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Original article

Novel cross-link breaker based on zwitterion structure: Synthesis, structure and druggability studies☆

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ABSTRACT

It has been universally acknowledged that the increase in cardiac and vascular stiffness is due to the formation of advanced glycosylation end-products (AGEs). Research on the stable form of 3-(carboxymethyl)-4-methylthiazol bromide sodium salt ($C_6H_7BrNNaO_2S$) showed that it had a notable effect on breaking the AGEs. Two compounds with novel structures, zwitterionic 3-(carboxymethyl)-4-methylthiazol ($C_6H_7O_2NS$) and a dipolymer ($C_{12}H_{15}O_4N_2S_2Br$) complex, were obtained. When compared with the forms of sodium salt and dipolymer, zwitterion had an obvious advantage in stability, solubility, synthesis and pH, which made the zwitterion a promising drug. The structure of sodium salt, dipolymer and zwitterion was comparatively analyzed by such methods as single crystal X-ray diffraction, ESI-MS, ^{1}H NMR, FT-IR and *in situ* IR.

1. Introduction

Excessive formation of advanced glycosylation end-products (AGEs) is a significant factor contributing to the development of cardiovascular diseases associated with aging, diabetes and hypertension as has been widely documented [1,2]. Glucose can react non-enzymatically with long-lived proteins, such as collagen and lens crystallin, linking them together to form cross-links, that will eventually generate advanced glycosylation end-products (AGEs). Formation of AGEs leads to increasing levels of oxidative stress, and their accumulation in serum, kidney and vascular tissues is a key risk marker for microvascular complications, especially in diabetic patients [3,4].

To decrease the number of these cross-links, some compounds have been discovered, the majority of which belong to a class of thiazolium derivatives such as phenacylthiazolium bromide (PTB) and ALT-711 [5–7]. These compounds can selectively cleave diketone bridges of two adjacent carbonyl groups that might form intermolecular cross-links with amino acid side chains and thus appears to reverse cross-linking both *in vitro* and *in vivo* [8–10].

Based on the principle of "cross-link breakers", we designed a series of *N*-(carboxymethyl) thiazolium derivatives [11,12]. By screening from these hundreds of betaines, some active candidates, having a common structure of ester, were identified. Subsequently, pharmacokinetic studies on these active candidates in our laboratory indicated that the functional ester group was predominantly metabolized into carboxyl *in vivo*. Therefore, we believe that these compounds play the role of "cross-link breaker".

According to the exact molecular mechanism, we reduced these *N*-(carboxymethyl) thiazolium derivatives, and designed the simplest representative of these derivatives, namely a 3-(carboxymethyl)-4-methylthiazol bromide sodium salt ($C_6H_7BrNNaO_2S$). The satisfactory efficacy of which *in vivo* had been confirmed by experiments performed in rats. However, to our great surprise, the results of X-ray analysis revealed that this compound including both sodium acetate and quaternary ammonium salt was not stable enough for isolation, possibly because the sodium and bromine cannot regularly exist in one molecule, a portion of which cohered in the form of NaBr. Subsequently, dipolymer ($C_{12}H_{15}O_4N_2S_2Br$) and zwitterion ($C_6H_7O_2NS$) were obtained by synthesis. After a series of

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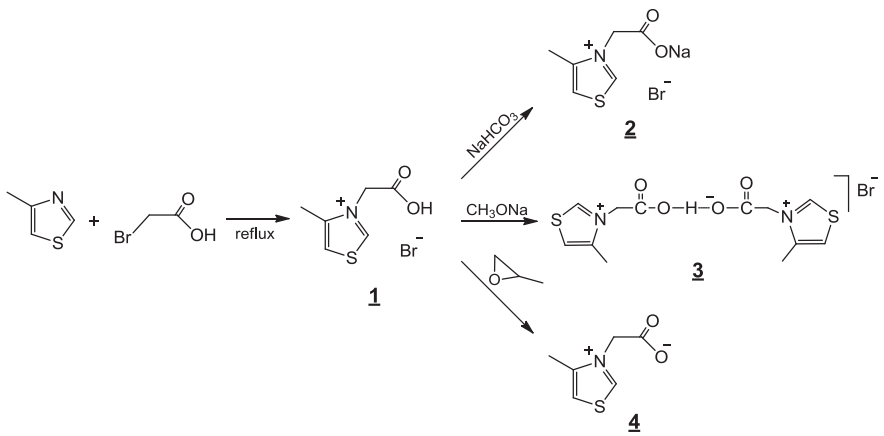

Scheme 1. Synthesis of compounds **2**–**4**.

comparative analyzes, we came to the conclusion that the zwitterion form has the most promising druggability of these potential "cross-link breakers".

To date although several zwitterions have been identified, an overwhelming majority of them stably exist in the form of coordination complexes [13,14]. Therefore, only a few of their structures have been determined in the solid state [15], so that there has been very little drug design based on zwitterionic structure appearing on the market. In this paper, a novel thiazole derivative, namely the zwitterionic 3-(carboxymethyl)-4-methylthiazol, which has a structure analogous to amino acid and a zwitterionic form containing a positively charged quaternary ammonium and a negatively charged carboxylate, is reported. Synthesis, structural characterizations and druggability studies of the three compounds will be described below.

2. Results and discussion

2.1. Synthetic studies

To determine the most stable structure of this "cross-link breaker", several syntheses are carried out and three forms of the target compounds (**2**–**4**) are obtained (Scheme 1). In the reaction to **2** and **3**, the quantity of base must be strictly restricted to the equimolar amount of the carboxylic derivation or else the redundant base would not be easily separated from the target sodium salt. In contrast to reactions 1 and 2, reaction 3 has the obvious predominance in outcome and refinement.

2.2. Structural studies

2.2.1. X-ray crystallography

The crystal data and details of structure refinement of compounds **2**–**4** are given in Table 1.

The asymmetric unit is shown in Fig. 1. In the single crystal structure of **2**, two donor sites of one ligand link to the sodium cation, Na2, via the coordination bond Na2–O3 and Na2–O4 (Na2–O3 = 2.530(6) Å and Na2–O4 = 2.410(6) Å), while the two donor sites coming from different ligands connect to Na1 via coordination bond Na1–O3 and Na1–O1 (Na1–O3 = 2.336(7) Å and Na1–O1 = 2.351(7) Å). Although the two bromine atoms in this molecule are apparently isolated, the distance from bromine to sodium (Br1–Na1 = 2.869(3) and Br2–Na2 = 2.864(3) Å) suggests that there is a great probability of bonding between them.

Table 1
Crystallographic data and details of structure refinement of compounds **2**–**4**.

Complex	**2**	**3**	**4**
Empirical formula	$C_{12}H_{14}Br_2N_2Na_2O_4S_2$	$C_{12}H_{15}BrN_2O_4S_2$	$C_6H_7NO_2S{\bullet}H_2O$
Formula weight	520.20	395.29	175.2
Crystal system	Triclinic	Monoclinic	Orthorhombic
Space group	*P*2(1)/n	*P*2(1)/n	*P*2(1)/n
a (Å)	8.9000(18)	5.1907(10)	5.6082(11)
b (Å)	9.4095(19)	12.092(2)	8.4615(17)
c (Å)	12.028(2)	13.052(3)	16.064(3)
α (°)	92.82	90	90
β (°)	109.00	97.96	90
γ (°)	104.11	90	90
V [Å^{-3}]	914.5(3)	811.3(3)	762.3(3)
Z	2	2	4
ρ_{calcd} [Mg/m^3]	1.904	1.618	1.527
F(000)	520	400	368
Crystal size (mm)	0.40 × 0.30 × 0.10	0.20 × 0.10 × 0.10	0.20 × 0.18 × 0.10
θ range for data collection	2.25–25.03°	2.31–25.01°	2.54–27.89°
Limiting indices	$-10 \le h \le 10, -11 \le k \le 11, -14 \le l \le 14$	$-6 \le h \le 6, -14 \le k \le 14, -15 \le l \le 14$	$-7 \le h \le 6, -11 \le k \le 11, -21 \le l \le 21$
Reflections collected/unique	5591/3046 [*R*(int) = 0.0303	5418/1424 [*R*(int) = 0.0303]	8477/1822 [*R*(int) = 0.0403]
Temperature (K)	293(2)	113(2)	143(2)
Data/restraints/parameters	3046/4/217	1424/1/102	1822/0/110
Final *R* indices [$I > 2\delta(I)$]	$R_1 = 0.0509$	$R_1 = 0.0336$	$R_1 = 0.0266$
R indices (all data)	$wR_2 = 0.1391$	$wR_2 = 0.0851$	$wR_2 = 0.0645$
Largest diff. peak and hole (Å^{-3})	0.960 and −0.676	0.463 and −0.989	0.232 and −0.211