



Note

A novel route for the synthesis of impurities C and G of antihypertensive drug timolol maleate

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In this short communication has been described the synthesis and characterization of C and G impurities of Timolol maleate. The recent study introduces the initial synthesis of these impurities with both high yield and high purity. The purity of the impurities have been validated through High-Performance Liquid Chromatography (HPLC), while their structures have been determined utilizing ¹H NMR and mass spectroscopy.

Keywords: Timolol maleate, Impurity, Synthesis

Timolol maleate is a non-selective beta-adrenergic antagonist having antihypertensive properties. It is the maleate salt of timolol; a propanolamine derivative. Timolol is a medicine that can be taken orally or as ocular drops. It is used to treat conditions like ocular hypertension and glaucoma, also it is used to prevent migraines, to treat high blood pressure and chest pain caused by a lack of blood flow to the heart¹. Timolol can also be used to treat infantile haemangiomas, hypertension, myocardial infarction, and atrial fibrillation in rare cases. The active isomer, timolol maleate, was previously obtained *via* chemical resolution and was assumed to have the S configuration based on the stereochemistry of compounds interacting with the adrenergic receptor².

Impurities are chemicals that coexist with the API or arise during the synthesis or development of both the API and the formulation. Even minute levels of these impurities can have an impact on a drug's effectiveness and safety. The safety of a drug is determined not only by the active drug substance's toxicological qualities but also by the impurities it

contains. Because impurities in APIs can impact the safety and quality of pharmaceutical products, API impurity profiling is becoming more important. As a result, identifying, isolating, and quantifying impurities are an important part of drug development and regulatory assessment³. There are two categories of impurities: (a) impurities linked with active medicinal components and (b) impurities formed during formulating, storage, or manufactured forms⁴. Various pharmacopeias, such as the BP (British Pharmacopeia), USP (United States Pharmacopeia), IP (Indian Pharmacopeia), and others, are gradually adding limitations to the authorized quantities of impurities present in active pharmaceutical ingredients (APIs) or formulations⁵. The reported impurities of timolol maleate in the European and British Pharmacopoeia are as given in Fig. 1; Impurity A, Impurity B, Impurity C, Impurity D, Impurity E, Impurity F, Impurity G, Impurity H and Impurity I.

In comparison to the tedious task of isolating impurities from the final API, which does not provide sufficient purity and yield, The synthesis of impurities enables the utilization of their pure forms in elucidating their biological effects and serves as a standardized reference for the discernment of analogous impurities within the final product. The synthesis of impurities enables the utilization of their pure forms in elucidating their biological effects and serves as a standardized reference for the discernment of analogous impurities within the final product. To date, there are no synthetic methods have been published in the literature for impurities C and G. Here we present the synthesis of these two impurities with detailed structural characterization.

Results and Discussion

The chemical synthesis of timolol maleate was reported by Bredikhina, Zemfira A. *et al.*⁶ as shown in Scheme 1.

Synthesis of impurity C, 18

Compound 14 was formed by reduction of compound 13 using KOH, HCl, dimethyl sulfoxide, and water. Compound 14 was treated with sodium hydride (NaH)

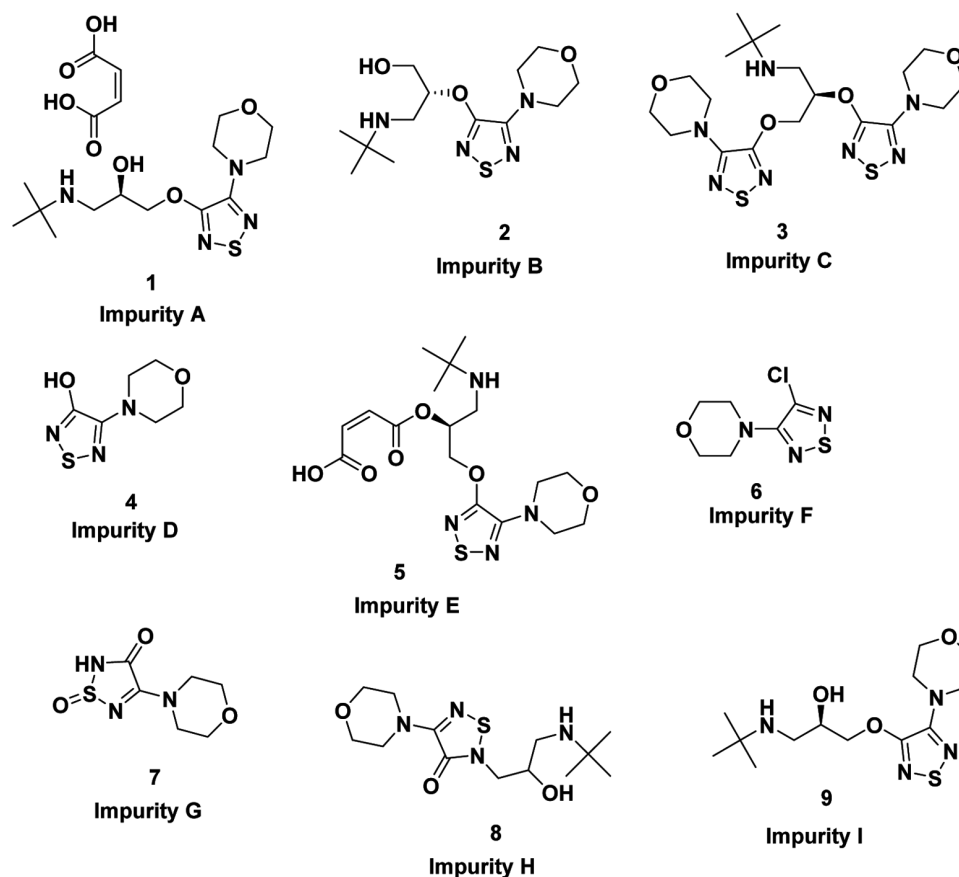
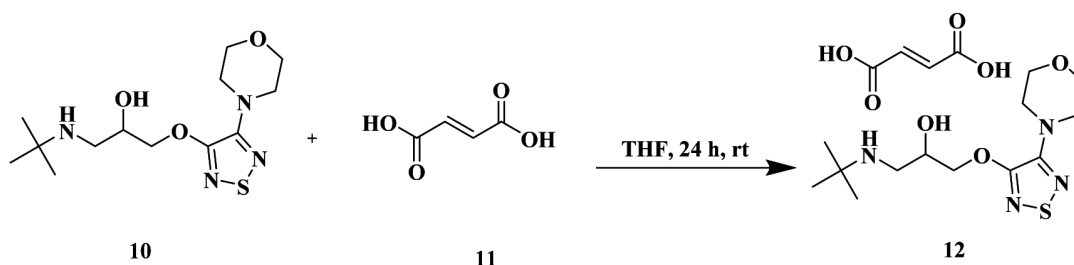


Fig. 1 — Timolol maleate impurities



Scheme 1 — Reported synthesis of Timolol maleate

and 3-chloro-1,2-propanediol, at 90-95°C for 20 h to get product **15**. Formation of compound **16** occurs by reacting **15** with thionyl chloride (SOCl₂) and triethylamine at 20°C. Compound **16** was treated with isobutylamine and dimethylformamide for 45 h at 60-70°C to get compound **17**. Compound **17** finally reacts with potassium carbonate (K₂CO₃) and 4-(4-chloro-1,2,5-thiadiazol-3-yl)morpholine by using DMF to get **18** (Scheme 2).

Synthesis of impurity G, **22**

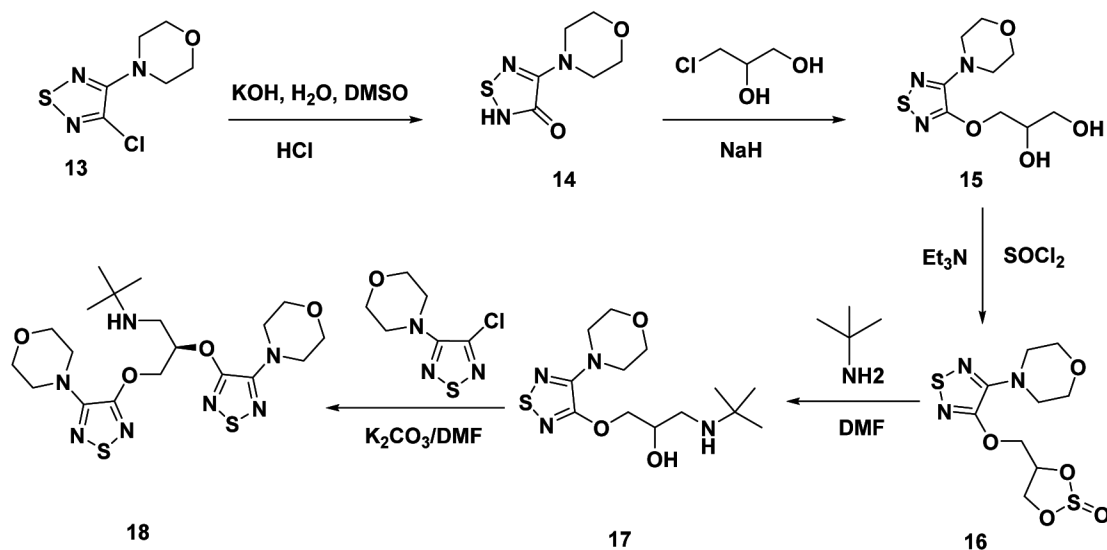
Compound **19** was treated with thionyl chloride (SOCl₂) and pyridine at RT for 2 h to get **20**.

Compound **20** was treated with pyrrolidine and ethanol to get **21**. Compound **22** (impurity G) was formed by reacting compound **21** with KOH and methanol (Scheme 3).

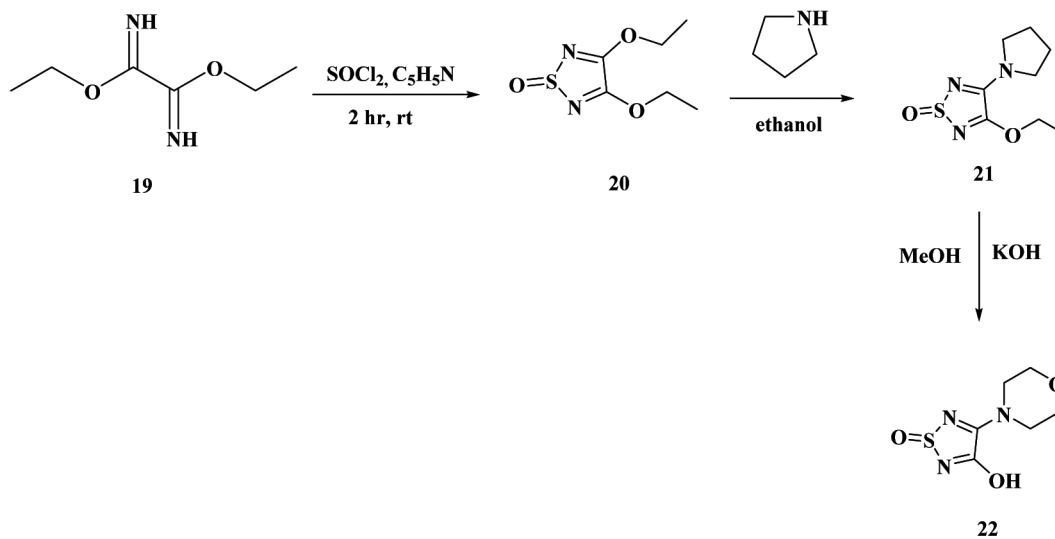
Experimental Section

Preparation of 4-morpholin-4-yl-1,2,5-thiadiazol-3-one, **14**

Compound **13** (5 g, 24.31 mmol) was mixed with DMSO (22 mL) and 4N aq. KOH (7.4 mL) at 25-28°C, and was allowed to reflux for 1-6 h with continuous stirring. The mixture was cooled to 0°C



Scheme 2 — Synthesis of Timolol maleate impurity C



Scheme 3 — Synthesis of Timolol maleate impurity G

and 25 mL of water was added. Using strong HCl, the reaction mixture was acidified, then filtered, and the white solid product was collected. Yield 3.75 g (82.4%). m.p.148-150°C. $^1\text{H NMR}$ (400 MHz, $\text{DMSO-}d_6$): δ 3.41 (4H, d), 3.64 (4H, d); MS: m/z Calcd for $\text{C}_6\text{H}_8\text{N}_3\text{O}_2\text{S}$ [M+H]: 188.21. Found: 188.22.

Preparation of (2S)-3-[(4-morpholin-4-yl)-1,2,5-thiadiazol-3-yl]oxy] propane-1,2-diol, 15

A suspension of sodium hydride (0.66 g, 16.5 mmol) was introduced to a dry dimethylformamide solution containing compound 14 (2.06 g, 11 mmol) under an argon atmosphere. A 1.5 h heating period was then

applied to the reaction mixture at 90–95°C, followed by subsequent cooling to RT. Mixture of (S)-3-chloropropane-1, 2-diol (S) (1.46 g, 13.2 mmol) in dimethylformamide (2 mL) was added to the the above cooled reaction mixture. Subsequently, the reaction mixture underwent heating and stirring for a duration of 20 h at 90-95°C. Following the completion of the reaction, the solvent was evaporated under reduced pressure. The obtained residue was diluted with 80 mL of water and subjected to extraction using ethyl acetate. Anhyd. MgSO_4 was used to dry the organic layers. The solvent was evaporated to get compound 15 (Ref. 6). (Yield 2.4 g, 86.34%). m.p.201.5-202.5°C. $^1\text{H NMR}$

(400 MHz, DMSO- d_6): δ 3.17-3.33 (4H, d), 3.43-3.55 (2H, d), 3.59-3.75 (4H, d), 3.90-4.10 (3H, q), 4.04 (2H,d); MS: m/z Calcd for $C_9H_{15}N_3O_4S$ [M+H]: 262.29. Found: 262.30.

Preparation of 4-(4-morpholino-1, 2, 5-thiadiazol-3-yl)oxy)methyl)-1,3,2-dioxathiolane 2-oxide, 16

Thionyl chloride $SOCl_2$ (1.19g, 10 mmol) was dissolved in 5 mL of dichloromethane. After adding the previously mentioned mixture to compound **15** (500 mg, 1.9 mmol), the mixture was stirred for 1 h at RT.

After completion of reaction, the solvent was distilled out to get product **16** (Ref. 7). Yield 321.3 mg (75.6%). m.p.205-207°C. 1H NMR (400 MHz, DMSO- d_6): δ 3.17-3.33 (4H,d), 3.59-3.75 (4H,d), 4.23 (1H,d), 4.32-4.43 (2H,d), 4.74-4.90 (2H,d); MS: m/z Calcd for $C_9H_{13}N_3O_5S_2$ [M+H]: 308.33. Found: 308.34.

Preparation of 2S-1-(tert-butylamino)-3-[(4-morpholin-4-yl-1,2,5-thiadiazol-3-yl)oxy]propan-2-ol, 17

Compound **16** (5 g, 16.27 mmol) was added to isobutylamine (1.6 g, 27 mmol) and dimethylformamide (6 mL) and the mixture was heated for 45 h at 60-70°C. After the reaction was finished, the mixture was washed with a 1N NaOH (20 mL) solution, and the residue was extracted into ethyl acetate. To obtain product **17**, the extract was dried over anhyd. $MgSO_4$ and the solvent evaporated. Yield 3.71 g (76.5%). m.p.201-202°C. 1H NMR (400 MHz, DMSO- d_6): δ 1.26 (9H, s), 2.73-2.85 (2H, d), 3.17-3.33 (4H, d), 3.59-3.75 (4H, d), 3.85 (1H, t), 4.02-4.14 (2H,d); MS: m/z Calcd for $C_{13}H_{24}N_4O_3S$ [M+H]: 317.42. Found: 317.45.

Preparation of (R)-N-(tert-butyl)-2,3-bis((4-morpholino-1,2,5-thiadiazol-3-yl)oxy)propan-1-amine, 18

Compound **17** (5 g, 15.8 mmol) was added to potassium carbonate K_2CO_3 (2.18 g) and dimethylformamide (20 mL). The reaction mixture was subjected to agitation for a period of 2 h under RT conditions. Product **18** was obtained after the residue was extracted into ethyl acetate. Yield 2.64 g, (81.3%). m.p.210-212°C. 1H NMR (400 MHz, DMSO- d_6): δ 1.08 (9H, s), 1.23 (2H,s), 2.74-2.85 (2H,d), 2.88 (1H,d), 3.37-3.38 (4H,d), 3.60-3.64 (8H,d), 4.67-4.69 (3H,t), 4.83 (1H,d), 5.26 (1H,m); MS: m/z Calcd for $C_{19}H_{31}N_7O_4S_2$ [M+H]: 486.19. Found: 486.20. HPLC purity 99.81%.

Preparation of 3, 4-diethoxy-1, 2, 5-thiadiazole 1-oxide, 20

To the compound **19** (5 g, 34.68 mmol), pyridine (7.8 mL, 98.6 mmol) was added. Then to above solution, thionyl chloride $SOCl_2$ (1.95 mL, 16.52 mmol) was added drop-wise. The reaction mixture was stirred for a duration of 2 h under ambient conditions. After completion of reaction, the product was washed with *n*-hexane, and dried to get compound **20** (Ref. 8). Yield 2.73 g (72%). m.p.74-78°C. 1H NMR (400 MHz, DMSO- d_6): δ 1.16 (6H, t), 4.15-4.27 (3H, q), 4.21 (1H, q); MS: m/z Calcd for $C_6H_{10}N_2O_3S$ [M+H]: 191.21. Found: 191.22.

Preparation of 3-ethoxy-4-pyrrolidin-1-yl-1, 2, 5-thiadiazole 1-oxide, 21

A solution of compound **20** (1.85 g,10 mmol) in ethanol (10 mL) was added to pyrrolidine (0.72 g,10 mmol). After being stirred for 0.5 h at RT, the mixture was concentrated to one-fourth of its original volume and then diluted with ether. The crystals were vacuum-dried after being filtered⁸. Yield 1.3 g (3%). m.p.62-64°C. 1H NMR (400 MHz, DMSO- d_6): δ 1.22 (3H, t), 1.89-2.09 (4H, d), 3.35-3.53 (4H,d), 4.19-4.31 (2H,q); MS: m/z Calcd for $C_8H_{13}N_3O_2S$ [M+H]: 216.27. Found: 216.30.

Preparation of 4-morpholin-4-yl-1-oxo-1,2,5-thiadiazol-3-one, 22

To compound **21** (1.15 g), 6 mL of 0.85 M KOH was added then methanol was added. The reaction mixture was then stirred for 30 min. After the completion of reaction, the precipitated product was separated from the rest of the mixture. Yield 1.0 g (82.5%). m.p.72-74°C. 1H NMR (400 MHz, DMSO- d_6): δ 3.41-3.43 (2H, d), 3.67-3.69 (4H, d), 4.28 (2H, d); MS: m/z Calcd for $C_6H_9N_3O_3S$ [M+H]: 204.21. Found: 204.00. HPLC purity: 99.33%.

Conclusion

Our current study demonstrates a simple way for obtaining a high-quality Timolol impurity reference for quality control of Timolol maleate. Two impurities of Timolol maleate, C, and G were effectively synthesized using a simple, fast protocol and characterized. This synthesis not only aids in the production of high-quality medicinal substances but also aids in the establishment of an impurity profile.

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Supplementary Information

The analytical spectral data is available in the website <http://nopr.niscpr.res.in/handle/123456789/58776>.

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