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A Comprehensive Synthesis and Standardisation of Nitrosamine Impurities of Mirabegron and Their Related Compounds

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ABSTRACT

Background: Impurities present in bulk API are a major issue now a days. The Mirabegron is one of the majorly consumed drugs worldwide for diabetes insipidus and bladder disorder. It is mainly consumed by all age group of patients especially in geriatrics. Nitrosamine impurities in Mirabegron have carcinogenicity. Synthesis and standardization of N-Nitroso-Mirabegron and related Nitrosamine impurities are of prime importance in this case. **Objective:** The objective of this study is to synthesize N-Nitroso-Mirabegron and related nitrosoamine impurities. **Methods:** Nitrosoamines were synthesized by optimization in various catalysts and development of synthetic routes for Mirabegron related nitrosoamine impurities, including isomeric nitrosoamine impurities. **Results:** The aim to synthesize N-Nitroso-Mirabegron and related Nitrosoamine was successful. The synthesis was confirmed by Mass spectroscopy and NMR analysis. **Conclusion:** The presence of Nitrosoamines were identified in Mirabegron previously. In current study, successful synthesis, characterization and optimal yield of defined Nitrosoamines of Mirabegron was done.

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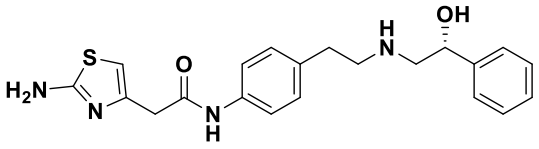
INTRODUCTION

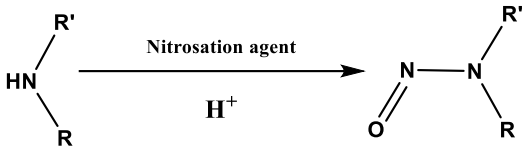
Mirabegron is API which is commonly used for all types of overactive bladder disorders, mainly in Diabetes insipidus. The drug was developed by Astellas (R)-2-(2-aminothiazol-4-yl)-40 - [2-[(2-hydroxy-2-phenylethyl) amino] ethyl acetanilide) and approved by FDA in 2012, the first drug of choice by clinicians for the treatment of hyperactive bladder disorder (Figure 1). The age group consuming Mirabegron ranges from 5-year-old to 95-year-old patients. Due to this wide age limits, the purity and safety become prime importance [1-2].



(*R*)-2-(2-aminothiazol-4-yl)-*N*-(4-(2-((2-hydroxy-2-phenylethyl)amino)ethyl)phenyl)acetamide

Figure 1. Mirabegron API

As we all know any API is not Hundred percent pure. Many relative impurities are present in the API such as isomeric, residual starting materials, oxidative products, Nitrosoamines and many more. Nitrosamines (NAs) are a class of compounds and are Mostly derived in Organic compound compounds containing secondary amines [3]**.** Various N-Nitrosamines are detected in various types of Active Pharmaceutical Ingredients (API). Firstly, (NDMA) N-Nitrosamine dimethyl amine was detected in valsartan in 2018 [4]. As Nitrosamines are highly carcinogenic for living organisms especially due to their alkylating efficiency after enzymatic activation to alkyl diazonium which results in DNA damage can lead to carcinogenicity, teratogenicity. Mostly it shows hepatotoxicity, neurotoxicity, cardiotoxicity, renal toxicity and toxicity to overall digestive system**.** A new challenging task for pharma industry to minimise the NAs in APIs is evolving [5]. Recently some years, FDA & Medical agencies step-up to discover presence of unacceptable levels of Nitrosamines in several popular medications and resulting to evoke and establishment some new guidelines. As a result, the Agencies provide guidelines to manufacturers of APIs and medicinal formulation to prevent risk assessments of their impurities [6]. Although, NAs are detected broadly from 2018, but their presence has been detected from 19th century in food, water, air and other materials in numerous forms. NAs are generally detected and formed in acidic environments. Also, they can be prepared by use of sodium nitrite ($NaNO_2$) and alkyl nitrates (Isoamyl nitrite) in compounds containing secondary, tertiary amines [7].



Scheme 1. The general method of synthesis of Nitrosoamines.

According to WHO, bladder cancer is the most prevalent type of cancer in the world, accounting for 3% of all occurrences. N-nitroso compounds (NOCs) are carcinogenic in all mammalian individuals, however, the target organ of cancer development, particularly bladder cancer, and scientists are exploring the exact cause till date [7-8]. Current research work focus on the synthesis of four major nitrosoamine impurities, i.e., N-Nitroso Mirabegron (N-MBG), S isomer of N- Nitroso Mirabegron (N-MBG-S), and the other two are Mirabegron related Nitrosoamines impurities derived from Mirabegron Key Starting Material for synthesis (N-MBG-I, N-MBG-II) [9-10]. The study includes optimisation of direct nitrosation of Mirabegron and related compounds and synthesis of S Mirabegron derivative for synthesis of N- Nitroso Mirabegron. The direct nitrosation was optimized for different nitrosation agent, different acidic condition, different solvents and different temperature conditions. All compounds were optimized for these reactions. The reaction time was variable for different substrates i.e., MBG, MBG-S, MBG-I. N-MBG-I was further converted to N-MBG-II by selective hydrogenation. [11-12]. To synthesize N-MBG-S, MBG-S was synthesized separately by taking Chemoselective Key Starting Material. The reason is isolation of MBG-S from crude MBG is very tedious [13].

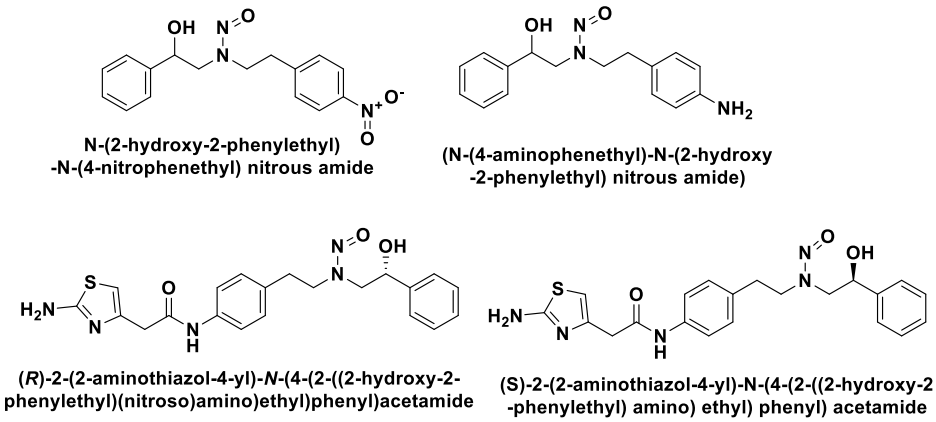


Figure 2. Nitrosamine impurities related to Mirabegron

MATERIALS AND METHODS:

Materials: Chemicals & reagents were procured from Sigma Aldrich, TCI, BLD Pharma, and were of synthetic grade. The 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, Ninhydrin reagent and iodine vapors. ^{1}H NMR was recorded on "Bruker Advance" Spectrometer at 400 MHz frequency in DMSO D_6 in the 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. Purification of synthesized Compounds done by BUCHI C-700 Prep-chromatographic technique.

Optimization of Direct N Nitrosation of Mirabegron related Nitrosoamine impurities: Starting Material (1 eq) was suspended in the appropriate solvent (10 Vol.). The reaction was set at 0˚C. The Nitrosating agent was added to the reaction mixture. The reactions were optimised for Various Nitrosating agents, various temperature range (0-85˚C), various equivalents and different time periods. Total optimization is summarised in Table 1. (Table 1, Table 2, Table 3) [14].

Table 1. Optimization of N Nitrosoamine synthesis of MBG

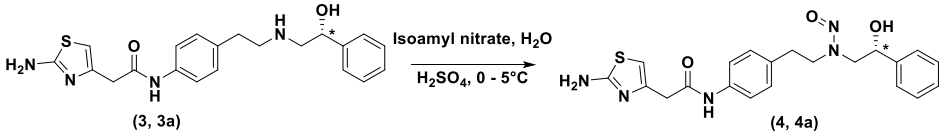
Sr. No.	Reagents	Temperature (°C)	Solvents	Time (Hrs)	Yield (%)
1	$NaNO_2$ + Conc. HCl	0-5	Water	20	10
		35		9	8
		80		4	6
2	$NaNO_2$ + Acetic Acid	0-5	Water	10	4
		35		10	30
		80		4	14
3	$NaNO_2$ +BiCl3	0-5	Acetonitrile	19	10
		35		16	14
		80		6	23
4	$NaNO_2$ +H2SO4	0-5	Water	3	27
		35		19	15
		80		9	25
5	IAN	0-5	Dichloromethane	35	32
		35		30	**62**
		80		9	10

Table 2. Optimization of N Nitrosoamine synthesis of MBG-S					
Sr. No.	**Reagents**	**Temperature (°C)**	**Solvents**	**Time (Hrs)**	**Yield (%)**
1	$NaNO_2$ + Conc. HCl	0-5	Water	22	5
		35		8	9
		80		3	7
2	$NaNO_2$ + Acetic Acid	0-5	Water	10	3
		35		10	10
		80		4	12
3	$NaNO_2$ +BiCl3	0-5	Acetonitrile	18	8
		35		16	14
		80		6	20
4	$NaNO_2$ +H2SO4	0-5	Water	2	10
		35		18	13
		80		8	21
5	IAN	0-5	Dichloromethane	25	23
		25		20	**60**
		45		8	3

Table 3. Optimization of N Nitrosoamine synthesis of MBG-I					
Sr. No.	**Reagents**	**Temperature (°C)**	**Solvents**	**Time (Hrs)**	**Yield (%)**
1	$NaNO_2$ + Conc. HCl	0-5	Water	5	82
		35		02:30	**91**
		80		2	78
2	$NaNO_2$ + Acetic Acid	0-5	Water	4	40
		35		03:30	54
		80		5	37
3	$NaNO_2$ +BiCl3	0-5	Acetonitrile	6	78
		35		4	72
		80		2	45
4	$NaNO_2$ +H2SO4	0-5	Water	3	69
		35		2	71
		80		02:30	56
5	IAN	0-5	Dichloromethane	05:30	37
		25		5	48
		45		2	21

1. **General procedure for the synthesis of N-Nitroso amine of S and R Isomer of Mirabegron:** 2-(2-amino-1,3-thiazol-4-yl)-N-[4-[2-[[(2R)-2-hydroxy-2-phenyl ethyl] amino] ethyl] phenyl] acetamide (7.8mmol,1eq) was suspended in THF (30ml). The reaction was set at 0°C. Isoamyl nitrite (9.8mmol, 1.34 eq) was added to the reaction

mixture followed by acidification using conc. sulfuric acid and the reaction was continued at 0°C. The reaction was monitored using TLC (Methanol: Dichloromethane 2: 8) using every 15 mins. Complete consumption of starting material was observed after 10 hrs. The reaction mixture was extracted with Dichloromethane to obtain a crude compound. The obtained crude compound was purified using flash chromatography. Obtained compound was converted to hydrochloride salt for stability (Scheme 2) [16-17].



Scheme 2. General procedure for the synthesis of N-Nitrosoamine of MBG AND MBG-S

2. Synthesis of S Mirabegron (S)-2-(2-aminothiazol-4-yl)-N-(4-(2-((2-hydroxy-2-phenylmethyl) amino) ethyl) phenyl) acetamide

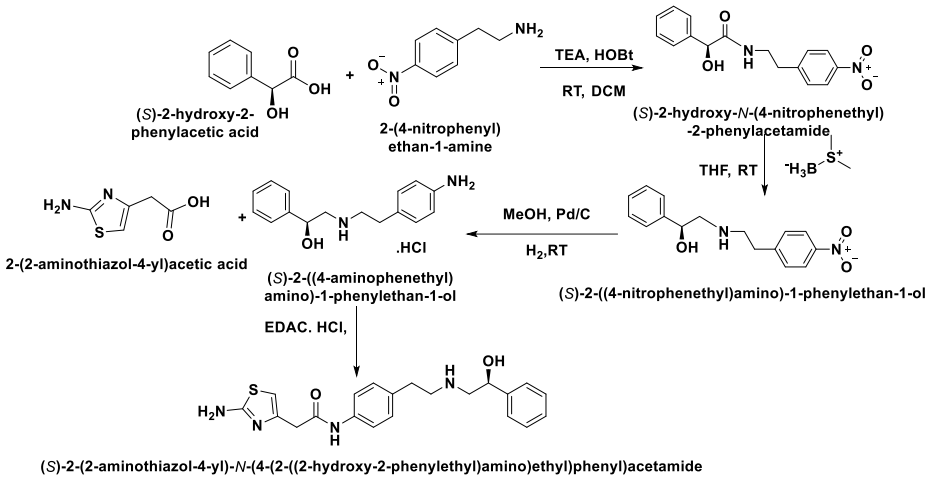
A) Synthesis of (S)-2-hydroxy-N-(4-nitrophenyl)-2-phenylacetamide: (S)-2-hydroxy-2-phenylacetic acid (131mmol, 1eq) was suspended in Dichloromethane (10 Vol). The reaction was set at RT. 2-(4-nitrophenyl) ethanamine (131mmol, 1eq), added in reaction mixture. Cooled reaction mixture to 0°C. Added Triethyl amine (262mmol, 2eq) & Hydroxybenztriazole (157mmol,1.2eq,) simultaneously. Solution of 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (197mmol, 1.5eq) was added slowly to the reaction mixture at 0°C. The reaction was monitored using TLC (Ethyl acetate) every 30 Mins. Complete consumption of starting material was observed after 4 hrs. The compound was quenched in cold water and extracted with Dichloromethane and dried over sodium sulphate. The compound was primarily purified using repeated washing using water to obtain pure product crude product. The obtained crude product was further purified using column chromatography to obtain (S)-2-hydroxy-N-(4-nitrophenyl)-2-phenylacetamide (40- 60 % Ethyl acetate: n-Hexane) [13].

B) **Synthesis of (S)-2-((4-nitrophenyl) amino)-1-phenylethanol:** (S)-2-hydroxy-N-(4-nitrophenyl)-2-phenylacetamide (89.9mmol, 1eq,) suspended in Tetrahydrofuran (10 Vol). The reaction mixture was set to 0°C under inert conditions. Borane Dimethyl Sulphide was added dropwise to the reaction mixture. The reaction was maintained at 0°C for the next 30 mins. Further, the reaction was maintained at room temperature for 2 hrs. The reaction was monitored using TLC (Ethyl acetate) every 30 mins. Chromatographic

representation of the development of product is observed. then the reaction was set at 0°C. Complete consumption of starting material was observed after 4 hrs. The reaction was cooled to room temperature. The reaction was quenched in equimolar mixture of Water and tetrahydrofuran. Further it was neutralised using 10 % aqueous NaOH. The mixture was stirred for 10 mins and the compound was extracted using Ethyl acetate and dried over sodium sulphate. The compound was further purified using excessive brine solution to obtain crude product. The obtained crude product was further purified using column chromatography to obtain (S)-2-((4-nitrophenyl) amino)-1-phenylethanol (40- 60 % Ethyl acetate: n Hexane) [13].

C) Synthesis of (S)-2-((4-aminophenethyl) amino)-1-phenyl ethanol: (S)-2-((4-nitrophenyl) amino)-1-phenylethanol (69mmol, 1eq,) was suspended in methanol (10 Vol) and it was transferred to a vial of Autoclave reactor. 5% Pd/C (69mmol, 1eq,) was added to the reaction mixture under inert conditions. The reaction was maintained for the next 10 mins. Hydrogen gas was then purged to the reaction mixture. The reaction was monitored using TLC every 10 Mins (Methanol: Dichloromethane 1:9). Complete consumption of starting material was observed after 5 hrs. The reaction was then discontinued and filtered through celite bed. Filtrate was then concentrated to get the crude product. The product was then purified using column chromatography (40- 60 % Ethyl acetate: n Hexane) to obtain (S)-2-((4-aminophenethyl) amino)-1-phenylethanol. The compound was converted to hydrochloride salt for stability [13].

D) Synthesis of (S)-2-(2-aminothiazol-4-yl)-N-(4-(2-((2-hydroxy-2-phenylethyl) amino) ethyl) phenyl) acetamide: (S)-2-((4-aminophenethyl) amino)-1-phenylethanol (76mmol, 1eq,) was suspended in water (10 Vol). The reaction was maintained at room temperature. 2-aminothiazol-4-yl acetic acid (76mmol, 1eq,) to the reaction mixture. Solution of 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (114mmol, 1.5eq,) in water (2vol) was added slowly to the reaction mixture at RT. The reaction was monitored using TLC (Methanol: Dichloromethane 1:9). Complete consumption of starting material was observed after 3 hrs. The reaction was then treated with 10% aqueous sodium hydroxide. the product was extracted with Ethyl acetate and dried over sodium sulfate to get the crude product. The compound was then purified using column chromatography to obtain (S)-2-(2-aminothiazol-4-yl)-N-(4-(2-((2-hydroxy-2-phenylethyl) amino) ethyl) phenyl) acetamide (Scheme 3) [13].

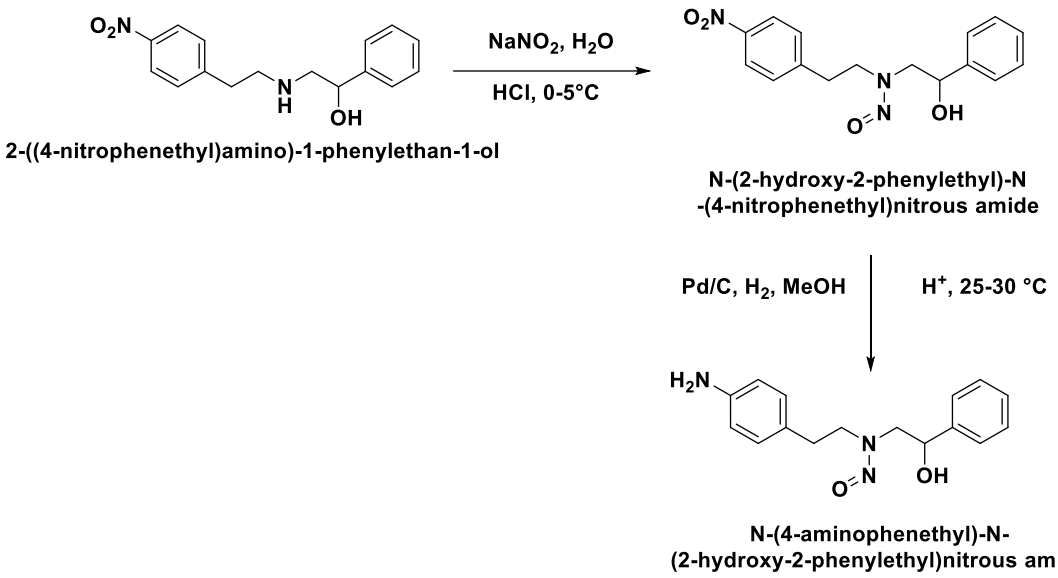


Scheme 3. Synthesis of S (S)-2-(2-aminothiazol-4-yl)-N-(4-(2-((2-hydroxy-2-phenylethyl) amino) ethyl) phenyl) acetamide

4. Synthesis of N-(4-aminophenethyl)-N-(2-hydroxy-2-phenylmethyl) nitrous amide:

A) Synthesis of N-(2-hydroxy-2-phenylethyl)-N-(4-nitrophenethyl) nitrous amide: 2-((4-nitrophenethyl) amino)-1-phenylethan-1-ol (24.4 mmol,1eq) was suspended in water and the reaction was set to 0°C for 15 Mins. conc. HCl was added dropwise till the pH get maintained between 2-3. Aqueous solution of Sodium Nitrate (31.72 mmol,1.3eq.) was added dropwise to the reaction. The reaction was monitored using TLC (Ethyl: Hexane, 4:6) every 15 Mins. Complete consumption of starting material was observed after 2 hrs. The compound was filtered and washed with acidic water to get the crude product. The product was purified using column chromatography to get compound N-(2-hydroxy-2-phenylethyl)-N-(4-nitrophenethyl) nitrous amide [14].

B) Synthesis of (N-(4-aminophenethyl)-N-(2-hydroxy-2-phenylethyl) nitrous amide): N-(2-hydroxy-2-phenylethyl)-N-(4-nitrophenethyl) nitrous amide (12.6 mmol,1eq) was suspended in methanol. The reaction was set at RT. Pd/C (10%, 0.8 gm) was added to reaction mixture. The acidic environment was maintained by the addition of Acetic acid. The reaction was stabilized for 15 Mins. H_2 gas was purged to reaction maintaining pressure at 5 Bar. The reaction was monitored using TLC (Ethyl: Hexane, 4:6) every 15 Mins. Complete consumption of starting material was indicated on TLC after 4 hrs. The reaction was then discontinued. The obtained reaction was filtered through flow bed and washed with methanol to obtain compound (N-(4-aminophenethyl)-N-(2-hydroxy-2-phenylethyl) nitrous amide) [14]. (Scheme 4) [15].



Scheme 4. Synthetic route of N-(4-aminophenethyl)-N-(2-hydroxy-2-phenylethyl) nitrous amide

RESULTS AND DISCUSSION:

RESULTS:

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

1. **2-(2-aminothiazol-4-yl)-N-(4-(2-((2-hydroxy-2-phenylethyl) amino) ethyl) phenyl)-N-nitroso acetamide: Chemical Formula:** $C_{21}H_{23}N_5O_3S$, **Molecular Weight**: 425.504 **Appearance:** Pale yellow to orange coloured hygroscopic solid, **1H NMR (400 MHz, $CDCl_3$):** 5.02 (s,2H,), 6.33 (s,1H) 3.92 (dd,1H) 3.77 (m,2H) 7.43 (d, 2H) 7.07 (d, 2H) 2.74 (t,1H)& 2.99 (t,1H)3.56 (d, 2H) 8.99 (s, 1H) 4.22 [(m, 1H) 4.09 (d, 1H) 5.0 (m,1H) 4.91 (d,1H) 7.34 (m, 5H). **Mass (Observed/Calculated):** 426. 38/ 426.14 **Yield:** 89.43% Purity**:** 94.95%.
2. **(S)-2-(2-aminothiazol-4-yl)-N-(4-(2-((2-hydroxy-2-phenylethyl) amino) ethyl) phenyl) acetamide: Chemical Formula**: $C_{21}H_{23}N_5O_3S$**, Molecular Weight**: 396.45, **Appearance:** Pale yellow to orange color solid, **1H NMR (400MHz, DMSO-D6):** 6.89 (s, 2H) 6.3 (s, 1H) 3.44 (s,2H) 10.0 (s,1H) 7.49(d,2H) 7.11 (d, 2H) 2.75 (m,2H) 1.7 (s,1H)

5.21(d,1H) 4.69 (m,1H) 7.21 (m,1H) 7.30 (m,4H) **Mass (Observed/Calculated):** 397.45/426.160 **Yield:** 83.45% **Purity:** 95.40%.

3. **(S)-2-(2-aminothiazol-4-yl)-N-(4-(2-((2-hydroxy-2-phenyl ethyl) (nitroso) amino) ethyl) phenyl) acetamide**: **Chemical Formula:** $C_{21}H_{23}N_5O_3S$, **Molecular Weight**: 425.504 **Appearance:** Pale yellow to orange colored hygroscopic solid, **1H NMR (400 MHz, DMSO-D6):** 8.14 (s,1H) 6.5 (s,1H) 4.27 (m,1H) 4.10 (m,1H) 10.17 (d,1H) 7.51 (t,2H) 7.14 (d, 2H) 3.60 (s,2H) 2.96 (t,1H) 2.67(s,1H), 3.83 (m,1H), 3.73 (m,1H), 5.70 (d,1H), 4.83 (dq,1H), 7.27 (t,1H), 7.37 (m,4H) **Mass (Observed/Calculated):** 425.50/ 425.54 **Yield:** 75.84% **Purity:** 94.23%.
4. **N-(2-hydroxy-2-phenylethyl)-N-(4-nitrophenethyl) nitrous amide: Chemical Formula:** $C_{16}H_{17}N_3O_4$, **Molecular Weight**: 315.324 **Appearance:** Off white to pale yellow solid, **1H NMR (500 MHz, DMSO-D6):**, 8.16 (m, 2H) 7.52 (t, 2H) 7.43 (t, 1H) 7.36pp m(q, 3H) 7.28 (m, 1H) 2.89 (m, 1H, 9), 5.72 (d, 1H), 4.85 (m, 2H), 4.38 (m, 2H), 4.19 (m, 1H), 3.77 (m, 2H), 3.21 (m, 1H) **Mass (Observed/Calculated):** 316.45/315.122, **Yield:** 85.43% **Purity:** 96.22%.
5. **(N-(4-aminophenethyl)-N-(2-hydroxy-2-phenylethyl) nitrous amide): Chemical Formula:** $C_{16}H_{19}N_3O_2$, **Molecular Weight**:285.34 **Appearance:** Pale yellow to orange colored hygroscopic solid. **1H NMR (400 MHz, DMSO-D6):** 3.62 (m,2H)7.37 (m,4H) 4.70 (m,1H) 4.11 (m,2H) 2.81 (t,1H) 4.89(d,2H) 4.93 (m,1H) 5.66 (d,1H) 6.84 (d,2H) 6.47 (t,2H) 7.27 (m,1H) **Mass (Observed/Calculated):** 286. 23/286.45 **Yield:** 78.97% **Purity:** 93.99%.

DISCUSSION:

The study aimed to synthesize nitrosoamine impurities of Mirabegron and to standardise them. The drug contains chiral center and the R isomer is standard Mirabegron drug. Hence, S isomer and its Nitrosoamine is considered as separate impurities amongst all impurities of Mirabegron. To synthesize and standardise Nitrosoamine of S isomer of Mirabegron, synthetic route of S Mirabegron was developed using Chemoselective starting materials to get optimal yield. Further synthesis of N-Nitroso-S-Mirabegron was done by various nitrosation agents. The two related Nitrosoamines of Mirabegron were attempted to synthesize by various routes out of which, direct nitrosation of key starting material used in the synthesis of Mirabegron and converting it to amino derivative using selective hydrogenation given successful results (Figure 3. Synthesis route attempted for N-nitroso derivatives of Mirabegron) [16].

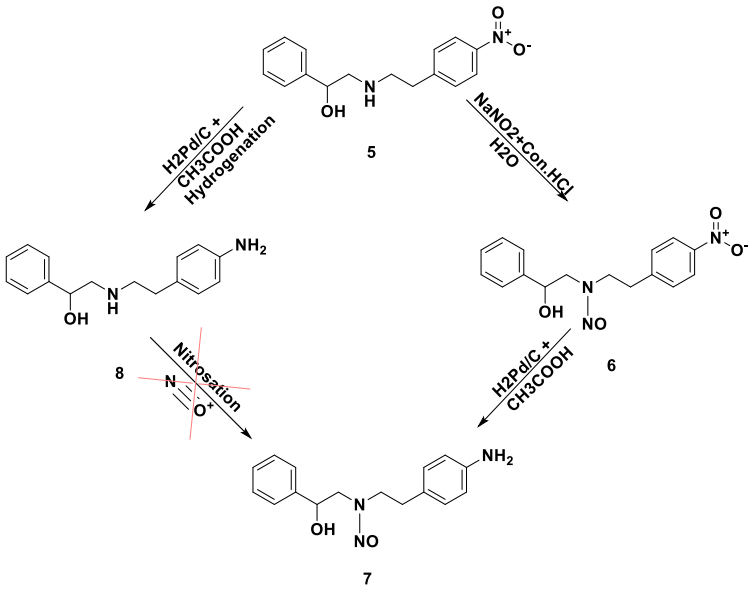


Figure 3. Synthesis route attempted for N-Nitroso Mirabegron related impurity

CONCLUSION:

The synthesis of *N*-Nitrosoamines of mirabegron was crucial and tedious, which has been attempted and made successful. As well as enhanced yield of nitrosoamine impurities was also attempted. The study also concluded that the synthesis of isomeric impurities is easier than isolation of them from crude drug is more successful. Based on this research, a sustainable method of synthesis for N Nitrosoamines can be established.

AVAILABILITY OF DATA AND MATERIALS

NMR spectra is available in a supplementary file.

CONFLICT OF INTEREST:

The Authors declare no conflict of interest.

ACKNOWLEDGEMENT:

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LIST OF ABBREVIATIONS:

MBG: Mirabegron

KSM: Key Starting Material

NA: Nitrosoamine

IAN: Isoamyl Nitrile

DCM: Dichloromethane

MeOH: Methanol

TEA: Trimethylamine

THF: Tetrahydrofuran

TLC: Thin Layer chromatography

Eq: Equivalent

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