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Structure–activity relationship study of novel tissue transglutaminase inhibitors

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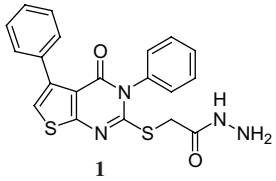
Abstract—Thieno[2,3-*d*]pyrimidin-4-one acylhydrazide derivatives were discovered as moderately potent inhibitors of TGase 2 (tissue transglutaminase) utilizing a fluorescence-based assay that measured TGase 2 catalyzed incorporation of the dansylated Lys derivative α-*N*-Boc-Lys-CH_2-CH_2-dansyl into the protein substrate *N*,*N*-dimethylated-casein. A SAR study revealed that the acylhydrazide thioether side-chain and the thiophene ring were critical to inhibitory activity.

Transglutaminases (TGases) are a family of Ca^{2+}-dependent enzymes that catalyze the formation of isopeptide bonds between the carboxamide group of protein/peptide-bound glutamine residues and the ε-amino group of protein/peptide-bound lysine residues to form N^{ε}-(γ-L-glutamyl)-L-lysine cross links with loss of ammonia. Currently, eight TGase isoforms have been identified. TGases are normally expressed at low levels in many different tissues and serve vital roles, such as in blood clotting and epithelia formation. However, it is becoming increasingly evident that some TGase isozymes are involved in diverse pathological conditions, such as celiac disease, inclusion body myositis, cataract formation, atherosclerosis and neurodegenerative disorders.[1]

The TGase 2 (i.e., tissue transglutaminase) isozyme is involved in several general biological functions, including apoptosis, cell adhesion and signal transduction.[1b] In addition, this particular isozyme has been most soundly linked to celiac disease,[2] Alzheimer's[3] and Huntington's[4] diseases. Therefore, potent and selective TGase 2 inhibitors are needed in order to further elucidate its role in various patho-physiologies and to provide lead compounds for therapeutic development.

Cystamine is a well known, albeit weak, inhibitor of TGases. Furthermore, it has shown positive effects in various cell-based and in vivo models of neurodegeneration.[1b] However, the relationship between its TGase inhibitory activity and its efficacy in cells and animals is complex and not entirely clear. All of the other reported TGase inhibitors to date do so irreversibly.[1b] In the course of screening for TGases 2 inhibitors utilizing a recently developed assay,[5] we discovered that the thieno[2,3-*d*]pyrimidin-4-one acylhydrazide derivative **1** was a moderately potent inhibitor. Herein we report an initial structure–activity relationship (SAR) study for this class of TGase 2 inhibitor.



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The 2-aminothiophene-3-carboxylates **3a** were prepared from aldehydes or ketones **2** using the Gewald reaction (Scheme 1). Depending on the starting material different reaction conditions were used (Method A:[6] R^1 = *i*-Pr or aryl, R^2 = H, Me or *i*-Pr; Method B:[7] for cyclohexanone derivatives and *N*-Boc-4-piperidone; Method C:[8] R^1 = H, R^2 = Ph). The 2-chlorothiophene **3b** was obtained from **3a** by sequential protection of the amine, chlorination of the thiophene ring with sulfuryl chloride[9] followed by amine de-protection. Reaction of

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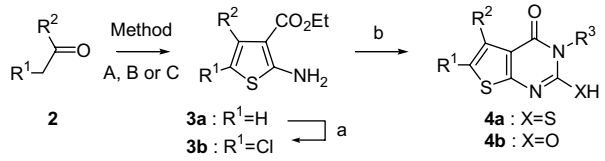


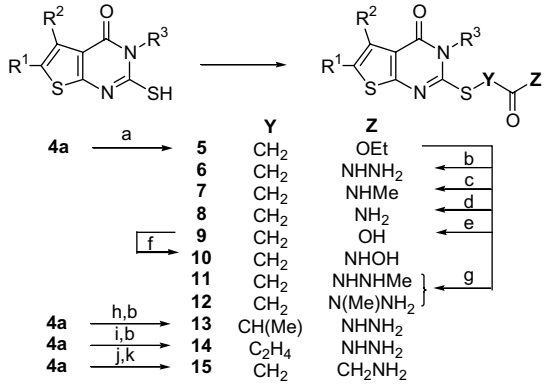
Scheme 1. Reagents and conditions: *Method A*: (i) EtO_2CCH_2CN, AcOH, $AcONH_4$, C_6H_6, Dean–Stark, Δ; (ii) sulfur, Et_2NH, EtOH, 50 °C; *Method B*: EtO_2CCH_2CN, sulfur, morpholine, EtOH, 50 °C; *Method C*: EtO_2CCH_2CN, sulfur, DBU, toluene, MW, 120 °C, 20 min; (a) TFAA, Et_3N, CH_2Cl_2 then SO_2Cl_2, CH_2Cl_2 then $NaBH_4$, EtOH; (b) R^3NCX (X = S or O), pyridine, 50 °C then MeONa, MeOH, rt.

3a–**b** with alkyl or aryl isothiocyanates (or isocyanates) in basic conditions afforded compounds **4a** (or **4b**).[10]

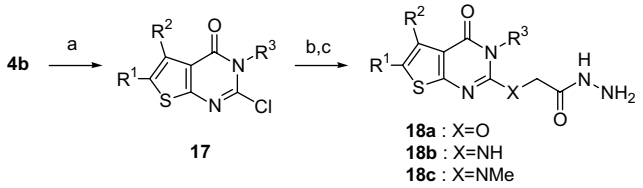
Alkylation of thiol **4a** with ethyl bromoacetate followed by aminolysis of the resulting ester **5** with hydrazine led to acylhydrazine **6** (Scheme 2). Compound **5** was also converted into amides **7** and **8**, acid **9** and hydroxamic acid **10**. Reaction of **5** with methylhydrazine led to a separable mixture of regioisomers **11** and **12**. Similarly, reaction of **4a** to methyl chloropropionate followed by aminolysis led to the acylhydrazine **13**. The homologated analog **14** was accessible by a Michael addition of **4a** with methylacrylate followed by aminolysis of the ester with hydrazine. Alternatively, alkylation of **4a** with the α-bromoketone $BrCH_2C(O)CH_2NHBoc$, **16**,[11] followed by de-protection of the amine led to the α-aminoketone **15**.

The oxygen and nitrogen analogs **18a**–**c** (Scheme 3) were obtained in three steps from **4b**. First, **4b** was converted to chloride **17** by treatment with $POCl_3$ under microwave (MW) irradiation. Addition of the requisite nucleophile to **17** followed by aminolysis of the ester gave **18a**–**c**.

Compounds **20a** and **20b**, in which the thiophene moiety has been replaced with a benzene ring, were prepared



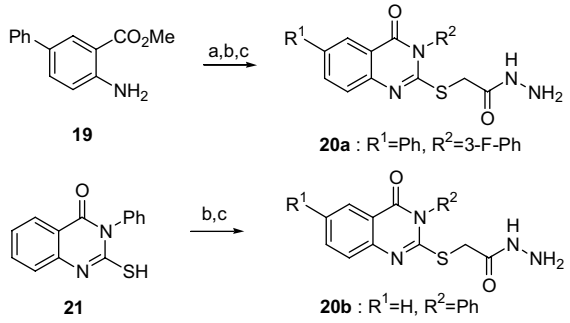
Scheme 2. Reagents and conditions: (a) $BrCH_2CO_2Et$, K_2CO_3, DMF, rt; (b) NH_2NH_2, EtOH, rt; (c) $MeNH_2$, MeOH, rt; (d) NH_3, MeOH, rt; (e) 10 N NaOH, THF/MeOH, 0 °C; (f) $(ClCO)_2$, DMF cat, CH_2Cl_2, then $NH_2OH{\cdot}HCl$, Et_3N, THF/water, rt; (g) $MeNHNH_2$, EtOH, 80 °C; (h) methyl 2-chloropropionate, K_2CO_3, THF, Δ; (i) methylacrylate, Et_3N, MeOH, 60 °C then rt; (j) **16**, K_2CO_3, DMF, rt; (k) HCl, Et_2O, rt.



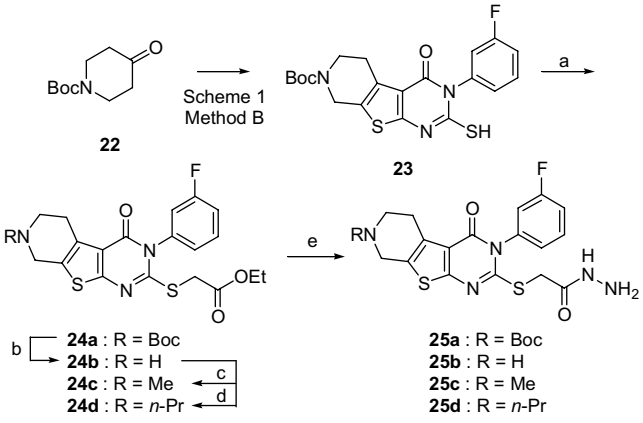
Scheme 3. Reagents and conditions: (a) $POCl_3$, MW, 150 °C, 1 h; (b) **18a**: EtO_2CCH_2OH, NaH, THF, Δ; **18b**: $MeO_2CCH_2NH_2{\cdot}HCl$, Et_3N, EtOH/MeCN, 60 °C; **18c**: $EtO_2CCH_2NHMe{\cdot}HCl$, Et_3N, EtOH/MeCN, 60 °C; (c) NH_2NH_2, EtOH, rt.

from **19** and **21** using the same procedures that were employed for the thiophene series (Scheme 4).

The *N*-Boc-protected thiophene derivative **23** (Scheme 5), prepared from *N*-Boc-4-piperidone, **22**, utilizing methodology described in Scheme 1/Method B, was alkylated with ethyl bromoacetate to afford **24a**. The amine was de-protected to give **24b**, which in turn was alkylated to afford **24c** and **24d**. Each of these derivatives (**24a**–**d**) was subsequently converted into acylhydrazines **25a**–**d**.



Scheme 4. Reagents and conditions: (a) 3-F-Ph-NCS, pyridine, 50 °C then MeONa, MeOH, rt; (b) $BrCH_2CO_2Et$, K_2CO_3, DMF, rt; (c) NH_2NH_2, EtOH, rt.



Scheme 5. Reagents and conditions: (a) $BrCH_2CO_2Et$, K_2CO_3, DMF, rt; (b) TFA, CH_2Cl_2, rt; (c) $H_2C{=}O$ 37%, $NaBH(OAc)_3$, AcOH, MeOH, CH_2Cl_2; (d) C_2H_5CHO, $NaBH(OAc)_3$, AcOH, MeOH, CH_2Cl_2; (e) NH_2NH_2, EtOH, rt.