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Potent transglutaminase inhibitors, dithio β-aminoethyl ketones

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ABSTRACT

Potent transglutaminase inhibitors were obtained from disulfide compounds, cystamine, dimethyl cystine, and dimethyl homocystine. The disulfide bond and thiophene ring play an important role in inhibitory activity of synthesized aryl β-amino ketones.

Transglutaminases (TGases)[1–5] are ubiquitous enzymes involved in various post-translational modifications, including protein cross-linking, amine incorporation, and deamidation. TGases catalyze protein cross-linking by forming isopeptide bonds between the carboxamide group of glutamine residues and the ε-amino group of lysine residues to produce *N*-(γ-glutamyl)-L-lysine upon loss of ammonia.[2,6,7] Strong intra- and intermolecular σ-bonds play an important role in biological phenomena, such as blood clotting upon fibrin cross-linking and cornified envelope formation.[8] In addition, unregulated high TGase activities result in numerous *N*-(γ-glutamyl)-L-lysine isopeptide bonds which may partly contribute to protein aggregates, such as amyloid β (Aβ) deposits,[9] tau protein,[10,11] polyglutaminylated proteins, and α-synuclein that are commonly observed in many neurodegenerative diseases, including Alzheimer's,[10,12–19] Huntington's,[3,20–25] and Parkinson's disease,[24] as well as progressive supranuclear palsy.[26,27] TGases are also involved in the pathogenesis of ear comedogenesis,[28,29] cataractous lens,[30] psoriasis,[31] cancer metastasis,[32] and injuries of the liver,[33] fibrin,[34,35] and immune system.[36] The deamidation function of TGase is also known to play a pathogenetic role in celiac disease by modifying the gluten peptide intake.[37–42] These cumulative reports suggest that the development of inhibitors for TGase may be clinically useful.

Many TGase inhibitors have been described, such as thienopyrimidonine-4-one acylhydrazides,[43] peptidyl methyl ketones,[44] dihydrooxazoles,[45] cinnamoyl derivatives,[46] 2-naphtyl 2-(N-isopropyl N-benzyl) aminoethyl ketone,[47] gluten peptide analogs,[48] epigallocatechin gallates,[49] chlorogenic acid,[50,51] peptide and peptidemimetic Michael systems,[52] glucosamine and its derivatives,[53] milk-derived inhibitors,[54] nordihydroguaiaretic acid,[55] cystamine,[56] IkBα-derived peptides,[57] and dihydroisoxazole and isatin derivatives.[58] We have also reported aryl β-amino ketones as potent transglutaminase inhibitors.[59]

As an extension of our previous report, we have synthesized aryl β-amino ketones starting from disulfide compounds, including cystamine, dimethyl cystine ester, and dimethyl homocystine ester, because disulfide compounds are well-known transglutaminase inhibitors,[60,61] in particular, cystamines.[22,23,62–64] In this report, we found that the resulting dithio aryl β-amino ketones (Fig. 1) were approximately 300 times more active than the starting disulfides.

Dithio β-aminoethyl ketone derivatives **1**, **5–41** (Table 1) were prepared using the conventional Mannich reaction.[59,65–67] Aryl methyl ketone derivatives **2**, disulfide compounds **3**, and paraformaldehyde were gradually heated from room temperature to 123 °C for 1 h and then kept at 123 °C for 10 min (Fig. 1). The resulting dithio β-aminoethyl ketones formed viscous oils[68] and were evaluated for TGase inhibitory activities using liver tissue transglutaminase (TGase 2), as reported previously.[59] Briefly, we monitored the velocity of the enzymatic reaction by measuring fluorescent intensity of monodansyl cadaverine using a Functional Drug Screening System (Hamamatsu Photonics, Shizuoka, Japan). IC_{50} values of the dithio β-aminoethyl ketones shown in Table 1 were calculated by fitting experimental data at 0.03, 0.1, 1, 3, 10, and 30 μM of compounds.[59]

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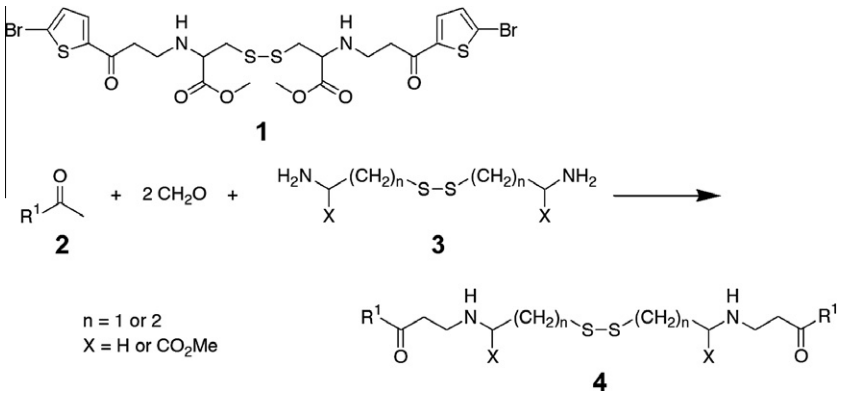


Figure 1. Representative structures of dithio β-aminoethyl ketones (**1** and **4**) and synthetic scheme.

Table 1
IC_{50} values of dithio β-aminoethyl ketones

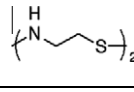
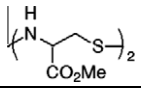
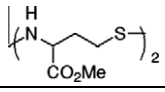
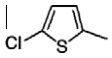
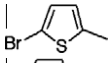
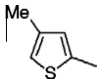
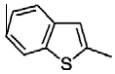
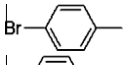
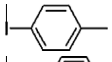
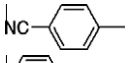
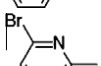
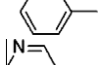
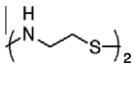
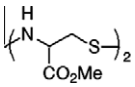
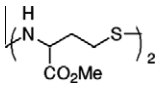
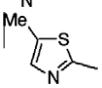
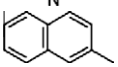
R^1	$(R^1COCH_2CH_2)_2R^2$		
	R^2		
			
	0.12 5	0.19 6	0.12 7
	0.12 **8**	0.18 1	0.099 9
	0.17 **10**	0.24 **11**	0.10 12
	0.51 **13**	0.40 **14**	3.6 **15**
	1.6 16		
			0.29 17
	0.21 **18**	5.7 **19**	0.33 **20**
	0.17 21	0.90 22	0.12 **23**
	0.42 **24**	2.9 **25**	
	2.2 26		
	0.12 **27**	11 **28**	1.6 **29**
	0.89 **30**		
	1.1 **31**	1.3 **32**	
		3.4 **33**	
	1.4 **34**		
	2.6 **35**	1.1 **36**	
		5.5 **37**	

Table 1 (*continued*)

R^1	$(R^1COCH_2CH_2)_2R^2$		
	R^2		
			
		9.7 **38**	
		4.0 **39**	
	2.0 **40**	19 **41**	

In each row, upper and lower values correspond to IC_{50} value (μM) and compound number, respectively.

Comparison of dithio β-aminoethyl ketones with the R^1 aryl group showed that the compound bearing the 2-thienyl group was more potent than phenyl, naphthyl, and other hetero aryl groups. The activities of the dithio β-aminoethyl ketones were not changed significantly by changing the substituents of the 2-thienyl group from hydrogen to chloro, bromo, iodo, methyl, and benzo groups. Furyl, 4-bromophenyl, and 4-iodophenyl groups were also effective, as shown by their IC_{50} values which were below 1 μM. Comparison of the disulfide backbones (R^2) revealed that cystamine (X = H, n = 1), dimethyl cystine (X = CO_2Me, n = 1), and dimethyl homocystine (X = CO_2Me, n = 2) derivatives exhibited almost the same inhibitory activities.

The IC_{50} values of cystamine, dimethyl cystine ester, and dimethyl homocystine ester were calculated as 31, 35, and 40 μM, respectively. Comparison between dithio β-aminoethyl ketones and starting disulfide compounds revealed that active compounds with IC_{50} values in the range of 0.1–0.2 μM (**1, 5–10, 12, 21**, and **23**) were approximately 300 times more effective than the starting disulfide compounds. These potencies were comparable to those of LDDN-80042 (IC_{50} = 0.13 μM), a thienopyrimidine derivative described by Duval et al.[43], and aryl β-aminoethyl ketones that we reported previously.[59] The disulfide bonds have previously been proposed to deactivate TGase by sulfide/disulfide interchange with an active site cysteine,[60] and the thienyl β-aminoethyl ketone moiety has been shown to strongly inhibit TGase.[59] Thus, incorporating these two elements within our synthesized compounds may additively contribute to the potent inhibition of TGase.

Hartley reported that a TGase inhibitor (LDDN-80042) blocked TGase-induced oligomerization of Aβ in concentration ranging from 0.1 to 10 μM,[9] suggesting that TGase might be a therapeutic target for slowing or blocking the progression of Alzheimer's disease. Mastroberardino reported that the knockout of tissue transglutaminase reduced neuronal death and prolonged survival in a model mouse having Huntington's disease.[20] Karpuj reported prolonged survival and decreased abnormal movements in transgenic models of Huntington's disease upon administration of cystamine.[22] These findings imply that our cystamine-derived TGase inhibitors may be more potent therapeutic drug candidates against Huntington's disease. In addition, the disulfide bond of compounds will be reduced to sulfide in vivo because of the high GSH/GSSG ratio. Thus, observing the effects of the drug produced using cystamine-derived TGase inhibitors in living cells will be an important step in future.

The dithio β-aminoethyl ketones described in this article are easy to synthesize from disulfide compounds already known to inhibit TGase. These ketones, which have IC_{50} values of approximately 0.1 μM, exhibit TGase inhibitory activities that are 300 times more potent than the starting disulfide compounds. Hence, they may be drug candidates for diseases caused by abnormal protein cross-links, such as cataract, Alzheimer's, Huntington's, Parkinson's, celiac, and skin diseases.

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68. Spectroscopic data for *N*,*N*-bis(2-(5-bromo-2-thiophenoyl)ethyl)cystine methyl ester **1**: ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.44 (m, 2H), 7.11 (m, 2H), 3.83 (m, 2H), 3.70 (s, 6H), 3.34 (m, 4H), 2.88 (m, 4H), 2.51 (m, 4H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 189.3, 163.9, 146.2, 132.4, 131.2, 122.7, 67.0, 53.4, 41.5, 38.5, 34.9. HRMS (FAB) (*m*/*z*): calcd for $C_{22}H_{27}Br_2N_2O_6S_4^+$ [M+H$^+$] 700.9119, found 700.9156.