An Expeditious Method for The Synthesis Of N-Nitroso N -Desmethyl Nintedanib

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***Abstract*—Impurities of N-Nitrosoamines have become a major problem for the pharmaceutical sector. These contaminants can inadvertently occur in bulk active pharmaceutical ingredients (APIs), presenting significant health concerns and regulatory issues, even though they are necessary to synthesize in controlled laboratory environments. There are many adverse effects of nitrosamines, carcinogenic qualities are prime. When it comes to anticancer medications, carcinogenicity become most dangerous. N-Nitroso N-Desmethyl Nintedanib is a significant worry as a strong nitrosoamine carcinogenic impurity that poses additional hazards in its therapeutic usage. Nintedanib is an API used to treat pulmonary fibrosis and some types of cancer. Because of its substantial mass, this molecule is difficult for synthesis in the lab, making direct nitrosation or demethylation hard. In this work, we used a variety of intermediates and bulk API to synthesize N-Nitroso N-Desmethyl Nintedanib. Synthesis using Boc-protected piperazine substitution proved the least time-consuming and most effective method out of all those attempted.**

***Index Terms*—Nintedanib, N-Nitroso N -Desmethyl Nintedanib, anticancer, pharmaceutical impurities, Nitroso, TBN etc.**

I. INTRODUCTION

N-Nitrosoamines are one of the most active oxygens scavenging moieties which on consumption leads to tissue damage and other functional complications. Some of the nitroso amines are carcinogenic, teratogenic and metabolic disorder inducing agents. However, if these impurities are not standardized and quantified in the bulk API the formulation may led to have undesirable adverse drug reactions which are not listed to the respective Pharmacopoeias. To regulate the quality of Pharmaceuticals it is now essential to identify, standardise, quantify and minimise the nitrosoamine impurities in every batch of Bulk API before formulating final product.[1-5]

In case of anticancer agents, presence of nitrosoamine impurities in the bulk API is very hazardous as minimal quantity of nitrosoamine can lead to decrease potency of the API. Nintedanib (NTD) is anticancer agent of tyrosine kinase inhibitors. The class of drug inhibits receptor tyrosine kinase and nonreceptor tyrosine kinase, which is responsible for protein activation by signal transduction cascade, which is one of the important steps in cell cycle. (Figure 1. Tyrosine Kinase Inhibitors)[6]

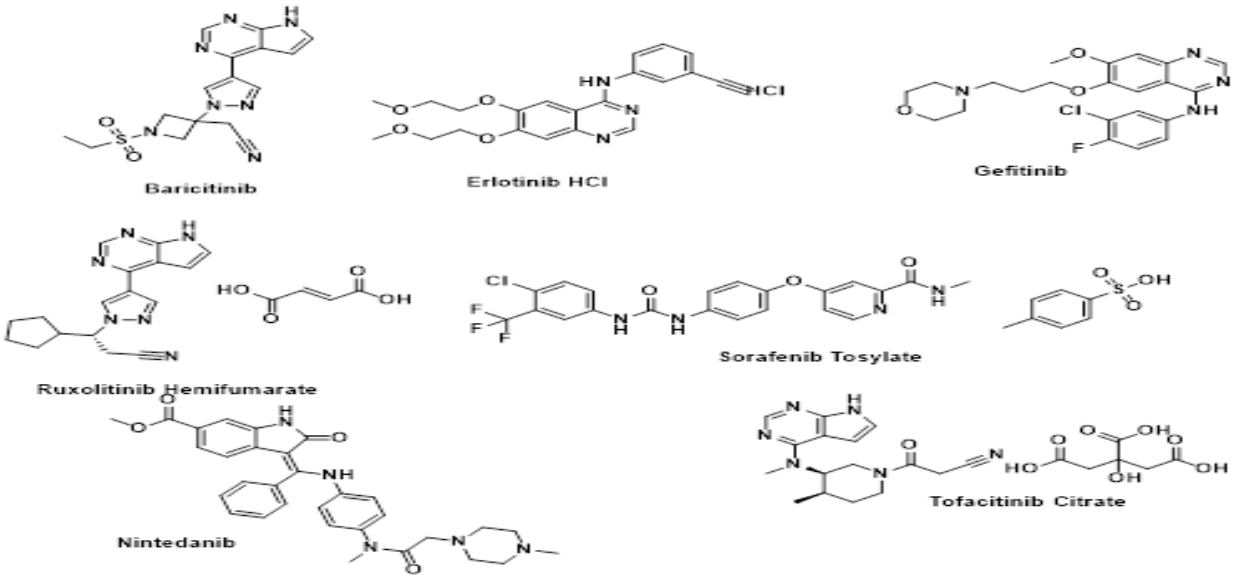
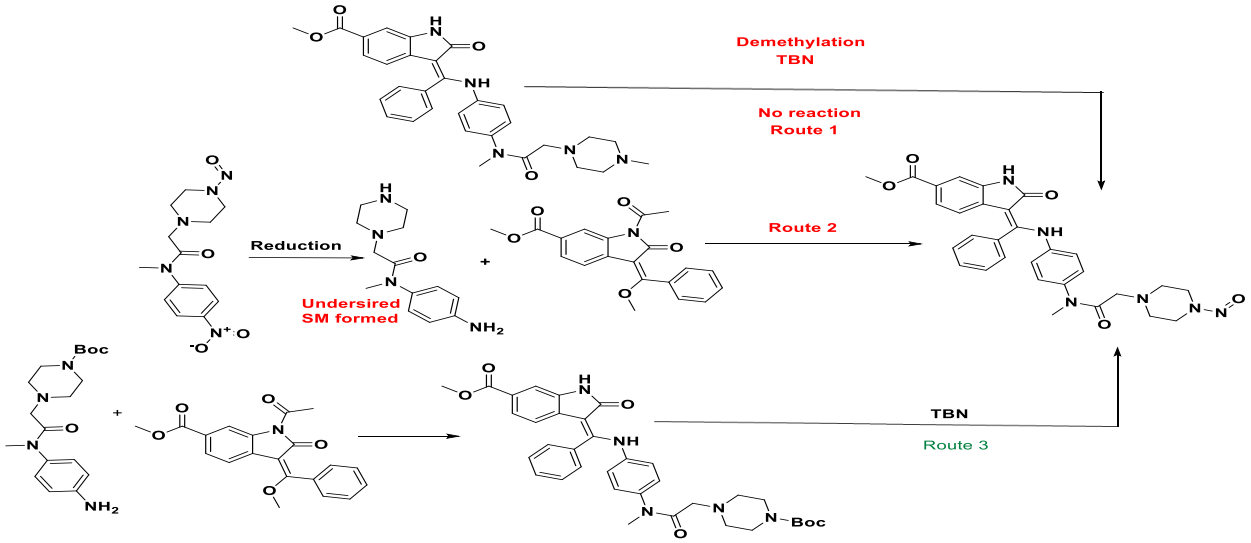


Figure 1. Drugs from Tyrosine Kinase inhibitor Class

Nintedanib (NTD) has reported about more than 60 reported impurities every impurity has some subclinical OR clinical adverse effect. Out of which, N-nitroso-N-desmethyl Nintedanib is one of the majorly observed impurity. the impurity causes significant nausea, tachycardia and seizures. its easily get developed in stock of bulk API but if we try to synthesize it in laboratory, it in very crucial to synthesize.

In this work, we attempted to synthesize N-nitroso-N-desmethyl Nintedanib via three different routes. The first route used was conversion of Nintedanib API to N Nitroso Nintedanib by various mechanisms. (Scheme 1, Route 1). Another route of was tried by synthesizing N-Methyl-N-(4-nitrophenyl)-2-(4-nitrosopiperazin-1-yl) acetamide and substituting it on Methyl-1-acetyl-3-(ethoxy(phenyl)methylene)-2-oxoindoline-6-carboxylate, but the desired second step material doesn't get synthesized (Scheme 1, Route 2). The last route was to attempt tert-butyl 4-(2-((4-aminophenyl) (methyl)amino)-2-oxoethyl) piperazine-1-carboxylate substitution on Methyl-1-acetyl-3-(ethoxy(phenyl)methylene)-2-oxoindoline-6-carboxylate which results in formation of N Boc Nintedanib which further converted to desmethyl Nintedanib which was easily converted to N-nitroso-N-desmethyl Nintedanib (Scheme 1, Route 3). [7-11]



Scheme 1. Synthesis route attempted for N-nitroso N desmethyl Nintedanib

II. MATERIALS AND METHODS

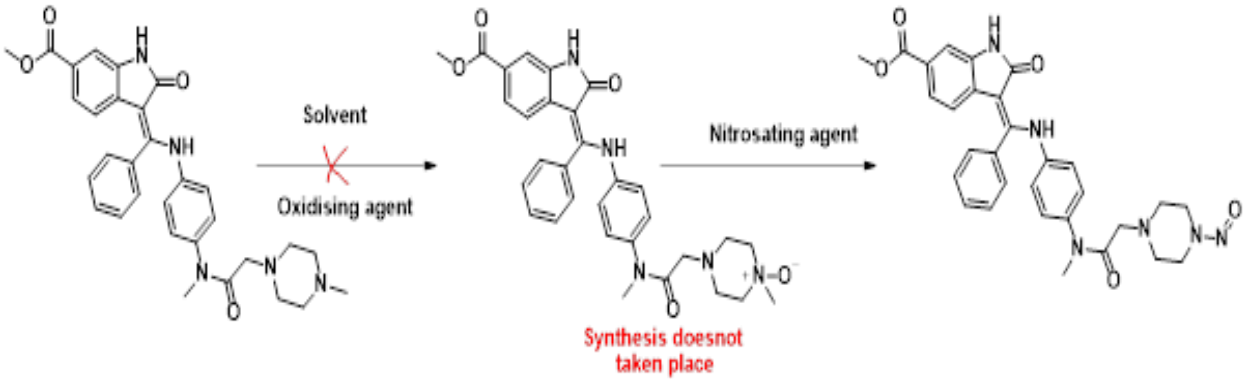
Materials:

Chemicals & reagents were procured from TCI, BLD Pharma, and were of synthetic grade. Solvents used were of commercial grade. Reaction procedures were optimized on SJI magnetic stirrer. Reactions were monitored by TLC using precoated aluminium plates procured from Merck silica gel 60F-254 using UV visualization technique and iodine vapours. ^{1}H NMR was recorded on "Bruker Advance" Spectrometer at 400 MHz frequency in DMSO D_6 in presence of TMS as internal standard (Chemical shift in ppm). Mass spectra were obtained from MD SCIEX API3200LC/MS/MS system equipped with electrospray ionization technique. HPLC was performed using Agilent 1100 series and C18 column.

Experimental:

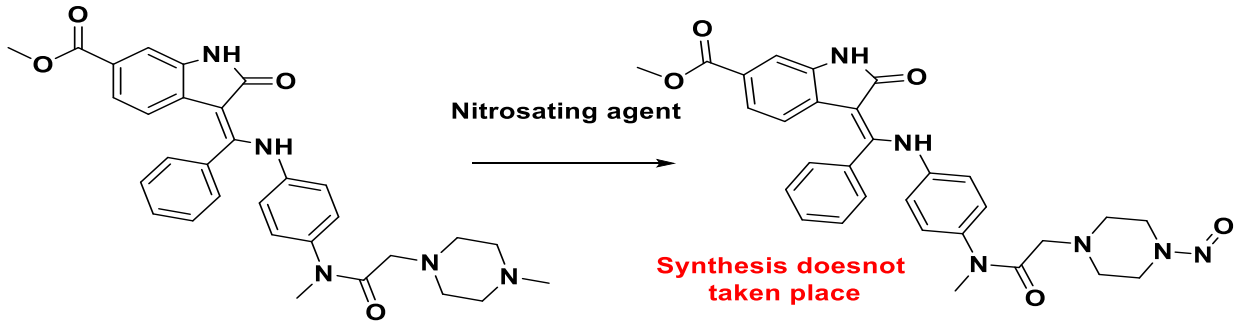
1. Optimisation of N-Nitroso N -Desmethyl Nintedanib synthesis using bulk NTD:

A) By synthesis of N-Oxide: NTD was (1gm, 10 mmol, 1 equiv) was dissolved in solvent (10 ml) at RT. Oxidising agent was added to the reaction mixture. The reaction was monitored using TLC every 30 mins. (Ethyl acetate: n Hexane 7: 3). The reaction was further carried out at different temperature conditions. The desired N oxide formation doesn't taken place. (Scheme 2).[8]



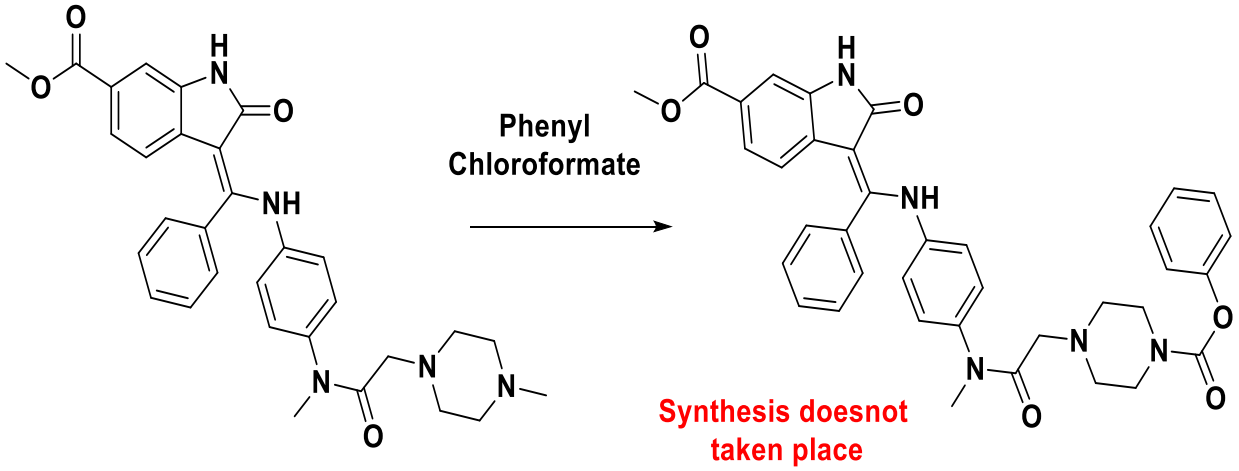
Scheme 2. N-oxide route of for N nitroso Nintedanib synthesis

B) By direct N-Nitrosoamine synthesis using direct Nitrosation agent: NTD was (1gm, 10 mmol, 1 equiv) was dissolved in solvent (10 ml) at RT. The direct Nitrosation agent were added to the reaction mixture. The reaction was monitored using TLC every 30 mins. (Ethyl acetate: n Hexane 7: 3:). The reaction was further carried out at different temperature conditions. The desired Nitrosation doesn't taken place. The various optimisation performed using nitrosating agent has been summarised in Table 2. (Table 2. Optimisation of Nitrosating agent). (Scheme 3)[8]



Scheme 3. Direct Nitrosation of Nintedanib

C) Demethylation followed by Nitrosation: NTD was (1gm, 10 mmol, 1 equiv) was dissolved in Toluene (30 ml) at RT. Phenyl chloroformate (0.574 gm, 10 mmol, 2 equiv) was added to reaction mixture followed by N, N-Diisopropylethylamine (5 ml, 10 mmol, 5 equiv). The reaction was refluxed at 110 ^{O}C. The reaction was monitored using TLC every 30 mins (Ethyl acetate: n Hexane 7: 3:). No reaction taken place after 24 Hrs (Scheme 4. Demethylation using Phenyl chloroformate). The reaction was discontinued, and synthesis of N nitroso Nintedanib was planned using synthetic route.

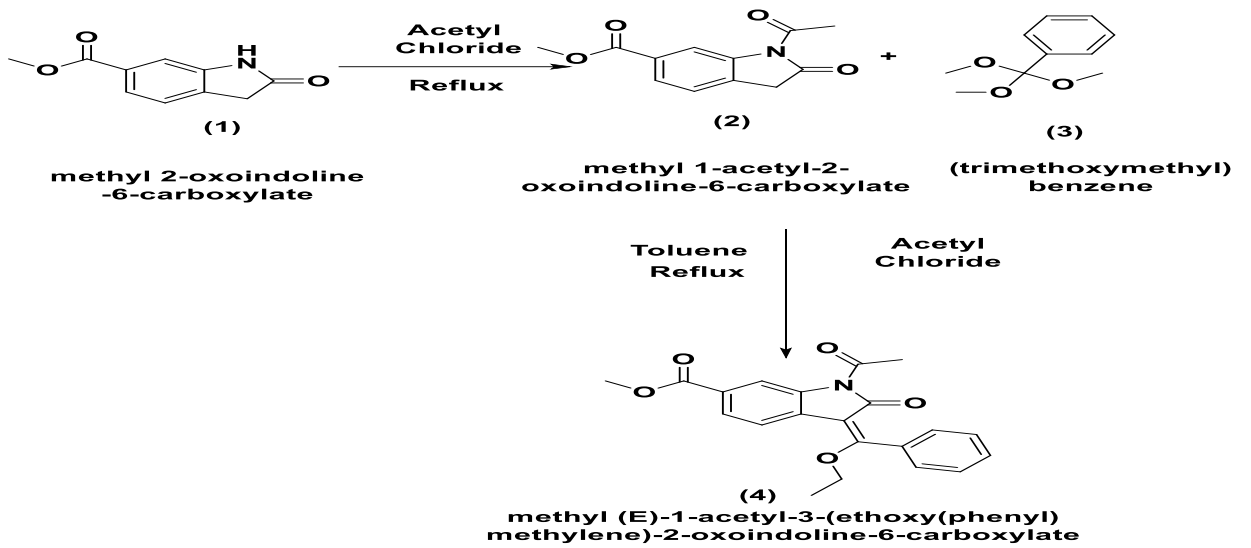


Scheme 4. Demethylation using Phenyl chloroformate

2. Synthesis of methyl (E)-1-acetyl-3-(Methoxy(phenyl)methylene)-2-oxoindoline-6-carboxylate (4):

A) Methyl 1-acetyl-2-oxoindoline-6-carboxylate (2): Methyl 2-oxoindoline-6-carboxylate (1) (3.80 gm, 10 mmol) was dissolved in Acetyl chloride (30 ml) at 0 ^{O}C. The reaction mixture was refluxed at 130 ^{O}C The reaction was monitored using TLC (Ethyl acetate: n Hexane 3:7) every 2 Hrs. The starting material get consumed after 16 Hrs. The reaction was then cooled and quenched in water to remove unreacted acetyl chloride. The compound was extracted with Dichloromethane, dried over sodium sulphate to get crude compound. Which was purified by column chromatography (Ethyl acetate: n Hexane 10% to 20% polarity) to obtain methyl 1-acetyl-2-oxoindoline-6-carboxylate (2). The product formation was confirmed by Mass analysis.

B) Synthesis of methyl (E)-1-acetyl-3-(Methoxy(phenyl)methylene)-2-oxoindoline-6-carboxylate (4): Methyl 1-acetyl-2-oxoindoline-6-carboxylate (2) (3.00 g, 9 mmol, 1.0 equiv) was then dissolved in Toluene (25 ml) and Acetyl chloride was added (6 ml). The mixture was refluxed at 110 ^{O}C and (trimethoxy methyl) benzene (3) (4.10 g, 22.5 mmol, 2.5 equiv) was added dropwise. The reaction was monitored using TLC (Ethyl acetate: n Hexane 3:7) every 2 Hrs. after 14 Hrs starting material get completely consumed. The reaction was cooled and quenched in water. The product was extracted using Dichloromethane and dried over sodium sulphate to get crude product. Which was purified and purified using column chromatography (Ethyl acetate: n Hexane 10% to 20% polarity) to get compound (4). The product formation was confirmed by Mass and NMR analysis (Scheme 5: Synthesis of methyl (E)-1-acetyl-3-(Methoxy(phenyl)methylene)-2-oxoindoline-6-carboxylate). [12]



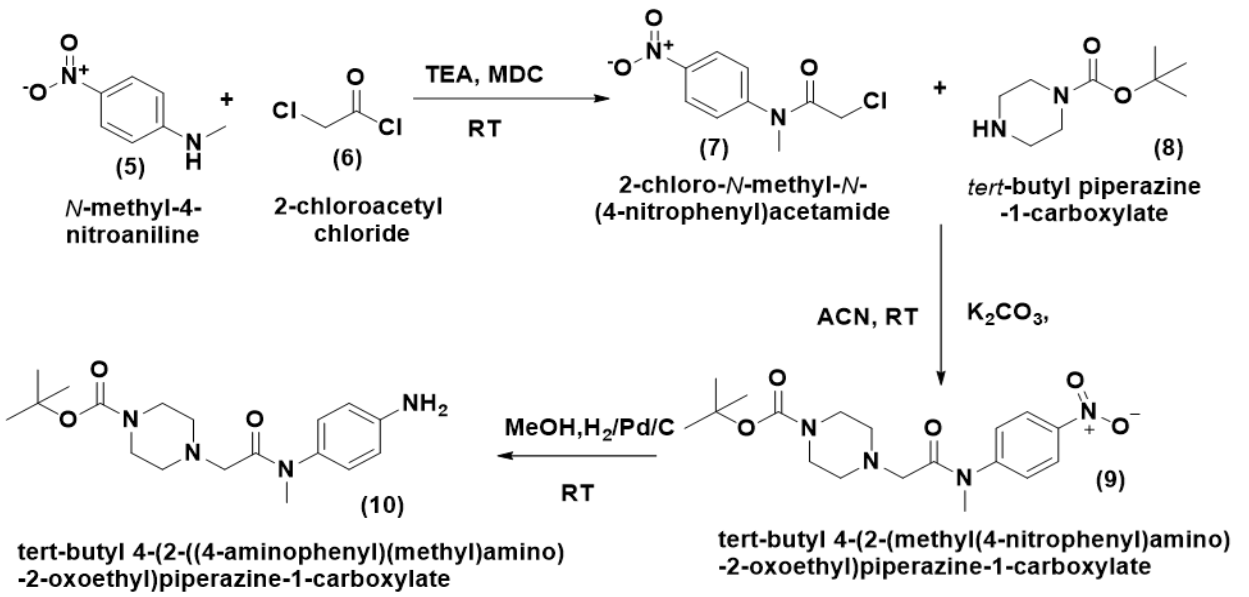
Scheme 5. Synthesis of methyl (E)-1-acetyl-3-(Methoxy(phenyl)methylene)-2-oxoindoline-6-carboxylate

2) Synthesis of tert-butyl 4-(2-((4-aminophenyl) (methyl)amino)-2-oxoethyl) piperazine-1-carboxylate (10):

A) 2-chloro-N-methyl-N- (4-nitrophenyl) acetamide (7): N methyl nitroaniline (5) (3.10gm,9 mmol, 1 equiv) was solubilised in Dichloromethane (30 ml) and reaction temperature was maintained at 0 ^{O}C. Triethylamine (3.25 ml, 9 mmol, 1.2 equiv) was added dropwise to the solution. Chloro acetyl chloride (6) (1.9 ml, 9 mmol, 1 equiv) was added dropwise to the reaction. The reaction was monitored using TLC (Ethyl acetate: n Hexane 6:4) every 30 mins. The reaction was continued for 2.5 hrs till starting material get fully consumed. The reaction mixture was quenched in water and extracted using Ethyl acetate and further purified using column chromatography (Ethyl acetate: n Hexane 20% to 30% polarity) to get compound (7). The compound was confirmed by Mass and 1 H NMR.

B) Tert-butyl 4-(2-(methyl(4-nitrophenyl) amino) -2-oxoethyl) piperazine-1-carboxylate (9): 2-chloro-N-methyl-N- (4-nitrophenyl) acetamide (7) (3 gm, 9mmol, 1 equiv) was dissolved in acetonitrile (30 ml). Tert-butyl piperazine -1-carboxylate (8) (2.45 gm, 9mmol, 1.2 equiv) was then added dropwise to the solution. K_2CO_3 (2.15 gm, 9mmol, 1.2 equiv) was further added to the solution. catalytic amount of KI (0.56gm, 9 mmol, 0.25 equiv). the reaction was monitored using TLC every 30 min (Ethyl acetate). After 2 Hrs, after complete consumption of starting material, reaction was stopped and quenched in water. The product was extracted using Dichloromethane, dried with sodium sulphate. Which was further purified using column chromatography (Ethyl acetate: n Hexane 50% polarity to get compound (9). The compound formation was confirmed by Mass analysis.

C) Tert-butyl 4-(2-((4-aminophenyl) (methyl)amino) -2-oxoethyl) piperazine-1-carboxylate (10): Tert-butyl 4-(2-(methyl(4-nitrophenyl) amino) -2-oxoethyl) piperazine-1-carboxylate (9) (2gm, 9 mmol, 1 equiv) was dissolved in Methanol. Pd/C (200mg, 9 mmol, 1.2 equiv) was added to the reaction. the reaction was continued at RT for 10 min. H_2 gas was purged in the reaction and reaction. Reaction was monitored using TLC (Ethyl acetate: n Hexane 8:2) for every 30 min. The reaction was continued for 4 hrs. after complete consumption of starting material the reaction mixture was filtered and the filtrate further concentrated and purified by recrystallisation using methanol to obtain compound (10) (Scheme 6 Synthesis of tert-butyl 4-(2-((4-aminophenyl) (methyl)amino) -2-oxoethyl) piperazine-1- carboxylate). The compound formation was confirmed by Mass analysis [14-16]

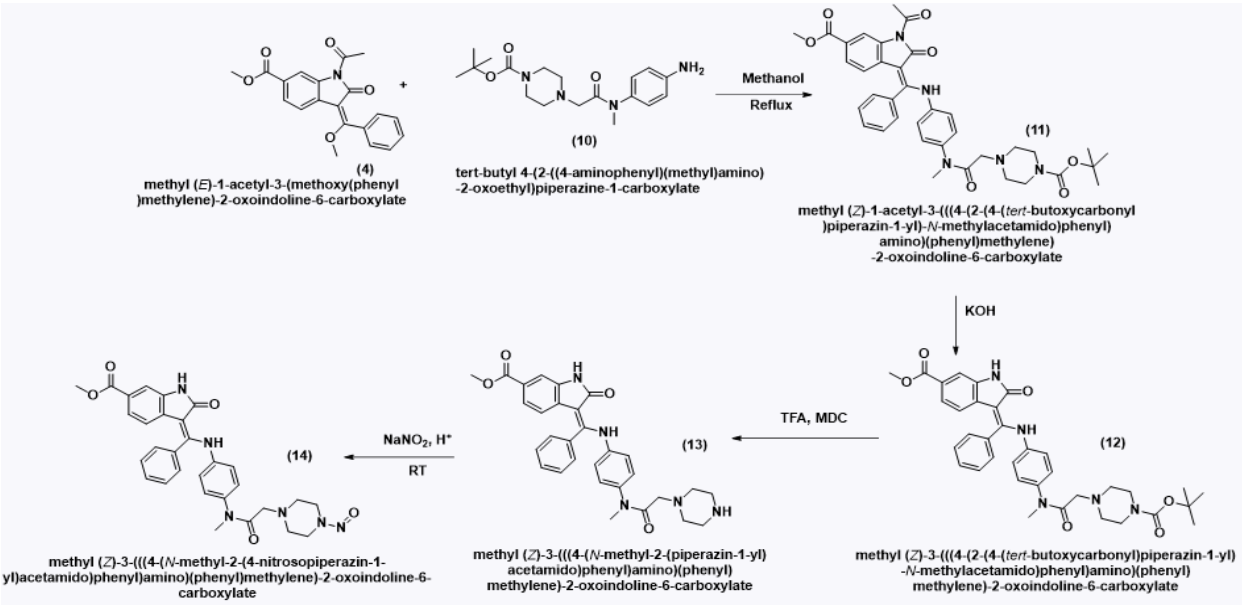


Scheme 6. Synthesis of tert-butyl 4-(2-((4-aminophenyl) (methyl)amino) -2-oxoethyl) piperazine-1- carboxylate

3) Synthesis of N Nitroso-Nintedanib:

A) Methyl (Z)-3-(((4- (2-(4-(tert-butoxy carbonyl) piperazin-1-yl) -N-methyl acetamido) phenyl) amino) (phenyl) methylene)-2-oxoindoline-6-carboxylate (12): methyl (E)-1-acetyl-3-(methoxy (phenyl) methylene)-2-oxoindoline-6-carboxylate (4) (3 gm, 9mmol, 1 equiv) was dissolved in Methanol (30 ml) and tert-butyl 4-(2-((4-aminophenyl) (methyl)amino) -2-oxoethyl) piperazine-1-carboxylate (10) was added dropwise by dissolving in small portion of methanol. the reaction was then set at 60 ^{O}C. The reaction was monitored using TLC for every 30 min. After 5 Hrs after complete consumption of starting material, the reaction indicates formation of compound (11). The reaction was charged with Potassium hydroxide (0.52 gm, 9 mmol, 0.5 equiv) the temperature was gradually reduced to Room Temperature and reaction was stirred for 20 min. Then the reaction temperature was maintained at 10 ^{O}C and it was continuously stirred for 1 Hr. The TLC examination was done an it indicated formation of compound. The reaction was quenched in ice water and compound was extracted with Dichloromethane and purified using column chromatography (Ethyl acetate: n Hexane 30% to 70% polarity) to get compound (12).

B) Methyl (Z)-3-(((4-(N-methyl-2-(4-nitrosopiperazin-1yl) acetamido) phenyl) amino) (phenyl) methylene)-2-oxoindoline-6-carboxylate (14): Methyl (Z)-3-(((4-(2-(4-(tert-butoxy carbonyl) piperazin-1-yl) -N-methyl acetamido) phenyl) amino) (phenyl) methylene)-2-oxoindoline-6-carboxylate (12) (1.00 gm, 9 mmol, 1 equiv) was dissolved in Dichloromethane and the reaction temperature was maintained at 0 ^{O}C. Trifluoracetic acid (0.20 ml, 9 mmol, 1.2 equiv) was added dropwise to the solution. the reaction was continued at Room Temperature. The reaction was monitored using TLC every 30 min. After 2 Hrs the complete consumption of Starting material was observed. The reaction was the concentrated to get compound (13), Which further used to synthesize last step compound. Methyl (Z)-3-(((4-(N-methyl-2-(piperazin-1-yl) acetamido) phenyl) amino) (phenyl) methylene)-2-oxoindoline-6-carboxylate (13) (0.9 gm, 9 mmol, 1 equiv) was dissolved in Dichloromethane and the reaction was maintained at 0 ^{O}C. Sodium Nitrite (0.21 ml, 9 mmol, 1.2 equiv) was then added dropwise to the reaction. The reaction was maintained at room temperature. The reaction was monitored by TLC (Ethyl acetate: TEA 9.5:0.5) every 10 mins. After 30 mins complete consumption of starting material is indicated on TLC. the reaction was quenched in ice water and the product was extracted using Dichloromethane and dried over sodium sulphate to obtain crude product. Purified using column chromatography (Ethyl acetate: n Hexane 50% to 70 % polarity) to obtain compound (Scheme 7. Synthesis of N Desmethyl N Nitroso Nintedanib) (14). [14-25]



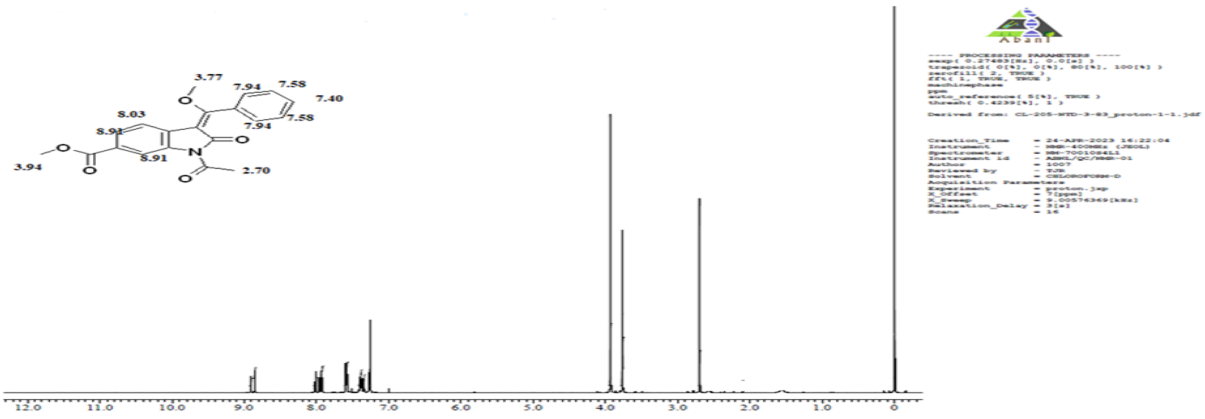
Scheme 7. Synthesis of N Desmethyl N Nitroso Nintedanib

III. RESULT AND DISCUSSION

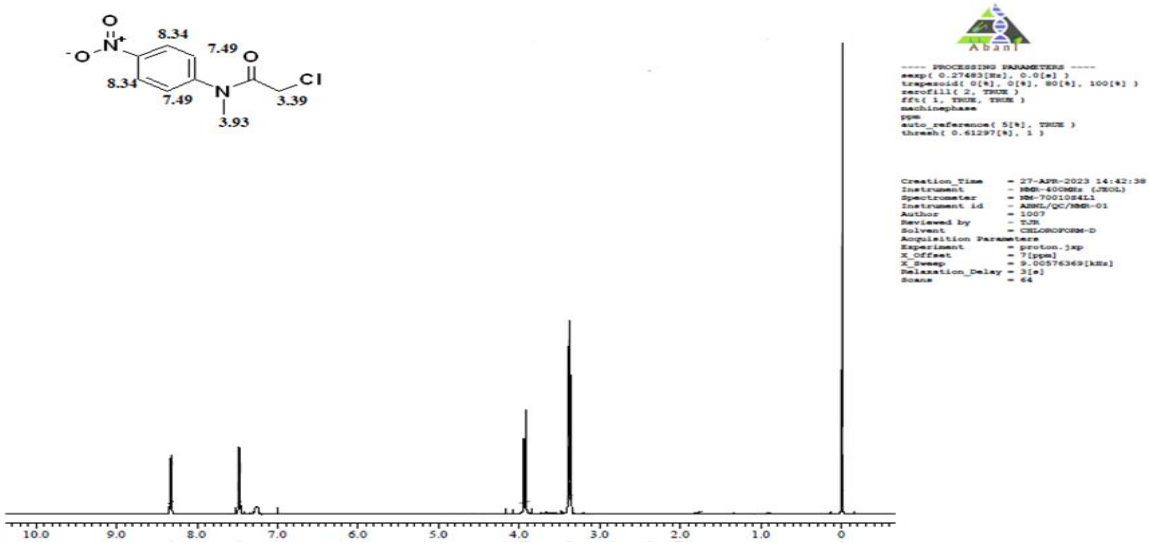
Results:

The synthesized products primarily characterized by chromatographic optimization (TLC for characterization & column chromatography for purification) & Mass Spectrometry. The observations were satisfactory as compared with literature findings. The final compounds were characterised by 1 H NMR analysis and purity was determined by HPLC analysis.[18]

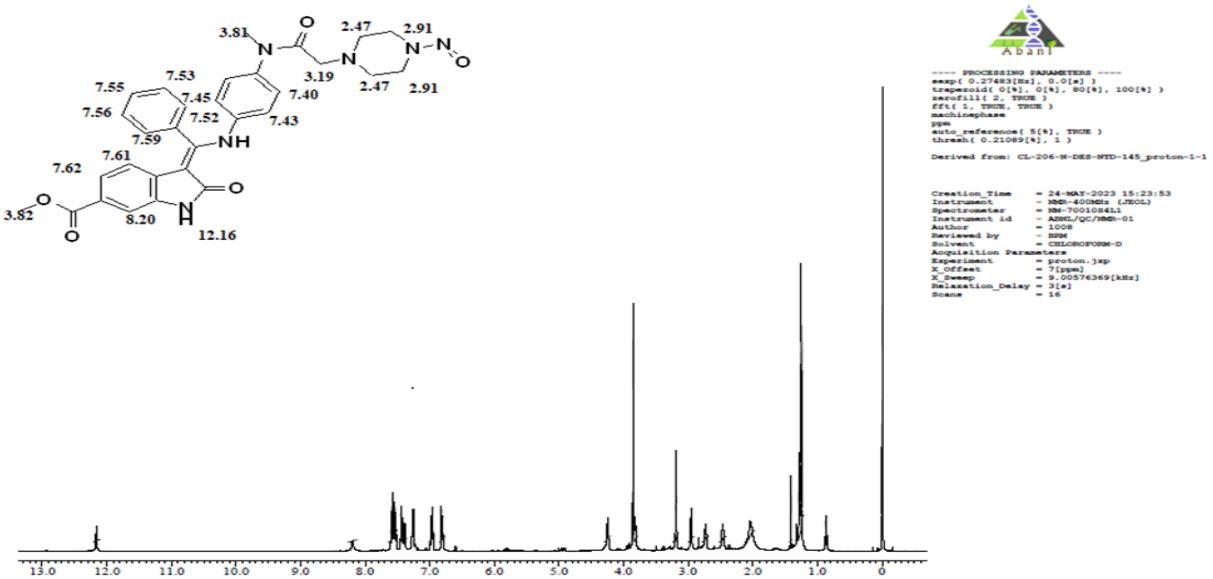
1) Methyl 1-acetyl-2-oxoindoline-6-carboxylate (3): Appearance: Pale orange to red amorphous solid, Yield: 3.8 gm (95%), Mass (observed /calculated): 234.08/234.07, Purity: 84.50%.
2) Methyl (E)-1-acetyl-3-(Methoxy(phenyl)methylene)-2-oxoindoline-6-carboxylate (4): Appearance: Pale yellow amorphous solid, Yield: 4.2 gm (92.96%), Mass (observed /calculated): 352.05/352.11, ^{1}H NMR (DMSO D_6): 8.91(d, 2H), 8.03 (d, 1H), 7.94 (d, 2H), 7.58 (m, 2H), 7.40 (m, 2H), 3.94 (s, 6H), 3.77 (s, 5H), 2. 70 (s, 3H). Purity: 83.24%.



3) 2-chloro-N-methyl-N- (4-nitrophenyl) acetamide (7): Appearance: Brownish Liquid, Yield: 2.30 gm (51.11 %), Mass (observed /calculated): 229.13/229.03, ^{1}H NMR (DMSO D_6): 8.34 (d, 2H), 7.49 (d, 2H), 3.93 (s, 2H), 3.39 (s, 3H). Purity: 95.60%.



4) Tert-butyl 4-(2-(methyl(4-nitrophenyl) amino) -2-oxoethyl) piperazine-1-carboxylate (9): Appearance: Yellowish Liquid, Yield: 3 gm, Mass (Observed/calculated): 380.02/380.20. (61.11%), Purity: 84.24%.
5) Tert-butyl 4-(2-((4-aminophenyl) (methyl)amino)-2-oxoethyl) piperazine-1-carboxylate (10): Appearance: Yellowish Liquid, Yield: 3 gm (85%), Mass (Observed/calculated): 348.02/348.20. (61.11%), Purity: 84.30%.
6) Methyl (Z)-3-(((4-(2-(4-(tert-butoxy carbonyl) piperazin-1-yl) -N-methyl acetamido) phenyl) amino) (phenyl) methylene)-2-oxoindoline-6-carboxylate (12): Appearance: White solid, Yield: 0.830 gm (82.10%) Mass (Observed/calculated): 348.02/348.20. (61.11%), Purity: 83.24%.
7) Methyl (Z)-3-(((4-(N-methyl-2-(4-nitrosopiperazin-1yl) acetamido) phenyl) amino) (phenyl) methylene)-2-oxoindoline-6-carboxylate (14): Appearance: Yellowish Sticky solid, Yield: 0.600 gm (69.04%) Mass (Observed/calculated): 555.45/556.23. (61.11%), ^{1}H NMR (DMSO D_6): 12.96(s, 1H), 8.20 (d, 1H), 7.62(d, 1H), 7.61 (d, 1H), 7.59 (m, 1H), 7.56 (m, 1H), 7.55 (m, 1H), 7.52 (m, 1H), 7.43 (d, 2H), 7.40 (d, 2H), 3.81 (s, 3H), 3.19 (s, 2H), 2.91 (d, 4H), 2.47 (d, 4H). Purity: 96.43%.



IV. CONCLUSION

In current study, we attempted to synthesize N-Nitroso N -Desmethyl Nintedanib. The API is complex molecule and having poor yield at last stage. Although in this study we attempted to have maximum possible yield and purity of synthesized molecules. The N nitrosoamine impurity is difficult to synthesize and it is highly unstable. In attempt to synthesize the impurity many organic reactions, various reagents and methods have been handled under various trials. To synthesize N-Nitroso N -Desmethyl Nintedanib it takes at least 10 steps. In this study we concluded that steady temperature conditions quick purification, and proper storage is important for the products. Further we are planning to conduct biological activities of the nitroso impurities, to identify exact mechanism of nitroso compounds on body. In attempt to study the synthesis pattern of Nintedanib and its nitroso impurities, we screened various reaction mechanisms, ways of synthesis of N Nitrosoamine impurities and purification out of which, synthesis of BOC protected Nintedanib and its further conversion to N-Nitroso N -Desmethyl Nintedanib is successful than other routes.

V. CONFLICT OF INTEREST:

The Authors declare no conflict of interest.

VI. ACKNOWLEDGEMENT

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