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# Acid-induced isomerization of ticagrelor: Systematic exploration on reaction condition and mechanism

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ABSTRACT

N1—N2 bond in molecules containing 1, 2, 3-triazoles framework is weakened and readily cleaved when N1 is attached to a strong electron-withdrawing group. However, ticagrelor, a pyrimidine-fused triazole without a strong electron-withdrawing group on N1 site still went through isomerization reaction via N1—N2 bond rupture in the presence of acid to produce an impurity **T3**. Its structure was precisely obtained via NMR spectral analysis, MS spectroscopy and X-ray crystallography. The optimal condition of the isomerization reaction was systematically studied. Moreover, the isomerization mechanism was investigated and it is likely that ticagrelor and its analogues go through isomerization reaction when the 4-NH group and electron-deficiency ring exist in their structures. Besides, the steric hindrance of the substituent on 4-NH has an effect on the reactivity of equilibrium. Under mimic *in vivo* condition, the isomerization reaction of ticagrelor also generated, indicating ticagrelor may be not stable in stomach.



## 1. Introduction

Ticagrelor (Fig. 1a) is an orally active, selective inhibitor of the P2Y12 receptor through preventing adenosine 5′-diphosphate (ADP) mediated platelet activation and aggregation to reduce the risk of atherothrombotic events [1–3]. Compared to other P2Y12 inhibitors such as clopidogrel, ticagrelor binds reversibly with P2Y12 receptor, generates quick absorption and inhibits rapid platelet aggregation [4,5]. This drug was approved for treating acute coronary syndrome (ACS) as an anti-platelet agent by the European Commission in 2010 and the Food and Drug Administration (FDA) in 2011. As a drug substance, the impurities may cause unknown side effects and should be below 1%, confirming the quality and safety of drug. Therefore, the impurity profile in drug substances is quite important for “regulatory” aspects for drug approval.

Recently, some impurities of ticagrelor were reported [6,7]. Kumar and his group found four new impurities of ticagrelor. The main impurity, TIC Imp-IV as proposed in Fig. 1b, was produced by acid-induced isomerization of ticagrelor at cyclopentane-1, 2-diol ring. They found a ticagrelor's Dimroth rearrangement impurity, an isomer of ticagrelor [8]. In our study, we also observed this acid-induced impurity of ticagrelor and it was confirmed that the isomerization was not occurred at cyclopentane-1, 2-diol ring but at triazole ring.

Molecules containing 1, 2, 3-triazoles framework (Fig. 1c) are found various applications in both materials science and medicinal chemistry because triazoles are exceedingly resistant to strong acid or base, as well as towards reductive or oxidative conditions [9]. However, when N1 is attached to a strong electron-withdrawing group (EWG), N1—N2 bond is weakened and readily cleaved [10–12]. For example, 1-cyano-1, 2, 3-triazole tautomerizes to α-diazo-*N*-cyanoethylidenimine through N1—N2 bond cleavage because of the existence of CN group (Scheme 1a). Another example is the tautomerization of N1-sulfonylbenzotriazoles with a 4-amino group via a Dimroth-type rearrangement under heating (Scheme 1b), and the rearrangement is controlled by solvents and substituents [13].

Ticagrelor is a pyrimidine-fused triazole without a N1 electron-withdrawing group, but we still found that it went through

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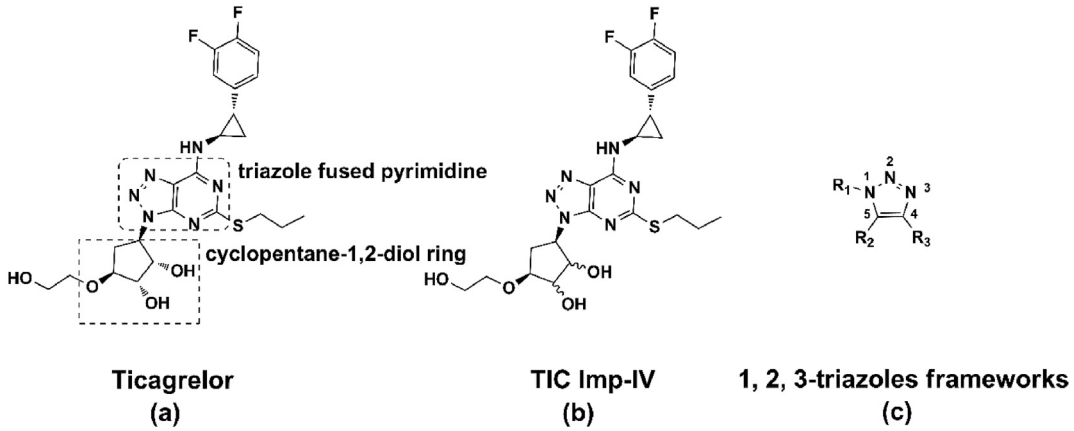
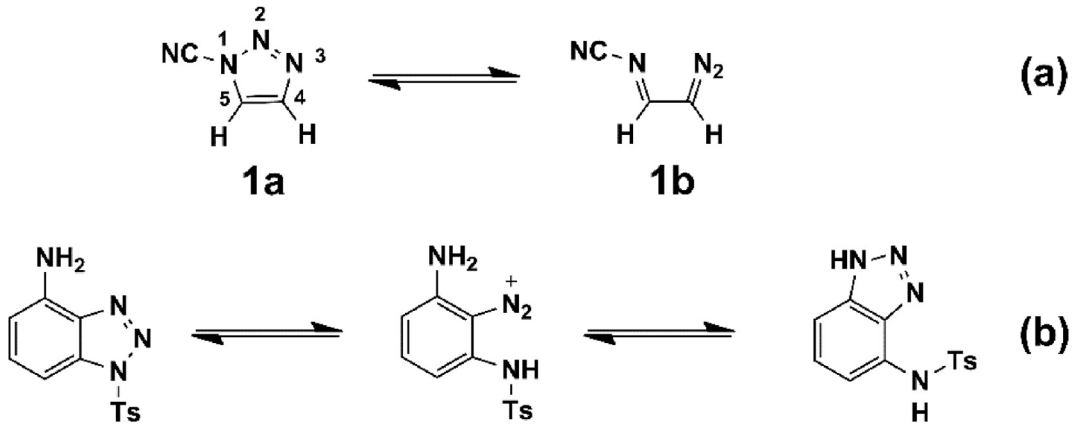



**Fig. 1.** Structures of ticagrelor (a), TIC Imp-IV (b), and 1, 2, 3-triazoles framework (c).




**Scheme 1.** Tautomerization of triazole with electron-withdrawing group on N1.

tautomerization under acidic conditions via N1—N2 bond cleavage and resulted in the production of a new impurity **T3**. Herein, we investigate the detailed isomerization mechanism of ticagrelor and its analogues without an electron-withdrawing group on N1 position under an acidic condition. The results revealed that a 4-NH substituent and an electron-deficient ring should be presented in their structures for the requirement of acid-induced isomerization on N1 position.

## 2. Experimental section

### 2.1. Chemicals and reagents

$CDCl_3$ or $CD_3OD$, isopropyl acetate (IPAc), HPLC grade acetonitrile were bought from Merck. Ltd. (Shanghai, China). The commercial grade solvents such as Methanol (MeOH), tetrahydrofuran (THF) N, *N*-dimethylformamide (DMF), Dimethyl sulfoxide (DMSO), etc. as well as commercial grade acid were purchased from Scinopharm Corporation (Shanghai, China). Purified water by Milli Q plus purification systemfrom Millipore (Bradford, PA, USA) was used during the course of experimental studies.

### 2.2. Preparation of ticagrelor

Ticagrelor was prepared according to the published route [14]. The experimental detail was described in Supplementary information. Briefly, the solution of the acetonide intermediate **M3** (100 g) in MeOH (600 mL) was drop-wise added 4 N HCl (300 mL) at room temperature under nitrogen atmosphere. The resulting mixture was stirred at room temperature for 6 h. The resulting mixture was added into 15% NaOH (160 g) and stirred at 20 °C for 20 min until obvious solid formed. Another part of 15% NaOH (160 g) was added drop-wise at 20 °C, adjusted pH to about 7.0 and stirred at 20 °C for 2 h. The mixture was filtered, washed with MeOH/water several times. The wet cake was dried under reduced pressure at 50 °C for 4 h. Ticagrelor was furnished as off-white solid (81.7 g, in 88% yield, with purity ratio>98%).

### 2.3. Preparation of *T3*

A mixture of ticagrelor (25 g), *n*-butanol (150 mL, 6P), 12 N HCl (15 mL, 3P) was stirred at 40 °C for 16 h. Two more days later, the resulting mixture was poured into ice-water, adjusted pH to 7.0 with 10% NaOH, extracted with IPAc (500 mL), washed with brine. The organic phase was dried, slurried in IPAc (300 mL) twice for 16 h, filtered, washed with IPAc (50 mL). The filter cake was dried under reduced pressure to give 2.4 g pale white solid with 95% purity.

### 2.4. Isomerization of ticagrelor under different conditions

A mixture of ticagrelor (0.1 g), solvent (0.8 mL) and acid (0.3 mL) was stirred at indicated temperature for required time. The resulting mixture was analyzed by UPLC.