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Synthesis of p-Chlorocelecoxib from 4-Methylacetophenone & its Spectral Characterization

Mark Edwards, Sowjanya Ratho*

The School of Science, University of Greenwich[Medway], United Kingdom

Address for Correspondance: soujanyaa28@gmail.com

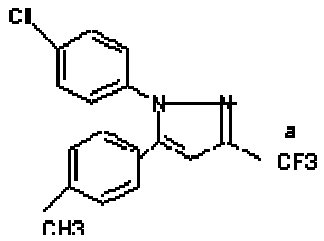
ABSTRACT: Celecoxib is a non-steroidal anti-inflammatory drug used in the treatment of pain and inflammation. The objective of this study is to synthesize p-chlorocelecoxib by the condensation of 4-methylacetophenone and ethyl trifluoroacetate, followed by reaction with 4-(2-chlorohydrazinly)benzenesulfanimide & identification of celecoxib analogue through several analytical methods like UV visible, IR, TLC, NMR etc. The methods used have been found to be fast, efficient, reproducible and suitable for the identification of celecoxib analogues. The purified β-keto-diester reaction with 4-chlorophenylhydrazine hydrochloric acid gave P-Cl - celecoxib in good yield.

KEYWORDS: NSAID(Non-Steroidal Anti-Inflammatory Drug); p-Chlorocelecoxib; 4-Methylacetophenone; β-Ketodiester.

INTRODUCTION

Celecoxib is among the most widely used drugs of the world [1]. Celecoxib, 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzene sulphonamide[empirical formula: C17H14F3N7O2S, molecular weight: 381, 37]belongs to a novel class of agent that selectively inhibits cyclooxigenase-2 (COX-2) enzymes [2]. It is a white powder soluble in chloroform and methanol, insoluble in water with a melting point of 157–158□C and is normally administered orally. Celecoxib has a pKa of 11.1 and has very less solubility in water (5µg/ml)[2]which contributes to high variability in absorption after oral administration. Thus it is very important to improve the solubility and dissolution rate of celecoxib to enhance overall oral bioavailability [2].

Non-Steroid Anti- Inflammatory Agents (NSAIDS) inhibit cyclooxygenase enzymes (COX-1 & COX-2) [4]. COX-1 is usually found in stomach, kidneys, intestine and platelets, whereas COX-2 is expressed during inflammation and pointed in spinal cord, pancreatic cells, kidneys and brain. Celecoxib and other coxibs such as rofecoxib, valdecoxib and etoricoxib are selective COX-2 inhibitors with low gastrointestinal side effects to traditional NSAIDs. As celecoxib has lower COX-1/ COX-2 selectivity, this research work has been aimed at synthesizing analogue of the celecoxib to obtain more effective celecoxib derivatives with less gastrointestinal side effects [4].



P-CL-CELECOXIB 19F

Figure 1

Celecoxib does not affect COX-1 when inhibiting COX-2. COX-2 is involved in the synthesis of prostaglandins whereas COX-1 is involved in the synthesis of thromboxane and prostaglandins. Therefore, by inhibiting COX -2, celecoxib inhibits only prostaglandin synthesis without effecting thromboxane (TXA2) and thus does not offer cardio protective effects that are offered by non-selective NSAIDs. In Celecoxib the SO_2Me and SO_2NH_2 chemical groups are believed to give COX-2 selectivity by insertion into secondary pocket of COX-2 which is absent in COX-1. The secondary pocket present in COX-2 has been attributed to the presence of isoleucine in COX-1 relative to the smaller valine in COX-2. Replacement of histidine in COX-1 by arginine COX-2 has been reported to play a key role in the hydrogen-bond network of the COX active site [5]. In the present study p-Cl Celecoxib is synthesized, purified by using column chromatography and characterized by thermal, spectroscopic and elemental analysis techniques.

MATERIALS & METHODS

Synthesis of β-ketodiester

To a10m.mol (1.3ml) of 4-methyl acetophenone,25% w/v solute of 11m.mol(2.4ml)sodium methoxide in methanol was added in round bottom flask equipped with reflux condenser and nitrogen gas supply tube. The mixture when magnetically stirred for 10 minutes at room temperature resulted in the appearance of green colour. To this mixture 12m.mol (1.3ml) of ethyl 1, 1, 1trifluro acetate was added drop by drop gradually and the reaction mixture was condensed for 1hour which gave diketone in good yield[4].

Synthesis of p-chlorocelecoxib

10m.mol(2.3gm) of diketone was then reacted with the 11m.mol (1.96gm) of 4(2-chlorohydrazinly)benzenesulfinamide hydrochloride and the resulting mixture is refluxed in methanol.12m.mol (0.98gm) of sodium acetate is added to the mixture in a round bottomed flask and mixed well until sample is dissolved. Then the flask is equipped to the water condenser and temperature is maintained at 70˚c for 20 min and kept under observation. After 20 minutes, a white colored precipitate was observed at the bottom of the flask. The precipitate was isolated through vacuum by using 2mm filter paper.

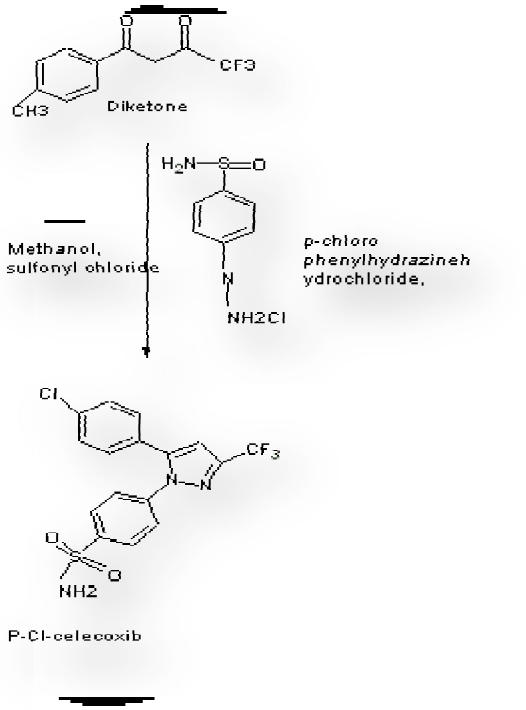


Figure 2

Purification and Analysis

The following analytical methods were used with good laboratory practice and were validated according to the ICH guidelines [7a, 7b].

Purification of the compound

The synthesized compound was weighed and 5gm of silica gel for 1gm of synthesized compound is added. The obtained compound was usedas stationary phase which allowed executing Column chromatography to get pure compound. Four different ratios of mobile phase were used to observe the flow rate i.e.hexane: CH_2CL_2(100:0),hexane:CH_2CL_2(80:20),hexane:CH_2CL_2(50:50) and hexane:CH_2CL_2(10:90). The fractions are collected and TLC is performed to getRF values. Based on the RF values obtained, the extracted liquid compound is mixed and evaporated using rota evaporator (127rppm)which after 35 minutes gave a fine white coloured powder.

Thin layer chromatography

In this procedure we used silica gel 60 F254 plates (15* 20 cm) with a thickness of 0.25 mm. The mobile phase used to develop the system consisted of dichloromethane and Hexane in the ratio 90:10 (V/V). TLC is performed in order to collect the identical RF value fractions drained after performing the column chromatography. TLC was performed by taking 5µg of the samples with a capillary tube and placing them on the TLS Plates which are then transferred to a developing tank containing mobile phase. The plates were then examined under UV short wavelength (245 to 254 nm).

Ultraviolet spectroscopy

For the identification trials a U-2900/2910 double beam spectrophotometer and 1 cm quartz cells were used with atomic wavelength between 400nm-200nm with the accuracy of 0.1nm. To identify the sample, a stock solution of 10µg/ml sample in methanol was used.

Infrared spectrophotometry

The FTIR infrared spectra were recorded on model Perkin Elmer Paragon 1000 FTIR spectrometer. Solid samples were examined by preparing KBr pellets, and spectra were recorded over the 4000-450 cm^{-1}region.The samples were prepared by triturating the KBr with 2mg of samples and placing the powder between the two discs, with the help of plunger the pressure is applied to the discs andKBr sample discs were prepared.

Nuclear Magnetic Resonance (NMR)

Proton NMR,13C NMR,19F NMR spectrums of di-ketones and final compound in the synthesis (p-cl Celecoxib) were carried out using JEOL ECA-500 (500MHz NMR) [8]. The samples were dissolved in $CDCl_3$ (20mg) solvent then placed into a borosilicate glass tube. The tube was loaded into instrument and spun at 15Hz. TMS was added as reference standard and the spectrum were recorded with the entire chemical shift in the range of 0-10 ppm.

RESULTS & DISCUSSION

The objective of the study was successfully achieved by synthesis of 4-(5-(4-chloro phenyl)-3-(trifluromethyl) pyrazol-1-yl) benzenesulphonamide (p-*Cl* Celecoxib) using beta-ketodiester to get 60% yield has been achieved. The compound is carried out for purification using column chromatography and identification of the compound by applying analytical methodologies (TLC, UV, FTIR and MNR spectroscopies). The spectrum of Celecoxib was found at maximum absorbance of 258nm. The IR spectrum of the compound showed very less absorption band at 3160 and 3260 cm-1, which were assigned to the –NH asymmetric and symmetric starching vibrations respectively [10].Hence, the prepared compound has very less –NH group present. The vibrations in the 1150 and 1340 cm-1 range shows =O symmetric and asymmetric stretching respectively. A very broad band obtained at 3500cm-1 is attributed to presence of water.

Table 1 Assignment of NMR floride chemical shifts to the p-Cl-Celecoxib

PEAK	CHEMICAL SHIFT(ppm)	INTEGRATION	SPLITTING PATTERN	ASSIGNMENT
A	-62.09	3	Singlet	CF3

Table 2 Assignment of NMR proton chemical shifts to the p-Cl-Celecoxib

PEAK	CHEMICAL SHIFT(ppm)	INTEGRATION	SPLITTING PATTERN	ASSIGNMENT
a 1	7.29	2	Singlet	NH2
a ,b	7.5	4	Paired doublet	Aromatic ring
C ,d	7.35	4	Paired doublet	Aromatic ring
E	7.1	1	Singlet	=CH-

Table 3 NMR Carbon chemical shifts are correlated to the functional groups

PEAK	CHEMICAL SHIFT (ppm)	ASSIGNMENT OF PEAKS
A	21.3	CH3
a 1	126	Aromatic
B	126.6	Aromatic
C	128.6	Aromatic
a 2	134	Aromatic
D	129.5	Aromatic
E	129.2	Aromatic

Table 4 FTIR Assignments of P-cl- Celecoxib

PEAK cm^{-1}	ASSIGNMENT
570.3	Chloro alkanes
695.4	Aromatic
1109.2	Trifluro alkanes
3160	NH2 Stretching
1500	N-H strechings
1132	S=0 streching
1378	CH3(Methyl group)

The spectrum of NMR analysis revealed peaks from 7.3 to 7.08ppm which is due to the proton of the aromatic group. The characteristic peak in range 2.5 to 2.3ppm can be used for the identification the extracted sample. A very small peak is obtained at 3ppm which belongs to the methyl and sulphonamide protons of the Celecoxib. The above analysis confirms the successful synthesis of Celecoxib.

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