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Identification and synthesis of impurities formed during Trametinib Dimethyl sulfoxide preparation.

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ABSTRACT

*Trametinib Dimethyl sulfoxide**(1)**,a typical anti neo plastic agent drug is used for the treatment of skin cancer. During the laboratory optimization, formation of various impurities wereobserved in the final Trametinib. Origin of these impurities were possible when key starting material 2-fluoro-4-iodo aniline contained2-fluoroaniline, leading to the formation of the des-iodo impurity. Another possibility of des iodo impurity was observed during the nitro reduction step using tin (II) chloride. A cyclic impuritywas also found during nitro reduction step using tin (II) chloride. These impurities were monitored and their structures were tentatively assigned on the basis of their fragmentation patterns in LC-MS. Further, these impurities weresynthesized andcharacterized. We describe here-in, the formation, synthesis and characterization of these impurities.Our study will be of immense help to others to obtain chemically pure Trametinib Dimethyl sulfoxide.*

INTRODUCTION

The safety of adrug product is not only dependent on the toxicological properties of the active drug substance (or API), but also on the impurities formed during the various chemical transformations. Therefore, identification, quantification, and control of impurities in the drug substance and drug product are important parts of the drug development for obtaining marketing approval. It is more challenging for an organic chemist to identify the impurities which are formed in very small quantities in drug substance. Since most of the time it is very difficult to identify and control impurities within acceptable levels in the process, extra purification steps may then become necessary thereby making the process less competitive. More often than not, the synthesis of impurities are not described in the literature which makes it even more difficult for the organic chemist, who must then design a synthesis, which is time consuming. The development of drug substance is incomplete without the identification of an impurity profile involved in the process. Furthermore, it is not mandatory to design synthetic routes for the impurities. Thus, in our study we explored the formation, identification, synthesis and characterization of impurities found in the preparation of Trametinib Dimethyl sulfoxide. This study will help organic chemistsin better understanding of the potential impurities in Trametinib Dimethyl sulfoxide synthesis and thereby in obtaining a pure compound.[1-3]

MATERIALS AND METHODS

2.1 Preparation of des iodoImpurity:

The des iodo Trametinib impurity **(2)** was prepared by using nitro phenyl pyrido pyrimidine ring **(15)** withRaney-Nickel, hydrazine hydrate in ethanol under reflux followed by reacting with acetic anhydride in presence of pyridine to give N-(3-{3-cyclopropyl-5-[(2-fluorophenyl)amino]-6,8-dimethyl-2,4,7-trioxo-3,4,6,7-tetrahydropyrido [4,3-d]pyrimidin-1 (2H)-yl}phenyl) acetamide (des iodo Trametinib) (2) under the Synthetic scheme -1

(Full scheme (**Synthetic scheme -3**) of Trametinib Dimethyl sulfoxide synthesis given below under results and discussion.)

Synthetic scheme -1

Scheme 1:

Reagents, conditions: ***(i)*** *Raney-Nickel, NH_2-NH_2 hydrate in ethanol reflux(ii) Acetic anhydride, pyridine, DCM at 25-30°c*

Mass m/z = 488 (M -1), UV (λ nm) 320, 248,202

IR Values:

3348(N-H stretching), 3026 (Aromatic C-H stretching), 1719, 1690 (C=O stretching), 1638, 1605, 1553 (Aromatic C=C stretching), 1483, 1421 (Aliphatic C-H bending), 1369, 1350, 1307 (C-N stretching), 1288, 1258, 1231, 1208, 1104, 1033 (C-F stretching), 788,755 (Aromatic C-H bending)

NMR (DMSO – d_6, 300 MHz)

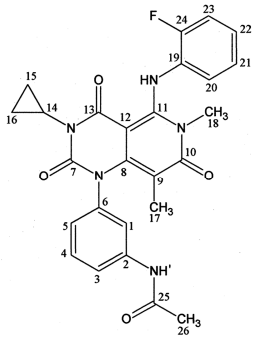


Table 1: NMR values

Position	1H	δ (ppm)	multiplicity	^{13}C, J(Hz)[1]	DEPT
1	1H	7.60-7.62	m	119.99	CH
2	-	-	-	140.32	-
3	1H	7.60-7.62	m	118.21	CH
4	1H	7.34-7.39	m	128.68	CH
5	1H	7.03-7.06	m	123.89	CH
6	-	-	-	139.55	-
7	-	-	-	151.01	-
8	-	-	-	145.00	-
9	-	-	-	101.31	-
10	-	-	-	164.28	-
11	-	-	-	151.59	-
12	-	-	-	89.64	-
13	-	-	-	162.87	-
14	1H	2.62-2.64	m	24.83	CH
15Ha, 16Ha	2H	0.68	br	8.17	CH_2
15Hb, 16Hb	2H	0.95-0.97	m		
17	3H	1.26	s	12.94	CH_3
18	3H	3.05	s	33.90	CH_3
19	-	-	-	127.87, d(8.6)	-
20	1H	7.13-7.25	m	123.99	CH
21	1H	7.13-7.25	m	126.32,d (6.6)	CH
22	1H	7.13-7.25	m	125.23,d (3.2)	CH
23	1H	7.34-7.39	m	116.25,d (18.9)	CH
24	-	-	-	154.66,d (243.5)	-
25	-	-	-	168.52	-
26	3H	2.05	s	23.97	CH_3
NH’	1H	10.13	s	-	-
NH	1H	11.25	s	-	-

s- singlet, d- doublet, t- triplet, m-multiplet, br- broad
$^{1.13}C$ – ^{19}F Coupling constant

2.2 Preparation of CyclicImpurity:

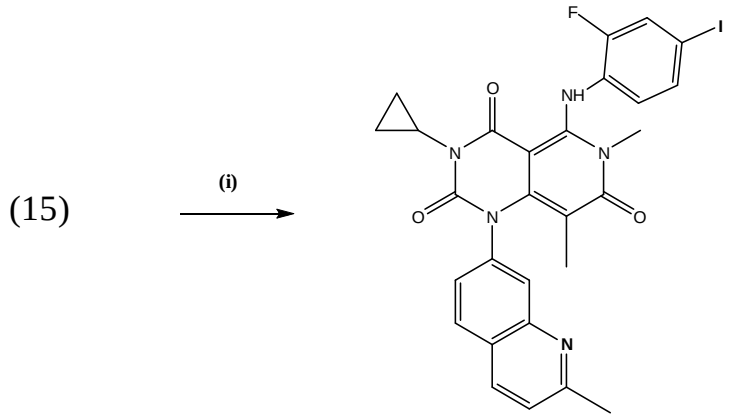
The Tin (II) chloride dihydrate reduction of nitro phenyl pyrido pyrimidine ring **(15)** is a critical reaction. A prolonged reaction time leads to a cyclic impurity.This contaminated material when reacted with acetic anhydride **(18)** in presence of pyridineresultsin Trametinib **(1)** contaminated with **(3).** The degree of contamination was 0.5-1.0%.It isdifficult to remove the impurity **(3)** from the Trametinib dimethyl sulfoxide **(1)**.

Scheme 2

The preparation of cyclic impurity is as illustrated here:

Nitro phenyl pyrido pyrimidine ring **(15)** was reacted with excess stannous chloride dihydrate under reflux in ethanol for 24 hours followed by reaction with acetic anhydride, in presence of pyridine to give 3-cyclopropyl-5-[(2-fluoro-4-iodophenyl)amino]-6,8-dimethyl-1-(2-methylquinolin-7-yl)pyrido[4,3-d]pyrimidine-2,4,7 (1H,3H,6H)-trione (3)(Cyclic impurity) under the Synthetic scheme -2, further purified by using preparative HPLC .

Synthetic scheme-2:



(3)

Scheme 2: Reagents and conditions: **(i)** stannous chloride dihydrate in ethanol reflux**,** Acetic anhydride, pyridine, DCM at 25-30°c

Mass m/z = 624 (M + H), UV (λ nm) 324, 254,211

IR values:

3430 (N-H stretching), 3092 (Aromatic C-H stretching), 2922 (Aliphatic C-H stretching), 1633 (C=O stretching), 1555(Aromatic C=C stretching), 1478, 1422 (Aliphatic C-H bending), 1351, 1305 (C-N stretching), 1281, 1244, 1204, 1134(C-F stretching), 834,790,720 (Aromatic C-H bending)

NMR (DMSO – d_6, 300 MHz)

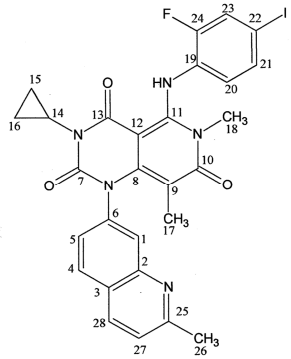


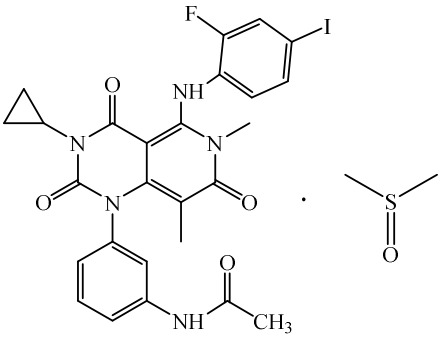
Table 2: NMR data

Position	1H	δ (ppm)	J (Hz)[1]	^{13}C, J(Hz)[3]	DEPT
1	1H	7.99	d (1.2) [1]	124.31	CH
2	-	-	-	142.84	-
3	-	-	-	125.32	-
4	1H	8.14	d (8.7) [1]	128.30	CH
5	1H	7.78-7.82	dd (10.4,1.7) [1]	128.90	CH
6	-	-	-	142.36	-
7	-	-	-	150.88	-
8	-	-	-	144.87	-
9	-	-	-	102.17	-
10	-	-	-	162.83	-
11	-	-	-	151.01	-
12	-	-	-	90.58	-
13	-	-	-	164.09	-
14	1H	2.63-2.66	m	24.86	CH
15Ha, 16Ha	2H	0.72	br	8.14	CH_2
15Hb, 16Hb	2H	0.97-0.99	m		
17	3H	1.13	s	13.63	CH_3

18	3H	3.10	s	33.90	CH_3
19	-	-	-	128.13, d(11.2)	-
20	1H	6.98	t (8.7) [1,2]	125.08	CH
21	1H	7.57	d (8.4)	134.01,d (3.2)	CH
22	-	-	-	88.36,d (7.2)	-
23	1H	7.71-7.75	dd (8.9,1.7) [1,2]	124.86,d (21.4)	CH
24	-	-	-	154.16,d (249.1)	-
25	-	-	-	159.38	-
26	3H	2.83	s	23.10	CH_3
27	1H	7.69	t (8.7)	123.46	CH
28	1H	8.61	t (9.0)	140.01	CH
NH	1H	11.02	s	-	-

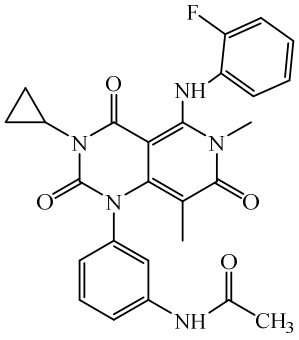
s- singlet, d- doublet, t- triplet, m-multiplet, br- broad
[1,1] H- [1] H Coupling constant
[2,1] H – [19] F Coupling constant
[3,13] C – [19] F Coupling constant

RESULTS AND DISCUSSION



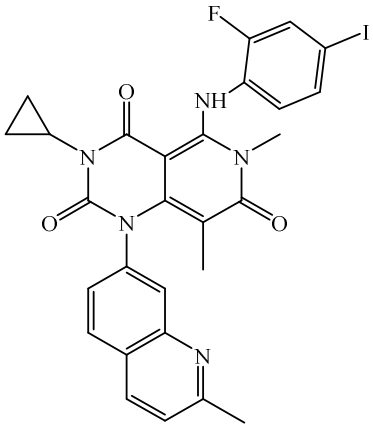
(1)

Trametinib Dimethyl Sulfoxide



(2)

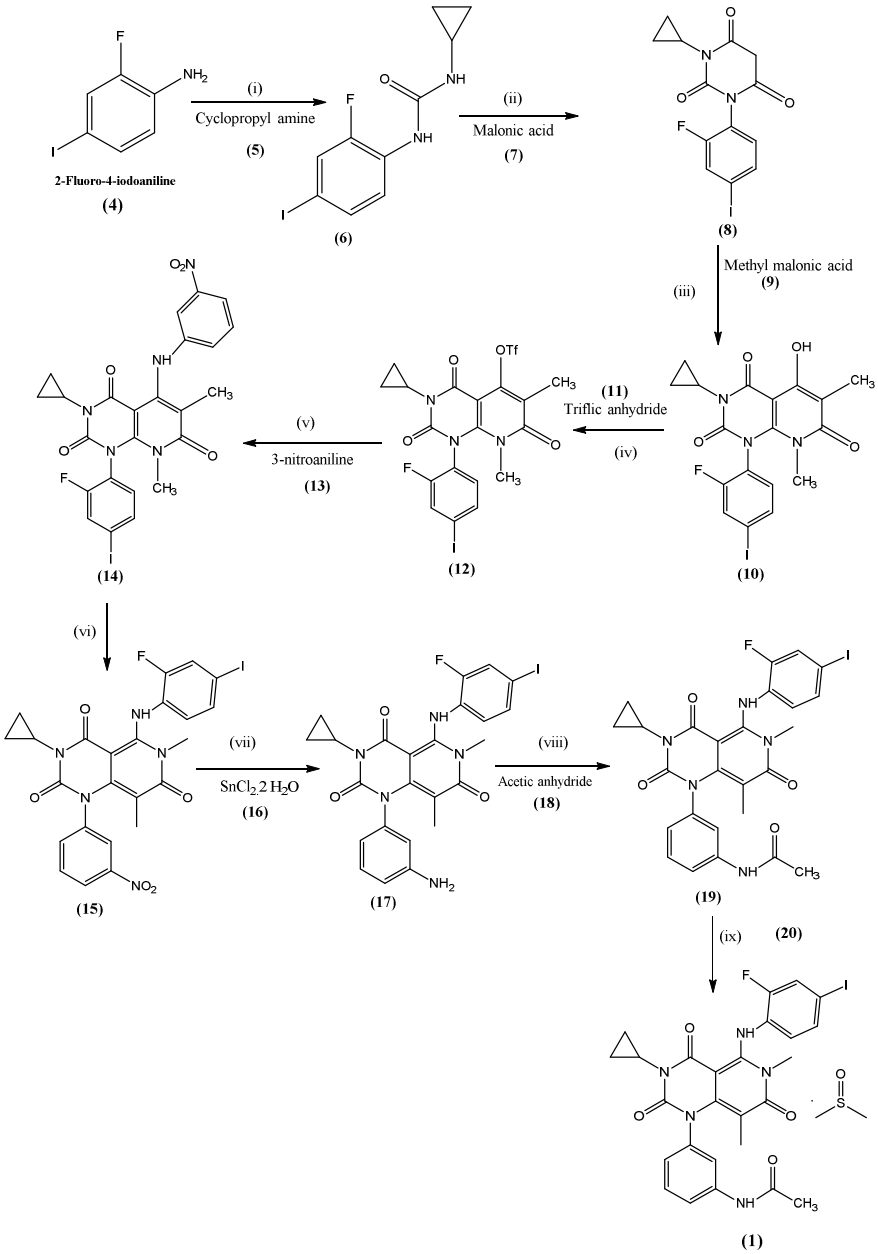
Des Iodo Trametinib



(3)

Cyclic Impurity

Synthetic scheme-3:



Scheme 3:

Reagents and conditions: (i) *N, N-Carbonyl diimidazole, Triethyl amine, DMF:* ***(ii)*** *Acetyl chloride, Acetic anhydride* ***(iii)*** *$POCl_3$, Dimethyl aniline, water, 95-100°c ,25% methyl amine solution in methanol at 25-30°c* ***(iv)*** *2,6 –lutidine, DCM at 25-30°c* ***(v)*** *DMF 130-135 °c (vi) Potassium carbonate, methanol, THF 60-65 °c,(vii) $Sncl_2$ H_2O (viii) Pyridine, DCM, (ix) DMSO.*

Trametinib Dimethyl sulfoxide is designated chemically as Acetamide,N[3-[3-cyclopropyl-5-[(2-fluoro-4-iodophenyl)amino]3,4,6,7-tetrahydro-6,8-dimethyl-2,4,7-trioxopyrido[4,3-d]pyrimidin-1(2H)-yl]phenyl], compound with 1,1'-sulfinylbis[methane] (1:1). Its literature synthesis[2]**(Scheme 1)** (1-9) involves reaction of 2-fluoro4-iodo aniline**(4)** with cyclopropyl amine**(5)**.The product1-cyclopropyl-3-(2-fluoro-4-iodo-phenyl) urea **(6)** on treatment with malonic acid **(7)**gives the product 1-cyclopropryl-3-(2-fluoro-4-iodo-phenyl) pyrimidine-2, 4, 6-trione**(8)**whichwhentreated with 2-methyl malonic acid**(9)**givesthe product 3-cyclo propyl-1-(2-fluoro-4-iodophenyl)-5-hydroxy-6, 8-dimethyl-1H, 8H-pyrido [2, 3-d] pyrimidine-2, 4, 7-trione,**(10)**which furtherreacts with Triflormethane sulfonic anhydride **(11)** to givethe product Trifluoromethanesulfonic acid 3-cyclo-propyl-1-(2-

fluoro-4-iodophenyl)-6,8-dimethyl-2,4,7-tri-oxo-1,2,3,4,7,8-hexahydro-pyrido[2,3-d]pyrimidin-5-yl ester**(12)**which was reacted with 3-Nitro aniline **(13)**to give rise to the product 3-Cyclopropyl-1-(2-fluoro-4-iodo-phenyl)-6,8-dimethyl-5-(3-nitro-phenylamino)-1H,8H-pyrido[2,3-d]pyrimidine-2,4,7-trione**(14),**which when reacted under basic condition affords the product 3-Cyclopropyl-5-(2-fluoro-4-iodo-phenylamino)-6,8-dimethyl-1-(3-nitro-phenyl)-1H,6H-pyrido[4,3-d]pyrimidine-2,4,7-trione**(15).** with stannous chloride dihydrate **(16)** affords the product1-(3-Aminophenyl)-3-cyclopropyl-5-[(2-fluoro-4-iodophenyl)amino]-6,8-dimethyl-2,4,7-trioxo-3,4,6,7-tetrahydro-2H-pyrido[4,3-d]pyrimidine**(17),** which is furtherreacted with acetic anhydride **(18**) to afford theproduct N-{3-[3-cyclopropyl-5-(2-fluoro-4-iodophenylamino)-6,8-dimethyl-2,4,7-trioxo-3,4,6,7-tetrahydro-2H-pyrido[4,3-d]pyrimidin -1-yl]phenyl}acetamide(19)which forms solvate with Dimethyl sulfoxide (20) to giveTrametinib Dimethyl sulfoxide(1).

During the laboratory optimization of theTrametinib Dimethyl sulfoxide **(1),**two process related impurities were identified. The guidelines recommend by ICH state that the acceptable levels for a known and unknown compound (impurity) in the drug should be less than 0.15 and 0.10%, respectively. In order to meet the stringent regulatory requirements, the impurities present in the drug substance must be identified and characterized.

CONCLUSION

The two process-related impurities identified during the preparation of TrametinibDimethyl sulfoxide, which were synthesized and characterized, will aid in better understanding of the impurity profile of TrametinibDimethyl sulfoxide. Complete characterization of the process related impurities also helps in better strategizing ways and means to remove them from the desired product.

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REFERENCES

[1] *Asian J. Chem*., **2011**, 23(10), 4536-4546
[2] US 7378423
[3] *Med. Chem. Lett*, **2011**, 2(4), 320-324.