Available online at www.joac.info

ISSN: 2278-1862

Journal of Applicable Chemistry

2014, 3 (3):1055-1058

(International Peer Reviewed Journal)

Investigation of Z-E Isomerism in Sunitinib Anticancer drug

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Accepted on 8th April 2014

ABSTRACT

Z-E isomerization in sunitinib, an important anticancer drug, has been investigated. The influence of light, heat and solvent was studied and the isomerizations were monitored by HPLC. As expected, the extent of Z-E isomerization (conversion of the Z-isomer into the undesired E-isomer) is found to depend on these parameters. In addition, both reversible and irreversible isomerizations were observed when the solutions were kept in dark following the exposures to light. These observations have implications in the analysis of sunitinib samples using HPLC methods.

Keywords: Sunitinib, Z-E isomerism, photo-isomerization and thermal-isomerization.

INTRODUCTION

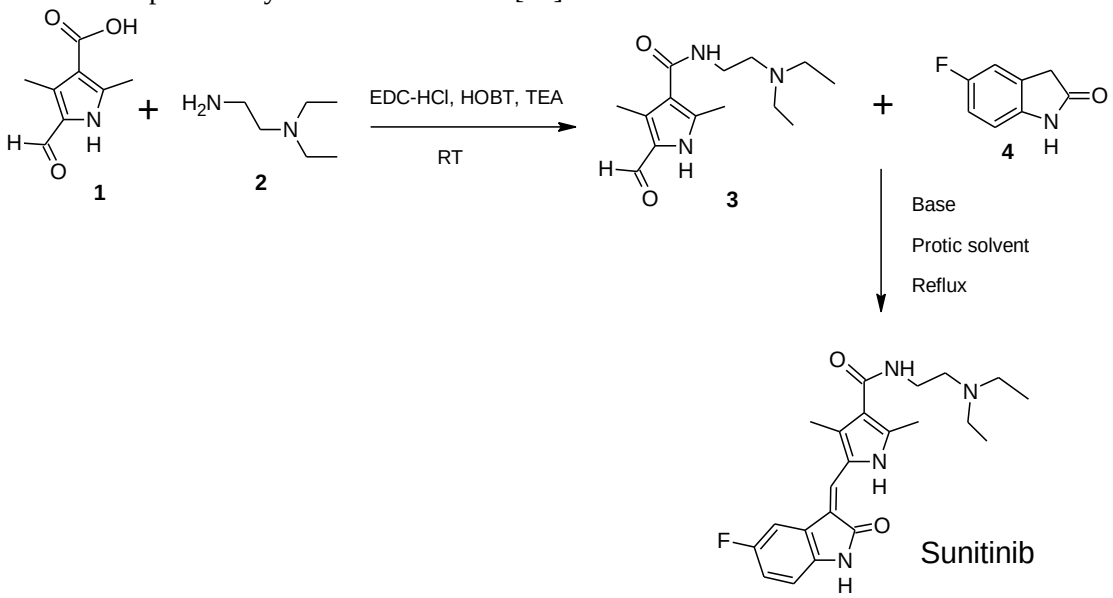
Sunitinib is a vascular endothelial growth factor receptor inhibitor which has demonstrated a high activity in renal cell carcinoma. This is currently used in the treatment of patients with metastatic disease[1-2]. It is sold as malate salt by Pfizer under the brand name Sutent. Sunitinib is a yellow to orange powder with a solubility of 25mg mL^{-1} in acidic solutions (pH 1.2 - 6.8) [3]. It has an exocyclic double bond at the 3-position of the oxindole ring and therefore can exhibit Z-E isomerism. It has been shown that SU5416, closely related to sunitinib, undergoes photo induced transformation into the less stable E isomer in solution[4]. Light[5-6] and heat[7-8] induced isomerization of compounds containing C=C and N=N double bonds is well documented in the literature[5-8]. Studies in the analytical solution, non-aqueous formulation and blood plasma have revealed that the extent of conversion of the Z-isomer into the E-isomer depends on the concentration, polarity and viscosity of the media. In addition, these authors have also shown that the Z-E isomerization of SU5416 is reversible. When the pre-exposed solutions are kept in dark, the E-isomer content was found to be less than 0.3%. In the present study, we report the synthesis of sunitinib and results of Z-E isomerization in sunitinib in various organic solvents.

MATERIALS AND METHODS

All the API intermediates and reagents were provided by Ranbaxy Laboratories Ltd., India. Waters HPLC 2695 alliance separation module (customized with syringe and loop volume of 2.5 mL) with PDA 2998 detector was used for. HPLC grade solvents were used as obtained from Rankem and Qualigens. Kromasil

C_8 (250 × 4.6 mm; 5 µm particle size) was procured from Akzonobel. The gradient elution consisted of potassium dihydrogen orthophosphate buffer (pH 4.5) and acetonitrile (1:1 v/v) with an injection volume and flow rate of 5 µl and 1 ml/min, respectively with the detection at 210 nm. Melting point was recorded on Buchi b 545. IR spectra were recorded on Perkin-Elmer FT-IR spectrometer. ^{1}H NMR spectra was recorded in DMSO-d_6 at 400 MHz using TMS as an internal standard. Mass/Ms-Ms data was generated by using QTRAP LC/MS/MS system (Applied Biosystems). All the chemicals were obtained from commercial suppliers and were used without any further purification. In the Z-E isomerization studies, the solutions (in 100 ml volumetric flasks) - prepared by dissolving 25 mg of sunitinib in 100 ml of each solvent - were either directly exposed to halogen lamp (150 W) or sunlight.

Synthesis: Sunitinib is prepared in two steps from imidazole derivative (**1**) as per the reported procedures (Scheme 1)[9]. Amidation of **1** with diethylethylenediamine (**2**) yielded imidazole-amide derivative (**3**). Intermediate **3** was then condensed with oxindole derivative (**4**) to give crude sunitinib. The crude sunitinib was then purified by acid-base treatment[10].



Scheme 1: Scheme for the synthesis of sunitinib.

M. P: 216.6 ^{o}C, IR (KBr) V_{max}(in cm^{-1})*:* 3424, 3339, 3042, 2955, 2470, 1968, 1870, 1675, 1637, 1563, 1194, 1520, 1476, 1463, 1440, 1377, 1322, 1286, 1253, 1193, 1151, 1096, 1071, 1047, 800, 666 and 586. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.00 (6 H, t, -CH_3, *J* = , 7.1 Hz), 2.43 (3H, s, -CH_3(Ar), 2.45(3H, s, -CH_3(Ar), 2.55-2.62 (6H, m, -N-CH_2-), 3.29-3.34(2H, -NH-CH_2, m), 6.83-6.86(1H, m, Ar-H), 6.90-6.95 (1H, m, Ar-H), 7.47 (1H, t, -N-H), 7.72 (1H,s, -CH (alkene), 7.76 (1H, d, Ar–H, *J* =9.4 Hz), 10.90 (1H, s, N-H), 13.69 (1H, s, N-H),); Mass: 398.4 $[M + H]^+$, 399.3; MS/MS: 326.1, 283.1.

RESULTS AND DISCUSSION

HPLC analysis of the lab batches of sunitinib exhibited large variation of the undesired E-isomer content that varied from 0.1% to 8%. That prompted us to investigate the possibility of the conversion of the Z-sunitinib into the undesired E-isomer in presence of heat and light. Reanalysis of the samples gave inconsistent E-isomer content which indirectly supported the said assumption. This was further confirmed when the solutions, prepared in 1:1 mixture of acetonitrile and water, were alternatively exposed to halogen lamp (20 h exposure) and sunlight (6 hours) at 10-15 ˚C during winter. The results are collected in

table 1. The conversions into the E-isomers were found to be about 3% and 28% upon exposure to halogen lamp and sunlight, respectively.

Table 1. Z-E Isomerization in sunitinib in presence of light.

Batch No.	Solvent	% E-isomer in halogen light and then in dark		% E-isomer in sunlight and then in dark	
		20 hrs exposure at 10-15 ˚C	Kept in dark for 42 hrs	6 hrs exposure at 10-15 ˚C	Kept in dark for 42 hrs
1	ACN-water (1:1)	2.35	Not detected	26.14	Not detected
2	ACN-water (1:1)	2.79	Not detected	28.58	Not detected

When the samples were kept in dark for about 40 h, the E-isomer could not be detected in the resulting solutions confirming that the Z-E isomerization in sunitinib is also reversible like that seen in its intermediate SU5416[4]. However, the situation was found to be different in organic solvents. The Z-E isomerization statistics for the solutions exposed to halogen lamp and sun light (during summer) for 1 hour at 25-30 ˚C and 35-40 ˚C respectively, are shown in table 2. It is evident from table 2 that the extent of formation of the E-isomer in the non-polar solvents is about 10% less than that seen in the polar solvents when the solutions were exposed to halogen light. However, when all these solutions were kept in dark for about 15 hours, the E-isomer content remained more or less the same in all the solvents, except for hexane, cyclohexane and heptanes. Further, the E-isomer almost completely converted back to the Z-form (sunitinib) in hexane and cyclohexane, while in heptane, only about 50% of the E-isomer converted back to the desired Z-isomer. The lower conversion statistics in DMSO may be due to high viscosity of DMSO or its ability to engage in strong hydrogen bonding interactions that stabilize the Z-form.

The trend is quite different for the solutions exposed to sunlight. The extent of isomerization is lower in the nonpolar solvents compared that in the polar solvents (Table 2). In the polar solvents, including DMSO, the extent of conversion is about 20-30% higher in sunlight than that noticed in halogen lamp and the conversion is found to be irreversible as well (Table 2). In addition, in the sunlight, the extent of conversion is more than that observed upon exposure to halogen light possibly due to the thermal and UV induced transformations. Thermal induced Z-E isomerization is confirmed by the conversion statistics of ACN-water solutions exposed to halogen light at 10-15˚C and 25-30 ˚C. In other words, the Z-E transformations of sunitinib are influenced by both heat and light.

The Z-E isomerization in the non-polar solvents is somewhat unexpected. In presence of sunlight and ,of course, at a higher temperature (35-40 ˚C), the Z to E conversion is about or more than two-fold to that observed under halogen lamp for hexane (19%) and toluene (37%), while in heptane and cyclohexane, the conversion is about 50% less than that seen in upon exposure to halogen lamp. However, surprisingly, the E-isomer content increased to about 40-60% from 5-7% even in dark in case of hexane, heptane and cyclohexane, while in toluene the trend was opposite.

Table 2.The effect of solvent on the Z-E isomerization in sunitinib.

S. No.	Solvent	Dipole moment	% E-isomer in halogen light and then in dark		% E-isomer in sunlight and then in dark	
			1 hr exposure at 25-30 ˚C	Kept in dark for 15 h	1 hr exposure at 35-40 ˚C	Kept in dark for 15 h
1	Hexane	0.00	10.21	0.26	19.54	39.01
2	n-Heptane	0.00	12.05	5.50	5.15	40.87
3	Cyclohexane	0.00	14.60	1.09	7.35	58.41
4	Toluene	0.36	11.38	9.09	37.25	5.39
5	Chloroform	1.04	26.76	27.52	57.37	57.09

6	MtBE	1.32	21.81	19.85	36.97	40.71
7	Dichloromethane	1.60	24.74	24.27	57.52	57.64
8	IPA	1.66	13.92	13.43	41.20	41.30
9	Ethanol	1.69	21.89	21.93	59.48	53.38
10	Methanol	1.70	22.75	26.60	57.80	51.96
11	THF	1.75	24.11	24.00	54.17	53.60
12	Ethyl acetate	1.78	21.78	18.56	54.32	53.86
13	Acetone	2.88	22.20	21.51	37.13	36.78
14	Acetonitrile	3.92	21.71	26.77	59.81	59.24
15	DMSO	3.96	10.16	8.03	54.76	51.97
16	ACN-water (1:1)	--	11.32	11.43	59.49	59.19

APPLICATIONS

The synthesized compound is an anticancer compound and the number of patients suffering from various kinds of cancer is on the increase[11].

CONCLUSIONS

Sunitinib is synthesized by the reported procedure and its isomerization is investigated. The study revealed that the Z-E isomerization in suntinib depends on the light source, time of exposure, temperature of the solution and polarity of solvent. The extent of conversion into the undesired E-isomer in polar solvents is found to be significantly more than that observed in non-polar solvents, and particularly, that extent is more pronounced in the presence of sunlight. Most of these conversions were found to be irreversible. No clear-cut trend could be noticed in the non-polar solvents. Significantly, these results suggest that care should be taken while handling the analytical solutions of sunitinib – particularly, protection from light and heat during preparation and storage - in the standard HPLC testing procedures for obtaining consistent and accurate data.

ACKNOWLEDGMENTS

SS thanks the Ranbaxy management for their support and encouragement.

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