 102 (2014); https://doi.org/10.1016/j.fitote.2014.03.003
17. Protein Preparation Wizard, Epik version 2.3, 2012; Impact version 5.7, Schrödinger, LLC, New York (2012).
18. W.L. Jorgensen, D.S. Maxwell and J. Tirado-Rives, *J. Am. Chem. Soc.*, **118**, 11225 (1996); https://doi.org/10.1021/ja9621760
19. W. Brand-Williams, M.E. Cuvelier and C. Berset, *LWT-Food Sci. Technol.*, **28**, 25 (1995); https://doi.org/10.1016/S0023-6438(95)80008-5
20. C.A. Lipinski, F. Lombardo, B.W. Dominy and P.J. Feeney, *Adv. Drug Deliv. Rev.*, **23**, 3 (1997); https://doi.org/10.1016/S0169-409X(96)00423-1
21. F. Ntie-Kang, *Springerplus*, **2**, 353 (2013); https://doi.org/10.1186/2193-1801-2-353
22. Qikprop 3.5 user manual Schrodinger, LLC, New York, NY (2012).