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Implications of *N*-capped urea/thiourea and *C*-capped 3-(1-piperazinyl)-1,2-benzisothiazole with bridging Gly-Val/Phe-Gly-Val-Pro as therapeutic targets

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ABSTRACT

A series of urea/thiourea derivatives were synthesized by using peptides conjugated to 3-(1-piperazinyl)-1,2-benzisothiazole and their structure was characterized by analytical and spectral (^1H, ^{13}C NMR and Mass) methods. These compounds were screened for antimicrobial and antiglycating activity as well as urease and H^+/K^+-ATPase inhibition. Preliminary structure-activity relationship studies revealed that the compounds possessing fluoro moiety were excellent antimicrobial agents. Furthermore, for other biological activities methoxy substituent was found to be the most active particularly upon substitution at para position.

1. Introduction

The design and synthesis of molecules having value as human therapeutic agents remain one of the main objectives of organic and medicinal chemistry. Several studies in the past few decades have established that bioactive peptides have certain biofunctionalities and may therefore serve therapeutic roles in body systems [1–4]. With a clear understanding of aspects of structural biology and drug metabolism pharmacokinetics, research into peptide/protein-based drugs has started to flourish, with the ability to deliver the drugs to specific sites, and the drugs offering high potency, and importantly, low toxicity. These key factors differentiate the peptide/protein therapeutics from more traditional 'small molecule' drugs [5]. Bioactive peptides may induce functionalities such as antioxidative, antimicrobial, antihypertensive, cytomodulatory and immunomodulatory effects in living systems and these multifunctionalities enhance their potential use as therapeutic aids. The role of bioactive peptides in modulating innate immune responses and boosting natural immunity while controlling microbial host invaders is well documented [6–8].

Thiazoles and their derivatives have found applications in drug development for the treatment of allergies [9], hypertension [10], inflammation [11], bacterial infections [12], HIV infections [13] etc. Thiazole nucleus is also an integral part of all the available penicillins which have revolutionized the therapy of bacterial diseases [14]. Several thiazole containing drugs are available such as; nizatidine, a histamine H_2-receptor antagonist that inhibits stomach acid production and commonly used in the treatment of peptic ulcer disease (PUD) and gastroesophageal reflux disease (GERD), niridazole as schistosomicidal, sulfathiazole as antibiotic, fanetizole as anti-inflammatory, combendazole as fungicidal. Urea and thiourea derivatives have been reported of considerable industrial importance and are linked to a series of biological activities including inhibition of nitric oxide, antimicrobial, anti-HIV, antiviral and analgesic properties [15–18].

Abbreviations: ANSA, 1-amino-2-naphthol-4-sulphonic acid; Bis-PNPC, bis(4-nitrophenyl)carbonate; Boc, *tert*-butyloxycarbonyl; DCM, dichloromethane; EGTA, ethylene glycol tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PBT, 3-(1-piperazinyl)-1,2-benzisothiazole; HOBt, *N*-hydroxy benzotriazole; IBCF, isobutyl chloroformate; NMM, *N*-methyl morpholine; TFA, trifluoroacetic acid.

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In recent years we have been engaged in the design, synthesis and biological evaluation of amino acids/peptides conjugated heterocycles and their derivatives [19–23]. Our earlier investigations revealed that among the peptides used, pentamers were found to be more active hence GVGVP and GFGVP were selected for this work. Recently we have reported the synthesis and biological evaluation of PBT conjugated glutamic acid and their urea/thiourea derivatives [24,25], in which compounds bearing fluoro and methoxy substituents exhibited excellent inhibitory potency. Encouraged by this and in order to further expand the scope of urea/thiourea derivatives of peptides conjugates as privileged medicinal scaffolds, herein we present our results on evaluation of novel PBT conjugated peptide (GVGVP or GFGVP) derivatives bearing urea/thiourea moieties as biologically active agents.

2. Results and discussion

2.1. Chemistry

Peptides, Boc-GVGVP-OH (**1**) and Boc-GFGVP-OH (**2**) were synthesized by solution phase method using Boc chemistry. Further, these were reacted separately with bis-PNPC to obtain Boc-GVGVP-ONp (**3**) and Boc-GFGVP-ONp (**4**). The esterified peptides were conjugated to PBT to obtain **5** and **6** using HOBt and NMM. Boc protection was removed using TFA. Urea/thiourea derivatives (**7**–**28**) were afforded by reacting with phenyl isocyanates/thiocyanates in the presence of NMM. All derivatives were obtained in high yield. The structures were confirmed by ^{1}H NMR, ^{13}C NMR, mass spectrometry and elemental analysis. The physical and analytical data of the derivatives are presented in Table S1 and S2 respectively in the supplementary data.

2.2. Biology

2.2.1. Antibacterial and antifungal activity

In this work, the synthesized compounds **5**–**28** were evaluated for their antibacterial and antifungal activities by both agar well diffusion method and microdilution method against human pathogens such as Gram negative bacteria (*Escherichia coli, Klebsiella pneumoniae and Xanthomonas oryzae*), Gram positive bacteria (*Coagulase positive staphylococcus*) and fungi (*Aspergillus niger, Aspergillus flavus, Fusarium moniliforme and Fusarium oxysporum*). Streptomycin and bavistin were used as reference drugs, respectively. The results were recorded for each test compound as the average diameter of inhibition zones of bacterial or fungal growth around the well in mm. The minimum inhibitory concentration (MIC) measurement was determined for those compounds which showed significant growth inhibition zones (Table 1).

The results revealed that most of the compounds displayed significant effects on the growth of the tested bacterial and fungal strains. The structure-antimicrobial activity relationship of the compounds revealed that GFGVP-containing series **18**–**28** are more active than the corresponding GVGVP-containing series **7**–**17**. This could be due to the presence of more hydrophobic Phe residue in the GFGVP series confirming the importance of hydrophobicity for the antimicrobial activity [19,20]. Among the analogues containing urea or thiourea, latter were found to be more potent as antimicrobials. This may be due to the divalent bioisosteric effect of sulphur[26]. It was also observed that compounds having fluoro atom were more effective than methoxy unit. Because of its strong electronegativity, the fluoro atom enhances the cell penetration and reduces plasma protein binding [27]. The combination of these effects might have resulted in an improved antimicrobial activity.

Regarding the pattern of the substituents, substitution at the para position seemed to be more decisive in increasing the antimicrobial activity than substitution at the ortho and meta positions. Furthermore, the results of antibacterial activity indicated that Gram-negative bacteria were more susceptible towards the tested compounds than Gram-positive ones.

2.2.2. Antiglycation and urease inhibition activities

The synthesized compounds were evaluated for antiglycating activity against bovine serum albumin and urease inhibition against jack bean urease using rutin and thiourea as reference standards, respectively. The results (IC_{50} values) are showed in Table 1 and represent the average values from triplicate runs. Both the peptides on conjugation with PBT were found to be inactive with IC_{50} values >200 μM. When Boc group of **5** and **6** was replaced by urea/thiourea derivatives, compounds showed enhanced activity than their conjugates. It seems interesting to point out that derivatives bearing the GFGVP skeleton **18**–**28** were more active then the GVGVP-containing **7**–**17**. This indicates that the presence of the more hydrophobic Phe plays a prominent role in increasing the activity. Results also revealed that replacement of oxygen with sulphur resulted in improved potency. This could suggest that the bigger size, the lower electronegativity, the variable oxidation states and the ability to form hydrogen bond (bioisosteric effect) of sulfur are important factors influencing the biological activity [26]. Urease is usually classed as a sulfhydryl enzyme and agents able to oxidize the sulfhydryl groups are known to inhibit it. Accordingly, sulfur-containing compounds were reported to act as urease inhibitors [28]. This could further explain the major activity of the thiourea-containing derivatives compared with their urea-containing analogues. Regarding the substituents, derivatives bearing a methoxy group showed to be more active respect to the fluoro-containing analogues. This could be attributed to the electron donating nature of the methoxy group. In addition, our findings also indicated that methoxy group located at the para position of the phenyl ring would be more beneficial for strong antiglycating and urease inhibitory activities, while shifting it to the meta or ortho position decreased the activity. Therefore, the preferential position of substituents for activity was found to be $p > o > m$.

2.2.3. H^+/K^+-ATPase inhibitory activity

Compounds **5**–**28** were further evaluated for their ability to inhibit purified H^+/K^+-ATPase using omeprazole as reference. The results (IC_{50} values) are reported in Table 1 and represent the average values from triplicate runs. A close inspection of the results suggests some interesting deductions regarding the structure-activity relationship: the urea/thiourea derivatization is important for inhibition of H^+/K^+-ATPase, which in turn is associated with the modification of mercapto groups in the enzyme to form disulphide adducts which are considered as models of the enzyme–inhibitor complex [29]. This could be the reason for higher activity of thiourea analogue compared to urea counterparts. Further it was witnessed that GVGVP series (**7**–**17**) have greater activity than GFGVP series (**18**–**28**). Based on our previous results [25] compounds with para substituents on the phenyl ring showed good activity while ortho and meta ones exhibited slightly reduced activity. The same trend has been noticed here. We also observed that the compounds which had the strong electron-withdrawing fluoro substituents only showed moderate inhibitory activity compared to methoxy substituted derivatives. Therefore, methoxy substituted derivatives particularly at para position had higher inhibition capacity.

This appears to be the first report on the development of benzothiazole conjugates as gastric antisecretory agents. We have