Research Article

Resolution of Clopidogrel Bisulfate's Racemic Precursor by Direct Crystallization: A Simple, Economical and Environment Friendly Approach for Clopidogrel Bisulfate

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ABSTRACT

The present work mainly deals with identification of (±)2-(2-chlorophenyl)-(6,7-dihydro-4H-thieno[3,2,c]pyridine-5-yl-acetamide (I) an important racemic precursor of Clopidogrel Bisulfate as a conglomerate. It was subsequently, successfully resolved by Direct Crystallization to afford (+)-2-(2-chlorophenyl)-(6,7-dihydro-4H-thieno[3,2,c]pyridine-5-yl-acetamide (II) and (+)-2-(2-chlorophenyl)-(6,7-dihydro-4H-thieno[3,2,c]pyridine-5-yl-acetamide (III). The desired enantiomer II was further converted to Clopidogrel Bisulfate, thus making it economical and environmental friendly approach.

Keywords: Clopidogrel Bisulfate, Conglomerate, Direct crystallization, Resolution.

INTRODUCTION

Many strategies are available for optical resolution of racemates. The methods include: a) Resolution by diastereomer formation, are well established over a period of time.[1-3] b) Kinetic resolution[2] and c) Chromatographic resolution.[1-2] Getting chiral purity through attrition that is Viedma Ripening[4, 5] is a recent development. Depending on the substrate, enantioselectivity and ease to handle at large scale, there differs use of resolution technology. Hence it is unfair to compare or draw conclusion about the superiority of one technology over the other. Separation of enantiomer by crystallization is rarely being considered as a tool of resolution. In spite of the usefulness, this method remained largely an empirical one based on the trial error approach. As per our literature study, these simple techniques of separation were least preferred for the resolution of chiral drugs or its chiral precursors, though this approach is very practical and scalable and its success is dependent on the physical properties of the racemic compound. There are few examples on separation of enantiomers by Direct or Spontaneous Crystallization reported in literature.[6-12] To carry out separation of enantiomers from a racemic compound by direct crystallization, it should behave as a conglomerate.[7] Conglomerate has a characteristic melting point behaviour[1,7] and solubility.[1,7] It has an identical solid-state Infra Red spectrum, X-Ray Powder Diffraction spectrum[1,7] with its enantiomers. Very few chiral compounds are identified as conglomerates and even fewer chiral drugs and chiral drug intermediates have been reported. The method of resolution by direct and preferential crystallization has been successful for the ethyl amine salt of Naproxen[13], hydroxyl acid intermediate of Diltiazem after N-acetylation[14], threo-chloramphenicol base[15], *S*-methyl DOPA[1] and glutamic acid.[16] These are few examples in the history of drug synthesis at commercial scale application. This approach of screening the compound for its conglomerate behavior, followed by resolution, if implemented will have a great impact on the cost of chiral life saving drugs. There is a very urgent need to identify chiral drugs and chiral drug intermediates as conglomerate, so that resolution by direct crystallization becomes an environment friendly, efficient and economical for the industrial scale operations. In view of these observations from literature, we have selected a recent generic drug Clopidogrel Bisulfate's (IV) racemic precursor (±)-2-(2-chlorophenyl)-(6,7-dihydro-4H-thieno [3,2,c] pyridine-5-yl-acetamide (I) (Figure 1) [17, 18] for screening of conglomerate behavior. Clopidogrel Bisulfate is an antithrombotic drug patented by Sanofi (France) and marketed as Plavix.[17,18] It is marketed worldwide in more than 110 countries with a sale of $6.6 billion in 2009 and may continue to grow by at least 20%. US FDA has extended the patent protection for six month exclusivity, which expired on May 2012.[19] Generic version of the drug has already hit the market. Major pharmaceutical giants are coming with innovative manufacturing route to compete and capture generic market, but none of them has given a thought for the molar efficiency of the reaction for this product, so that the impact of waste generated on the environment is minimized.

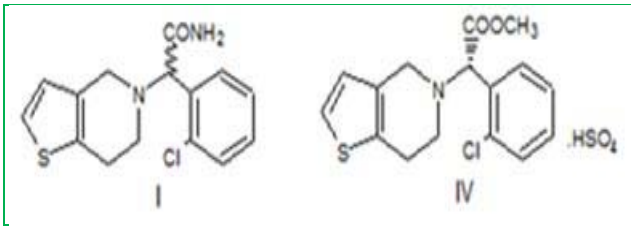


Figure 1: Clopidogrel Bisulfate's precursor–I, Clopidogrel Bisulfate-IV.

MATERIALS AND METHODS

All solvents were obtained from commercial sources and dried or purified by standard procedures before use. Melting points were measured on Gallenkamp melting

point apparatus and are uncorrected. Optical rotations were measured using a sodium D line on Jasco DIP 370. IR spectra recorded on Shimadzu FTIR-IR-4200 spectrophotometer in KBr discs. X-Ray Powder Diffraction were measured using PANalytical X'Pert Pro X-ray diffractrometer - Cu-K_α radiation (λ = 1.54184 Å) using Ni filter. Chiral HPLC analysis was carried out on a Perkin Elmer 2000 series Chiral AGP: 100mm X 4mm. packing 5micron scanned at 215nm.

Resolution of (±)-2-(2-chlorophenyl)-(6,7-dihydro-4H-thieno [3, 2-c] pyrid-5-yl)-acetamide (I) by Direct Crystallization

Compound I (40 gm, 0.130moles) was dissolved in 2-propyl alcohol (200ml) by heating and filtered hot through filter bed. The clear filtrate was stirred at 60 – 70ºC for 1 hour with over-head stirrer at low speed. It was gradually cooled to 35 - 40°C, and then a seed of compound II (0.400gm) was added. On further stirring at low speed, solid separated. This on filtration afforded crude white solid of compound II, (10.28gm. yield: 25.7%), m.p.138 - 141ºC, $^{25}[\alpha]_D$ +19º, (c=1, methanol). The mother liquor or the filtrate was taken for the isolation of compound III.

After separation of compound II, 2-propyl alcohol (20ml) was added to the above mother liquor. The reaction mass was made clear by heating followed by gradual cooling under low speed with over-head stirring for 90min. The separated solid on filtration afforded crude white solid of compound III, (8.82gm, yield: 22.05%), m.p.138 - 143ºC, $^{25}[\alpha]_D$ -20º, (c=1, methanol). The mother liquor or the filtrate was taken for the isolation of compound II.

To the filtrate was again added 2-propyl alcohol (20ml). The reaction mass was made clear by heating. It was then gradually cooled to 35 - 40°C under stirring at low speed with the help of over-head stirrer. The separated solid on filtration afforded crude white solid of compound II, (7.24gm, yield: 18.1%), m.p.137 - 141ºC, $^{25}[\alpha]_D$ +20º, (c=1, methanol). The mother liquor or the filtrate was taken for the isolation of compound III.

Now the solvent from the mother liquor was evaporated under vacuum to get a thick slurry followed by filtration to afford crude compound III, (12.34gm, yield: 30.85%), m.p.134 - 138ºC, $^{25}[\alpha]_D$ -14º, (c=1, methanol).

The crude compounds II and III obtained in the above separation were combined separately and crystallized in 2-propyl alcohol.

Purification of crude (+)-2-(2-chlorophenyl)-(6, 7-dihydro-4H-thieno [3, 2-c] pyrid-5-yl) acetamide (II) by crystallization

Crude compound II (15gm, 0.0488mole) was heated in 2-propyl alcohol (125ml) for 1 hr under stirring to get a clear solution. The reaction mass was gradually cooled to 30 - 35°C under slow over-head stirring. The separated solid on filtration afforded white solid of compound II (12.775gm, yield: 85.17%), m.p.151 - 153ºC, (lit.[20-22] m.p.152 - 155ºC), $^{25}[\alpha]_D$ +39º, c=1, methanol, (lit.[20, 21, 22], $^{25}[\alpha]_D$ +40º, c=1, methanol). Chiral HPLC purity, 99.44%.

Purification of crude (-)-2-(2-chlorophenyl)-(6, 7-dihydro-4H-thieno [3, 2-c] pyrid-5-yl) acetamide (III) by crystallization

Crude compound III (15 gm, 0.0488mole) was heated in 2-propyl alcohol (125ml) under stirring for 1 hr to get a clear solution. The reaction mass was gradually cooled to 30 - 35°C under slow over-head stirring. The separated solid on filtration afforded white solid of compound III, (12.75gm, yield: 85%), m.p.151-153ºC, (lit. [20-22] m.p.152 - 155ºC), $^{25}[\alpha]_D$ -39º, c=1, methanol, (lit. [20-22], $^{25}[\alpha]_D$ -40º, c=1, methanol). Chiral HPLC purity, 99.38%.

Thus for the first time compound **I** has been characterized as a conglomerate and resolved into enantiomers II and III by direct crystallization successfully with good chemical yield and optical purity.

Preparation[21, 22] of Clopidogrel Bisulfate: (+)-2-(2-chlorophenyl)-(6,7-dihydro-4H-thieno [3, 2-c] pyrid-5-yl)-acetic acid methyl ester bisulfate (IV)

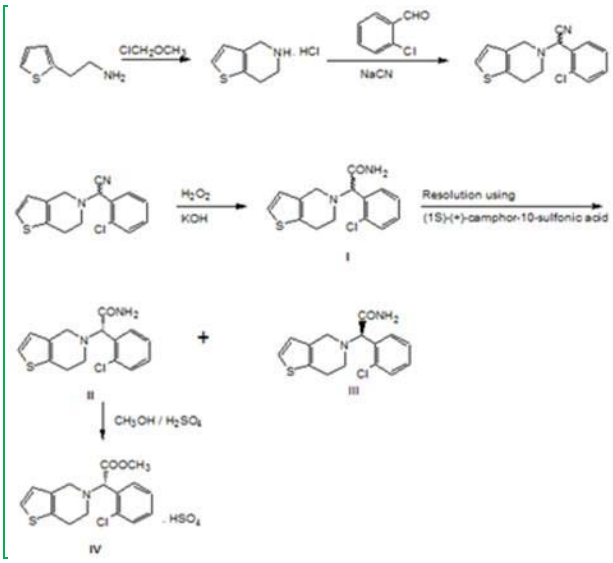
The reaction was carried out using (+)-2-(2-chlorophenyl)-(6,7-dihydro-4H-thieno[3, 2-c] pyrid-5-yl)-acetamide (II) (98 gm, 0.32mole), dimethyl sulfate (0.5volumes) and conc. sulfuric acid (1 volume) in methanol (7 volumes) as per the reported procedure, which on workup followed by isolation from acetone and sulfuric acid to afford white solid of (+)-2-(2-chlorophenyl)-(6,7-dihydro-4H-thieno [3, 2-c] pyrid-5-yl)-acetic acid methyl ester bisulfate (IV), (42.6 gm, 0.101mole), m.p.184 - 186°C, (lit[21] m.p.186 - 187°C), $^{25}[\alpha]_D$ = +54°, (c=1.89, methanol), (lit[21] . $^{25}[\alpha]_D$ = +55°, (c=1.89, methanol)). The spectral data (IR, 1H-NMR) of this compound was also in agreement with reported data.[21, 22]

RESULTS AND DISCUSSION

The screening of (±)-2-(2-chlorophenyl)-(6,7-dihydro-4H-thieno[3,2,c]pyridine-5-yl-acetamide (I) as a conglomerate was carried out using simple analytical tools and understanding its physical properties. For the study we synthesized (±)-2-(2-chlorophenyl)-(6,7-dihydro-4H-thieno[3,2,c]pyridine-5-yl-acetamide (I) (melting point 123 -124°C, and $^{25}[\alpha]_D$ = 0º, (c=1, methanol)), (+)-2-(2-chlorophenyl)-(6,7-dihydro-4H-thieno[3,2,c]pyridine-5-yl-acetamide (II) (melting point 154°C, and $^{25}[\alpha]_D$ = +39º, (c=1, methanol)), and (-)-2-(2-chlorophenyl)-(6,7-dihydro-4H-thieno[3,2,c]pyridine-5-yl-acetamide (III) (melting point 153ºC, and $^{25}[\alpha]_D$ = -38º, (c=1, methanol)), according to the literature procedure and is depicted in Scheme 1.[20-22]

Thus the melting point difference between compound I and II or I and III is 27-28°C. This is suggestive of compound I being a conglomerate.[7] The melting point of admixture was used to plot binary melting point diagram. The 'V' shape binary melting point diagram curve depicted in Figure 2, is as expected for a conglomerate.[7] The solubility of compound I, II and III was investigated in

different solvents. It reveals that compound I (0.501mole / L) and compound II and III (0.232mole / L) has appreciable solubility difference in 2-propanol at ambient temperature. This solubility difference also points to the conglomerate behavior.[7] The FT IR data (Figure 3, Figure 4, Figure 5) and X-Ray Powder Diffraction (Figure 6, Figure 7, Figure 8) of compound I, II and III are almost identical, which is as expected for a conglomerate.[7]



Scheme 1: Synthesis of compounds I, II, III and IV.

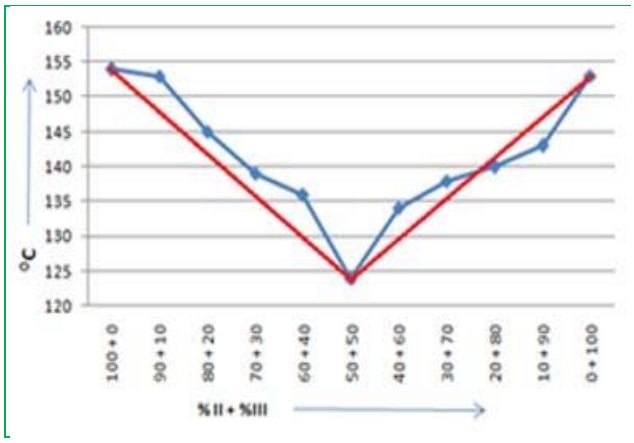


Figure 2: Binary melting point diagram-Admixture[a] of compounds % II + % III (w/w).

a. Melting point admixture was prepared by physical mixing of dry crystals in the ratios mentioned above. This was further confirmed by mixing the crystals physically and then getting it dissolved in methylene chloride solvent. On evaporation of solvent afforded solid, which was checked for its melting point.

Based on these evidences, compound I have been identified as a conglomerate for the first time. The compound behaving as a conglomerate essentially can be resolved by direct crystallization. Hence we have attempted its resolution by direct crystallization to confirm the claim. Based on the solubility data 2-propyl alcohol was preferred as solvent for crystallization. The process sequence is depicted in Figure 9 afforded enantiomers II and III from racemic mixture I as described in the experimental section. The compound II has been converted to Clopidogrel Bisulfate (**IV**) in a single step.[20-22]

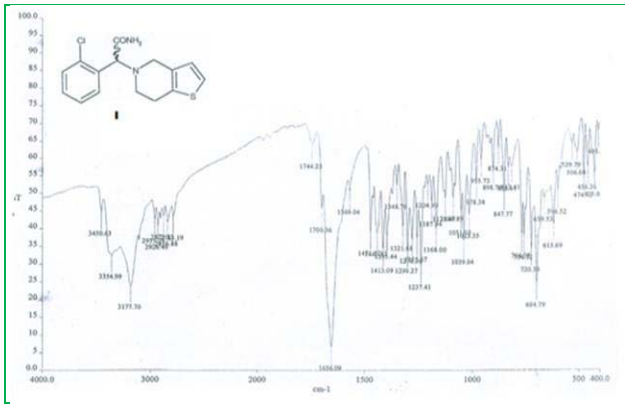


Figure 3: Infra Red Spectrum in cm^{-1} in KBr for compound I

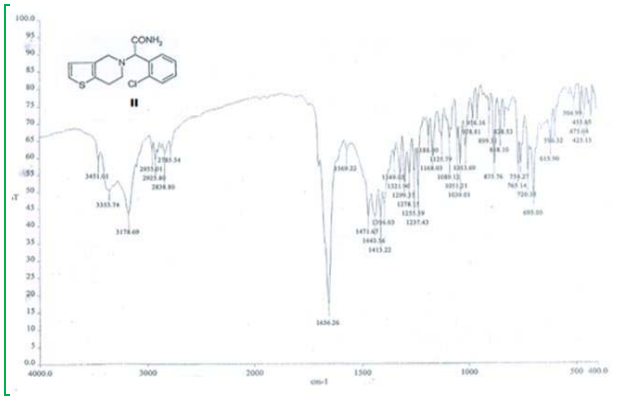


Figure 4: Infra Red Spectrum in cm^{-1} in KBr for compound II

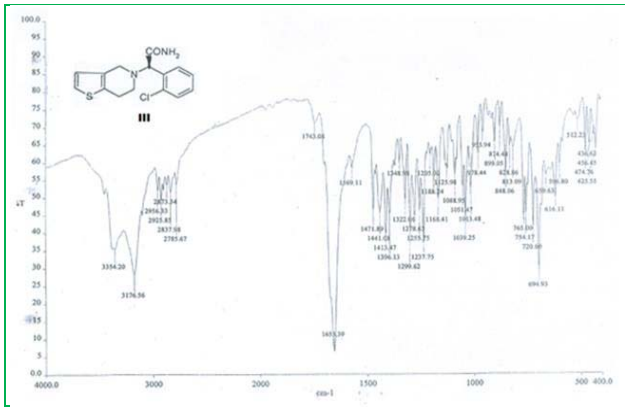


Figure 5: Infra Red Spectrum in cm^{-1} in KBr for compound III

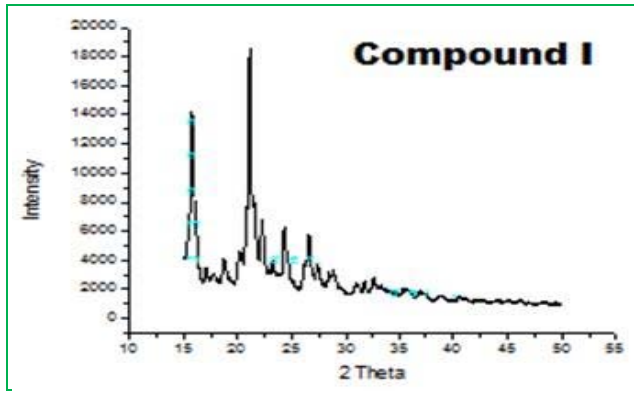


Figure 6: X-Ray Powder Diffraction Spectrum for compounds I.

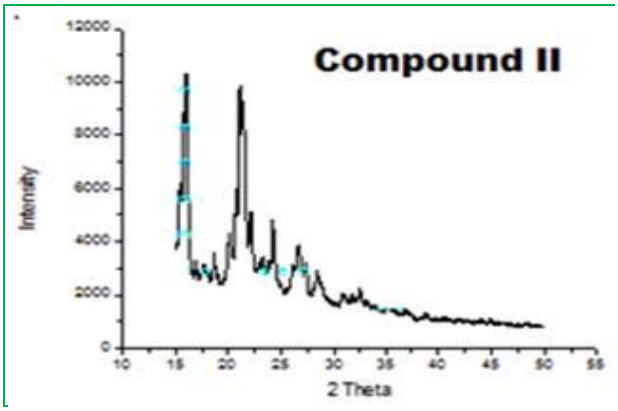


Figure 7: X-Ray Powder Diffraction Spectrum for compounds II.

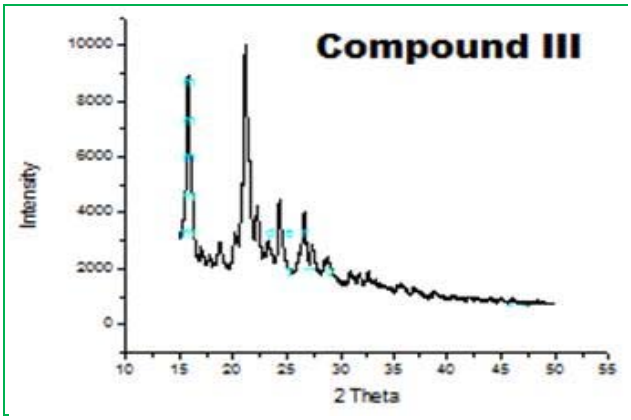


Figure 8: X-Ray Powder Diffraction Spectrum for compounds III.

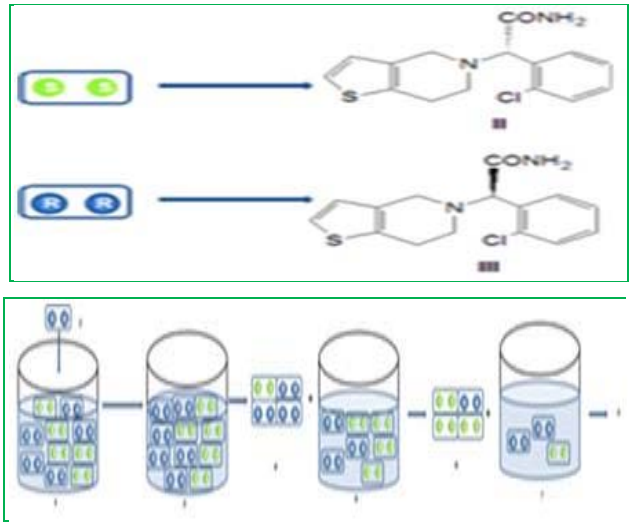


Figure 9: 1- Conglomerate compound I dissolved in solvent at elevated temperature. 2- Seed of pure compound III added to the saturated solution for crystal growth at ambient temperature. 3- Saturated solution with seed of pure compound III at ambient temperature. 4- First crop of crude compound III was filtered from the saturated solution. 5- First filtrate was heated to get clear solution and allowed to cool. 6- Crystals of crude compound II collected by filtration. 7- Second filtrate was heated to get clear solution. 8- The steps 3 to 6 are repeated, till the compound I get exhausted.

It is also significant to note that compound III could be racemized to compound I by treatment with sodium hydroxide.[20-22] In this manner undesired enantiomer that is compound III after racemization to compound I, can be further subjected to resolution by direct crystallization to afford compound II, thus making the process highly efficient and economical.

CONCLUSION

It was for the first time an important chiral drug intermediate (±)-2-(2-chlorophenyl)-(6,7-dihydro-4H-thieno [3, 2-c] pyrid-5-yl-acetamide (I) of Clopidogrel Bisulfate has been identified as a conglomerate and subsequently resolved successfully by direct crystallization to afford (+)-2-(2-chlorophenyl)-(6,7-dihydro-4H-thieno [3, 2-c] pyrid-5-yl-acetamide (II) and (-)-2-(2-chlorophenyl)-(6,7-dihydro-4H-thieno [3, 2-c] pyrid-5-yl-acetamide (III) in good chemical yield with excellent optical purity. Compound II on esterificaion[21,22] gave Clopidogrel Bisulfate (IV).

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