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Letter

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SAR Development of Lysine-Based Irreversible Inhibitors of Transglutaminase 2 for Huntington's Disease

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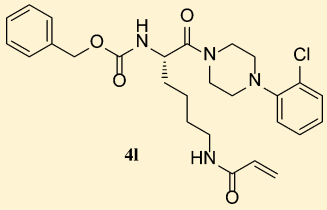
Supporting Information

ABSTRACT: We report a series of irreversible transglutaminase 2 inhibitors starting from a known lysine dipeptide bearing an acrylamide warhead. We established new SARs resulting in compounds demonstrating improved potency and better physical and calculated properties. Transglutaminase selectivity profiling and in vitro ADME properties of selected compounds are also reported.



KEYWORDS: *plasma stability, polar surface area, acrylamides, celiac disease, in vitro ADME*

Tissue transglutaminase 2 (TG2) is a multifunctional enzyme primarily known for its calcium-dependent protein cross-linking activity.[1] Less well-studied functions include simple amidase, GTPase, ATPase, and protein disulfide isomerase activities.[2−4] TG2 has been characterized in at least three forms, including open,[5] closed,[6] and an open-inactive form.[7] Genetic deletion of TG2 in mice suggests a role for TG2 activity in mitochondrial energy function,[8] and its overactivity has been most closely associated with celiac disease and Huntington's disease (HD). In addition, there is growing support for roles in inflammation and cancer.[9−12]

HD is an autosomal dominant, progressive, neurodegenerative disease that is characterized clinically by motor, cognitive, and behavioral deficits.[13] TG2 expression and transglutaminase activity have been shown to be increased in the brains of HD patients,[14] and in vitro and in vivo models have implicated TG2 in HD pathophysiology,[15−18] although more recent contradictory animal data have appeared.[19]

The subject of irreversible inhibitors of TG2 has been recently reviewed.[20] Our studies have focused on irreversible inhibitors bearing an acrylamide warhead,[21,22] during which we became interested in dipeptides **A** and **B**[23] (Figure 1) as leads due to their attractive potency and specificity for TG2. In a prior report,[21] we established that an excellent correlation exists for several transglutaminase isoforms between the IC_{50} values using a 30 min compound incubation and the irreversible inhibition constants, k_{inact}/K_i. With this correlation in hand, we relied on the IC_{50} values to guide our medicinal chemistry effort. We began by benchmarking dipeptide **A**, resulting in the selectivity profile illustrated in Figure 1. We also resynthesized and tested several compounds from the Marrano paper,[23] the

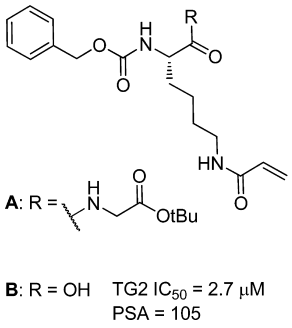


Figure 1. Lysine-based irreversible inhibitors of TG2 from Marrano et al.[23]

results of which are found in the Supporting Information. To summarize, the results confirmed that dipeptide **A** was one of the most potent TG2 inhibitors from this report. As shown in Figure 1, ADME profiling studies on this compound indicated good solubility, low permeability potentially accompanied by efflux, and rapid metabolism in mouse liver microsomes (mLM). Our goal was to identify a tool molecule from this series for in vivo proof of concept studies in HD, where brain is the target organ. Therefore, we focused on increasing potency, improving the absorption profile, and increasing microsomal stability. Lowering the polar surface area (PSA), the number of hydrogen bond donors, and the number of rotatable bonds

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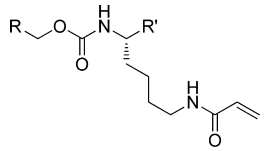
were the main tactics employed. The synthesis of new compounds for this report was accomplished using well-known literature methods, the details of which may be found in the Supporting Information.

Because of its lower molecular weight, lower PSA, and equipotency, we selected compound **B** from the Marrano paper as our starting point. Our prior reports described a structural biology/computational chemistry-driven approach to structure−activity relationship (SAR) construction;[21,22] however, in the present series, we were unable to successfully use these models to explain the known (or new) SAR. We instead focused on an empirical approach based on improvement of calculated properties. As the carboxylic acid was viewed as an impediment to permeability, we began by replacement with a hydroxmethyl (**1**) or deletion of this group entirely as in **2** (Table 1). While these modifications resulted in approximately

Table 1. TG2 Activity of Carbamate Derivatives[a]



compd no.	R	R′	PSA (Å^2)[b]	TG2 IC_{50} ± SD (μM)
B	C_6H_5	CO_2H	105	2.7 ± 0.32
1	C_6H_5	CH_2OH	88	5.4 ± 0.54
2	C_6H_5	H	67	7.6 ± 0.78
3a	H	CO_2H	105	>80
3b	2−Cl-C_6H_4	CO_2H	105	1.9 ± 0.19
3c	2-CF_3-C_6H_4	CO_2H	105	3.5 ± 0.62
3d	3-CF_3-C_6H_4	CO_2H	105	10 ± 0.028
3e	4-CF_3-C_6H_4	CO_2H	105	33
3f	4-NO_2-C_6H_4	CO_2H	148	62
3g	4−F-C_6H_4	CO_2H	105	7.9 ± 0.075
3h	4-CH_3-C_6H_4	CO_2H	105	14 ± 0.064
3i	4-*n*-Bu-C_6H_4	CO_2H	105	5.9 ± 0.68
3j	4-*t*-Bu-C_6H_4	CO_2H	105	21
3k	2,6-F,F-C_6H_3	CO_2H	105	10 ± 3.7
3l	1-naphthyl	CO_2H	105	14 ± 0.39
3m	2-naphthyl	CO_2H	105	9.1 ± 1.3

[a]Values accompanied by standard deviations were averaged from at least two independent experiments; they were otherwise obtained in a single determination. [b]PSA calculations were obtained using the Dotmatics mol2image property calculation engine (http://www.dotmatics.com/).

2- and 4-fold losses in potency relative to **B**, the PSAs of these compounds (88 and 67, respectively) were significantly improved and in a range more compatible with blood−brain barrier (BBB) permeability. In addition, the relatively modest loss in potency suggested that the carboxylate was not making a productive interaction with TG2.

We next established the SAR with respect to the carbamate moiety (Table 1). With **B** again as a comparator, the methyl carbamate (**3a**) resulted in a large loss in potency. Turning again to the benzyl carbamate motif, the 2-chloro (**3b**) was slightly more potent than **B**, the 2-trifluoromethyl (**3c**) was equipotent, and the 3- or 4-trifluoromethyl (**3d** and **3e**, respectively) resulted in 3- and 10-fold losses in potency, respectively. Continuing with 4-substituted benzyl carbamates, a 4-nitro substituent, as in **3f**, resulted in a 20-fold loss in potency, while the 4-fluoro (**3g**) was 2−3-fold less potent. A series of 4-alkyl substituents were next examined (**3h−j**); however, these were also of lower potency relative to **B**. Similar results were found with the 2,6-difluoro (**3k**) and with the 1- and 2-naphthyl derivatives **3l** and **3m**.

After preliminary investigation of the SAR with respect to the carbamate moiety, we chose to keep the benzyl carbamate constant and turned our attention to replacing the carboxylate with a series of tertiary amides (Table 2). Relatively low molecular weight aliphatic amides as exemplified by **4a−h** nearly all showed significantly improved potency relative to **B**, with several compounds showing submicromolar potency. It was noteworthy that this improvement did not require an increase in PSA, bulking up the molecular weight, or the addition of hydrogen bond donors relative to **1**. Observing that a more lipophilic pyrrolidine (**4i**) afforded 2-fold improved potency relative to **4e**, we next prepared 4-phenylpiperidine **4j**, which had a TG2 IC_{50} of 0.054 μM, and proved to be one of the more potent compounds from this series. Phenylpiperidine **4j** also had a favorable selectivity profile, with IC_{50} values of 9.3, > 80, and 21 μM against TG1, TG3, and FXIIIa, respectively.

We followed this with a series of piperazines, which had superior synthetic accessibility facilitating SAR development, but at the cost of increased PSA. Judicious choice of functionality could, in principle, keep the PSA of the piperazines in a more acceptable range. The potency of methylpiperazine **4k** (IC_{50} = 0.61 μM) was in line with the earlier simple tertiary aliphatic amides, indicating that addition of the basic piperazine nitrogen atom was well tolerated. The additional substitution on the terminal phenyl moiety of piperazines **4l** and **4m** did not affect potency relative to **4j**, while a slight loss of potency was observed with 2-naphthyl **4n**. Next investigated was a series of 2-piperazinylpyridines (**4o−s**). As a baseline, pyridine **4o** recorded an IC_{50} of 0.26 μM. The addition of a methyl group at either the 6- (**4p**) or the 3-position (**4q**) resulted in improved potency, with **4p** having an IC_{50} of 0.055 μM. Substitution of **4o** with a trifluoromethyl group at either the 3- (**4r**) or the 5-position (**4s**) resulted in approximately 2-fold improvement in potency relative to **4o**. Interestingly, the 3-methyl (**4q**) and electron-withdrawing 3-trifluoromethyl (**4r**) and 5-trifluoromethyl (**4s**) analogues all had similar potency. Favorable transglutaminase selectivity was also retained; **4l** had IC_{50} values of 0.062, 12.9, >80, 69, and 67 μM against TG2, TG1, TG3, TG6, and FXIIIa, respectively. Further profiling also demonstrated that compounds from this series were 2−7-fold less potent against mouse TG2 than they were against the human form (shown in the Supporting Information). This species difference may be an indication of a somewhat different binding mode as compared with our earlier chemotypes,[21,22] which did not show this effect.

At this point, we had significantly improved potency while keeping transglutaminase selectivity and the hydrogen bond donor count in favorable ranges. However, the large number of rotatable bonds inherent in the lysine-based scaffold and PSA exceeding 100 for some potent compounds (particularly the 2-piperazinylpyridines) were concerns for oral bioavailability.[24] A key tactic employed to reduce the number of rotatable bonds was lysine scaffold replacement. Early attempts resulted in 4-aminophenylalanine-based **5** and piperidinylalanine-based **6** (Figure 2); however, both were inactive against TG2 when tested at 80 μM, and comparisons with the potencies of **B**, **4p**, and **8a** argued against synthesis of additional analogues. Improved potency was observed for proline-based **7** (IC_{50} =

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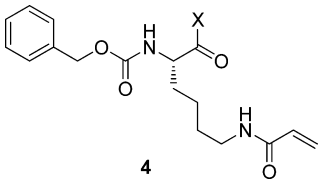
Table 2. TG2 Activity of Tertiary Amide Derivatives[a]



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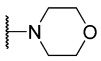
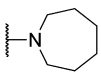
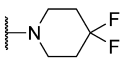
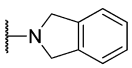
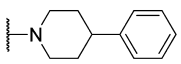
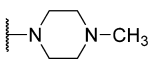
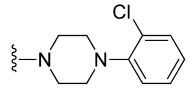
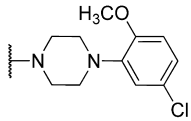
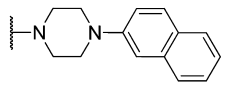
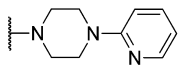
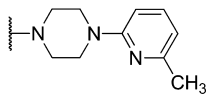
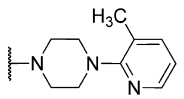
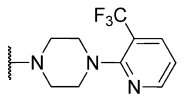
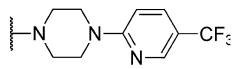
Cmpd No.	X	PSA (Å²)[b]	TG2 IC_{50} ± SD (μM)
B	OH	105	2.7 ± 0.32
4a		88	1.1 ± 0.0042
4b		88	1.4 ± 0.43
4c		97	0.73 ± 0.012
4d		88	1.2 ± 0.029
4e		88	0.58 ± 0.049
4f		88	2.5 ± 0.20
4g		88	0.59 ± 0.043
4h		88	0.66 ± 0.082
4i		88	0.24 ± 0.038
4j		88	0.054 ± 0.029
4k		91	0.61 ± 0.046
4l		91	0.062 ± 0.0050
4m		100	0.078 ± 0.015
4n		91	0.23 ± 0.083
4o		104	0.26 ± 0.011
4p		104	0.055 ± 0.013
4q		104	0.17 ± 0.057
4r		104	0.16 ± 0.016
4s		104	0.11 ± 0.029

[a]Values accompanied by standard deviations were averaged from at least two independent experiments; they were otherwise obtained in a single determination. [b]PSA calculations were obtained using the Dotmatics mol2image property calculation engine (http://www.dotmatics.com/).

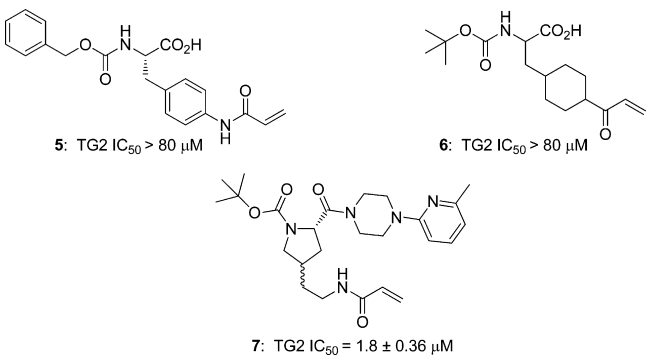


Figure 2. Lysine scaffold replacements **5**–**7**.

1.8 μM), which was prepared as a diastereomeric mixture. However, given this potency, that it shared many structural features with **4p** and would be expected to carry similar liabilities in terms of permeability/efflux and microsomal stability, we elected not to continue the SAR development required to optimize this scaffold.

Another round of SAR development led to the series of 2-piperazinyl-6-methylpyridines shown in Table 3. The bulky Boc derivative **8a** was ca. 15-fold less potent than benzyl carbamate **4p**. A further loss in activity resulted from incorporation of a 2-naphthylamide (**8b**). Interestingly, improved potency was seen with acetamide **8c**, and an additional improvement was observed from phenylacetamide **8d**, which was the most potent compound of the series. Favorable transglutaminase selectivity was also retained; **8d** had IC_{50} values of 0.014, 15, >80, >80, and 35 μM against TG2, TG1, TG3, TG6, and FXIIIa, respectively. Benzamide **8e** was equipotent with **8c**, demonstrating the importance of the methylene linker in positioning and orienting the phenyl substituent.

Illustrated in Table 4 are the results of in vitro metabolism profiling of **4l** and **8d**. In a kinetic solubility assay, both showed

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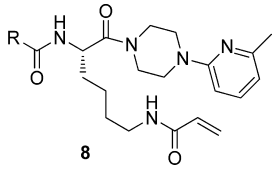
Table 3. TG2 Potency of 2-Piperazinyl-6-methylpyridine Derivatives[a]



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compd no.	R	PSA (Å^2)[b]	TG2 IC_{50} ± SD (μM)
4p	$PhCH_2O$	104	0.055 ± 0.013
8a	*tert*-BuO	104	0.85 ± 0.34
8b	2-naphthyl	95	1.6 ± 0.021
8c	CH_3	95	0.19 ± 0.064
8d	$PhCH_2$	95	0.014 ± 0.0082
8e	Ph	95	0.19 ± 0.073

[a]Values accompanied by standard deviations were averaged from at least two independent experiments; they were otherwise obtained in a single determination. [b]PSA calculations were obtained using the Dotmatics mol2image property calculation engine (http://www.dotmatics.com/).

good solubility and were stable in both mouse and human plasma with a half-life >24 h. In addition, **4l** showed no evidence of conjugation to the prototypical biological nucleophile glutathione (GSH) when tested in vitro over a period of 68 h (data not shown).[25] In liver microsomal stability testing, metabolic stability was poor, with a short half-life and rapid intrinsic clearance in both mouse and human. The high rate of metabolism was likely due to the benzyl and piperazinyl moieties, which are susceptible to oxidation at multiple sites.

To be effective agents for HD, it is preferable that compounds possess a high rate of permeability and a low efflux rate. P-glycoprotein (P-gp) is one of the main efflux transporters in brain; the potential for **4l** and **8d** to be effluxed by P-gp was assessed in an MDCK-MDR1 transfected cell line. The results of this study indicated that both had good permeability but suffered high active efflux via P-gp. Combining this result with the results of microsomal stability testing indicated that these compounds were not suitable candidates for in vivo evaluation in the context of HD. The result of this profiling suggests that they may be better suited as treatments for celiac disease, where BBB permeability would be a disadvantage and 24 h systemic coverage may not be required for efficacy.

In summary, we have developed a series of irreversible TG2 inhibitors based on a known lysine scaffold having improved potency and favorable selectivity against closely related transglutaminase isoforms. The series exhibited low GSH reactivity, improved PSA, and excellent plasma stability but showed a species-dependent loss in potency against mouse TG2. Additionally, compounds exhibited a high rate of oxidative metabolism and high P-gp efflux, signifying that further optimization of the ADME profile will be required to achieve brain exposure in vivo.

■ ASSOCIATED CONTENT

Supporting Information

Table of in-house-generated TG2 activity of compounds from Marrano et al.;[22] synthetic disclosures comprising schemes and full experimental procedures; and characterization of key compounds, supporting figures, details of the ADME profiling assays, and transglutaminase selectivity profiling data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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Table 4. In Vitro ADME Properties of Selected Lysine Amides[a]

			μL/min/mg[a]		$T_{1/2}$ (min)[a]			
compd no.	TG2 IC_{50} (μM)	aq. sol. (mg/mL)[a]	mLM Clint	hLM Clint	mouse plasma	human plasma	MDCK-WT P_{app} A−B (nm s^{-1})[a]	MDCK-MDR1 EER
8d	0.014	0.87	NT[b]	>1386	>1440	>1440	142	118
4l	0.06	0.06	>1386	>1386	>480	>480	303	7.1

[a]See the Supporting Information for experimental details. [b]NT indicates that the compound was not tested.

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