Medicinal Chemistry and Properties of 1,2,4-Thiadiazoles

Tim Fat Tam*, Regis Leung-Toung, Wanren Li, Michael Spino and Khashayar Karimian

Apotex Research, Inc. Medicinal Chemistry Department, 400 Ormont Drive, Toronto, ON, M9L 1N9, CANADA

Abstract: 1,2,4-Thiadiazole is a distinctive class of small heterocyclic thiol trapping agents that serve as an interesting pharmacophore in the design of inhibitors targeting the cysteine residues of proteins. X-Ray crystal structures of enzyme-inhibitor complex indicate that the cysteine thiol reacts with the N-S bond of the thiadiazole moiety to form a disulfide bond resulting in the inactivation of the enzymes.

This review addresses the medicinal chemistry and various properties of 1,2,4-thiadiazoles in their potential as new electrophilic "warheads" for targeting the cysteine residues of biomolecules (*e.g*, H^+/K^+ ATPase), and cysteine-dependent enzymes (*e.g*., cathepsin B and transglutaminase).

Keywords: Thiadiazole, proton pump, cathepsin B, Factor XIIIa, transglutaminase, cysteine thiol, inhibitors.

1. INTRODUCTION

Cysteine dependent enzymes are involved in a variety of diverse biological functions. The topic of thiol dependent enzymes and their inhibitors has recently been reviewed [1]. A large number of chemical functionalities has been used for the irreversible modification of the cysteine thiol of enzymes, thus resulting in their inactivation [1-6]. Examples of functionalities commonly cited in the literature include chloromethyl ketones, aryloxymethyl ketones, epoxides and vinyl sulfones. Most of these functional groups driven inhibitors have limitations because they react with other nucleophiles (*e.g*., amines, alcohols). Usually, compounds with reactive chemical functionalities are chemically, metabolically and pharmacologically unstable. These inhibitors invariably fail during pharmacological evaluation in pre-clinical studies and/or in clinical trials due to their highly reactive nature. To overcome these difficulties, a program using small heterocycles was initiated to develop active, but stable cysteine protease inhibitors in our laboratory.

Karimian *et al*. reported that three different families of 1,2,4-thiadiazoles (THDs, Fig. **1**) reacted with enzyme cysteine residue to form a disulfide adduct, thus inhibiting the enzyme [7-10]. The stable chemical classes of THDs, shown in Fig. (**2**), are the monocyclic 1,2,4-thiadiazole (**1**), the bicyclic imidazo[1,2-d][1,2,4]thiadiazole (**2**), and the tricyclic benzo[4,5]imidazo[1,2-d][1,2,4]thiadiazole (**3**).

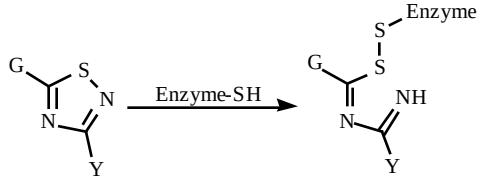


Fig. (1). Mechanism of inhibition of cysteine dependent enzymes by 1,2,4-thiadiazoles.

The lack of reactivity (S-N bond cleavage) of derivatives of **1-3**, with other nucleophiles such as amines and alcohols [11-14], makes the 1,2,4-thiadiazole heterocycle an excellent pharmacophore to be incorporated into lead molecules for the inhibition of enzymes by targeting the cysteine residue [1,15]. The design of inhibitors based on the monocyclic 1,2,4-thiadiazole scaffold (**1**, Fig. **2**) involves the use of substituent G at the C5 position as a recognition arm for enzyme binding, and the C3 substituent Y for tuning the reactivity of the ring opening of the THD [7]. The use of a fused ring in bicyclic THD (**2**) and tricyclic THD (**3**) is preferred for the inhibition of certain enzymes such as H^+/K^+ ATPase [8-10]. In these cases, the C3 substituent Y can be used to tune both enzyme affinity and reactivity of the N-S bond towards the incoming thiol nucleophile. As a result of this interesting property, THD can be used to target the cysteine residues of enzymes via the formation of an enzyme-inhibitor complex/adduct through the formation of a disulfide bond with the cysteine residue. This review will focus on the medicinal chemistry and properties of 1,2,4-thiadiazoles with some illustrated examples in the inactivation of enzymes through the modification of the cysteine residues.

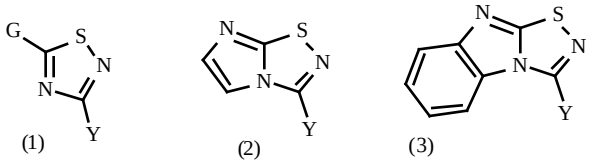


Fig. (2). Monocyclic (**1**), bicyclic (**2**) and tricyclic (**3**) 1,2,4-thiadiazoles.

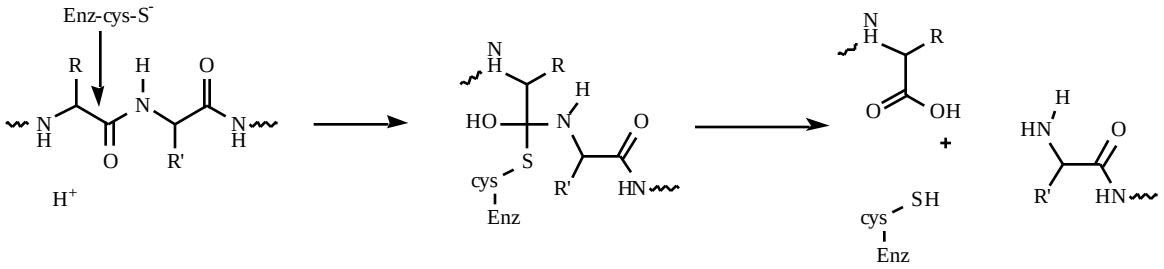
2. MECHANISM OF ACTION OF SELECTED CYSTEINE DEPENDENT ENZYMES

Within the family of cysteine dependent enzymes, cysteine proteases (*e.g*., the lysosomal cathepsins) serve a catalytic function that involves the cleavage of an amide bond. The mechanism is outlined in Fig. (**3**).

In contrast, enzymes of the transglutaminase family are involved in the transamidation of amide bonds. Transglutaminase is a cysteine dependent enzyme that catalyzes the formation of amide bonds between the γ-carboxamide group of peptide-bound glutamine residues and the ε-amino group of peptide-bound lysine residues. The cysteine residue in the active site of tranglutaminase initially

*Address correspondence to the author at the Apotex Research, Inc., Medicinal Chemistry Department, 400 Ormont Drive, Toronto, ON, M9L 1N9, Canada; Tel: 416-749-9300 ext. 7384; E-mail: ttam@apotex.com

1389-5575/05 $50.00+.00



Fig, (3). Mechanism of amide bond hydrolysis by cysteine protease.

forms a thioester acyl enzyme intermediate with the glutamyl side chain. Further reaction of the thioester with the lysine residue of the protein results in the formation of a covalent amide bond as shown in Fig. (**4**).

Hence, it is conceivable that chemical compounds capable of modifying the active site cysteine of these families of enzymes can prevent their normal catalytic function. The ideal inhibitor must not only be enzyme specific but also active site directed. 1,2,4-Thiadiazole meets this requirement as it can serve as a thiol trapping agent, and the C3 and/or C5 substitutent can be used as a recognition arm to fit into the enzyme to ensure the THD warhead is active site directed.

to give the ring-opened compound (**6**) and diphenyl disulfide (**7**), presumably via the disulfide intermediate (**5**) (Scheme 1).

Similarly, the 1,2,4-thiadiazoline derivative (**8**) reacted with the sulfide nucleophile (**9**) to afford the disulfide compound (**11**) and the ring-opened product (**12**), via the disulfide intermediate (**10**) (Scheme 2) [16].

Also, Δ^3-1,2,4-thiadiazoline (**13**) reacted with benzyl mercaptan to give the ring-opened product (**14**) and dibenzyl

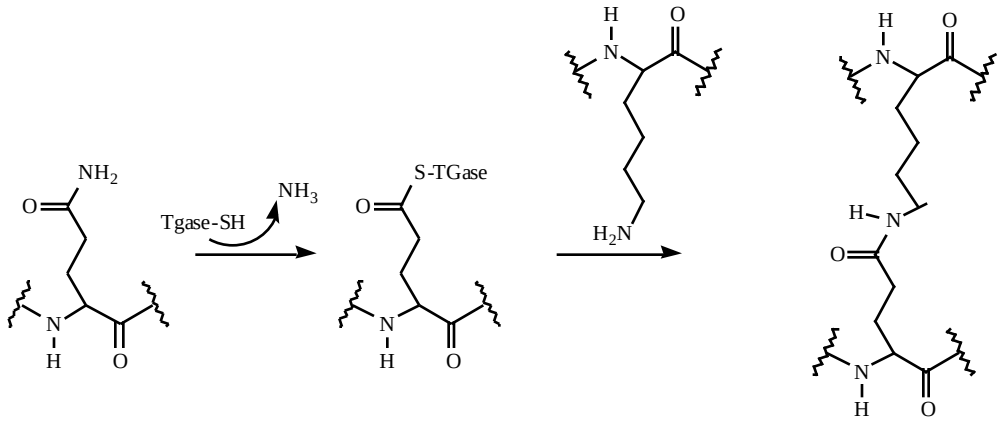


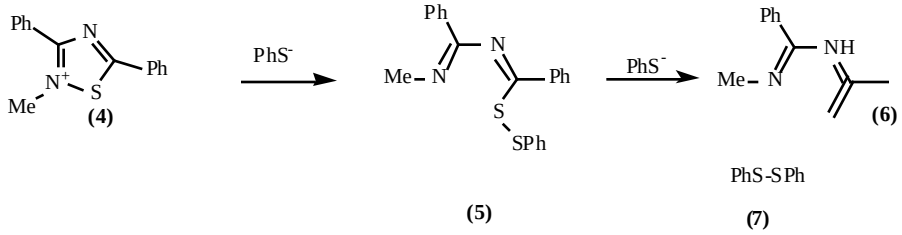
Fig. (4). Mechanism of amide bond formation by transglutaminase.

disulfide (**15**) (Scheme 3). Interestingly, the simple 1,2,4-thiadiazole derivative (**16**) 4-chlorophyenyl-3-phenyl-[1,2,4]-thiadiazol-5-yl-amine did not react with benzyl mercaptan [17].

In a biological system, Hagiwara and his coworkers reported that **13** showed fungicidal activity against cucumber downy mildew, *P. cubensis*, through modification of the cysteine residue of the enzyme, while **16** did not show any activity. The inactivation of alcohol dehydrogenase (ADH) by **13** has also been suggested to occur in a similar fashion [17]. To our knowledge, these "*activated* 1,2,4-thiadiazoles"

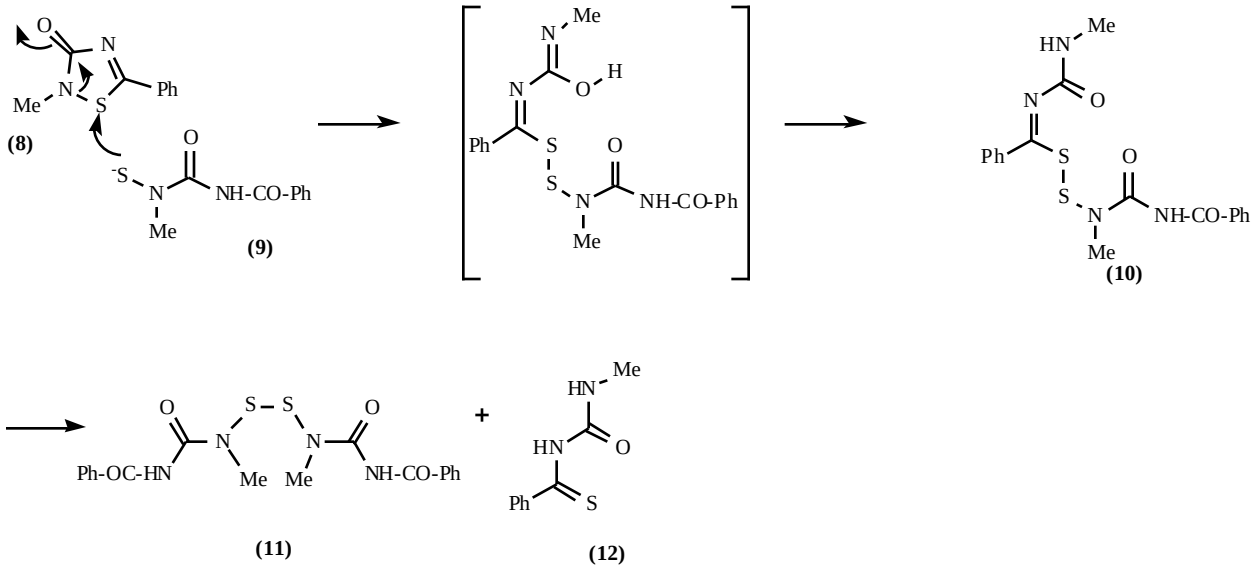
2.1. Heterocycles and Their Scissile N-S Bonds

Heterocyclic compounds possessing N-S bonds that are cleaved through their reaction with chemical thiols to form disulfide intermediates or adducts have been previously reported. In 1977, Crook and Sykes [14] proposed that *N*-methyl-1,2,4-thiazolium salt (**4**) reacted with benzenethiolate

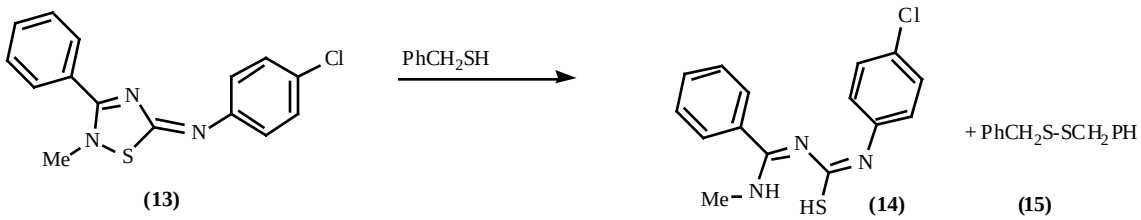


(4) **(5)** **(6)** **(7)**

Scheme 1. Reaction between thiolphenolate and *N*-methyl-1,2,4-thiazolium salt (**4**).



Scheme 2. Ring opening reaction of 1,2,4-thiadiazoline (**8**) with sulfide nucleophile (**9**).



Scheme 3. Ring opening reaction of Δ^3-1,2,4- thiadiazoline (**13**) with benzyl mercaptan.

(**4**, **8** and **13**) have not been used as scaffolds for structural activity investigation as N-S cysteine thiol trapping agents in enzyme systems.

The use of 2-sulfonylimino-2*H*-[1,2,4]thiadiazolo[2,3-a]pyridine derivatives (**17**, Fig. **5**) as compounds possessing platelet aggregation inhibitory and cardiotonic actions, has been reported in a Japanese patent [18]. However, the mechanism of action of this class of compounds at the molecular level has not been disclosed.

2.2. 1,2,4-Thiadiazoles (THDs)

There are numerous disclosures on a variety of potential uses of monocyclic 1,2,4-thiadiazole in the patent literature. The majority of the claims relate to the use of such compounds as insecticides, herbicides and fungicides [11-13]. For example, the THD derivative **18** shown in Fig. (**5**) is commercially available as etridazole, and is used as a soil fungicide [11].

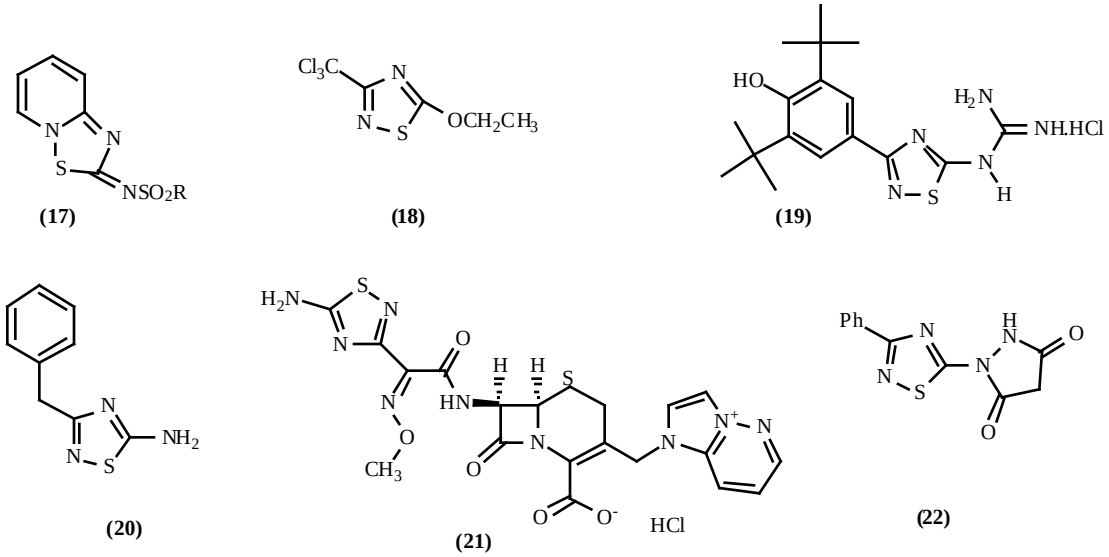


Fig. (5). Structures of some biologically active 1,2,4-thiadiazole analogues.

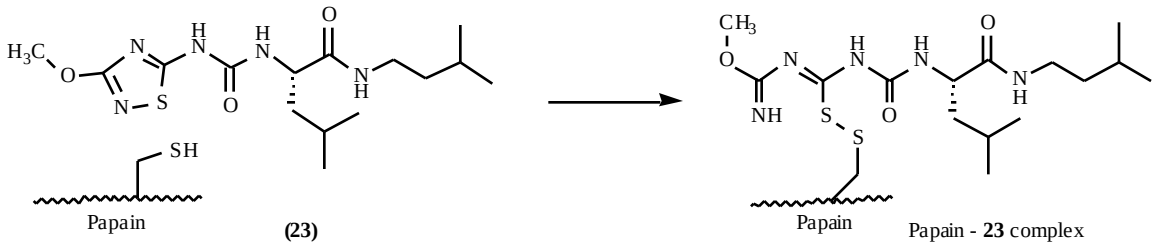


Fig. (6). Enzyme-inhibitor adduct of Apo1073 (**23**) and papain.

The structure of some biologically active THDs exhibiting interesting medicinal properties are shown in Fig. (**5**). Although a simple compound such as (4-chlorophenyl)-(3-phenyl-[1,2,4]thiadiazol-5-yl)-amine (**16**) did not show any fungicidal activity [17], a similar analogue (**19**) has been reported to have dual lipoxygenase and cyclooxygenase inhibitory activities [19,20]. Compound (**20**) showed biological activity in an adjunct arthritis model in rats [21]. Cefozopran hydrochloride (**21**), an injectable cephalosporin product, was launched in August 1995 in Japan under the trade name of *Firstcin* [22]. It is a 1,2,4-thiadiazole cephem antibiotic with antimicrobial activity against a wide spectrum of Gram-positive and Gram-negative organisms. Interestingly, the simple THD analogue (**22**) showed antibacterial activity [23]. Analogues of 1,2,4-thiadiazole have also been studied as potential adenosine receptor antagonists [24], as β-adrenergic antagonists [25], and for their muscarinic activities [26]. Recently, a 1,2,4-thiadiazole derivative has been reported to bind in the benzodiazepine binding site of human γ-aminobutyric acid receptor ion channels [27].

In all these cases, the role and importance of the THD moiety of these compounds or inhibitors with regards to the mechanism of the biological response of the targeted enzymes are not clearly understood at the molecular level, thus providing an excellent opportunity for further investigation of chemistry mechanism at the molecular level.

2.3. Proof of Principle

There is ample evidence that the N-S bond of the 1,2,4-thiadiazole moiety is cleaved by thiols, and the intermediacy of a disulfide adduct has been suggested [13,14,28,29]. Early in our research, we reasoned that a similar type of reaction may occur in a biological system. For example, it is conceivable that a 1,2,4-thiadiazole analogue such as Apo1073 (**23**) can react with the active site cysteine of papain as shown in Fig. (**6**). A disulfide adduct is formed and consequently the cysteine protease enzyme is inactivated.

The proof of principle comes from the protein crystal structure of inhibitor Apo1073 (**23**) and the papain adduct as

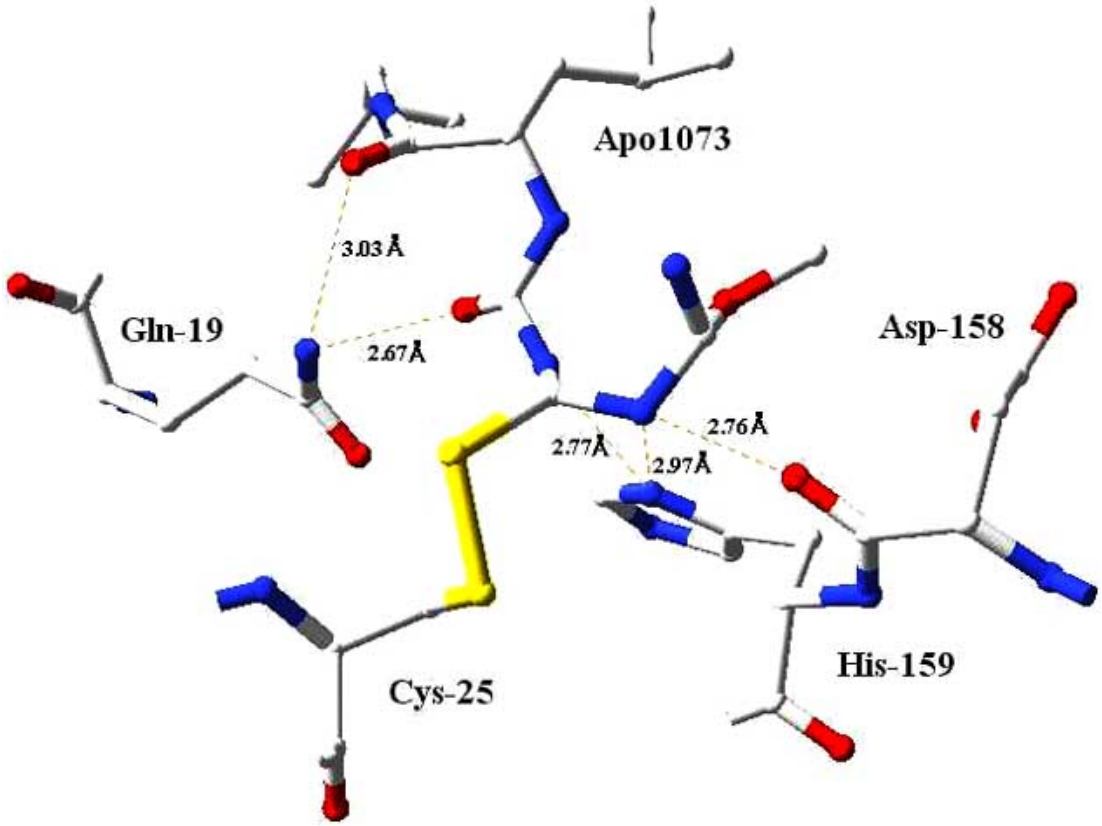
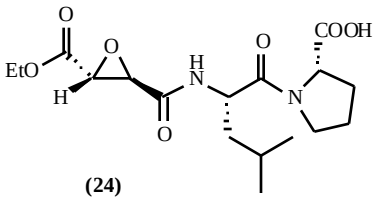
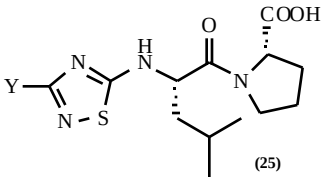


Fig. (7). Crystal structure of Papain-Apo1073 (**23**) adduct.



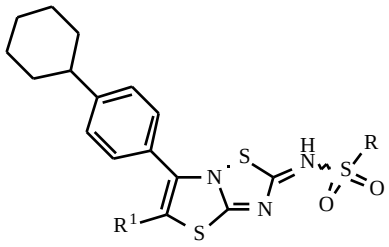
(24)



(25)

Cat. B inhibitory activity [7]

	Y	$K_i/K_i(M^{-1}s^{-1})$
a.	MeO	5629
b.	Ph	175
c.	Me	55
d.	COOH	293



(26) R = p-tolyl; $R^1 = (CH_2)_3CO_2Et$

(27) R = 3-pyridyl; R^1 = Me

Fig. (8). Cathepsin B inhibitors.

shown in Fig. (**7**). The presence of a disulfide bond between papain and **23** is clearly demonstrated. This is the first example in the literature that confirms the formation of a disulfide bond between a 1,2,4-thiadiazole compound and a member of the cysteine protease family [7,30].

2.4. Cathepsin B Inhibitor

Having established that a 1,2,4-thiadiazole analogue could inactivate the active site cysteine residue of papain, we directed our efforts in the design of potential inhibitors of cathepsin B, a lysosomal cysteine protease which is believed to play a critical role in a number of important diseases that include cancer, and arthritis [1].

The group G at the C5 position of the monocyclic 1,2,4-thiadiazoles can be designed to incorporate a recognition sequence specific to the active site of the enzyme. The group Y at the C3 position may be designed to tune the reactivity of the 5-substituted-[1,2,4]-thiadiazoles towards the enzyme by activating the adjacent bonds. The substrate P1'-P2' sequence for cathepsin B is Leu-Pro-OH [1], and the extensively studied epoxysuccinyl derivative **24** (Fig. **8**), with Leu-Pro-OH recognition sequence, is a potent inhibitor of cathepsin B [31].

Apo501 (**25a, Y = OMe**, Fig. **8**) was designed based on this principle. The preliminary crystal structure of **25a** and cathepsin B adduct was published as proof of principle in a patent disclosure [7]. A congener series of analogues of **25a-d** (analogue of (**1**) with G = Leu-Pro-OH; Y = MeO, Ph, Me and COOH) were evaluated for their potency against cathepsin B in an effort to establish a structure-activity relationship. As shown in Fig. (**8**), the most potent inhibitor was the 3-MeO analogue **25a (Y = OMe)**, which had a second order rate constant of $k_i/K_i = 5629\ M^{-1}s^{-1}$ [7].

Interleukin-1 (IL-1) is a pro-inflammatory cytokine produced by human rheumatoid arthritis synovium that contribute to joint destruction. The effect of Apo501 (**25a, Y = OMe**) on IL-1 induced proteoglycan degradation of articular cartilage from normal calf joints was investigated in

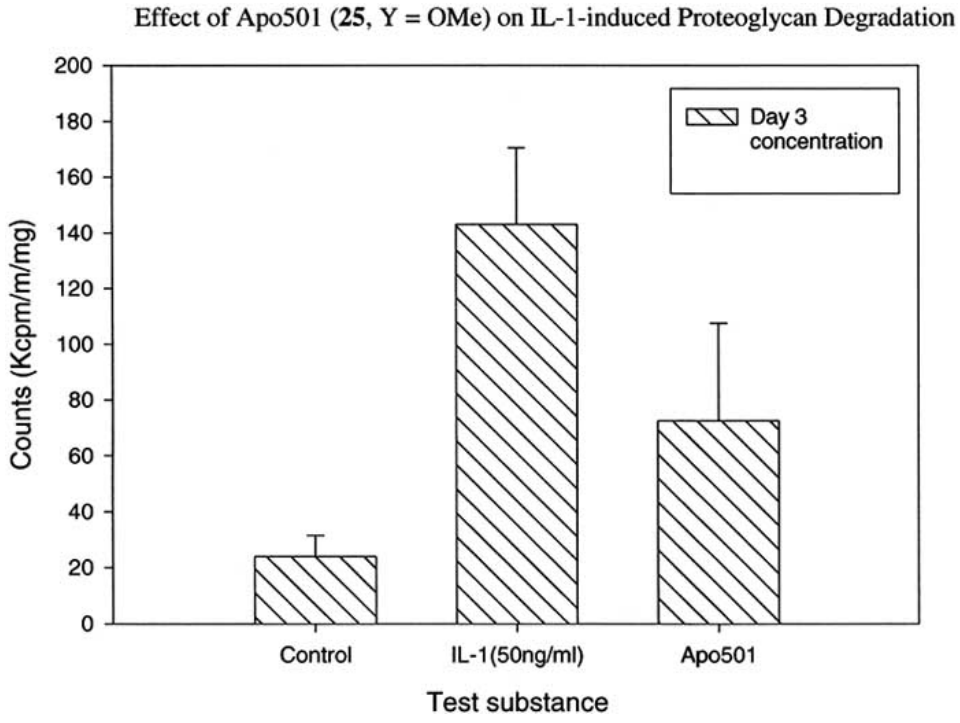


Fig. (9). Inhibitory effect of Apo501 (25, Y = OMe) on the IL-1 induced proteoglycan degradation.

vitro [7]. The extent of proteoglycan degradation in each cartilage treated with (a) 1 x 10^{-6}M Apo501 + 50 ng/ml IL-1, (b) control (no drug & IL-1), (c) 50 ng/ml IL-1 was determined by measuring the radioactivity of S^{35} sulfate-glycosaminoglycans. Proteoglycan degradation was expressed as radiolabelled glycosaminoglycans released into the media (counts per min per ml) per dry weight (mg) of cartilage after 72 hrs. As shown in Fig. (**9**), Apo501 (**25a**) demonstrated an inhibitory effect on the IL-1 induced proteoglycan degradation. Apo501 resulted in a significant reduction (60%) of IL-1 (50 ng/ml)-induced degradation of newly synthesized proteoglycan (p=0.023) at 1 μM.

Sohda *et al*. disclosed a new class of heterocyclic compounds such as **26** and **27** (Fig. **8**) which exhibit endothelin receptor antagonistic action, cathepsin B inhibitory action and bone resorption inhibitory action [32]. Both **26** and **27** inactivated cathepsin B with IC_{50}s of 3.5 and 1.1 μM, respectively. In an *in-vitro* assay using bone culture samples labeled with Ca^{45} from a Sprague-Dawley rat [32], **27** showed an 85% inhibition of bone resorption when compared to control experiment without test compound.

It is not clear if the mechanism of action of these compounds (**26** and **27**) is derived from the ring cleavage of the N-S bond of the heterocyclic moiety to form a disulfide bond with the active site cysteine during the inactivation of cathepsin B or due to some other mechanism. Although there are numerous claims to the potential uses of cathepsin B inhibitors in the treatment of disease such as rheumatoid arthritis, none of the known cathepsin B inhibitors is currently in clinical development [1].

2.5. Transglutaminase Inhibitors

The role and activation of transglutaminases in a variety of pathological conditions such as Celiac disease, Huntington's disease, Alzheimer's disease and Parkinson's disease have recently been reviewed [33,34]. Also, in some cases of wound healing, the formation of hypertrophic scar has been attributed to an increased amount of type III procollagen. The Tgase competitive inhibitor, putrescine, prevents the formation of isopeptide cross-linking of procollagen type III in the wound matrix. Fibrostat™ cream (topical putrescine hydrochloride) has been shown to regulate collagen production by controlling Tgase activity in the healing area [35]. Therefore, the use of inhibitors based on the 1,2,4-THD scaffold for the inactivation of these enzymes is another area of potential interest.

The approach used by Keillor *et al*. for the design of Tgase inhibitors such as **28** as shown in Fig. (**10**), involved

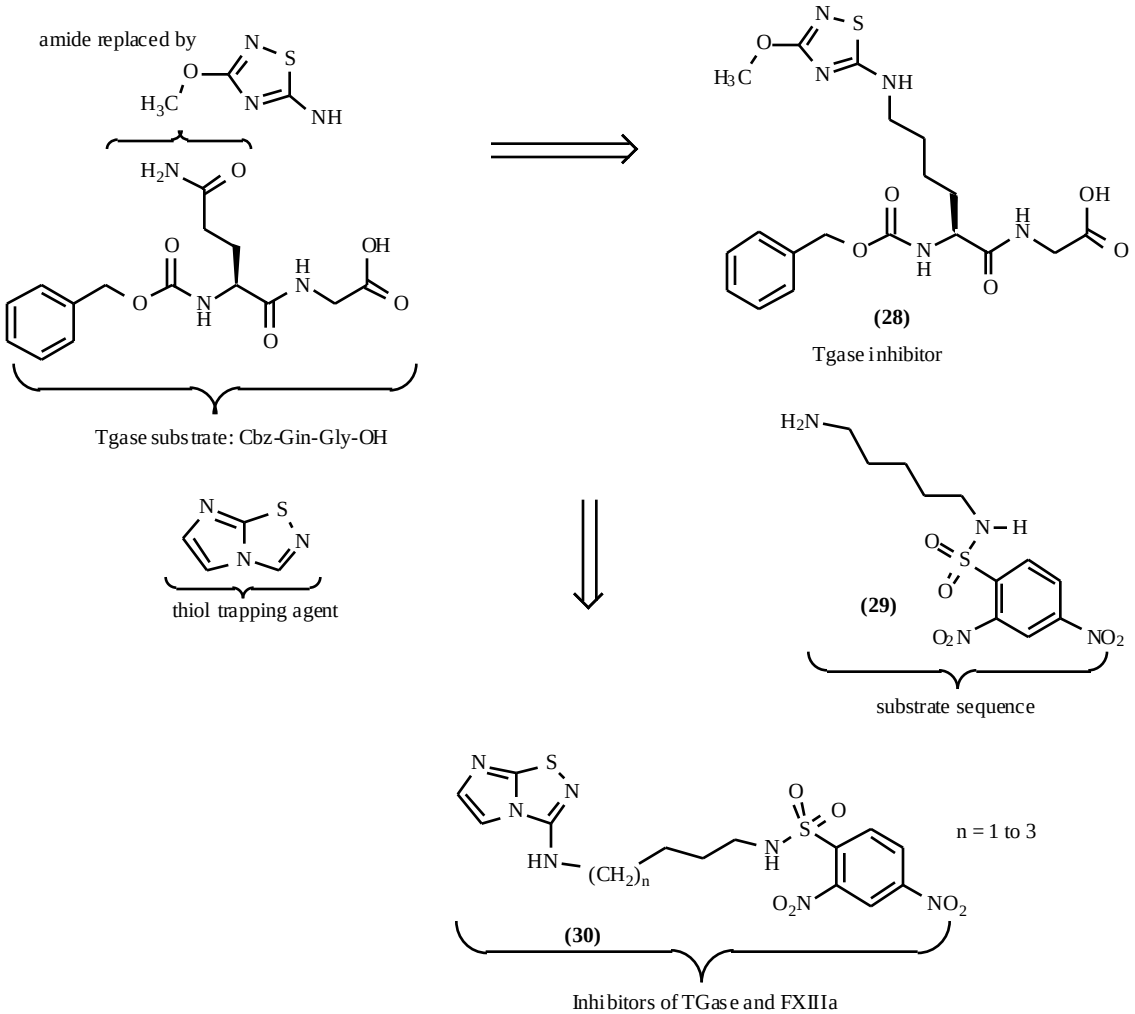
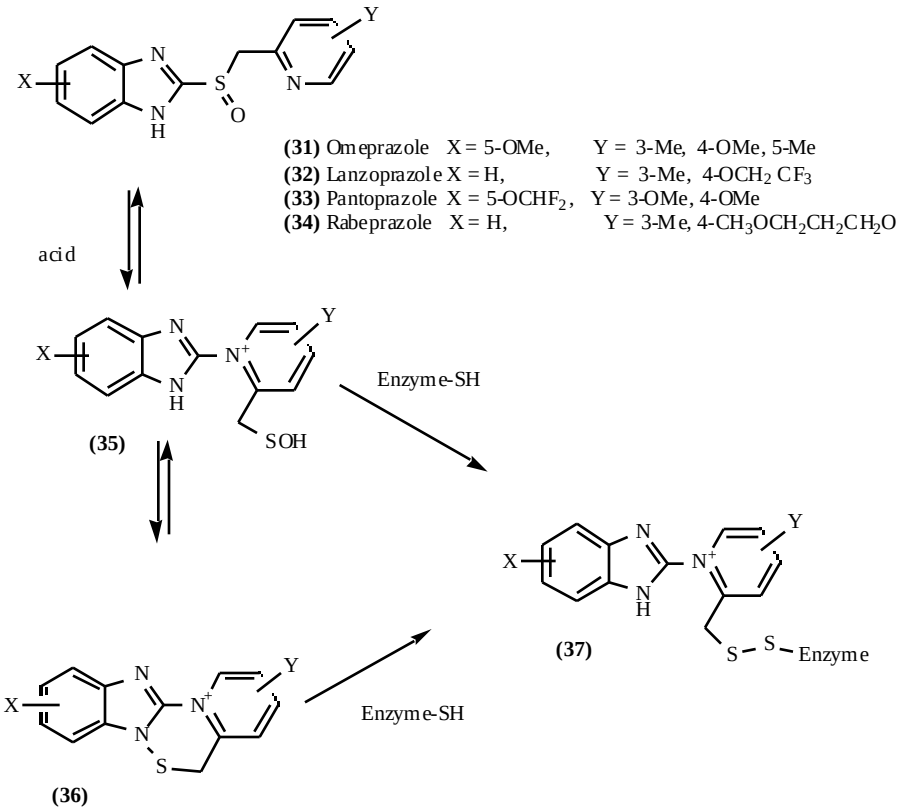


Fig. (10). Potential Tgase and FXIIIa inhibitors based on the 1,2,4-thiadiazole scaffold.



Scheme 4. Mechanism of cysteine thiol modification by proton pump inhibitors.

the attachment of a 1,2,4-THD moiety to replace the amide portion of the Tgase substrate Cbz-Gln-Gly-OH [36]. This class of heterocyclic compounds are potent inactivators of guinea pig liver Tgase, but they have not been evaluated against other members of the Tgase family.

Plasma transglutaminase FXIIIa catalyzes the covalent cross-linking reactions of fibrins, to give additional structural stability to the clot and thus provide resistance to plasma-mediated degradation. It is suggested that the inhibition of Factor XIIIa may render thrombi more susceptible to tissue plasminogen inactivator thrombolysis *in vivo* [37]. Therefore, a Factor XIIIa inhibitor can conceivably be used as a therapeutic agent towards the prevention of fibrin cross-linking.

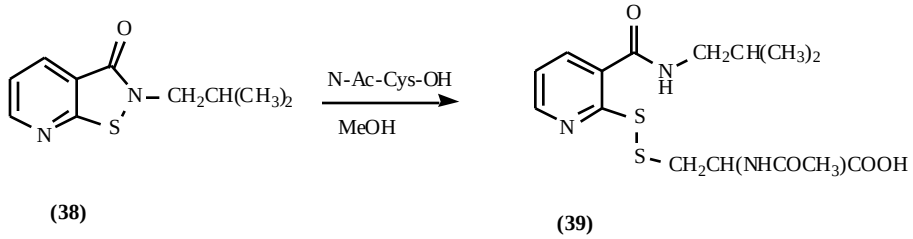
Tam *et al*. used a non-peptido approach, as illustrated in Fig (**10**), towards the inhibition of plasma Tgase [38]. Thus, a new class of FXIIIa inhibitors (*e.g.*, **30**) based on the attachment of the bicyclic imidazo[1,2-d][1,2,4]thiadiazole moiety to the 1-amino group of 2,4-dinitrophenylsulfonyl cadaverine (**29**), a substrate for guinea pig liver Tgase [39], was designed. Compound (**30**) inactivated both liver Tgase and FXIIIa [38], but the pharmacological and biochemical properties of (**30**) and its analogues have not yet been reported.

3. CYSTEINE RESIDUES OF BIOMOLECULES AS TARGET

The H^+/K^+ ATPase Enzyme or The Proton Pump

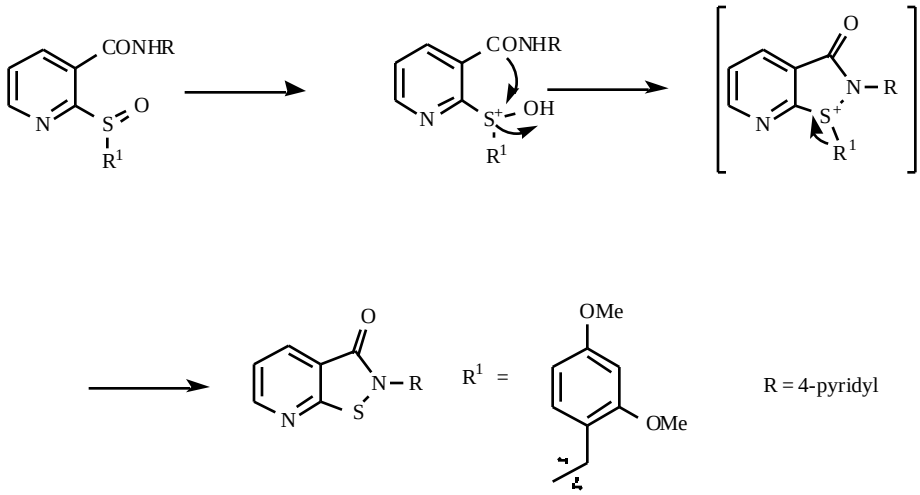
The proton pump inhibitors or PPIs are probably the best known class of thiol trapping agents [40]. PPIs developed on the PSB, 2-[(2-pyridylmethyl)sulfinyl)benzimidazole, scaffolds have enjoyed huge commercial successes as antiulcer drugs, with annual sales exceeding 4 billion USD [41]. Examples of those are omeprazole (**31**), lansoprazole (**32**), pantoprazole (**33**) and rabeprazole (**34**) (Scheme 4).

The H^+/K^+ ATPase enzyme (proton pump) is a trans-membrane protein with 32 cysteine residues. Sachs *et al*. have demonstrated that modification of two and/or three of the cysteine residues (Cys-813 or Cys-822 and Cys-892) in the loop between transmembrane segment TM7 and TM8 of



(38) (39)

Scheme 5. Reaction of *N*-acetyl cysteine with **38** to give product **39** via S-S bond formation.



Scheme 6. Conversion of pro-drug **40** to the active sulfenamide **41** under acidic conditions.

the enzyme H^+/K^+ ATPase through disulfide bond formation by the PSB class of PPIs led to the inactivation of the enzyme, and hence resulted in the suppression of gastric acid secretion (Scheme 4) [42,43].

H^+/K^+ ATPase is located at the apical membrane of the parietal cell and plays a major role in acid secretion. In order to suppress acid secretion, compounds such as (**31-34**) serve as prodrugs and accumulate in the acidic compartment of the parietal cell. In the acidic medium, the PPI rearranges to the sulfenic acid derivative (**35**) and then to the cyclic sulfenamide intermediate (**36**) via the mechanism shown in Scheme 4. The targeted cysteine residues of the trans-membrane bound protein H^+/K^+ATPase are then modified either by the sulfenic acid (**35**) or sulfenamide (**36**), or by both, to form a tight S-S bond adduct (**37**).

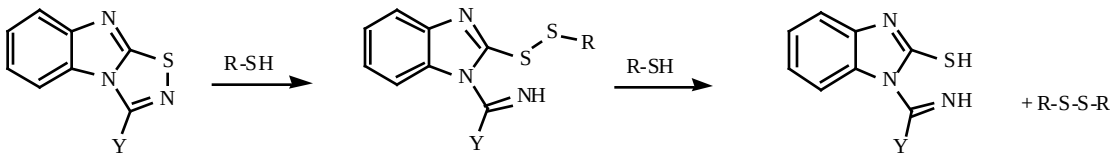
Terauchi *et al*. reported that the cyclic sulfenamide derivative (**38**) inactivated porcine gastric H^+/K^+ATPase with an IC_{50} = 0.53 μM [44]. He also showed that in a chemical system, compound (**38**) reacted with *N*-acetyl cysteine to give the disulfide adduct (**39**) (Scheme 5). This finding accentuates the possibility that a similar mechanism may be operating in the biological system, and that sulfenamide **38** is a thiol trapping agent.

In another example, under acidic condition the prodrug (**40**) is converted to the sulfenamide derivative (**41**), an analogue of **38** (Scheme 6). Compound (**41**) has been reported to be a selective inhibitor of H^+/K^+ ATPase, and is equipotent to omeprazole (**31**) in its inhibitory activity against [^{14}C]aminopyrine stimulated by db-cAMP in isolated rabbit parietal cells. It suppresses gastric acid secretion in histamine-induced gastric acid secretion in pylorus ligated rats by intraduodenum administration. Compound (**40**) is reported to have better acid stability than pantroprazole (**33**) at both neutral and acidic pH.

All PSB core structure-based compounds (**31-34**) are acid unstable and require an enteric coated formulation for effective systemic drug delivery. In principle, one can design an acid-stable cyclic sulfenamide (*e.g*., **3**) structurally not very different from (**36**, Scheme 4), to modify the cysteine residues on the proton pump. The use of an acid-stable sulfenamide such as 1,2,4-thiadiazolo[4,5*a*]benzimidazole (**3**) as a chemical thiol trapping agent is illustrated in Scheme **7** [8]. Depending on the nature of the C3 substituent Y, compound (**3**) can be tuned for its basicity and its thiol affinity, thus allowing its accumulation in the acid compartment of the parietal cell, and hence its access to the targeted cysteine residues of the protein.

The use of compounds based on the THD scaffold (**3**) as potential antiulcer agents was first disclosed in 1994 [8]. In an attempt to prepare the sulfoxide derivative (**46**) by mCPBA oxidation of compound (**45**), it was serendipitously found that a 1:1 mixture of two ketone regioisomers OME-II-57A (**47**) and OME-II-57B (**48**) was obtained instead, as shown in Fig. (**11**).

The cyclic sulfenamide structure of **48** was confirmed by single X-ray crystal structure determination [8]. Compound **48** is reminiscent of the cyclic sulfenamide intermediate (**36**) of omeprazole (**31**). OME-II-57B (**48**) showed similar potency to omeprazole at low concentrations in the suppression of gastric acid secretion in the pylorus ligated rat model. The gastric acid output (μmol/min) after administration of vehicle and 4 doses of **48** (0.3, 3, 30 and



(3) **(42)** **(43)** **(44)**

Scheme 7. Reaction of acid stable tricyclic 1,2,4-thiadiazoles with mercaptans.

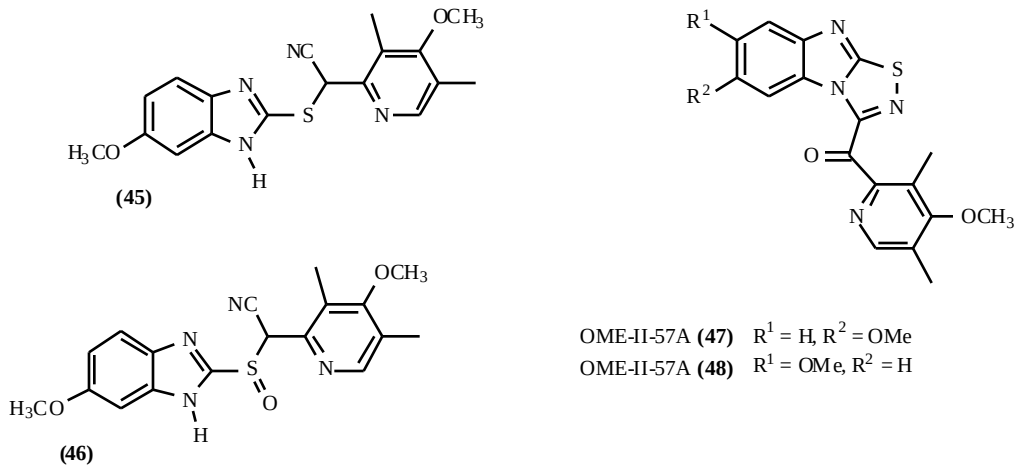


Fig. (11). Chemistry of OME-II-57B (**48**).

300 μmol/kg) in anesthetized rats are shown in Fig. (**12**). Thus, **48** showed significant inhibition of histamine-stimulated acid secretion at 3, 30 and 300 μmol/kg doses.

However, the ketone derivative such as **48** has several shortcomings: (a) the compound is extremely insoluble in buffer or water; (b) the compound decomposed rapidly in sodium hydroxide solution to give 2-mercaptobenzimidazole (**49**) and the acid (**50**) as shown in Scheme **8**); and (c) the manufacturing cost is high.

In order to address these issues, the tricyclic THD (**3**), was used as a scaffold for the preparation of new analogues with different Y groups (*e.g.*, **51**-**58** are analogues of **3**, *c.f.*, Fig. **2** and Table **1**) [8]. A simple chemical assay was used to screen compounds against thiols prior to the more expensive *in vitro* and *in vivo* efficacy studies. Each THD analogue of **3** was reacted with 2.5 equivalents of 2-mercaptoethanol in methanol (5μmol substrate, 12.5μmol reagent /mL of solvent). The disappearance of starting material (**3**) was monitored against time by using a HPLC method, and the time taken (T) for the disappearance of 50% of the starting materials (**51**-**58**) are shown in Table **1**. *While these numbers (T) are not derived from kinetic experiments, they do provide an estimate of the relative reactivities of the differently Y-substituted THDs (**51-58**) towards 2.5 equivalents of chemical thiols in methanol (5μmol substrate/mL of solvent).* The 3-methoxy analogue (**51**, T = 0.31h) is the most reactive, while either the 3-methyl (**55**, T = 59h) or the methylene derivative (**57**, T = 98h) are relatively very stable to 2.5 equivalents of thiols in methanol (Table **1**) [8]. In this model study, the Y substituent has a profound effect on the stability of THD (**3**) with thiols. The analogues of **3** have also been evaluated for their chemical stabilities. Thus, THDs (**3**) are generally acid stable, and inert to chemical nucleophiles such as amines and alcohols. Only the ketone derivatives (*e.g.*, **48**) are unstable in sodium hydroxide solution at pH > 12, and 2-mercaptobenzimidazole is the decomposed product.

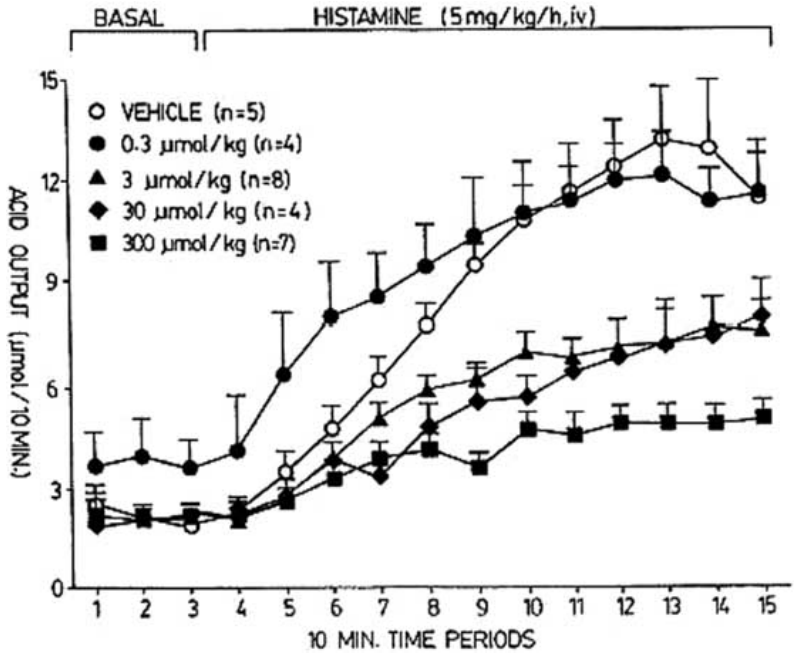
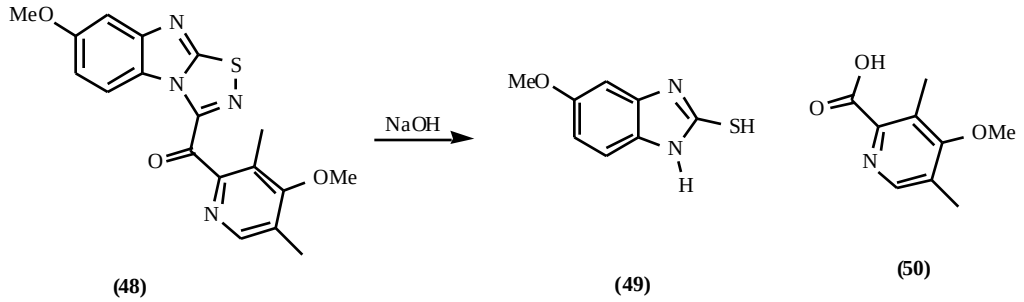


Fig. (12). Experimental data on the Acid Output (μmol/min) v/s Time (10 min periods) after administration of vehicle and 4 doses of **48**.



(48) **(49)** **(50)**

Scheme 8. Decomposition of ketone analogue of tricyclic 1,2,4-thiadiazole (**48**) with base.

THD such as **53** was found to be less stable towards organic thiols in acidic media. For example, the time taken (T) for the disappearance of 50% of **53** with 2.5 fold of mercaptoethanol in methanol was 6.7h at a concentration of 5 μmol substrate and 12.5 μmol reagent/mL solvent (Table **1**), and T drops to 0.17h when the same reaction is carried out in a mixture of 0.1M HCl solution and methanol (10/1 ratio) as solvent.

These results indicate that cleavage of the S-N bond of the thiadiazole moiety by the cysteine residues in the acidic medium of the parietal cell is a possibility. Pursuing on this reasoning, **53** is not expected to readily react with other protein cysteines until it reaches the acidic compartment of the parietal cell where it can interact with the exposed cysteine residues of the H^+/K^+ ATPase enzyme.

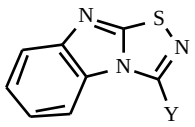
Hence, from the perspective of compound design, a cheap and simple chemical method has been developed for the initial screening of compounds based on the THD (**3**) scaffold for the selective targeting of cysteine residues in acidic media. These compounds should ideally be stable to mercaptans in methanol for a prolonged period of time and then degrade more rapidly in aqueous acidic solution.

In a biological assay, compound **53** was evaluated against omeprazole (**31**) in the inhibition of hog gastric H^+/K^+ATPase *in vitro* [45,46]. Compound **53** (EC_{50} = 2 μM) was slightly more potent than omeprazole (EC_{50} = 7 μM) [47]. Selected analogues of THD (**3**) [compounds Type I (**53**, **58**-**60**) and compounds Type II (**61**-**66**)] shown in Table **2** were then evaluated in a mouse gland model [48]. Omeprazole (**31**), a known PPI and anti-ulcer drug was used as a reference standard and it inhibits cAMP stimulated acid secretion with IC_{90} of 0.6 μM. Compounds **53**, **58**-**63** (Table **2**) are significantly less potent (IC_{90}s = 30-100 μM) than omeprazole (**31**). Substitution of the methyl group at the 4-position of the piperazine of **62** (IC_{50} = 50 μM) with a pyridine ring (**64**, IC_{50} = 4 μM) afforded a twelve-fold increase in the antisecretory activity in favor for **64**. The IC_{90}s of **64** and **66** are in the 8 μM range, which is about 12-fold less potent than omeprazole.

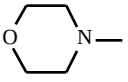
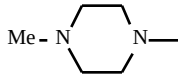
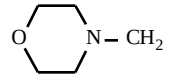
H^+/K^+ATPase operates in the low pH (1-2) environment, at the tubulovesiclar and canalicular structures of the parietal cell. Omeprazole (**31**) is a known weak base (pKa = 4) that penetrates the parietal cell membrane and accumulates in the acidic region. Most of the PPIs that possess pKas < 5 accumulate in the acidic compartment of the parietal cell [49]. Compounds with high pKas, for example **55** and **62** (pKa = 6.9) are less potent than compounds with pKa of *ca.* 4, and conversely compounds **64** and **66** with pKas of 4.3 and 4.9, respectively, are significantly more potent than compounds with higher pKas (*ca.* > 5) in the mouse gland assay. Therefore, potent inhibitors can be designed by tuning the pKa through the judicious choice of the C3 substituent.

The 1,2,4-thiazolo[4,5a]thiadiazoles **64** and **66** are potent antisecretory agents and have further been evaluated in the pylorus ligated rat model [50]. Oral administrations of compound **64** and **66** show that they inhibit the volume of

Table 1. The Time Taken (T) for the Disappearance of 50% of Starting Material (51-58) Upon Treatment with 2.5 Equivalents of 2-Mercaptoethanol in Methanol (5μmol Substrate, 12.5μmol Reagent /mL of Solvent)[a]

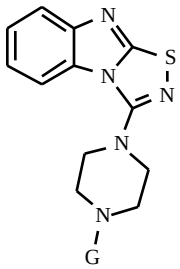


THD (3)

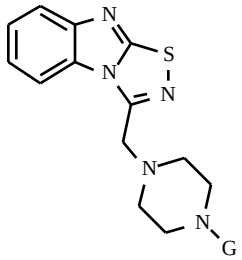
Compound	51	52	53	54	55	56	57	58
Y	MeO			Ph	Me	$Me_2NCH_2CH_2$		2-pyridyl
T (h)	0.31	1.5	6.7	45	59	64	98	134

[a] While these numbers are not derived from kinetic experiments, they do provide an estimate of the relative reactivities of the differently Y-substituted THDs (**51**-**58**) towards 2.5 equivalents of chemical thiols in methanol (5μmol substrate, 12.5μmol reagent /mL of solvent).

Table 2. Inhibition of Gastric Acid Secretion in Mouse Gland Model



Compounds Type I: 53, 58-60



Compounds Type II: 61- 66

#	G	IC_{50} (μM)	IC_{90} (μM)	#	G	IC_{50} (μM)	IC_{90} (μM)
31[a]		0.07	0.6	**61**	H	>1000	nd
53	Me	10	30	**62**	Me	50	100
58	2-pyridyl	20	100	**63**	Et	40	80
59	H	40	nd	**64**	2-pyridyl	4	8
60	Et	30	70	**65**	5-amino-2-pyridyl	5	30
				66	3-amino-2-pyridyl	3	8

[a]Reference test compound is omeprazole (**31**).

gastric juice, total acid output (expressed in μEq HCl) and total acid (expressed in μEq HCl.ml). The present *in vivo* data support that 1,2,4-thiazolo[4,5-*a*]benzimidazole with low pKas are potent antisecretory agents with activities equipotent to that of omeprazole (**31**) at 8 mg/kg in this animal model (Table **3**).

These observations parallel the *in vitro* results in such that the inhibition mechanism is likely due to the ability of compounds of types I and II (*e.g*., **58**-**66**, Table **2**) to trap the SH group of the enzyme at the parietal cell. Like the PSBs, these THD compounds penetrate the cell membrane, accumulate in the parietal cell, and are activated in the acidic compartment to effect the suppression of gastric acid secretion. However, other mechanism(s) of inhibition cannot be discounted. For example, the inhibitors could modify other cysteine residues of the proton pump through covalent S-S bond formation, or they could behave as reversible potassium channel antagonists [51].

Apo199 (**67**) [a type II compound with G = 3-isopropylamino-2-pyridyl] was tested in acute toxicity study for 14 days at a single oral dose of 500 mg/kg in rats (5 males and 5 females). All animals appear normal and healthy after 14 days of observation. Apo199 (**67**) showed parallel dose response to pantroprazole, when administered intravenously at 0.1, 0.5, 1 and 5 mg/kg (Fig. **13**). Compound (**67**) remains an attractive lead for further development as an I.V. drug for the treatment of peptic ulcer.

One major advantage of 1,2,4-thiazolo[4,5-a]benzimidazole derivatives of types I and II (*e.g*., **58**-**67**, Table **2** over the existing PPIs is that they are acid stable.

Table 3. Inhibition of Total Acid in Pylorus Ligated Rats (Number of Animals = 4 or 5)

#	Dose (mg/kg)	% Inhibition of volume[a]	% Inhibition of TA[b]	% Inhibition of TAO[c]
31[d]	8	34	12	45
64	8	39	11	49
64	32	50	32	67
66	8	27	35	52
66	32	61	30	75

[a] Volume refers to volume of gastric secretion in mL
[b] TA is the titratable acidity - μEq HCl/mL
[c] TAO is the total acid output - μEq HCl/2 hr; TAO = volume × titratable acidity
% Inhibition - % Inhibition of parameter compared to vehicle control
= (Control treatment)/control
[d] Reference compound is omeprazole (**31**)

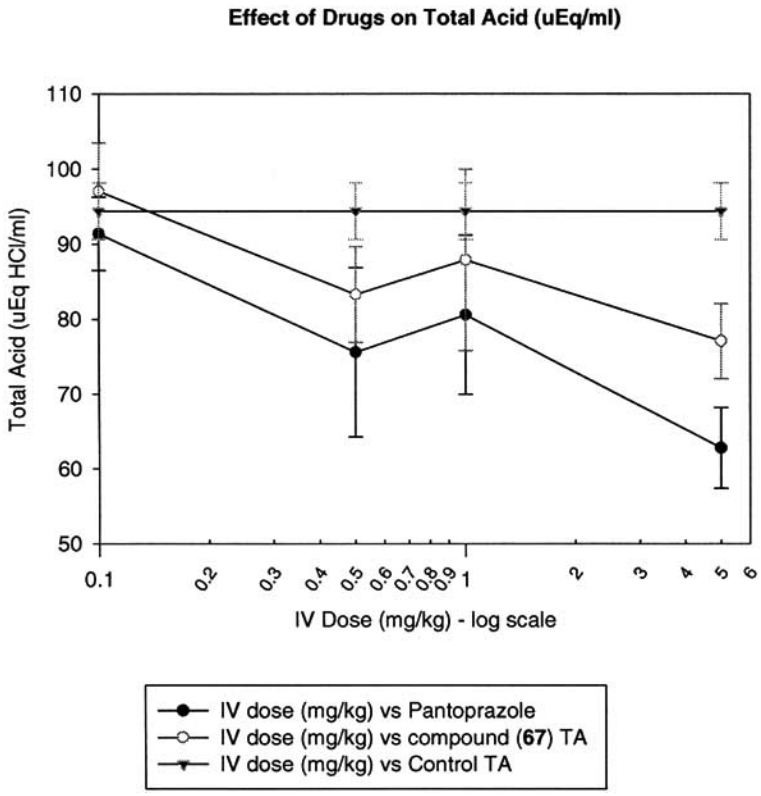


Fig. (13). Intravenous dose response study of compound (**67**) *vs* pantroprazole at 0.1, 0.5, 1 and 5 mg per kg in pylorus ligated rats. Although the graph shows control at each dose, only one single set of control is used as reference.

By tuning the pKa of the C3 substituent to *ca.* < 5, these compounds inhibit gastric acid secretion in the same potency range as omeprazole (**31**). Nevertheless, further lead refinement of these compounds is required to clarify the nature of the inhibition and the duration of action in animal models for THDs.

4. CONCLUSION

Cross-linking of proteins through disulfide bond formation increases their stability, and this is a common occurrence in biological systems. Its disruption through the use of THD may be a viable strategy in the modification of enzyme cysteine residues. There is ample evidence to show that 1,2,4-thiadiazole derivatives form a disulfide bond with organic thiols, as well as enzyme cysteine residues. At present, the only commercial 1,2,4-thiadazole drug is the antibiotic cefozopran, but the role of the thiadiazole moiety in cefozopran is not known. The other thiadiazole compounds are still in the discovery and biological evaluation stages. Further medicinal chemistry research is required to promote the understanding of these 1,2,4-thiadiazole heterocycles as new electrophilic "warheads" for targeting the cysteine residues of proteins.

5. ACKNOWLEDGEMENT

The anti-ulcer program was partially funded by the National Research Council Canada-Industrial Research Assistance Program (Project #25323U, 1995-1998). The authors thank Dr. Richard Hunt and Dr. Rene Padol for the evaluation of compounds as antisecretory agents in the mouse gland assay.

6. REFERENCES

[1] Leung-Toung, R.; Li, W; Tam, T. F.; Karimian, K. *Curr. Med. Chem.* **2002**, *9*, 979-1002.
[2] Powers, J. C.; Asgian, J. L.; Ekici, Ö. D.; James, K. L. *Chem. Rev.* **2002**, *12*, 4639-4750.
[3] Otto, H-H.; Schirmeister, T. *Chem. Rev*. **1997**, *97*(1), 133-171.
[4] Leung, D.; Abbenante, G.; Fairlie, D. P. *J. Med. Chem*. **2000**, *43*(3), 305-341.
[5] Talanian, R. V.; Brady, K. D.; Cryns, V. L. *J. Med. Chem*. **2000**, *43*(18), 3351-3371.
[6] Donkor, I. O. *Curr. Med. Chem*. **2000**, *7*, 1171-1188.
[7] Karimian, K.; Tam, T. F.; Leung-Toung, R. C. S. H.; Li, W.; Bryson, S. P.; Wodzinska, J. M. *US6,468,977*, **2002**.
[8] Karimian, K.; Tam, T. F.; Desilets, D.; Lee, S.; Cappelletto, T.; Li, W. *US6,114,537*, **2000**.
[9] Karimian, K.; Tam, T. F.; Desilets, D.; Lee, S.; Cappelletto, T.; Li, W. *US6,093,738*, **2000**.
[10] Karimian, K.; Tam, T. F.; Leung-Toung, R.; Li, W. *US6,060,472*, **2000**.
[11] Franz, J. E.; Dhingra, O. P. *Comp. Heterocycl. Chem. I* **1984**, *6*, 463-511.
[12] Wilkins, D. J.; Bradley, P. A. *Comp. Heterocycl. Chem. II* **1996**, *4*, 307-354.
[13] Kurzer, F. *Adv. Heterocycl. Chem*. **1982**, *32*, 285-398.
[14] Crook, S; Sykes, P. *J. Chem. Soc. Perkin I* **1977**, 1791-1796.
[15] Scozzafava, A.; Casini, A.; Supuran, C. T. *Curr. Med. Chem*. **2002**, *9*, 1167-1185.
[16] Newton, C. G.; Ollis, D. W. *J. Chem. Soc. Perkin 1* **1984**, 75-84.
[17] Hagiwara, K.; Hashimoto, S.; Shimoda, S. *J. Pestic. Sci*. **1992**, *17*, 251-259.
[18] Takeuchi, E. *JP60246389*, **1985**.
[19] Unangst, P. C.; Shrum, G. P.; Connor, D. T.; Dyer, R. D.; Schrier, D. J. *J. Med. Chem.*, **1992**, *35*, 3691-3698.

[20] Song, Y.; Connor, D. T.; Sercel, A. D.; Sorenson, R. J.; Doubleday, R.; Unangst, P. C.; Roth, B. D.; Beylin, V. G.; Gilbertsen, R. B.; Chan, K.; Schrier, D. J.; Guglietta, A.; Bornemeier, D. A.; Dyer, R. D. *J. Med. Chem*. **1999**, *42*, 1161-1169.

[21] Smith, C. W.; Chakrabarti, J. K.; Williamson, W. R. N. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1673-1678.

[22] Cheng, X-M. In *Ann. Rep. Med. Chem*.; Bristol, J.A., Ed; Academeic Press: London **1996,** Vol. *31*, Chapter 34, pp. 337-355.

[23] Yousif, N. M. *Egypt. J. Chem*. **1989**, *32*, 597-605.

[24] van Muijlwijk-Koezen, J. E.; Timmerman, H.; Vollinga, R. C.; Frijtag von Drabbe Kunzel, J.; de Groote, M.; Visser, S.; Ijzerman, A. P. *J. Med. Chem.* **2001**, *44*, 749-762.

[25] Antonio, Y.; Camargo, C.; Galeazzi, E.; Iriarte, J.; Guzman, M.; Muchowski, J. M.; Gerrity, K.; Liu, F.; Miller, L. M.; Strosberg, A. M. *J. Med. Chem*. **1978**, *21*, 123-126.

[26] MacLeod, A. M.; Baker, R.; Freedman, S. B.; Patel, S.; Merchant, K. J.; Ros, M.; Saunders J. *J. Med. Chem*. **1990**, *33*, 2052-2059.

[27] Collins, I.; Moyes, C.; Davey, W. B.; Rowley, M.; Bromidge, F. A.; Quirk, K.; Atack, J. R.; McKernan, R. M.; Thompson, S. A.; Wafford, K.; Dawson, G. R.; Pike, A.; Sohal, B.; Tsou, N. N.; Ball, R. G.; Castro, J. L. *J. Med. Chem*. **2002**, *45*, 1887-1900.

[28] Goerdeler, J. *Chem. Ber*. **1954**, *87*, 57-67.

[29] Hennrich, G.; Sonnenchein, H.; Resch-Genger, U. *J. Am. Chem. Soc*. **1999**, *121*, 5073-5074.

[30] Bryson, S.; Pai, E. F.; Desilets, D.; Li W.; Tam T.; Karimian K. *Evidence for the formation of a disulfide bond between cysteine proteases and 1,2,4-thiadiazoles*; PENCE AGM **2000** Meeting, Montebello, Quebec, Canada, June 24-26; PENCE Inc. National Business Centre, 750 Heritage Medical Research Centre, Edmonton, AB T6G 2S2, Canada.

[31] Schaschke, N; Assfalg-Machleidt, I.; Machleidt, W.; Moroder, L. *FEBS Lett.* **1998**, *421*, 80-82.

[32] Sohda, T; Terashita, Z-i.; Momose, Y.; Fujisawa, Y.; Mizoguchi, J. *US 5,550,138*, **1996**.

[33] Kim, S. Y.; Jeitner, T. M.; Steinert, P. M. *Neurochem. Int.* **2002**, *40*, 85-103.

[34] Lorand, L.; Graham, R. M. *Nat. Rev. Mol. Cell Biol.* **2003**, *4*, 140-156.

[35] Dolynchuk, K. N.; Ziesmann, M.; Serletti, J. M. *Plast. Reconstr. Surg*. **1996**, *97*, 117-125.

[36] Marrano, C.; Macedo, P.; Gagnon, P.; Lapierre, D.; Gravel, C.; Keillor, J. W. *Bioorg. Med. Chem*. **2001**, *9*, 3231-3241.

[37] Dufner, G. S.; Marbet, G. A. *Hamostaseologie* **2002**, *22*, 11-19.

[38] Tam. T. F.; Leung-Toung, R.; Li, W.; Karimian, K.; Zhao, Y.; Wodzinska, J.; Lowrie, J. *Can. Patent Application* 2,379,375.

[39] Lorand, L.; Conrad, S. M. *Mol. Cell. Biochem.* **1984**, *58*, 9-35.

[40] Tam, T.; Li, W.; Leung-Toung, R.; Karimian, K. *IDrugs* **2000**, *3*, 1064-1074.

[41] Kube, D. M. *Drug Discov. Develop*. **2003**, *6*, 73-76.

[42] Lainbrecht, N.; Corbett, Z.; Bayle, D.; Karlish, S. J. D.; Sachs, G. *J. Biol. Chem*. **1998**, *273*, 13719-13728.

[43] Sachs, G.; Wallmark, B. *J. Gastroenterol. Hepatol*. **1989**, *4*, 7-18.

[44] Terauchi, H.; Tanitame, A.; Tada, K.; Nakamura, K.; Seto, Y. Nishikawa, Y. *J. Med. Chem*. **1997**, *40*, 313-321.

[45] Rabon, E. C.; Bin Im, W., Sachs, G. *Methods Enzymol.* **1988**, *157*, 649-654.

[46] Matsuya, H.; Okamoto, M.; Ochi, T.; Nishikawa, A.; Shimizu, S.; Kataoka, T.; Nagai, K.; Wasserman, H. H.; Ohkuma, S. *Biochem. Pharmacol*. **2000**, *60*, 1855-1863.

[47] Yoda, A.; Hokin, L.E. *Biochem. Biophys. Res. Comm.* **1970**, *40*, 880-886.

[48] Berglindh T. *Methods Enzymol*. **1990**, *192*, 93-107.

[49] Lindberg, P.; Brandstrom, A.; Wallmark, B.; Mattsson, H.; Rikner, L.; Hoffmann, K.-J. *Med. Res. Rev.* **1990**, *10*, 1-54.

[50] Kinoshita, M.; Saito, N.; Tamaki, H. *Eur. J. Pharmacol.* **1997**, *321*, 325-332.

[51] Leach, C. A.; Brown, T. H.; Life, R. J.; Keeling, D. J.; Parsons, M. E.; Theobold, C. J.; Wiggall, K. J. *J. Med. Chem.* **1995**, *38*, 2748-2762.