Bioorganic & Medicinal Chemistry Letters 20 (2010) 1141–1144

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Potent transglutaminase inhibitors, aryl β-aminoethyl ketones

Shoichiro Ozaki [a], Etsuko Ebisui [a], Kozo Hamada [a,b], Jun-Ichi Goto [a], Akinobu Z. Suzuki [a], Akiko Terauchi [a,b], Katsuhiko Mikoshiba [a,b,*]

[a] Laboratory for Developmental Neurobiology, Brain Science Institute, Institute of Physical and Chemical Research (RIKEN), 2-1 Hirosawa, Wako, Saitama 351-0198, Japan
[b] Calcium Oscillation Project, ICORP-SORST, Japan Science and Technology Agency (JST), 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, Japan

ARTICLE INFO

Article history:
Received 20 October 2009
Revised 30 November 2009
Accepted 3 December 2009
Available online 22 December 2009

Keywords:
Transglutaminase inhibitor
Protein cross-link inhibitor
Alzheimer's disease
Huntington's disease
Parkinson's disease
Celiac disease

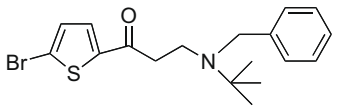
ABSTRACT

Aryl β-aminoethyl ketones were discovered as potent inhibitors of tissue transglutaminase. Heteroaryl-like thiophene groups and *N*-benzyl *N*-*t*-butyl aminoethyl group are critical to the strong inhibitory activity of aryl β-aminoethyl ketones.

Transglutaminases (TGases)[1] are calcium-dependent enzymes that catalyze various post-translational modifications including protein cross-linking, amine incorporation, and deamidation. Protein cross-links are formed by the creation isopeptide bonds between the carboxamide group of glutamine residues and the ε-amino group of lysine residues to form *N*-(γ-glutamyl)-L-lysine with the loss of ammonia.[2] The intra- and inter-molecular cross-links with the σ-bond are physically robust to serve physiological roles. These include the blood clotting by fibrin cross-linking and the cornified envelope formation in which TGase1 (a genetic factor for lamellar ichthyosis disease) cross-links insoluble amalgam of proteins.[3] Tissue TGase is also implicated in various neurodegenerative diseases including Alzheimer's disease,[4,5] Huntington's disease,[6–9] Parkinson's disease, and progressive supranuclear palsy. Unregulated high activity of TGase and many *N*-(γ-glutamyl)-L-lysine isopeptide bonds in protein aggregates are observed in these neurodegenerative diseases[10–12] as well as in vitro amyloid β deposits,[13] tau protein,[4,12] polyglutaminylated proteins, and α-synuclein are all potential substrates of tissue TGase. TGase is also implicated in the pathogenesis of ear comedogenesis,[14,15] cataractous lenses,[16] psoriasis,[17] cancer metastasis,[18] liver injury,[19] fibrin injury,[20,21] and damage to the immune system.[22] The deamidation of ingested gluten peptide by TGase is a pathogenic modification in celiac disease.[23,24] Altogether, these reports suggest that TGase regulation should have pivotal pathophysiological roles. Therefore, the development of TGase inhibitors could result in novel preventions or curing for these implicated diseases.

Many chemical inhibitors of TGase such as thienopyrimidonine-4-one acylhydrazides,[25] peptidylmethyl ketone,[26] dihydrooxazoles,[27] and cinnamoyl derivatives,[28] have been reported. Lai et al.[29] recently carried out a study on TGase inhibitors by screening existing drug libraries in which 2-naphtyl 2-(*N*-isopropyl *N*-benzyl)-aminoethyl ketone showed the highest activity. This report helped us to postulate that the important functional groups that are responsible for the inhibitory activity of TGase are carbonyl and amine moieties proximal to the aromatic ring.

We synthesized about 200 amino ketones and discovered that 5-bromo-2-thienyl-(*N*-*t*-butyl *N*-benzyl)-aminoethyl ketone (**1**) was a potent TGase inhibitor.



1

In the course of synthesis, we found that only β-aminoethyl ketones strongly inhibited TGase; α- and γ-aminoalkyl ketones showed very weak TGase inhibition (IC_{50} >30 μM). Therefore, we present the TGase inhibitory activities of only β-aminoethyl ketones in this Letter.

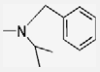
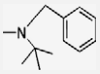
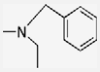
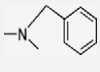
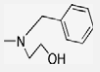
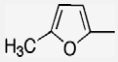
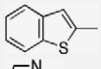
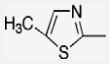
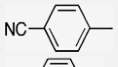
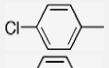
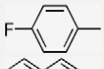
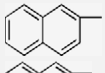
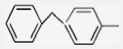
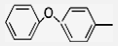
* Corresponding author. Tel.: +81 48 467 9745; fax: +81 48 467 9744.
E-mail address: mikosiba@brain.riken.jp (K. Mikoshiba).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.12.011

Table 1
IC_{50} values of aryl β-aminoethyl ketones (**4**)

$R^1CO\text{-}CH_2CH_2R^2$

4

R^1	R^2									
										
	0.83 **5**	1.4 **6**	1.4 **7**	2.0 **8**	2.5 **9**	4.8 **10**	2.5 **11**	3.5 **12**	11 **13**	7.9 **14**
	0.83 **15**	0.47 **16**	1.7 **17**	4.0 **18**	2.3 **19**	7.4 **20**	2.7 **21**	3.3 **22**	7.8 **23**	5.2 **24**
	1.1 **25**	1.0 **26**	2.7 **27**	17 **28**	4.9 **29**	5.4 **30**	10 **31**	5.1 **32**	12 **33**	>30 **34**
	0.53 **35**	0.57 **36**	0.67 **37**	1.2 **38**	1.5 **39**	6.9 **40**	1.7 **41**	1.2 **42**	2.7 **43**	9.9 **44**
	0.92 **45**	1.7 **46**			18 **47**	7.7 **48**		15 **49**		
	0.32 **50**	0.65 **51**	0.55 **52**	1.8 **53**	1.3 **54**	0.12 **55**	0.84 **56**	0.36 **57**	4.5 **58**	1.2 **59**
	0.32 **60**	2.3 **61**	2.8 **62**	4.3 **63**	6.6 **64**	0.42 **65**	0.80 **66**	6.5 **67**	0.68 **68**	1.4 **69**
	1.4 **70**	0.44 **71**	2.1 **72**	10 **73**	10 **74**	6.5 **75**	19 **76**	8.3 **77**	6.8 **78**	4.0 **79**
	0.19 **80**	0.081 1	0.61 **81**				1.4 **82**			
	0.23 **83**	0.10 **84**	0.42 **85**	1.0 **86**	1.5 **87**	5.4 **88**		1.9 **89**		
	8.6 **90**	11 **91**	12 **92**	10 **93**	12 **94**	11 **95**	12 **96**	13 **97**		14 **98**
	0.98 **99**				2.7 **100**	2.5 **101**				
	0.77 **102**	0.21 **103**				9.4 **104**				
	3.2 **105**	1.4 **106**	2.6 **107**	4.3 **108**	8.5 **109**	16 **110**		6.9 **111**	10 **112**	4.7 **113**
	0.85 **114**			1.8 **115**			2.3 **116**			
	5.6 **117**	1.7 **118**			11 **119**	10 **120**				
	0.59 **121**	0.17 **122**				6.1 **123**			10 **124**	
	1.4 **125**	0.7 **126**				2.2 **127**				
	10 **128**	1.1 **129**	13 **130**			10 **131**				
	4.0 **132**			21 **133**		16 **134**		22 **135**		
	1.7 **136**				1.5 **137**		1.9 **138**			
	2.6 **139**	3.6 **140**		26 **141**						
	12 **142**			18 **143**			7.4 **144**			

In each box, the upper and lower values are the IC_{50} value and compound number, respectively.

Aryl (*N*,*N*-disubstituted) β-aminoethyl ketone **4**, **5–144** (Table 1) were prepared by the conventional Mannich reaction[30–32] of the corresponding aryl methyl ketones (**2**), secondary amines (**3**) and formaldehyde (paraformaldehyde) by heating from room temperature to 133 °C in 1 h, and then keeping at 133 °C for 15 min.

$$\underset{\mathbf{2}}{R^1COCH_3} + HCHO + \underset{\mathbf{3}}{HNR^3R^4} \longrightarrow \underset{\mathbf{4}}{R^1CO\text{-}CH_2CH_2R^2}$$

$R^2 = N(R^3)(R^4)$

The products were purified by alumina column and were obtained as viscous oils. The inhibitory activity against tissue TGase was evaluated by our improved assay.[33] The resultant IC_{50} values of aminoketones are summarized in Table 1.

Among β-aminoketones, when R^1 of compound **4** was aliphatic, TGase inhibitory activity was very weak (IC_{50} >30 μM). We therefore showed only TGase inhibitory activities of aryl β-aminoketones.

Among aryl β-aminoketones, when a single hydrogen atom was attached at *N*,*N*-positions of the amino group (NHR), TGase inhibitory activities were very weak (IC_{50} >30 μM). Therefore we present only aryl *N*,*N*-disubstituted β-aminoethyl ketone **4** as shown in this Letter.

When we compared the aryl group R^1 of **4** by looking vertically at the top left line of Table 1, heteroaryls were more potent than phenyls and naphthyls. Among heteroaryl groups, thienyl appeared the most potent. Among thienyls, 5-bromo-2-thienyl was the most potent, followed by 5-chloro-2-thienyl and 2-benzothienyl, 2-thienyl in that order. The second most potent in the heteroaryls was the furyl group. Among the furyl group, the 2-furyl group was better than the 3-furyl group and the 5-methyl-2-furyl group. The pyridyl group was also effective. The 3-pyridyl group was potent, followed by 4-pyridyl and 2-pyridyl. The 2-pyrazinyl group and 6-ethyl 2-pyrazinyl group were also effective. 2-Thiazolyl groups were not very effective. Phenyl groups were quite effective. 4-Cyanophenyl had potent activity, followed by phenyl, 4-chlorophenyl, and 4-fluorophenyl in that order. 2-Naphtyl, chloronaphtyl, diphenyl methane, phenoxyphenyl groups also gave active compounds.

When we compared R^2 (NR^3R^4 in the reaction scheme) by looking horizontally at the top line of Table 1, *N*-*t*-butyl *N*-benzyl amino showed the most potent activity followed by *N*-isopropyl *N*-benzylamino, *N*-isopropyl, and *N*,*N*-bis-2-hydroxyethylamino groups. The *N*,*N*-dibenzylamino, and *N*,*N*-diphenylamino group showed very weak activity (IC_{50} >30 μM). Therefore these compounds are not shown in Table 1.

We synthesized 10 types of *N*-monosubstituted β-aminoethyl ketones. These compounds showed very weak activities (IC_{50} >30 μM). Therefore these compounds are also not shown in this Letter.

Duval et al.[25] reported that thienopyrimidines LDDN-80042 has an IC_{50} value of 0.13 μM. This was the highest value that was found in our literature search. Griffin et al.[26] reported on peptidyl methyl ketones which had an IC_{50} value of 3 μM. Watts et al.[27] had reported on dihydroisozazoles earlier, but the IC_{50} value was not reported. Pardin et al.[28] studied cinnamoyl benzotriazoyl amide which had an IC_{50} value of 18 μM. Lai et al.[29] reported that 2-naphtyl (*N*-isopropyl *N*-benzyl)-aminoethyl ketone was the most potent in existing drug libraries. The IC_{50} value of this compound (**132**) was 4 μM by our assay. The most potent compound presented in this Letter, 5-bromo-2-thienyl-(*N*-*t*-butyl *N*-benzyl)-aminoethyl ketone (**1**),[35] had an IC_{50} value of 0.081 μM. This value is currently the best.

Interestingly, our best compound had a thiophene ring. This supports the theory that the thiophene ring is critical to inhibitory activity, as reported by Duval et al.[25] Our best compound had a bromine atom. Watts et al.[27] also reported that compounds with bromine atoms were potent. In this way, these two structures make it feasible for us to obtain the strongest TGase inhibitors.

Hartley et al.[13] reported that TGase induced Aβ oligomerization. The TGase inhibitor LDDN-80042 (a gift from Cuny laboratory) was highly effective in attenuating Aβ aggregation. Aggregation was blocked at concentrations ranging from 0.1 to 10 μM, suggesting that TGase may constitute a specific therapeutic target for slowing (or blocking) the progression of Alzheimer's disease.

The TGase inhibitors described in this Letter are readily synthesized and yet are very strong inhibitors of TGase. Therefore, they may be strong candidates as therapeutic drugs for many diseases caused by abnormal protein cross-linking (isopeptide bonds). They may slow or block the progression of diseases (e.g., Alzheimer's, Huntington's, celiac, and cataract), and various disorders in the skin, liver, and immune system.

References and notes

1. Griffin, M.; Casadio, R.; Bergamini, C. M. *Biochem. J.* **2002**, *368*(Pt 2), 377.
2. Lorand, L.; Graham, R. M. *Nat. Rev. Mol. Cell Biol.* **2003**, *4*, 140.
3. Candi, E.; Schmidt, R.; Melino, G. *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 328.
4. Norlund, M. A.; Lee, J. M.; Zainelli, G. M.; Muma, N. A. *Brain Res.* **1999**, *851*, 154.
5. Selkoe, D. J.; Abraham, C.; Ihara, Y. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 6070.
6. Mastroberardino, P. G.; Iannicola, C.; Nardacci, R.; Bernassola, F.; De Laurenzi, V.; Melino, G.; Moreno, S.; Pavone, F.; Oliverio, S.; Fesus, L.; Piacentini, M. *Cell Death Differ.* **2002**, *9*, 873.
7. Jeitner, T. M.; Matson, W. R.; Folk, J. E.; Blass, J. P.; Cooper, A. J. *J. Neurochem.* **2008**, *106*, 37.
8. Karpuj, M. V.; Becher, M. W.; Springer, J. E.; Chabas, D.; Youssef, S.; Pedotti, R.; Mitchell, D.; Steinman, L. *Nat. Med.* **2002**, *8*, 143.
9. Dedeoglu, A.; Kubilus, J. K.; Jeitner, T. M.; Matson, S. A.; Bogdanov, M.; Kowall, N. W.; Matson, W. R.; Cooper, A. J.; Ratan, R. R.; Beal, M. F.; Hersch, S. M.; Ferrante, R. J. *J. Neurosci.* **2002**, *22*, 8942.
10. Lorand, L. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 14310.
11. Hoffner, G.; Djian, P. *Front. Biosci.* **2005**, *10*, 3078.
12. Grierson, A. J.; Johnson, G. V.; Miller, C. C. *Neurosci. Lett.* **2001**, *298*, 9.
13. Hartley, D. M.; Zhao, C.; Speier, A. C.; Woodard, G. A.; Li, S.; Li, Z.; Walz, T. *J. Biol. Chem.* **2008**, *283*, 16790.
14. De Young, L.; Ballaron, S.; Epstein, W. *J. Invest. Dermatol.* **1984**, *82*, 275.
15. Malorni, W.; Farrace, M. G.; Rodolfo, C.; Piacentini, M. *Curr. Pharm. Des.* **2008**, *14*, 278.
16. Azari, P.; Rahim, I.; Clarkson, D. P. *Curr. Eye Res.* **1981**, *1*, 463.
17. Schroeder, W. T.; Thacher, S. M.; Stewart-Galetka, S.; Annarella, M.; Chema, D.; Siciliano, M. J.; Davies, P. J.; Tang, H. Y.; Sowa, B. A.; Duvic, M. *J. Invest. Dermatol.* **1992**, *99*, 27.
18. Choi, K.; Siegel, M.; Piper, J. L.; Yuan, L.; Cho, E.; Strnad, P.; Omary, B.; Rich, K. M.; Khosla, C. *Chem. Biol.* **2005**, *12*, 469.
19. Tatsukawa, H.; Fukaya, Y.; Frampton, G.; Martinez-Fuentes, A.; Suzuki, K.; Kuo, T. F.; Nagatsuma, K.; Shimokado, K.; Okuno, M.; Wu, J.; Iismaa, S.; Matsuura, T.; Tsukamoto, H.; Zern, M. A.; Graham, R. M.; Kojima, S. *Gastroenterology* **2009**, *136*, 1783.
20. Lorand, L.; Rule, N. G.; Ong, H. H.; Furlanetto, R.; Jacobsen, A.; Downey, J.; Oner, N.; Bruner-Lorand, J. *Biochemistry* **1968**, *7*, 214.
21. Stenberg, P.; Nilsson, J. L.; Eriksson, O.; Lunden, R. *Acta Pharm. Suec.* **1971**, *8*, 415.
22. Fesus, L. *Surv. Immunol. Res.* **1982**, *1*, 297.
23. Piper, J. L.; Gray, G. M.; Khosla, C. *Biochemistry* **2002**, *41*, 386.
24. Hovhannisyan, Z.; Weiss, A.; Martin, A.; Wiesner, M.; Tollefsen, S.; Yoshida, K.; Ciszewski, C.; Curran, S. A.; Murray, J. A.; David, C. S.; Sollid, L. M.; Koning, F.; Teyton, L.; Jabri, B. *Nature* **2008**, *456*, 534.
25. Duval, E.; Case, A.; Stein, R. L.; Cuny, G. D. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1885.
26. Griffin, M.; Mongeot, A.; Collighan, R.; Saint, R. E.; Jones, R. A.; Coutts, I. G.; Rathbone, D. L. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5559.
27. Watts, R. E.; Siegel, M.; Khosla, C. *J. Med. Chem.* **2006**, *49*, 7493.
28. Pardin, C.; Pelletier, J. N.; Lubell, W. D.; Keillor, J. W. *J. Org. Chem.* **2008**, *73*, 5766.
29. Lai, T. S.; Liu, Y.; Tucker, T.; Daniel, K. R.; Sane, D. C.; Toone, E.; Burke, J. R.; Strittmatter, W. J.; Greenberg, C. S. *Chem. Biol.* **2008**, *15*, 969.
30. Arend, M.; Westermann, B.; Risch, N. *Angew. Chem., Int. Ed.* **1998**, *37*, 1044.
31. Brown, G. R.; Bamford, A. M.; Bowyer, J.; James, D. S.; Rankine, N.; Tang, E.; Torr, V.; Culbert, E. J. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 575.
32. Mannkind cooporation. Intracellular Kinase Inhibitors. PCT WO 2007/136790 A2.
33. The inhibitory activities of compounds against TGase were evaluated by our improved high-throughput screening. We applied the method established for the measurement of TGase activity[34] to a high-throughput screening using the functional drug screening system, FDSS 3000 (Hamamatsu Photonics, Shizuoka, Japan). The synthesized compounds were added to 0.1 ml enzyme reaction solutions containing 100 mM HEPES-NaOH (pH 7.5), 1 mM $CaCl_2$, 20 μM monodansyl cadaverine, 0.05 mg/ml *N*,*N*-dimethylcasein, 5 μg/ml guinea-pig liver TGase in a 96-well plate (Nunc, 96-Well Black Plate with clear bottom). We measured fluorescence emissions at 510 nm after excitation at 340 nm. We calculated the velocity of the fluorescent increase to evaluate enzyme activity. Compounds were dissolved and diluted with DMSO to prepare final concentrations of 3.0, 10, and 30 μM. When the compounds were effective at these concentrations, we diluted the compounds to determine the IC_{50} values at submicromolar concentrations. DMSO was added to the reaction mixture as a control in the same 96-well plate and enzyme activity expressed as a percentage of control. The IC_{50} values were calculated by nonlinear

regression analysis to fit experimental data to the logistic function with 4 or 3 parameters using SigmaPlot.
34. Lorand, L.; Lockridge, O. M.; Campbell, L. K.; Myhrman, R.; Bruner-Lorand, J. *Anal. Biochem.* **1971**, *44*, 221.
35. Spectroscopic data for 5-bromo-2-thienyl-(*N*-*t*-butyl *N*-benzyl)-aminoethyl ketone (**1**). ^{1}H NMR ($CDCl_3$, 270 MHz) 7.0–7.8 (m, 7H), 3.73 (m, 2H), 2.97 (t, *J* = 7.5 Hz, 2H), 2.60 (t, *J* = 7.5 Hz, 2H), 1.18 (s, 9H). ^{13}C NMR ($CDCl_3$, 270 MHz) 189.5, 145.9, 142.7, 132.4, 131.9, 131.2, 128.4, 128.3, 128.1, 127.8, 122.7, 54.6, 51.0, 46.7, 40.8, 28.9; HREMS *m*/*e*; found 380.0694; calcd for $C_{18}H_{22}BrNOS$, 380.0684.