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SYNTHESIS AND CHARACTERIZATION OF ISOMERS OF COX-2 INHIBITOR, CELECOXIB

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ABSTRACT

Celecoxib is chemically known as 4-[5-(4-Methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1yl] benzene sulfonamide. The present work describes the identification, origin, synthesis, characterization and control of isomeric compounds of Celecoxib.

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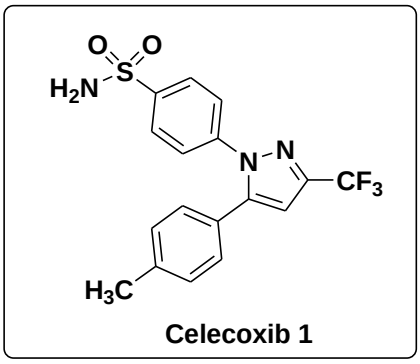
INTRODUCTION

Celecoxib **1** is a COX-2 selective non steroidal anti inflammatory drug (NSAID).



Celecoxib 1

Selective inhibitors of cyclooxygenase-2 (COX-2) have demonstrated effective anti-inflammatory activity with reduced gastrointestinal side effects, as compared to other anti-inflammatory agents, e.g., NSAIDs, which inhibit both the constitutive form of cyclooxygenase (COX-I), and the inducible form of the enzyme (COX-2). Particularly effective structural classes of selective COX-2 inhibitors are the 1,5-diarylpyrazoles. 4-[5-(4-Methylphenyl)-3-(trifluoromethyl)-l*H*-pyrazol-l-yl]benzenesulfonamide (Celecoxib) is a 1,5-diarylpyrazole compound, which has been approved by the US Food and Drug Administration for the treatment of rheumatoid arthritis and osteoarthritis. Celecoxib is the active ingredient used in the Celebrex®, which is marketed by Pharmacia Corporation.

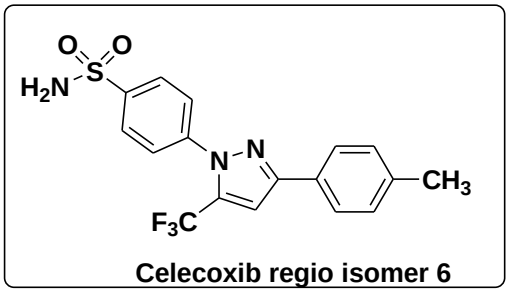
Impurities presence in an Active Pharmaceutical Ingredient (API) will influence drug effectiveness by change the quality and safety. Impurities more than 0.1% [1-2] should be identified and characterized as per the International Conference on Harmonization (ICH) guidelines. To performance the co-injection studies and analytical performance characteristic studies for example specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), system suitability testing and relative retention factor [3] impurities are required.

In view of regulatory importance of the related substances and analogues in the API, a detailed study on all possible isomers in Celecoxib was conducted.

During the development studies of Celecoxib **1** in the laboratory, we prepared possible isomers of Celecoxib. In the present work, the isomers of Celecoxib were synthesized and characterized by various spectroscopic techniques.

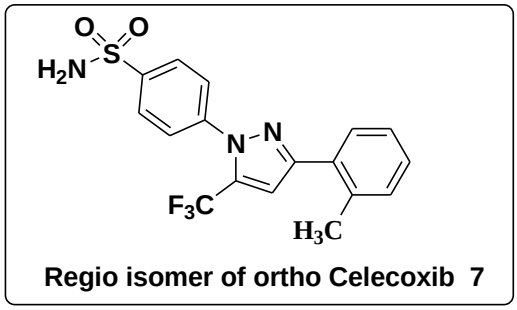
The chemical names and structures of Isomers of Celecoxib are given below.

4-[3-(4-Methylphenyl)-5-(trifluoromethyl)-l*H*-pyrazol-l-yl]benzenesulfonamide [**Celecoxib regio isomer, 6**]



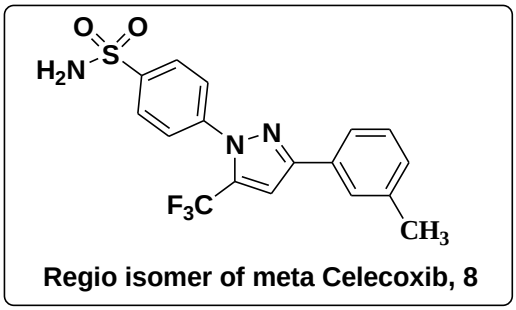
Celecoxib regio isomer 6

4-[3-(2-Methylphenyl)-5-(trifluoromethyl)-l*H*-pyrazol-l-yl]benzenesulfonamide [**Regio isomer of ortho Celecoxib, 7**]



Regio isomer of ortho Celecoxib 7

4-[3-(3-Methylphenyl)-5-(trifluoromethyl)-l*H*-pyrazol-l-yl]benzenesulfonamide [**Regio isomer of meta Celecoxib, 8**]

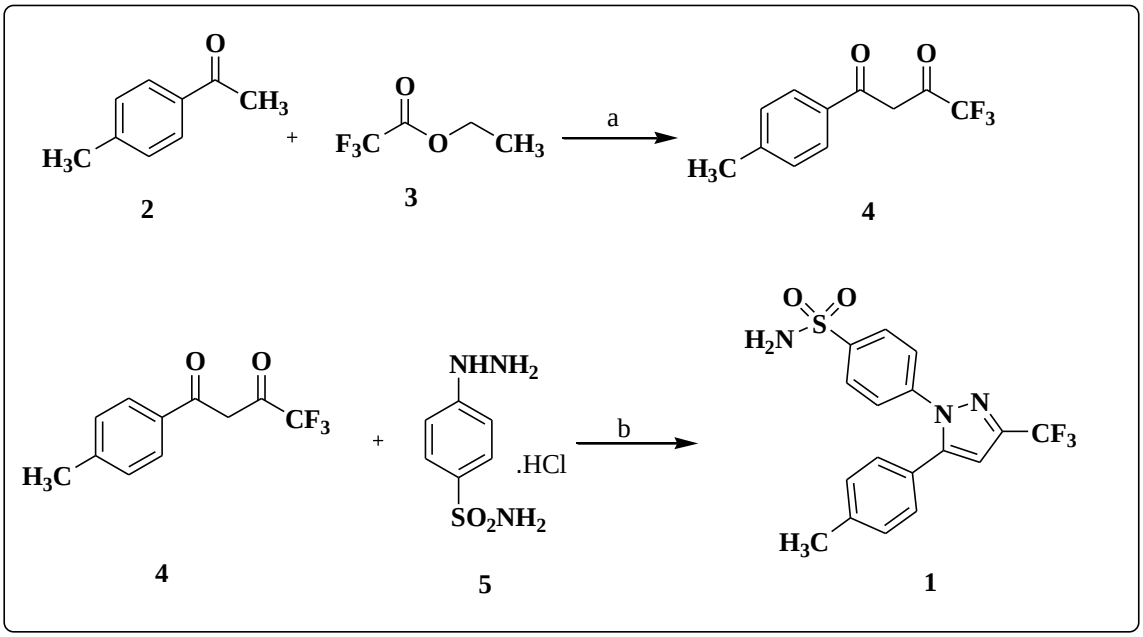


A number of impurities and analogues of Celecoxib were reported in literature [4-8]. To the best of my knowledge identification, synthesis and characterization of these isomers (**6**, **7** & **8**) are not reported anywhere in the literature till date.

RESULTS AND DISCUSSION

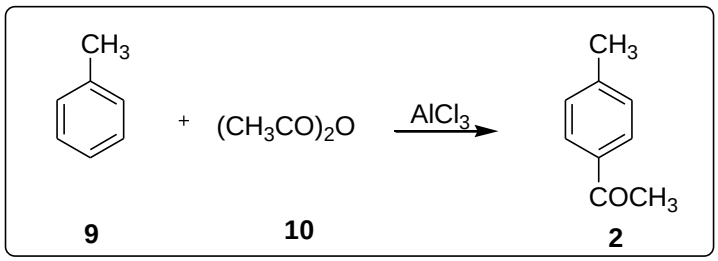
Celecoxib **1** has been synthesized by known literature methods [9-10]. Our route of synthesis of Celecoxib **1** is shown in scheme-1**.**

The reported synthesis of Celecoxib [10] (scheme-1) involves reduction of para methyl acetophenone with ethyl trifluoroacetate in presence of sodium methoxide in toluene gives 1-(4-methylphenyl)-4,4,4-trifluoro-butane-1,3-dione **4**. Condensation of compound **4** with 4-hydrazinobenzenesulfonamide hydrochloride in water and hydrochloric acid to give celecoxib.



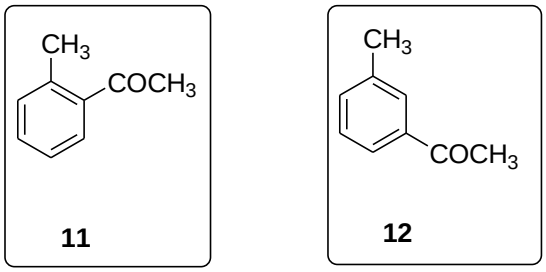
Scheme-I: Reported synthetic scheme of Celecoxib 1.
Reagents and conditions: (a) sodium methoxide, toluene (b) DM water, Hydrochloric acid

Para methylacetophenone and 4-Hydrazinobenzenesulfonamide hydrochlorides are the key raw materials for the preparation of Celecoxib. The isomers and related compounds present in these raw materials will influence the quality of the Celecoxib drug substance.



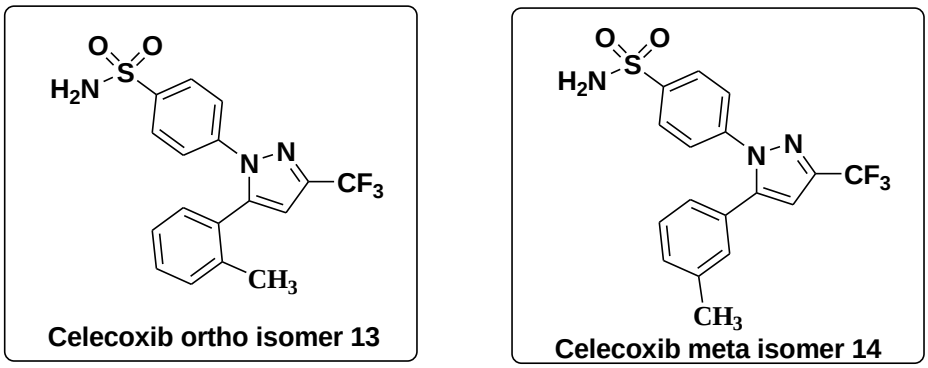
Scheme-2: Synthetic scheme of Para methylacetophenone 2.

Based on the route of synthesis of Para methylacetophenone (as shown in scheme-2), the following isomers are possible during the synthesis.

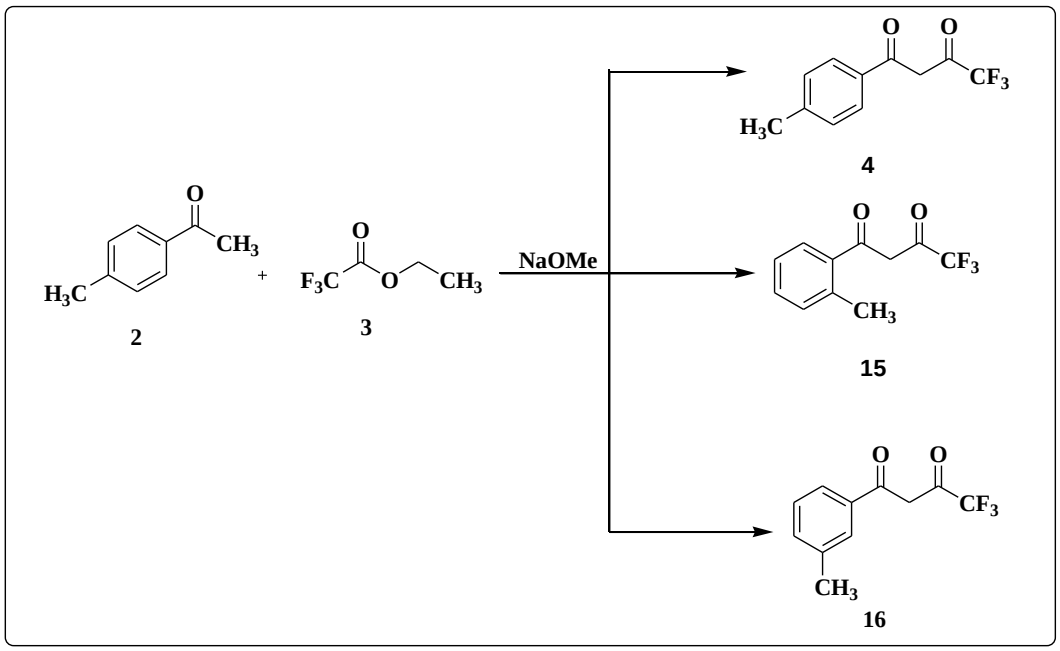


Structures of Ortho methylacetophenone 11 & Meta methylacetophenone 12.

The presence of these isomers (11 &12) in Para methylacetophenone leads to the formation of Celecoxib Ortho isomer and Celecoxib Meta isomer in Celecoxib drug substance.



Structures of Celecoxib Ortho isomer 13 & Celecoxib Meta isomer 14.



Scheme-3: Synthetic scheme of Celecoxib state-I 4 and its isomers.

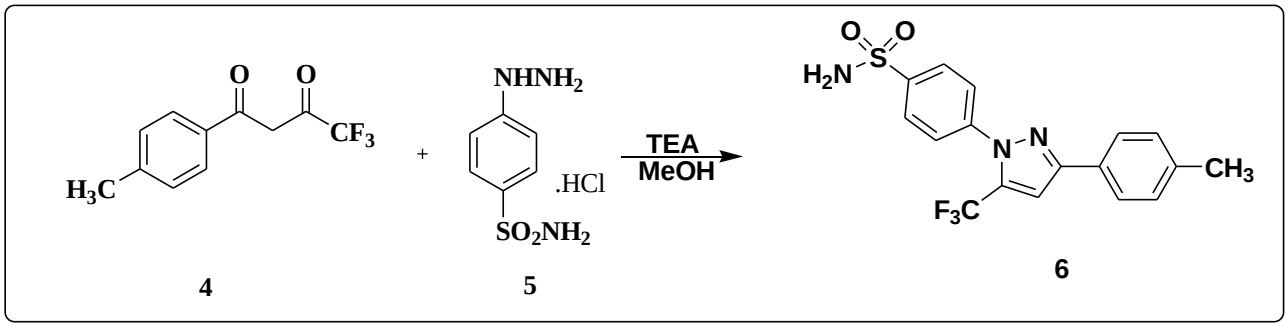
Origin, synthesis and control of Celecoxib Isomers:

Celecoxib ortho isomer (**13**) and Celecoxib meta isomers (**14**) are reported in literature [4-8].

These isomers are originated from the raw material ortho methyl acetophenone. Hence we should control these isomers in raw material itself to control corresponding isomers in Celecoxib drug substances.

Celecoxib regio isomer (6):

Celecoxib regio isomer **6** was one of the possible isomer during the preparation of Celecoxib. It was independently prepared by condensation of 1-(4-methylphenyl)-4,4,4-trifluoro-butane-1,3-dione with 4-hydrazinobenzenesulfonamide hydrochloride in methanol in presence of triethylamine base (as shown in scheme-4).



4 5 6

Scheme-4: Synthetic scheme of Celecoxib regio isomer 6.

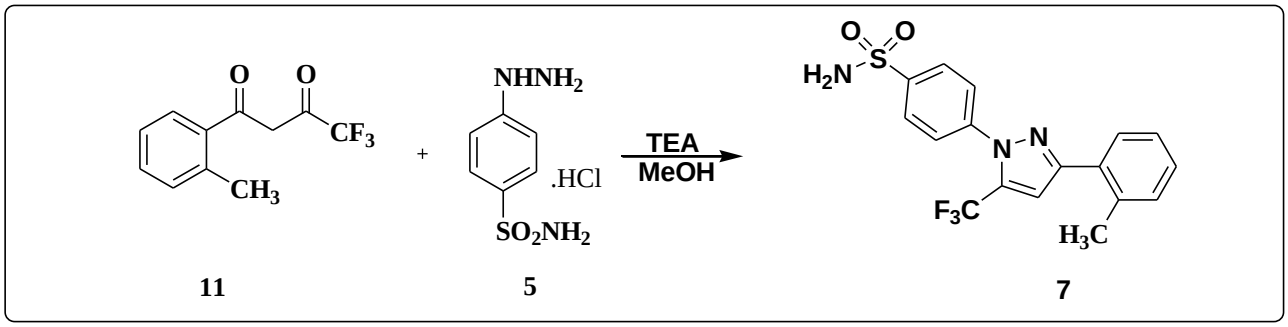
The mass spectrum showed a molecular ion at m/z 382 amu [(M+H) $^{+}$]. NMR spectrum showed a singlet at δ 2.35, corresponding to the CH_3, confirming the assigned structure **6**.

Origin: Celecoxib regio isomer formed during the condensation of compound **4** and 4-hydrazinobenzenesulfonamide.

Control: Celecoxib regio isomer formed during the preparation will eliminate in the mother liquors during the purification.

Regio isomer of ortho Celecoxib (7):

Regio isomer of ortho Celecoxib **7** was one of the possible isomer during the preparation of Celecoxib. It was prepared by condensation of 1-(2-methyl phenyl)-4,4,4-trifluoro-butane-1,3-dione with 4-hydrazinobenzenesulfonamide hydrochloride in methanol in presence of triethylamine base (as shown in scheme-5).



11 5 7

Scheme-5: Synthetic scheme of Regio isomer of ortho Celecoxib 7.

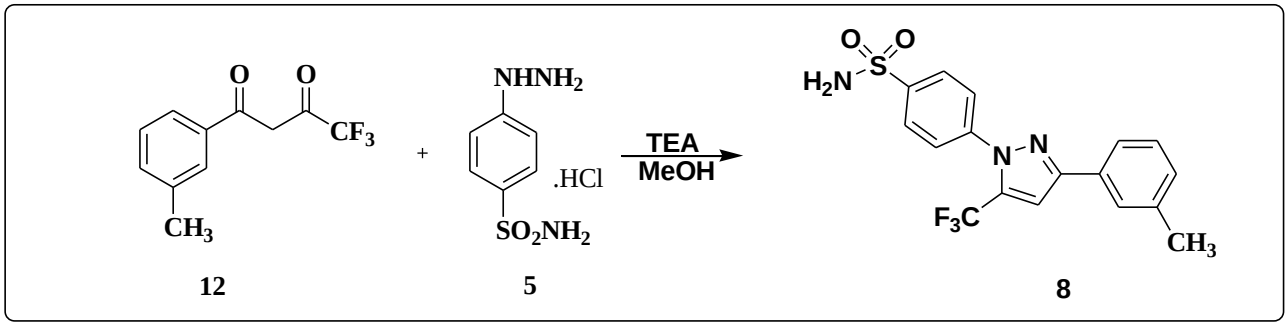
The mass spectrum showed a molecular ion at m/z 382 amu [(M+H) $^{+}$]. NMR spectrum showed a singlet at δ 2.52, corresponding to the CH_3, confirming the assigned structure **7**.

Origin: Regio isomer of ortho Celecoxib is formed due to the presence of 1-(2-methylphenyl)-4,4,4-trifluoro-butane-1,3-dione **11** in compound **4**.

Control: Regio isomer of ortho Celecoxib is controlled by limiting the ortho-methylacetophenone in Para-methylacetophenone and further the compound **7** will eliminate in the mother liquors during the purification.

Regio isomer of meta Celecoxib (8):

Regio isomer of meta Celecoxib 8 was one of the possible isomer during the preparation of Celecoxib. It was prepared by condensation of 1-(3-methyl phenyl)-4,4,4-trifluoro-butane-1,3-dione with 4-hydrazinobenzenesulfonamide hydrochloride in methanol in presence of triethylamine base (as shown in scheme-6).



Scheme-6: Synthetic scheme of Regio isomer of meta Celecoxib 8.

The mass spectrum showed a molecular ion at m/z 380 amu [(M-H)$^-$]. NMR spectrum showed a singlet at δ 2.37, corresponding to the CH_3, confirming the assigned structure **8**.

Origin: Regio isomer of meta Celecoxib formed due to the presence of 1-(3-methylphenyl)-4,4,4-trifluoro-butane-1,3-dione **12** in compound **4**.

Control: Regio isomer of meta Celecoxib is controlled by limiting the meta-methylacetophenone in Para-methylacetophenone and further the compound **8** will eliminate in the mother liquors during the purification.

EXPERIMENTAL

Solvents and reagents were obtained from commercial sources and used without purification. ^{1}H NMR and ^{13}C NMR spectral data were performed on Bruker-Avance 300 MHz, 500 MHz spectrometer in DMSO-d_6 & $CDCl_3$. The chemical shift values reported on the δ scale in parts per million (ppm), downfield from tetramethylsilane (TMS) as an internal standard. IR spectra were recorded in the solid state as KBr pellet using a Perkin-Elmer FT-IR spectrophotometer. Mass spectrum was recorded by using a Perkin-Elmer PE SCIEX-API 2000, equipped with ESI source used online with a HPLC system after the ultraviolet (UV) detector.

Preparation of 4-[3-(4-Methylphenyl)-5-(trifluoromethyl)-l*H*-pyrazol-l-yl]benzene sulfonamide [Celecoxib regio isomer, 6]

Para-methylacetophenone (50 g, 373 mmol) was dissolved in toluene (250 mL) and 30% methanolic sodium methoxide solution (80.6 g, 447 mmol), followed by ethyl trifluoroacetate (63.58 g, 447 mmol) were added at 25-30 °C. Temperature of the reaction mass was raised to 55-60 °C and stirred for 4 h to complete the reaction. The reaction mass was cooled to 20-25 °C and washed with 10% aqueous hydrochloric acid (200 mL). The layers were separated and concentrated the organic layer at 50-55 °C under reduced pressure to produce 80 g of l-(4-methylphenyl)-4,4,4-trifluorobutane-1,3-dione (**4**) as an oily mass. Dissolved compound **4** in methanol (800 mL) and added 4-hydrazino benzenesulfonamide hydrochloride (77.73 g, 347.8 mmol), triethylamine (35.2 g, 347.8 mmol) at room temperature. Refluxed the reaction mass for 10 h at 60-65 °C. Then concentrated the reaction mass at 45-50 °C under reduced pressure. The resulting residue dissolved in 10% aqueous hydrochloric acid and raised the mass temperature to 65-70 °C. Maintained the reaction mass for 1 h at 65-70 °C. Then cooled the reaction mass to room temperature and added ethyl acetate (800 mL) and DM water (800 mL). Separated the organic layer and washed with brine solution (500 mL). Concentrated the organic layer under reduced pressure at 50-55 °C. The resulting residue was crystallized with toluene (800 mL) to give 48 g of crude Celecoxib reigo isomer. This crude isomer is further purified by column chromatography to give 23 g (16%) of pure Celecoxib regio isomer.

IR (KBr pellet, cm^{-1}): 3368, 3078, 2923, 2626, 2322, 1921, 1595, 1443, 1419, 1346, 1291, 1238, 1189, 1162, 765, 740, 691; ^{1}H-NMR (DMSO-D_6, 300 MHz): 2.35 (s, 3H), 7.29 (d, 2H), 7.58 (brs, 2H), 7.75 (s, 1H), 7.83 (2d, 4H), 8.02 (d, 2H). MS *m/z*: 382 [(M+H)$^+$]

Preparation of 4-[3-(2-Methylphenyl)-5-(trifluoromethyl)-l*H*-pyrazol-l-yl]benzene sulfonamide [Regio isomer of ortho Celecoxib, 7]

Ortho-methylacetophenone (50 g, 373 mmol) was dissolved in toluene (250 mL) and 30% methanolic sodium methoxide solution (80.6 g, 447 mmol), followed by ethyl trifluoroacetate (63.58 g, 447 mmol) were added at 25-30 °C. Temperature of the reaction mass was raised to 55-60 °C and stirred for 4 h to complete the reaction. The reaction mass was cooled to 20-25 °C and washed with 10% aqueous hydrochloric acid (200 mL). The layers were separated and concentrated the organic layer at 50-55 °C under reduced pressure to produce 80 g of l-(2-methylphenyl)-4,4,4-trifluorobutane-1,3-dione (**15**) as an oily mass. Dissolved compound **15** in methanol (800 mL) and added 4-hydrazinobenzenesulfonamide hydrochloride (77.73 g, 347.8 mmol), triethylamine (35.2 g, 347.8 mmol) at room temperature. Refluxed the reaction mass for 10 h at 60-65 °C. Then concentrated the reaction mass at 45-50 °C under reduced pressure. The resulting residue dissolved in 10% aqueous hydrochloric acid and raised the mass temperature to 65-70 °C. Maintained the reaction mass for 1 h at 65-70 °C. Then cooled the reaction mass to room temperature and added ethyl acetate (800 mL) and DM water (800 mL). Separated the organic layer and washed with brine solution (500 mL). Concentrated the organic layer under reduced pressure at 50-55 °C. The resulting residue was crystallized with toluene (800 mL) to give 45 g of crude Celecoxib reigo isomer. This crude isomer is further purified by column chromatography to give 21 g (15%) of pure regio isomer of ortho Celecoxib (**7**).

IR (KBr pellet, cm^{-1}): 3393, 3070, 2929, 1918, 1595, 1446, 1420, 1349, 1289, 1229, 1196, 1160, 766, 739, 695; ^{1}H-NMR (DMSO-D_6, 300 MHz): 2.52 (s, 3H), 7.29-7.35 (m, 3H), 7.57 (s, 2H), 7.59 (s, 1H), 7.66 (d, 1H), 7.84 (d, 2H), 8.02 (d, 2H). MS *m/z*: 382 [(M+H)$^+$].

Preparation of 4-[3-(3-Methylphenyl)-5-(trifluoromethyl)-l*H*-pyrazol-l-yl]benzene sulfonamide [Regio isomer of meta Celecoxib, 8]

Meta-methylacetophenone (50 g, 373 mmol) was dissolved in toluene (250 mL) and 30% methanolic sodium methoxide solution (80.6 g, 447 mmol), followed by ethyl trifluoroacetate (63.58 g, 447 mmol) were added at 25-30 °C. Temperature of the reaction mass was raised to 55-60 °C and stirred for 4 h to complete the reaction. The reaction mass was cooled to 20-25 °C and washed with 10% aqueous hydrochloric acid (200 mL). The layers were separated and concentrated the organic layer at 50-55 °C under reduced pressure to produce 80 g of l-(3-methylphenyl)-4,4,4-trifluorobutane-1,3-dione (**16**) as an oily mass.

Dissolved compound **16** in methanol (800 mL) and added 4-hydrazinobenzenesulfonamide hydrochloride (77.73 g, 347.8 mmol), triethylamine (35.2 g, 347.8 mmol) at room temperature. Refluxed the reaction mass for 10 h at 60-65 °C. Then concentrated the reaction mass at 45-50 °C under reduced pressure. The resulting residue dissolved in 10% aqueous hydrochloric acid and raised the mass temperature to 65-70 °C. Maintained the reaction mass for 1 h at 65-70 °C. Then cooled the reaction mass to room temperature and added ethyl acetate (800 mL) and DM water (800 mL).

Separated the organic layer and washed with brine solution (500 mL). Concentrated the organic layer under reduced pressure at 50-55 °C. The resulting residue was crystallized with toluene (800 mL) to give 46 g of crude Celecoxib reigo isomer. This crude isomer is further purified by column chromatography to give 22 g (15%) of pure regio isomer of ortho Celecoxib (**8**).

IR (KBr pellet, cm^{-1}): 3352, 3108, 2920, 2643, 2321, 1911, 1597, 1455, 1419, 1351, 1291, 1218, 1194, 1159, 764, 741, 692; ^{1}H-NMR (DMSO-D_6, 300 MHz): 2.37 (s, 3H), 7.24 (d, 1H), 7.37 (dd, 1H), 7.58 (s, 2H), 7.73-7.84 (m, 5H), 8.03 (d, 2H). MS *m/z*: 380.2 [$(M-H)^{+}$].

CONCLUSION

A detailed study on various isomers of Celecoxib was conducted. Different process related isomers of Celecoxib were identified, synthesized, and characterized by using various spectroscopic techniques like liquid chromatography-mass spectrometry (LCMS), mass, ^{1}H NMR and FT-IR. These efforts to synthesize and characterize the isomers of Celecoxib effectively have proved to be beneficial.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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