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SYNTHESIS AND CHARACTERIZATION OF FIVE IMPURITIES OF RANOLAZINE

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ABSTRACT

Ranolazine (Ranexa) is a piperazine derivative, used as a second-line treatment in patients with stable or poorly controlled chronic angina and unresponsive to other drugs. Five known impurities of ranolazine were synthesized individually and characterization of these five impurities were done using the spectral data (IR, NMR and mass). The structure of all five impurities were assigned as *N*-(2,6-dimethylphenyl)-2-(piperazin-1-yl) acetamide, 4-(2-methoxyphenoxy) butane-1,3-diol, 2,2'-(4,4'-(2-hydroxypropane-1,3-diyl) bis(piperazine-4,1-diyl)) bis(N-(2,6-dimethylphenyl) acetamide), 2-((2-methoxyphenoxy) methyl) oxirane and *N*-(2,6-dimethylphenyl)-2-(4-(2-hydroxy-3-(4-methoxyphenoxy) propyl) piperazin-1-yl) acetamide respectively. The present work describes synthesis and characterization of these impurities of ranolazine.

KEYWORDS: Characterization, Impurities, Ranolazine, Synthesis.

INTRODUCTION

Chronic stable angina is the most common manifestation of ischaemic heart disease in the developed world and is associated with impaired quality of life and increased mortality.[1] Ranolazine (Ranexa) is a piperazine derivative [RS-43285; (6)-N (2,6-dimethyl-phenyl)-4[2-hydroxy-3(2-methoxy-phenoxy) propyl] 1-piperazine acetamide)]. Ranolazine is used as a second-line treatment in patients with stable or poorly controlled chronic angina pectoris and unresponsive to other drugs. This non-hemodynamic anti-angina agent, patented in 1986 and approved by the Food and Drug Administration in 2006, is prescribed in the United States, Japan, and some European countries. However, ranolazine has other potential beneficial therapeutical effects in various cardiovascular pathologies, including post-operative, new-onset, paroxysmal, and chronic atrial fibrillation (AF), ventricular arrhythmias (VA), revascularization, coronary artery disease, diastolic and microvascular dysfunction, metabolic diseases, and diabetes.[2] The major mechanism of action of ranolazine is to inhibit late I_{Na} thus preventing sodium overload of the cell. As a consequence, ranolazine prevents reverse mode sodium–calcium exchange and thus diastolic accumulation of calcium possibly resulting in improved diastolic tone and improved coronary blood flow. Accordingly, ranolazine has been shown to decrease post-ischemic contracture in rabbit isolated perfused hearts subjected to ischemia and reperfusion.[3]

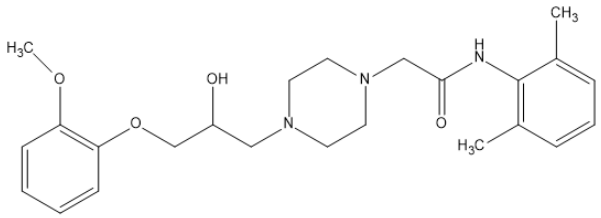

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Fig 1: structure of ranolazine.

Pharmaceutical impurities are those substances which co-exist with the API or they may develop during synthesis or ageing of both API and formulation. The presence of these impurities even in minor amounts can influence the efficacy and safety of drug.[4] Most active pharmaceutical ingredients (API) are produced by organic chemical synthesis. Various components, including residual solvents, trace amounts of inorganic, and organic components can be generated during such a process. Those components remaining in the final API are considered as impurities.[5] The ICH quality guidelines note that impurities can arise from a variety of places including: starting materials, by-products, intermediates, degradation products, reagents, ligands and catalysts.[6]

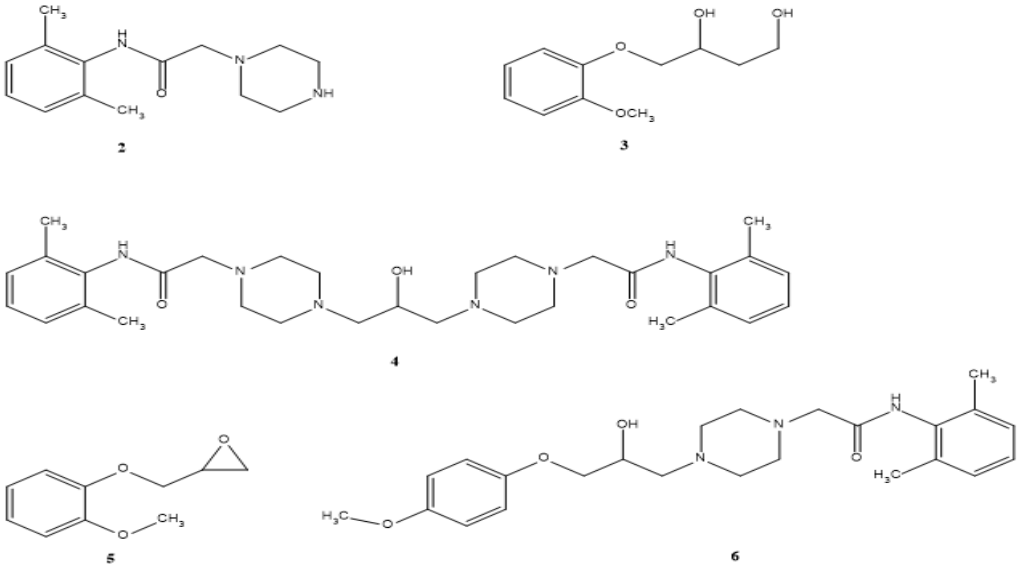


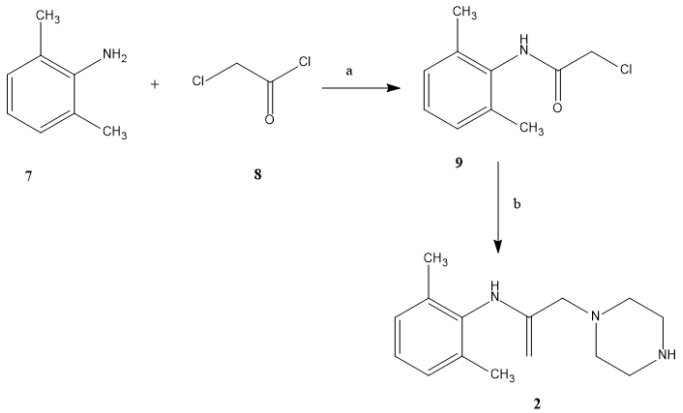
Fig 2. structure of impurities.

MATERIALS AND METHOD

Melting points of the synthesized compounds were determined using open microcontroller-based Melting point apparatus, CL 725/726 and were found uncorrected. Purity of the compound was checked by thin layer chromatography using silica gel 60 F254 recoated silica gel G (20 × 20 cm) as stationary phase and various combinations solvent as mobile phase. These plates procured from E-Merc, Darmstadt, Germany. These spots resolved were visualized as dark coloured spots by using UV chamber. The techniques employed for the characterization of the synthesized compounds were IR spectra, 'H-NMR, Mass spectra. The IR spectra of the synthesized compounds were recorded using KBr pellets in range of 4000-400 cm:' on a Fourier transform IR spectrometer (model Shimadzu 8700) and the frequencies were recorded in Wave numbers. H-NMR (400 MHz) spectra were recorded in chloroform-d and in DMSO-d in Amx-400 MHz liquid state NMR spectrometer BRUCKER. Chemical shifts (8) are reported in parts per million downfield from internal reference Tetra methyl Silane (TMS) as well as Mass spectra also taken for identification of derivatives. The chemicals and reagents used in the present project were of AR grade and LR grade, purchased from S.D. Fine chem. Ltd., Merck, LOBA Chemicals, Mumbai, India.

1. Synthesis of *N*-(2,6-dimethylphenyl)-2-(piperazin-1-yl) acetamide (2).

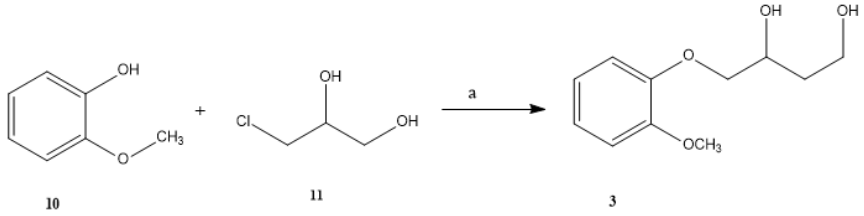
In a 250 ml RBF, 1 gm of 2,6-Dimethylaniline **(7)** was dissolved in 40 ml of DCM, and then added 0.17g of TEA at Room temperature. After 10 minutes 0.093g of chloroacetyl chloride **(8)** was added slowly with constant stirring. Then reaction was kept for stirring over 30 minutes at RT. After completion of the reaction, (reaction was monitored by TLC/LCMS) reaction mass was quenched with water and extracted with DCM. The organic layer was collected, concentrated. In a 250 ml RBF, Stage-01 product (2.0g, 0.010152mole) was dissolved with ACN (40 ml), then added K_2CO_3 (4.2g, 0.03043 mole) at Room temperature. After 30 minutes piperazine (0.8 g, 0.010152 moles) was added slowly with constant stirring. Then reaction was kept for stirring over 3 hours at 80^{o}C. After completion of the reaction, (reaction was monitored by TLC/LCMS) reaction mass was filtered off, filtrate was collected and concentrated and directly taken to purification. Purified by re-crystallization by using methanol and hexane.



Scheme 1: reagents and conditions a) TEA, DCM b) K2CO3, CAN.

2. Synthesis of 4-(2-methoxyphenoxy) butane-1,3-diol (3).

In a 250 ml RBF, 1gm of guaiacol (**10**) was dissolved in ACN. 1.2 equivalence of K2CO3 was added. Stir the reaction mixture for few minutes. Add 1.2 equivalence of 3-chloro-1,2-propanediol (**11**) was added drop wise. Reaction mixture was heated to 80°C for 6 hours. Completion of the reaction is monitored by TLC/LC-MS. Finally, the reaction mixture is filtered and concentrated under reduced pressure.

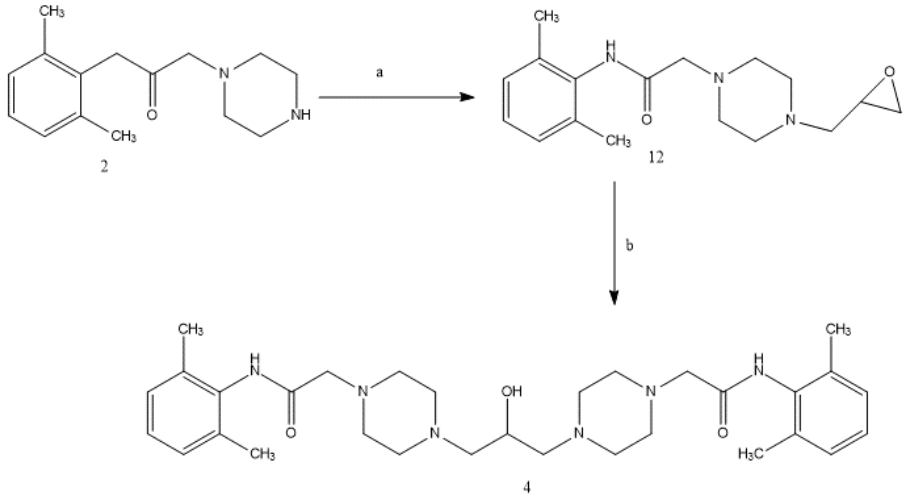


10 11 3

Scheme 2: reagents and conditions a) K2CO3 and CAN.

3. Synthesis of 2,2'-(4,4'-(2-hydroxypropane-1,3-diyl) bis(piperazine-4,1-diyl)) bis(*N*-(2,6-dimethylphenyl) acetamide) (4).

In a 250 ml RBF, 1 gm of ranolazine impurity 1 **(2)** was dissolved with water (20 mL), then added epichlorohydrin (0.749 g, 0.008095mokes) and TBAB (0.79g, 0.00201 moles) at 0-15°C. Then NaOH solution was added slowly over 30 minutes with constant stirring at 0-15°C. Then reaction was kept for stirring over 1 hour at 0°C and stand over 3 hours at room temperature. After completion of the reaction, (reaction was monitored by TLC/LCMS) reaction mass extracted with toluene 3 times. The organic layer was collected and concentrated and taken to next step. In a 250 ml RBF,2 gm of stage-01 product **(12)** was dissolved with ACN (40 mL), and then added TEA (4.2g, 0.03043 moles) at Room temperature. Then reaction was kept for stirring over 24 hours at 80°C. After completion of the reaction, (reaction was monitored by TLC/LCMS) reaction mass was concentrated and directly taken to purification. Purification done by column chromatography using ethyl acetate and hexane.

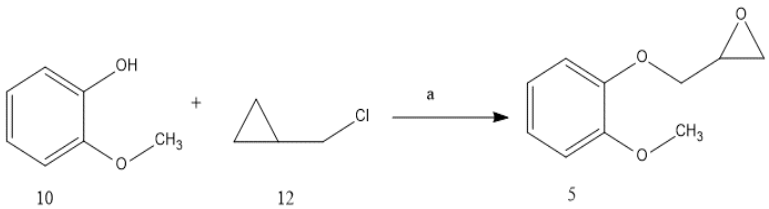


2 12 4

Scheme 3: Reagent and condition a) epichlorohydrin, TBAB and H2O b) TEA and CAN.

4. Synthesis of 2-((2-methoxyphenoxy) methyl) oxirane.[5]

In a 250 ml RBF, 1 gm of 2-methoxyphenol **(10)** was dissolved in ACN. 1.2 equivalence of K2CO3 was added. Stir the reaction mixture for few minutes. 1.2 equivalence of epichlorohydrin **(12)** was added drop wise with continuous stirring. The reaction mixture was heated to 80°C for 6 hours. Completion of the reaction was monitored by TLC/LC-MS. Finally, the reaction mixture was filter and concentrated under reduced pressure. It is purified using column chromatography using methanol and DCM.

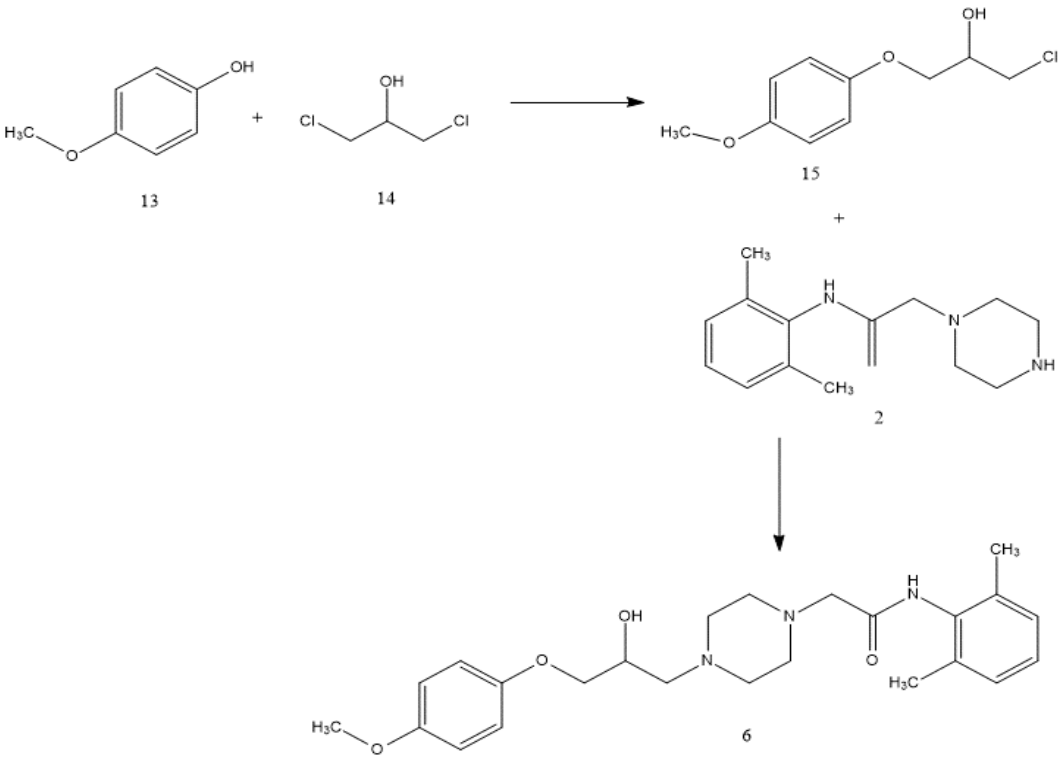


10 12 5

Scheme 4: Reagent and condition a) K2CO3 and ACN

5. Synthesis of *N*-(2,6-dimethylphenyl)-2-(4-(2-hydroxy-3-(4-methoxyphenoxy) propyl) piperazin-1-yl) acetamide.[6]

In a 250 ml RBF, 1 gm of 4-methoxy phenol **(13)** was dissolved in ACN. 1.2 of K2CO3 was added. Stir the reaction mixture for few minutes.1.2 equivalence of 1,3-dichloropropan-2-ol **(14)** with continuous stirring. The reaction mixture was heated to 80°C. Reaction completion was monitored by TLC. Filter the reaction mixture and concentrated under reduced pressure and taken to next stage. In an RBF stage 1 product **(15)** was dissolved in ACN with stirring. 1.2 equivalence of K_2CO_3. Stir the reaction for few minutes. 1.2 equivalence of ranolazine impurity was added with continuous stirring. The reaction mixture is heated to 80°C for 6 hours. Completion of the reaction was monitored by TLC/LC-MS. Finally, the reaction mixture is filtered and concentrated under reduced pressure.



Scheme 5: Reagent and condition a) K2CO3 and ACN b) K2CO3 and CAN.

RESULT AND DISCUSSION

Physical characterization and spectral data

***N*-(2,6-dimethylphenyl)-2-(piperazin-1-yl) acetamide (2)** is a white colour solid; molecular weight-247.34g/mol; solubility- Methanol; melting point range-174-176 °C; percentage yield-93.13%; TLC solvent system-20 % ethyl acetate and hexane; Rf value-1.0.

4-(2-methoxyphenoxy) butane-1,3-diol (3) is an off-white colour solid; molecular weight-198.10 g/mol; Solubility- Methanol; melting point range-195-197 °C; Percentage yield-93.3%; TLC solvent system-5% methanol and DCM; Rf value-1.6.

IR (ν cm−1): 3216 cm^{-1}; broad peak hydrogen bonded OH stretching of OH group, 3073 cm^{-1}; aromatic CH stretching, 2962, 2883, 2932, 2837 cm^{-1}; CH stretching of CH2 moiety, 2941 & 2857 CH3 of OCH3 & CH2 of OCH2 groups both asymmetric and symmetric, 1593, 1507 & 1454 cm^{-1}; C=C ring stretching, 1454 & 1376, 1439 & 1346, 1408 & 1328 cm^{-1}; CH bending of CH3 of OCH3, CH2 of OCH2 & CH2 groups both, 1295 cm^{-1}; CO stretching, 1253 cm^{-1} OH bending, 1295 cm^{-1}; CO stretching, 1126 &1020 cm^{-1}; COC stretching, 741 & 667 cm^{-1}; 1,2 disubstituted benzene.

^{1}H-NMR (DMSO) $\delta\delta$ ppm: The compound shows its NMR signals in the following regions.
δ3.3; 2H, s, CH2 of CH2OH, δ3.8; 5H, m, 3H of OCH3, δ3.9; 1H, s, hydrogen of OH of CH2OH, δ4.9; 1H, s, H of OH of CHOH, δ6.8-7.0; 4H multiplate, Aromatic proton.
Mass (ESI-MS): Molecular weight of the compound is 198 g/mol. Molecular ion peak M+1 is observed at m/z value 198. Hence the molecular ion peak value agrees with molecular weight of the compound.

2,2'-(4,4'-(2-hydroxypropane-1,3-diyl) bis(piperazine-4,1-diyl)) bis(N-(2,6-dimethylphenyl) acetamide) (4) is a White colour solid; soluble in methanol; Molecular weight-550.74 g/mol; Melting point range-265-267 °C; Percentage yield-22.72%; TLC solvent system-5% methanol and DCM; Rf value-0.2.

IR (ν cm−1): 3336 cm^{-1}; OH stretching of OH group, 3290 cm^{-1}; NH stretching of NH group, 3023 cm^{-1};

aromatic CH stretching, 2948 & 2878, 2938 & 2819 cm^{-1}; CH stretching of CH3 & CH2 groups, 1673 cm^{-1}; C=O stretching of CO-NH, 1592, 1533, 1497, 1475 cm^{-1}; C=C ring stretching, 1465, 1434, 1359, 1329 cm^{-1}; bending of CH2 & CH3 groups, 1279 cm^{-1}; CO stretching, 1260 cm^{-1}; OH bending, 769 & 815 cm^{-1}; substituted benzene ring.

2-((2-methoxyphenoxy) methyl) oxirane (5) is a white to off-white colour solid; soluble in methanol; Molecular weight-180.20 g/mol; melting point range-220-220 °C; Percentage yield-45.27%; TLC solvent system-5% methanol and DCM; Rf value-1.6.

IR (ν cm−1): 3045 cm^{-1}; aromatic CH stretching, 2967 & 2891, 2936 & 2842 cm^{-1} CH stretching of CH3 of OCH3 & CH2 groups both symmetric and asymmetric, 1636,1616,1558,1539 cm^{-1}; C=C ring stretching, 1465, 1362, 1449 & 1345 cm^{-1}; CH bending of CH3 of OCH3 & CH2 groups both asymmetric and symmetric, 1256 cm^{-1}; CO stretching, 1022 cm^{-1}; COC stretching, 776 cm^{-1}; 2 substituted benzene ring.

^{1}H-NMR (DMSO) $\delta\delta$ ppm: The compound shows its NMR signals in the following regions.

δ 2.62; 3H, s, 2H of CH2 & H of ring, δ3.8 & 4.2; 5H, m, 3H of OCH3 and 2H of OCH2, δ6.9; 4H, m, aromatic proton.
Mass (ESI-MS): Molecular weight of the compound is 124 g/mol. Molecular ion peak M+1 is observed at m/z value 124 which satisfy the molecular structure of the compound.

***N*-(2,6-dimethylphenyl)-2-(4-(2-hydroxy-3-(4-methoxyphenoxy) propyl) piperazin-1-yl) acetamide (6)** is a white colour solid; soluble in methanol; Molecular weight-427.54 g/mol; Melting point range-185-187 °C; Percentage yield-43.6%; TLC solvent system-20% ethyl acetate and hexane; Rf value-0.2.

IR (ν cm−1): 3338 cm^{-1}; NH stretching of NH hydrogen bonded, 3036 cm^{-1}; aromatic CH stretching, 2953 & 2872, 2938 & 2829 cm^{-1}; CH stretching of CH3 & CH2 groups both symmetric and asymmetric, 1683 cm^{-1}; C=O of CO-NH, 1622, 1595, 1558, 1507 cm^{-1}; C=C ring stretching, 1462 & 1362, 1436 & 1334 cm^{-1}; CH bending of CH3 & CH2 group both asymmetric and symmetric, 1310 cm^{-1}; CN stretching, 1289 cm^{-1}; CO stretching, 1229 cm^{-1}; OH bending, 1022 cm^{-1} ; COC stretching, 712 & 740 cm^{-1}; mono & disubstituted phenyl ring.

CONCLUSION

Five impurities of ranolazine such as *N*-(2,6-dimethylphenyl)-2-(piperazin-1-yl) acetamide, 4-(2 methoxy phenoxy) butane-1,3-diol, 2,2'-(4,4'-(2-hydroxypropane-1,3-diyl) bis(piperazine-4,1-diyl)) bis(N-(2,6-dimethylphenyl) acetamide), 2-((2-methoxyphenoxy) methyl) oxirane and *N*-(2,6-dimethylphenyl)-2-(4-(2-hydroxy-3-(4-methoxyphenoxy) propyl) piperazin-1-yl) acetamide were synthesised and characterized using respective spectral data.

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