INTRODUCTION

Prasugrel hydrochloride (1), an important platelet inhibitor is used for the reduction of thrombotic cardiovascular events. During laboratory optimization and later its bulk synthesis the formation of various impurities was observed. The impurities formed were monitored and their structures were tentatively assigned on the basis of their fragmentation patterns in LC-MS. Most of the impurities were synthesized and their assigned constitutions confirmed by co-injection in HPLC. We describe herein the formation, synthesis and characterization of these impurities. Present study will be of immense help to others to obtain chemically pure prasugrel hydrochloride.

Key Words: Impurity profile, Prasugrel, Related substances.

During laboratory optimization of prasugrel hydrochloride (1), many process related impurities were identified. The guidelines recommended by ICH state that the acceptable levels for a known and unknown compound (impurity) in the drug should be less than 0.15 and 0.10 %, respectively, also EMEA document state that the acceptable levels for a 3-fluro prasugrel and desacetyl prasugrel in the drug should be less than 0.20 %. In order to meet the stringent regulatory requirements, the impurities present in the drug substances must be identified and characterized. Literature reports include impurities formed due to either incomplete acetylation (e.g., 4) or during conversion of prasugrel hydrochloride (e.g., 7). However, no synthetic details have been reported. In this context, the present study described the identification, synthesis and characterization of impurities formed during prasugrel hydrochloride synthesis.
**EXPERIMENTAL**

The $^1$H and $^{13}$C NMR spectra were recorded on a Bruker 300 MHz Avance NMR spectrometer. The chemical shifts were reported ppm relative to either residual solvent or tetramethylsilane. The FT-IR spectra were recorded on Perkin-Elmer spectrum one FT-IR spectrometer and only prominent peaks are reported. Mass spectra were recorded on a Agilent 1100 series LC-MSD-TRAP-SL system. HPLC analysis was carried out on Waters Alliance 2487 or Waters Alliance 2695 systems. All the reagents used were of LR grade and used without further purification. All the anhydrous reactions were carried out under a nitrogen atmosphere. Silica gel (120-200 mesh) was used for column chromatography.

**General experimental procedure**

5-(2-Cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl)-6,7,7a-tetrahydrothieno[3,2-b]pyridin-2(4H)-one (4): To a suspension of 5,6,7,7a-tetrahydrothieno[3,2-b]pyridin-2(4H)-one hydrochloride (10 g, 52.17 mmol) (1) in DMF (40 mL) was added and the mixture was stirred until clear solution was obtained. Cool thereaction mixture to 0-5 ºC, charged anhydrous potassium carbonate (14.45 g, 104.55 mmol) and maintain until the TLC revealed the completion of reaction. Charged toluene (100 mL) and water (100 mL). The clear biphasic system was stirred for 15 min and then the layers were separated. The aqueous phase was extracted with toluene (3 × 50 mL) and the combined organic phases washed with water (4 × 20 mL). The organic phase was distilled under vacuum and purified by silica column chromatography. Elution with 8-10 % ethyl acetate in hexane (v/v) afforded pale yellow oily crude. The resulting crude was dissolved in acetone (60 mL) and slowly added conc. HCl (1.8 mL) at 25-30 ºC and stirred for 4 h. The obtained material was removed by filtration and washed with acetone (10 mL). The filtrate acetonitrile was removed by distillation under vacuum at 40-45 ºC to afford desacetyl prasugrel 4 as an brown oily liquid. The resulting oily crude was recrystallized from diisopropylether (100 mL) to afford desacetyl prasugrel 4 (4.0 g, 23.2 % yield, HPLC purity 95.82 %) as a off-white solid. IR (KBr, $v_{max}$, cm$^{-1}$): 3053, 2968, 2812, 1700, 1640, 1584, 1486, 1440, 1397, 1384, 1222, 1171, 1076, 1055, 877, 845, 761, 645. $^1$H NMR (DMSO-d$_6$): δ 7.37-7.44 (m, 1H), 7.24-7.30 (m, 1H), 6.22 (s, 1H), 4.86 (s, 1H), 4.42-4.48 (m, 1H), 3.94 (d, $J$ = 12.0, 1H), 3.05 (d, $J$ = 12.0, 1H), 2.96-3.00 (m, 1H), 2.36-2.42 (m, 1H), 2.24-2.32 (m, 2H), 1.62-1.67 (m, 1H), 0.83-0.93 (m, 4H). $^{13}$C NMR : 201.74, 167.73, 160.82 (d, $J$ = 293.9), 206.72, 198.17, 170.11, 160.60 (d, $J$ = 244.0), 131.36 (d, $J$ = 3.8), 130.29 (d, $J$ = 8.5), 125.40 (d, $J$ = 124.55), 121.08 (d, $J$ = 14.8), 115.71 (d, $J$ = 22.5), 70.95, 53.22, 50.50, 47.84, 33.71, 17.94, 11.18, 11.39. ESI-MS: m/z 332 ([M + H]$^+$, C$_{18}$H$_{18}$NO$_2$S$^+$ calcd. (%) 332.1).

**Scheme-I**: Reagents and conditions: (i) K$_2$CO$_3$, acetonitrile, 0-5 ºC; (ii) K$_3$CO$_3$, acetic anhydride, DMF, 0-5 ºC; (iii) acetonitrile, HCl, 40-45 ºC

5-(5-Chloro-1-(2-fluorophenyl)-2-oxyethyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl acetate hydrochloride (8): To a suspension of 5-(2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl)-5,6,7,7a-tetrahydrothieno[3,2-c]pyridin-2(4H)-one (4) (10 g, 30.17 mmol) in water (50 mL), hydrochloric acid (13.5 mL) was added and the mixture stirred for 0.5 h at 80-85 ºC. The pH was adjusted to 7.5 with 10 % NaOH solution at 25-30 ºC and extracted with toluene (2 × 50 mL) and the combined organic phases washed with water (2 × 20 mL), concentrated under reduced pressure afford desacetylchloro prasugrel 6 (6.2 g, 55.8 % yield) as a brown yellow liquid. Its mass (ESI-MS) displayed the required molecular ion peak at m/z 368 and the characteristic chlorine isotopic pattern.

To the residue (6), DMF (40 mL) was added and the mixture was stirred until clear solution was obtained. Cool the reaction mixture to 0-5 ºC, charged anhydrous potassium carbonate (5.24, 37.9 mmol) and slowly added acetic anhydride (3.45 g, 33.7 mmol) and maintain until the TLC revealed the completion of reaction. Charged toluene (100 mL) and water (100 mL). The clear biphasic system was stirred for 15 min and then the layers were separated. The aqueous phase was extracted with toluene (3 × 50 mL) and the combined organic phases washed with water (4 × 20 mL). The organic phase was distilled under vacuum and purified by silica column chromatography. Elution with 8-10 % ethyl acetate in hexane (v/v) afford pale yellow oily crude. The resulting crude was dissolved in acetone (60 mL) and slowly added conc. HCl (1.8 mL) at 25-30 ºC and stirred for 4 h. The obtained material was removed by filtration and washed with acetone (10 mL). The wet material was dried under vacuum at 40-45 ºC for 5 h to afford CATP hydrochloride 7 (7:). To a suspension of 5-(2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl)-5,6,7,7a-tetrahydrothieno[3,2-c]pyridin-2(4H)-one (4) (10 g, 30.17 mmol) in water (50 mL), hydrochloric acid (13.5 mL) was added and the mixture stirred for 0.5 h at 80-85 ºC. The pH was adjusted to 7.5 with 10 % NaOH solution at 25-30 ºC and extracted with toluene (2 × 50 mL) and the combined organic phases washed with water (2 × 20 mL), concentrated under reduced pressure afford desacetylchloro prasugrel 6 (6.2 g, 55.8 % yield) as a brown yellow liquid. Its mass (ESI-MS) displayed the required molecular ion peak at m/z 368 and the characteristic chlorine isotopic pattern.
To a suspension of 5,6,7,7a-tetrahydrothieno[3,2-c]pyridin-2(4H)-one hydrochloride 3 (10 g, 52.17 mmol) in acetonitrile (60 mL) was cooled to 0-5 °C, anhydrous potassium carbonate (14.4 g, 104.45 mmol) was added and charged 2-bromo-1-cyclopropyl-2-(3-fluorophenyl)ethanone 8 (10.75 g, 41.7 mmol) at 0-5 °C and the mixture was stirred for 4 h at 0-5 °C. The salt was removed by filtration and then washed with acetonitrile (10 mL). The filtrate acetonitrile was removed by distillation under vacuum at 40-45 °C to afford desacetyl 3-fluoroprasugrel 9 (14 g) as a brown oily liquid. To the oily crude (14 g, 42.2 mmol), DMF (120 mL) was charged and stirred until clear solution was obtained. The resulting solution was cooled to 0-5 °C, anhydrous potassium carbonate (12.84 g, 92.9 mmol) was added and slowly added acetic anhydride (10.75 g, 105.60 mmol) at 0-5 °C and then stirred for 1 h, when analytical TLC revealed the amount of desacetyl 3-fluoroprasugrel was below 2 %. The reaction mixture was cooled to 20-25 °C then charged toluene (90 mL) and water (150 mL). The mixture was stirred for 15 min and then separated the layers. The aqueous layer was extracted with toluene (2 × 30 mL). The combined organic layer was washed with water (4 × 20 mL) and then washed with acetonitrile (5 mL). The filtrate acetonitrile was removed by distillation under vacuum at 40-45 °C and purified by silica column chromatography using 10 % ethyl acetate in hexane (v/v) afforded 3-fluoroprasugrel 10 as a off-white solid (5 g, 25.6 % yield, HPLC purity 98.82 %) IR (KBr, νmax, cm⁻¹): 3009, 2942, 1761, 1602, 1435, 1373, 1221, 1200, 1158, 898, 832, 653. ¹H NMR (DMSO-d₆): δ 7.47-7.52 (dd, J = 5.7, 8.7, 2H), 7.23 (t, J = 8.9, 2H), 6.43 (s, 1H), 4.39 (s, 1H), 3.36-3.42 (2H), 2.72 (s, 2H), 2.48-2.48 (1H), 2.26 (s, 3H), 0.70-0.91 (m, 2H), 0.70-0.91 (m, 2H). ¹C NMR: 208.19, 167.54, 149.86, 128.31, 115.59 (d, J = 14.7, 1H), 121.22, 78.90, 50.21, 48.22, 24.40, 20.40, 17.49, 11.80, 11.15. ESI-MS: m/z 374 ([M + H]⁺), C₁₃H₁₂NO₃S, calcd. (%) 374.1.

5-(2-Cyclopropyl-1-(3-fluorophenyl)-2-oxoethyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl acetate (10): To a suspension of 5,6,7,7a-tetrahydrothieno[3,2-c]pyridin-2(4H)-one hydrochloride 3 (10 g, 52.17 mmol) in acetonitrile (70 mL) was cooled to 0-5 °C, anhydrous potassium carbonate (21.65 g, 156.7 mmol) was added and slowly added acetic anhydride (16 g, 156.7 mmol) at 0-5 °C. The reaction mixture was stirred for 1 h at 0-5 °C, analytical TLC revealed the completion of reaction. The reaction mass ethyl acetate (100 mL) and water (60 mL) were charged and then stirred for 15 min. The layers were separated, the aqueous layer was extracted with ethyl acetate (2 × 20 mL). The combined organic layer was washed with water (4 × 20 mL). The solvent was completely removed under vacuum at 45-50 °C. The residue was purified by silica column chromatography using 10 % methanol in dichloromethane (v/v) to afford diacetyl prasugrel acetate (10 g) as a brown oily liquid. The oily crude (10 g) was added to stirred suspension of 5,6,7,7a-tetrahydrothieno[3,2-c]pyridin-2-yl aceta te (17): Anhydrous potassium carbonate (7.2 g, 52.11 mmol) was added to stirred suspension of 5,6,7,7a-tetrahydrothieno[3,2-c]pyridin-2(4H)-one hydrochloride 3 (5 g, 26.1 mmol) in acetonitrile (30 mL) at 0-5 °C and then charged 1-bromo-1-cyclopropyl-2-(4-fluorophenyl)ethanone 11 (12.7 g, 49.6 mmol) at 0-5 °C and the mixture was stirred for 4 h at 0-5 °C. The salt was removed by filtration and then washed with acetonitrile (10 mL). The filtrate acetonitrile was removed by distillation under vacuum at 40-45 °C to afford desacetyl 4-fluoro prasugrel 12 (14 g, 42.2 mmol) as a brown oily liquid. To the oily crude (14 g), DMF (120 mL) was charged and stirred until clear solution was obtained. The resulting solution was cooled to 0-5 °C, anhydrous potassium carbonate (12.84 g, 92.9 mmol) was added and slowly added acetic anhydride (10.8 g, 105.60 mmol) at 0-5 °C and then stirred for 1 h, when analytical TLC revealed the amount of desacetyl 4-fluoroprasugrel 12 was below 2 %. The reaction mixture was cooled to 20-25 °C then charged toluene (90 mL) and water (150 mL). The mixture was stirred for 15 min and then separated the layers.
separated the layers. The aqueous layer was extracted with toluene (2 × 15 mL). The combined organic layer was washed with water (4 × 25 mL) and concentrated under vacuum at 40-45 °C. The resulting residue was triturated with methanol (10 mL) for 1 h. The solid obtained was removed by filtration and washed with methanol (5 mL). The wet material was dried under vacuum to afford methyl keto prasugrel impurity 17 (1.0 g, 11.6 % yield, HPLC purity 89.82 %) as a pale yellow solid. IR (KBr, v, cm⁻¹): 2954, 2844, 1759, 1613, 1488, 1372, 1217, 1134, 826, 760, 612. "H NMR (DMSO-d₆): δ 7.73-7.47 (m, 1H), 7.37-7.47 (m, 1H), 7.27 (d, J = 7.8, 1H), 7.22 (d, J = 3.0, 1H), 6.41 (s, 1H), 4.72 (s, 1H), 3.40 (s, 2H), 2.62-2.81 (m, 1H), 7.37-7.47 (m, 1H), 7.27 (d, J = 7.8, 1H), 7.22 (d, J = 3.0, 1H), 6.41 (s, 1H), 4.72 (s, 1H), 3.40 (s, 2H), 2.62-2.81 (m, 2H), 2.62-2.81 (m, 2H), 2.25 (s, 3H), 2.14 (s, 3H), 1.85 (m, 2H), 0.88-1.07 (m, 2H). "C NMR: 205.50, 167.77, 160.58 (d, J = 14.5), 121.72, 115.72, 68.81, 49.95, 47.51, 21.97, 12.69, 12.34, 12.04. ESI-MS: m/z 348 ([M + H]+), C₂₀H₁₉NO₂S, calcd. (%) 348.1, m/z 370 ([M + Na]+).

1-Cyclopropyl-2-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)-2-(2-fluorophenyl)ethanone hydrochloride (19): To a suspension of 50.34, 117.82, 105.50, 50.32, 48.32, 24.42, 20.42, 17.31, 11.62, 11.03. ESI-MS: m/z 1315 ([M + Na]+), C₂₀H₁₇NO₂S, calcd. (%) 1315.2.

RESULTS AND DISCUSSION

Desacetyl prasugrel (4), a possible contaminant in prasugrel hydrochloride that can be formed by incomplete acetylation of desacetyl prasugrel or may be deacetylation of prasugrel base. The desacetyl prasugrel impurity (4) was synthesized by condensation of (2) with 5,6,7,7a-tetrahydrothieno[3,2-c]pyridin-2(4H)-one hydrochloride (3) in the presence of base and acetonitrile (Scheme-I).

During hydrochloride conversion of prasugrel (5), formation of 0.3-0.5 % of the chloro prasugrel (7) is observed, the level is reduced to less than 0.1 % during its isolation and purification. The CATP impurity (7) was prepared by reaction of HCl/water with (4) and the desacetyl chloro prasugrel formed (6) was acetylated with acetic anhydride in the presence of DMF and potassium carbonate, followed by purification by column chromatography and further treatment with conc. hydrochloric acid in the presence of acetone (Scheme-II).

In some of commercial samples, 2-bromo-1-cyclopropyl-2-(2-fluorophenylethanone (2) was found to be contaminated with traces of 2-bromo-1-cyclopropyl-2-(3-fluorophenyl)-ethanone (8), although the amount of contamination of the fluoro analogue (10) in prasugrel hydrochloride (1) was never more than 0.2 %. The fluoro analogue of prasugrel (10) was synthesized from 2-bromo-1-cyclopropyl-2-(3-fluorophenyl)-ethanone (8) following the reaction sequence used to synthesize (5), followed by purification by column chromatography (Scheme-III).

In some of commercial samples, 2-bromo-1-cyclopropyl-2-(2-fluorophenylethanone (2) was found to be contaminated with traces of 2-bromo-1-cyclopropyl-2-(4-fluorophenyl)-ethanone (11), although the amount of contamination of the fluoro analogue (13) in prasugrel hydrochloride (1) was never more than 0.15 %. The fluoro analogue of prasugrel (13) was synthesized from 2-bromo-1-cyclopropyl-2-(4-fluorophenyl)-
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Scheme-II: Reagents and conditions: (i) HCl, water, 80-85 °C; (ii) (a) K₂CO₃, acetic anhydride, DMF, 0-5 °C; (b) column chromatography; (c) acetone, conc. HCl

Scheme-III: Reagents and conditions: (i) K₂CO₃, acetonitrile, 0-5 °C; (ii) (a) K₂CO₃, acetic anhydride, DMF, 0-5 °C; (b) column chromatography

Ethanone (11) following the reaction sequence used to synthesize (5), followed by purification by column chromatography (Scheme-IV).

During the acetylation reaction there is always the probability of diacetylation due to presence of excess 5,6,7,7a-tetrahydrothieno[3,2-c]pyridin-2(4H)-one hydrochloride (3), hence the diacetyl thieno pyridine (14) was synthesized by acetylation of thienopyridine (3) with acetic anhydride, followed by purification by column chromatography (Scheme-V).

In some of commercial samples, 2-bromo-1-cyclopropyl-2-(2-fluorophenyl)ethanone (2) was found to be contaminated with traces of 1-bromo-1-(2-fluorophenyl)propan-2-one (15), although the amount of contamination of methylketone impurity (17) in prasugrel hydrochloride (1) was never more than 0.15 %³. Methylketone impurity (17) was synthesized from 1-bromo-1-(2-fluorophenyl)propan-2-one (15) following the reaction sequence used to synthesize 5 (Scheme-VI).

In some of the commercial samples, 5,6,7,7a-tetrahydrothieno[3,2-c]pyridin-2(4H)-one hydrochloride (3) was found to be contaminated with traces of 4,5,6,7-tetrahydrothieno[3,2-c]pyridine hydrochloride (18), although the amount of contamination of thiophene impurity (19) in prasugrel hydrochloride (1) was never more than 0.15 %³. Thiophene impurity (19) was synthesized from 4,5,6,7-tetrahydrothieno[3,2-c]pyridine hydrochloride (18) by alkylation with 2-bromo-1-cyclopropyl-2-(2-fluorophenyl)ethanone (2) in presence of potassium carbonate and acetonitrile followed by treatment with ethanolic HCl in the presence of acetone (Scheme-VII).

In some of the commercial samples, 2-bromo-1-cyclopropyl-2-(2-fluorophenyl)ethanone (2) was found to be contaminated with traces of 2-bromo-1-cyclopropyl-2-phenylethanone (20), although the amount of contamination of the desfluoro analogue (22) in prasugrel hydrochloride (1) was never more than 0.15 %³. The desfluoro analogue of prasugrel (22) was synthesized from 2-bromo-1-cyclopropyl-2-phenylethanone (20) following the reaction sequence used to synthesize 5 (Scheme-VIII).
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Conclusion

For better knowledge of the synthetic path way of an active pharmaceutical ingredient (API) it is mandatory to identify all the impurities formed/anticipated. In this context we have synthesized and characterized different potential process related impurities of prasugrel hydrochloride.
REFERENCES