Tert-Butyl 1,5-bis(4-(benzo[d]isothiazol-3-yl)piperazin-1-yl)-1,5- dioxopentan-2-ylcarbamate urea/thiourea derivatives as potent H +/K+-ATPase inhibitors

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ABSTRACT

Amino acids are known to possess variable efficacy against ulceration. Considering the good antiulcer activity of amino acids, a series of urea/thiourea derivatives of glutamic acid conjugated benzisothiazole analogue **3a**–**u** with various substituents on aryl ring were synthesized, spectroscopically characterized and evaluated for in vitro H^+/K^+-ATPase inhibition. Majority of the compounds possessed potency compared to that of omeprazole, a reference drug. In particular, methoxy derivatives **3p**–**u** were the most active compounds possessing a significant 15-fold increase for *para* substituent thus, contributing positively to gastric H^+/K^+-ATPase inhibition.

Gastric and duodenal ulcers are commonly occurring diseases. Ulcers are believed to result from an imbalance between the aggressive (acid, pepsin) and defensive forces (bicarbonate, mucous) in the stomach and duodenum. Reduction of acid secretion, especially by antagonism of the H_2 receptor, has proven to be a useful means for promoting the healing of ulcers, particularly those of the duodenal mucosa.[1] Recently, agents have been identified that completely suppress acid secretion by inhibition of the gastric proton pump H^+/K^+-ATPase. Such inhibition leads to a profound and prolonged achlorhydria, which results in ulcer healing rates substantially more rapid than those achievable by H_2 antagonists. H^+/K^+-ATPase catalyzes the terminal step in gastric acid whereas histamine is but one of three key messengers which stimulate acid secretion (the other two being acetylcholine and gastrin). As a result, inhibition of H^+/K^+-ATPase can provide an intrinsically greater reduction in gastric acid secretion.[2–7] Although several efficient drugs are available for their treatment with different mechanisms of action there is always a need for new agents with even better efficacy and safety profiles.[8]

Intraduodenal administration of amino acid solution has been reported to significantly inhibit gastric acid secretion and gastrin release, stimulated by intragastric perfusion of peptone.[9] However, studies conducted with individual amino acids have yielded varying results. Amino acids such as leucine, isoleucine and proline augment the acid release[10,11] where as amino acids including serine, tryptophan, arginine, glycine, tyrosine and glutamic acid[12] have been shown to provide protection against gastric ulcers. Although the mechanism of antiulcer action of individual amino acids is not clear, their derivatives have been shown to have direct in vitro H^+/K^+-ATPase inhibitory activity as well as in vivo efficacy in pylorus ligation model.[13]

Small and simple heterocyclic structures often have surprisingly varied biological properties. Compounds containing thiazole nucleus and their derivatives were found to have pharmacological activities like antiviral,[14] antibacterial,[15] analgesic, anti-inflammatory and antiulcer.[16] Urea/thiourea derivatives display a wide range of biological activities including antibacterial, antifungal, antithyroid, antiulcer, herbicidal and plant growth regulator properties.[17–19]

This apart, glutamic acid-heterocycle conjugate derived ureas and thioureas were synthesized in our laboratory and evaluated their antiglycation and urease inhibitory activities.[20] Compounds showed interesting bioactivity. The present investigation was aimed at the evaluation of these compounds for H^+/K^+-ATPase inhibitory properties since the groups present in the analogues fit best within the framework of antiulcer properties also.

Hence, with this background and previous investigations from our group,[21–24] the present work was undertaken with a view to evaluate the antiulcer efficacy of the title compounds. Synthesis of the title compounds was carried out as reported in literature.[20]

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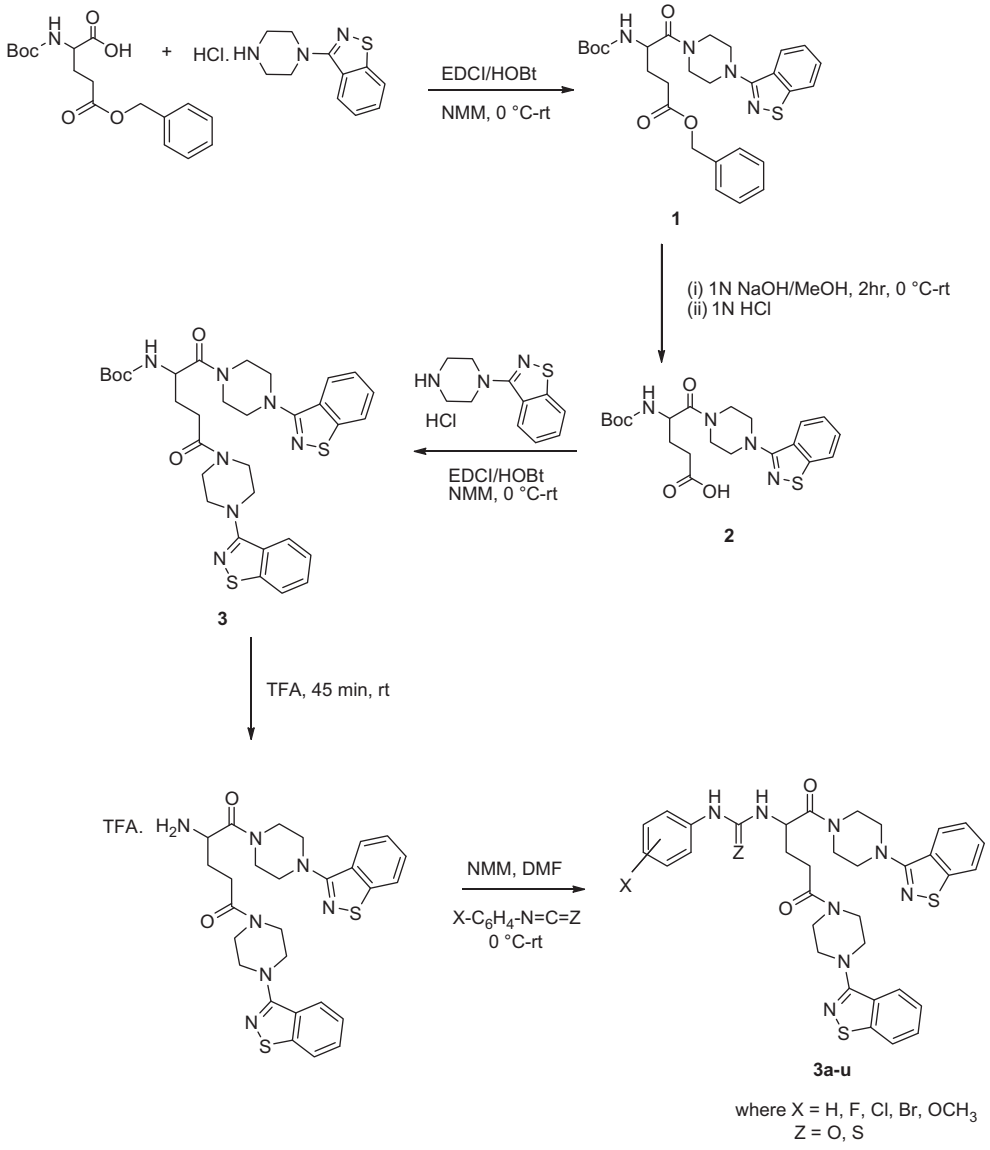
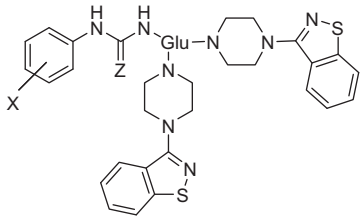
Synthesis of glutamic acid–benzisothiazole conjugate and their urea/thiourea derivatives were accomplished according to the steps illustrated in Scheme 1. Glutamic acid was conjugated to 3-(1-piperazinyl)-1,2-benzisothiazole using 1-ethyl-3-(3-dimethyllaminopropyl)carbodiimide (EDCI)/1-hydroxybenzotriazole (HOBt) as coupling agent and *N*-methyl morpholine (NMM) as base to get **1**. The side chain benzyl group of **1** was deprotected with 1 N NaOH/MeOH to get **2** which was conjugated again with 3-(1-piperazinyl)-1,2-benzisothiazole to get **3**. Boc group of **3** was deblocked using trifluoroacetic acid (TFA) and further reacted with isocyanates and isothiocyanates to get urea and thiourea derivatives respectively. All the derivatives were obtained in high yield. The structures of all the newly synthesized compounds were identified by IR, ^{1}H NMR, ^{13}C NMR, mass and elemental analysis. IR spectrum showed strong absorption bands at 3304–3399 cm^{-1}, 2029–2048 cm^{-1} and 1629–1642 cm^{-1} for –NH–, –C═S, and –C═O groups, respectively. ^{1}H NMR spectrum revealed the appearance of peaks multiplets for the aromatic protons at δ 7.21–7.93 and urea/thiourea NH protons at δ 8.03–8.25 and 8.23–8.77, respectively. ^{13}C NMR spectrum confirmed the carbonylic and thiocarbonylic structure due to peak appearance in the range δ 154.49–155.36 and δ 180.62-180.86, respectively. The physical and spectroscopical data of the compounds **1**, **2**, **3** and **3a**–**u** have been provided in Tables 1 and 2, respectively (Supplementary data).

All the synthesized compounds were evaluated for gastric H^{+}/K^{+}-ATPase activity (assay procedure provided as Supplementary data). The results are tabulated in Table 1. Omeprazole was used as the reference compound. Glutamic acid earlier has been shown to provide protection against gastric ulcers.[12] Boc-Glu(OBzl)-OH after conjugation with 3-(1-piperazinyl)-1,2-benzisothiazole resulted in compound **1** which is also biologically inactive. Removal of benzyl ester of γ-carboxyl protection of **1** and further conjugation with another molecule of 3-(1-piperazinyl)-1,2-benzisothiazole showed no effective increase in activity. On the other hand, it has been reported that the urea/thiourea structure was closely correlated with its potent antiulcer activity.[25] This prompted us to synthesize urea/thiourea derivatives of **3**. Eventually, compounds **3a**–**u** showed better activity compared with their precursors **1**–**3**, indicating that urea/thiourea derivatization seems to be in favor of enhancing the inhibitory activity. As the H^{+}/K^{+}-ATPase inhibition is associated with the modification of the mercapto groups in the



Scheme 1. Urea/ thiourea derivatives of double 3-(1-piperazinyl)-1,2-benzisothiazole conjugated glutamic acid.

Table 1
Antiulcer activity of the synthesized compounds



3a-u
For 1, 2, 3 refer Scheme

Entry	Z	X	Antiulcer activity[a] IC_{50} (μM)
1	—	—	Inactive
2	—	—	Inactive
3	—	—	Inactive
3a	O	H	94.0 ± 0.52
3b	S	H	90.0 ± 0.66
3c	S	2-Br	44.0 ± 0.82
3d	O	3-Br	50.0 ± 0.79
3e	S	3-Br	48.0 ± 0.97
3f	O	4-Br	46.0 ± 0.84
3g	S	3-Cl	60.0 ± 0.99
3h	O	3-Cl	56.0 ± 0.75
3i	S	4-Cl	54.0 ± 0.63
3j	O	4-Cl	52.0 ± 0.81
3k	S	2-F	80.0 ± 0.53
3l	S	2-F	74.0 ± 0.74
3m	O	3-F	78.0 ± 0.67
3n	S	4-F	72.0 ± 0.99
3o	O	4-F	70.0 ± 0.57
3p	O	2-OCH_3	19.0 ± 0.74
3q	S	2-OCH_3	17.0 ± 0.89
3r	O	3-OCH_3	11.0 ± 0.73
3s	S	3-OCH_3	10.0 ± 0.58
3t	O	4-OCH_3	6.2 ± 0.52
3u	S	4-OCH_3	5.4 ± 0.64
Boc-Glu(OBzl)-OH	—	—	Inactive
Omeprazole	—	—	84.0 ± 0.61

[a] Values are mean of three determinations, the ranges of which are <5% of the mean in all cases.

enzyme, thiourea containing analogues were found to be more potent compared to urea counterparts, which binds covalently to the cysteine residues of the H^+/K^+-ATPase thus increasing acid neutralization capacity.[26]

Among the derivatives, phenyl urea **3a** and phenyl thiourea **3b** substituents displayed activity with IC_{50} = 94 μM and IC_{50} = 90 μM respectively which is less potent than omeprazole IC_{50} = 84 μM. It can be inferred that compounds without substitution on the aryl ring showed lesser activity. Therefore, the effect of substituents on the phenyl ring was further investigated. When a methoxy group was placed on the phenyl ring, derivatives exhibited highly potent activity. Compound **3u** bearing methoxy group at C_4 resulted as being most potent showing IC_{50} = 5.4 μM which is nearly 15-fold more potent than that of the reference standard. Ortho and *meta* substituents were less active compared to *para* ($p > m > o$). Further, the substitution of halogens (F, Cl and Br) for methoxy yielded compounds **3c–o** that resulted as being marginally active antiulcer agents. Amongst, bromo derivatives were endowed with significant antiulcer activity against H^+/K^+-ATPase compared to fluoro and chloro derivatives. The preferential order of halogens was found to be Br > Cl > F. This revealed that electronegativity plays an important role in the activity for electron withdrawing groups. As the electronegativity decreases, increase in activity was significantly observed.

From the above results, some broad generalizations could be drawn. Due to electron releasing methoxy derivatives, an increase in the activity was witnessed. Among electron withdrawing groups, bromo derivative seems to be more active compared to chloro and fluoro derivatives. Therefore, electron donating group was found to be the most favorable which significantly contribute in enhancing the antiulcer activity of the synthesized compounds.

In summary, the compounds presented in this Letter clearly differ in their corresponding antiulcer activity depending on the type of substituent. In course of the study, derivatives possessing electron donating group such as methoxy is identified as exhibiting potent antiulcer activity. These results make urea/thiourea derivatives of benzisothiazole glutamic acid conjugate not only interesting and simplified leads for further chemical optimization but also potentially interesting for future scope to study their mechanism of action and would be worthy of additional structure–activity relationship investigation.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.05.043.

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