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

ARTICLE *in* EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY · OCTOBER 2014

Impact Factor: 3.45 · DOI: 10.1016/j.ejmech.2014.09.098 · Source: PubMed

5 AUTHORS, INCLUDING:

R. Suhas

University of KwaZulu-Natal

59 PUBLICATIONS **134** CITATIONS

SEE PROFILE

Channe D Gowda

University of Mysore

247 PUBLICATIONS **3,467** CITATIONS

SEE PROFILE

European Journal of Medicinal Chemistry 87 (2014) 657–661

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

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

Anamika Sharma [a], R. Suhas [b], Syeda Hajira Banu [c], S. Chandrashekar [a], D.Channe Gowda [a, *]

[a] Department of Studies in Chemistry, University of Mysore, Manasagangotri, Mysore 570 006, Karnataka, India
[b] Postgraduate Department of Chemistry, JSS College of Arts, Commerce and Science, Ooty Road, Mysore 570 025, Karnataka, India
[c] Postgraduate Department of Biochemistry, JSS College of Arts, Commerce and Science, Ooty Road, Mysore 570 025, Karnataka, India

ARTICLE INFO

Article history:
Received 4 July 2013
Received in revised form
4 August 2014
Accepted 30 September 2014
Available online 2 October 2014

Keywords:
Peptides
Conjugation
Antimicrobial
Antiglycation
Urease inhibition
H^+/K^+-ATPase activity

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.

* Corresponding author.
E-mail address: dchannegowda@yahoo.co.in (D.Channe Gowda).

http://dx.doi.org/10.1016/j.ejmech.2014.09.098
0223-5234/

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

Table 1
Biological activities (antimicrobial, antiglycation, urease inhibition and H^+/K^+-ATPase inhibition) of the synthesized compounds.

Entry	Antibacterial			Antifungal					Antiglycation	Urease inhibition	H^+/K^+-ATPase inhibition
	Zone of inhibition in mm (MIC values in μg)[a]										
	EC	XO	KP	CPS	AF	An	FM	FO	IC_{50} (μM) ± SEM[a]		
5	6 ± 0.23	4 ± 0.51	4 ± 0.28	5 ± 0.26	4 ± 0.19	5 ± 0.28	5 ± 0.14	6 ± 0.29	250 ± 0.83	295 ± 0.82	Inactive
6	8 ± 0.21	7 ± 0.24	7 ± 0.36	6 ± 0.19	7 ± 0.26	6 ± 0.26	7 ± 0.44	7 ± 0.28	235 ± 0.56	255 ± 0.79	Inactive
7	25 ± 0.43 (15.75)	23 ± 0.64 (16.75)	24 ± 0.26 (17.00)	25 ± 0.25 (16.75)	24 ± 0.63 (17.75)	25 ± 0.46 (16.75)	26 ± 0.62 (15.50)	22 ± 0.36 (16.75)	54 ± 0.34	42 ± 0.37	100.0 ± 0.68
8	27 ± 0.32 (14.25)	24 ± 0.21 (15.25)	26 ± 0.37 (16.00)	27 ± 0.27 (15.50)	29 ± 0.62 (16.50)	30 ± 0.62 (15.25)	31 ± 0.61 (13.00)	30 ± 0.47 (15.25)	50 ± 0.52	40 ± 0.41	92.0 ± 0.83
9	24 ± 0.34	22 ± 0.41	23 ± 0.47	23 ± 0.63	25 ± 0.34	27 ± 0.37	27 ± 0.51	24 ± 0.36	52 ± 0.36	44 ± 0.36	88.0 ± 0.67
10	30 ± 0.64 (12.75)	27 ± 0.25 (10.50)	28 ± 0.42 (11.25)	29 ± 0.35 (13.25)	32 ± 0.46 (14.50)	33 ± 0.52 (11.75)	34 ± 0.56 (13.50)	30 ± 0.44 (14.50)	40 ± 0.57	39 ± 0.63	78.0 ± 0.94
11	32 ± 0.78 (10.25)	29 ± 0.56 (9.50)	31 ± 0.57 (10.00)	31 ± 0.74 (10.75)	34 ± 0.52 (12.75)	35 ± 0.54 (10.50)	35 ± 0.46 (11.00)	35 ± 0.48 (13.25)	34 ± 0.61	35 ± 0.45	76.0 ± 0.79
12	19 ± 0.28	18 ± 0.38	19 ± 0.41	19 ± 0.38	21 ± 0.46	23 ± 0.70	23 ± 0.67	21 ± 0.62	24 ± 0.27	22 ± 0.23	46.0 ± 0.47
13	21 ± 0.52	19 ± 0.41	17 ± 0.36	20 ± 0.57	22 ± 0.30	20 ± 0.48	23 ± 0.48	22 ± 0.39	20 ± 0.42	20 ± 0.46	40.0 ± 0.83
14	15 ± 0.41	13 ± 0.36	12 ± 0.56	14 ± 0.52	17 ± 0.74	15 ± 0.44	18 ± 0.36	18 ± 0.37	25 ± 0.36	25 ± 0.42	38.0 ± 0.49
15	17 ± 0.33	16 ± 0.25	18 ± 0.46	18 ± 0.43	18 ± 0.47	16 ± 0.25	19 ± 0.41	19 ± 0.61	23 ± 0.16	23 ± 0.37	36.0 ± 0.76
16	23 ± 0.43	22 ± 0.46	21 ± 0.38	22 ± 0.72	26 ± 0.24	24 ± 0.56	24 ± 0.36	28 ± 0.46	21 ± 0.27	21 ± 0.32	34.0 ± 0.86
17	24 ± 0.29	25 ± 0.36	23 ± 0.72	24 ± 0.37	25 ± 0.76	24 ± 0.57	26 ± 0.61	26 ± 0.29	18 ± 0.42	18 ± 0.47	32.0 ± 0.69
18	30 ± 0.71 (12.75)	28 ± 0.37 (12.25)	29 ± 0.58 (12.25)	29 ± 0.42 (12.25)	28 ± 0.62 (14.25)	29 ± 0.35 (14.00)	26 ± 0.25 (10.00)	29 ± 0.39 (14.00)	53 ± 0.71	41 ± 0.52	110.0 ± 0.57
19	32 ± 0.69 (11.00)	31 ± 0.71 (11.00)	31 ± 0.72 (10.75)	31 ± 0.37 (11.25)	30 ± 0.39 (13.00)	31 ± 0.75 (12.75)	28 ± 0.63 (9.25)	31 ± 0.52 (13.00)	48 ± 0.46	38 ± 0.57	102.0 ± 0.49
20	28 ± 0.44	26 ± 0.32	27 ± 0.37	29 ± 0.26	29 ± 0.62	30 ± 0.36	31 ± 0.46	29 ± 0.28	50 ± 0.41	43 ± 0.73	92.0 ± 0.73
21	33 ± 0.74 (11.00)	31 ± 0.62 (8.75)	32 ± 0.27 (9.25)	32 ± 0.74 (11.25)	31 ± 0.58 (11.00)	30 ± 0.62 (10.50)	31 ± 0.26 (11.00)	32 ± 0.16 (12.25)	44 ± 0.37	35 ± 0.35	80.0 ± 0.82
22	35 ± 0.78 (9.00)	34 ± 0.48 (7.50)	34 ± 0.25 (8.50)	35 ± 0.56 (9.00)	36 ± 0.82 (10.25)	35 ± 0.34 (9.75)	36 ± 0.37 (9.75)	35 ± 0.35 (11.00)	42 ± 0.42	33 ± 0.62	72.0 ± 0.74
23	23 ± 0.48	22 ± 0.58	22 ± 0.46	23 ± 0.57	25 ± 0.27	24 ± 0.60	26 ± 0.49	23 ± 0.51	22 ± 0.27	21 ± 0.25	54.0 ± 0.80
24	24 ± 0.25	22 ± 0.25	21 ± 0.64	24 ± 0.35	25 ± 0.48	24 ± 0.25	26 ± 0.41	24 ± 0.36	19 ± 0.19	18 ± 0.54	46.0 ± 0.62
25	19 ± 0.19	18 ± 0.61	17 ± 0.58	20 ± 0.51	21 ± 0.53	20 ± 0.49	22 ± 0.34	22 ± 0.52	23 ± 0.43	23 ± 0.36	48.0 ± 0.74
26	21 ± 0.38	19 ± 0.38	21 ± 0.31	20 ± 0.48	23 ± 0.60	24 ± 0.61	24 ± 0.52	23 ± 0.63	20 ± 0.52	19 ± 0.34	38.0 ± 0.90
27	26 ± 0.27	23 ± 0.34	24 ± 0.48	26 ± 0.32	28 ± 0.31	28 ± 0.48	29 ± 0.35	27 ± 0.53	19 ± 0.35	17 ± 0.42	38.0 ± 0.73
28	28 ± 0.48	26 ± 0.54	27 ± 0.51	28 ± 0.43	29 ± 0.52	30 ± 0.71	31 ± 0.64	29 ± 0.62	16 ± 0.26	15 ± 0.23	34.0 ± 0.86
Streptomycin	14 ± 0.43 (22.00)	11 ± 0.73 (18.00)	12 ± 0.48 (20.00)	13 ± 0.49 (21.00)	–	–	–	–	–	–	–
Bavistin	–	–	–	–	13 ± 0.38 (24.00)	14 ± 0.31 (22.00)	15 ± 0.48 (20.00)	13 ± 0.51 (24.00)	–	–	–
Rutin	–	–	–	–	–	–	–	–	41.9 ± 0.27	–	–
Thiourea	–	–	–	–	–	–	–	–	–	21.5 ± 0.35	–
Omeprazole	–	–	–	–	–	–	–	–	–	–	84.0 ± 0.69

EC: *Escherichia coli*; XO: *Xanthomonas oryzae*; KP: *Klebsiella pneumoniae*; CPS: *Coagulase positive staphylococcus.*
AF: *Aspergillus flavus*; AN: *Aspergillus niger*; FM: *Fusarium moniliforme*; FO: *Fusarium oxysporum.*
[a] Values are mean of three determinations, the ranges of which are <5% of the mean in all cases.

made a preliminary attempt to discuss the possible mechanism of action of the synthesized compounds in comparison with omeprazole, a benzimidazole derivative connected *via* S═O to a pyridyl ring and also contain Me/OMe groups [30]. In parallel, derivatives presented in this study also contain similar features like methoxy group on the phenyl ring, a benzothiazole moiety (an isostere of benzimidazole). G. Sachs [31] has discussed that the sulphur atom of omperazole reacts with thiol of cysteine (present in the protein of the enzyme ATP-ase) thereby reducing the power of H^+ secretion. In this sense, we envisage that the thiourea containing compounds may also behave in a similar fashion thereby rendering high inhibition compared to the standard.

3. Conclusion

In the present study we synthesized two pentapeptides *viz.* GVGVP and GFGVP, covalently conjugated to PBT and further derivatized with urea or thiourea derivatives bearing substituents at different positions. Compounds **5**–**28** were tested as antimicrobial and antiglycation agents, as well as urease and H^+/K^+-ATPase inhibitors. All these compounds exhibited promising activity. Furthermore, the following findings can be drawn: replacement of O by S in the urea derivatives enhances the biological activity; among pentapeptides, GFGVP is more active than GVGVP for antimicrobial, antiglycation, and urease inhibition activity whereas GVGVP containing analogues enhanced activity for H^+/K^+-ATPase; methoxy derivatives particularly at para position showed interesting antiglycating and urease inhibitory activities; the presence of fluoro in the molecule was found to be more favorable for antimicrobial efficacy; finally, the new compounds presented here clearly differ in their biological activity depending on the substituent. Hence, the current investigation warrants to take this project much forward to study the mechanism of action, further SAR, etc.

4. Experimental

4.1. Materials

All Boc-amino acids, HOBt and TFA were purchased from Advanced Chem. Tech. (Louisville, Kentucky, USA). All chiral amino acids had *L*-configuration unless otherwise mentioned. IBCF, NMM and phenyl isocyanates/isothiocyanates were from Sigma Chemical Co. (St. Louis, MO). All other chemicals and reagents were from

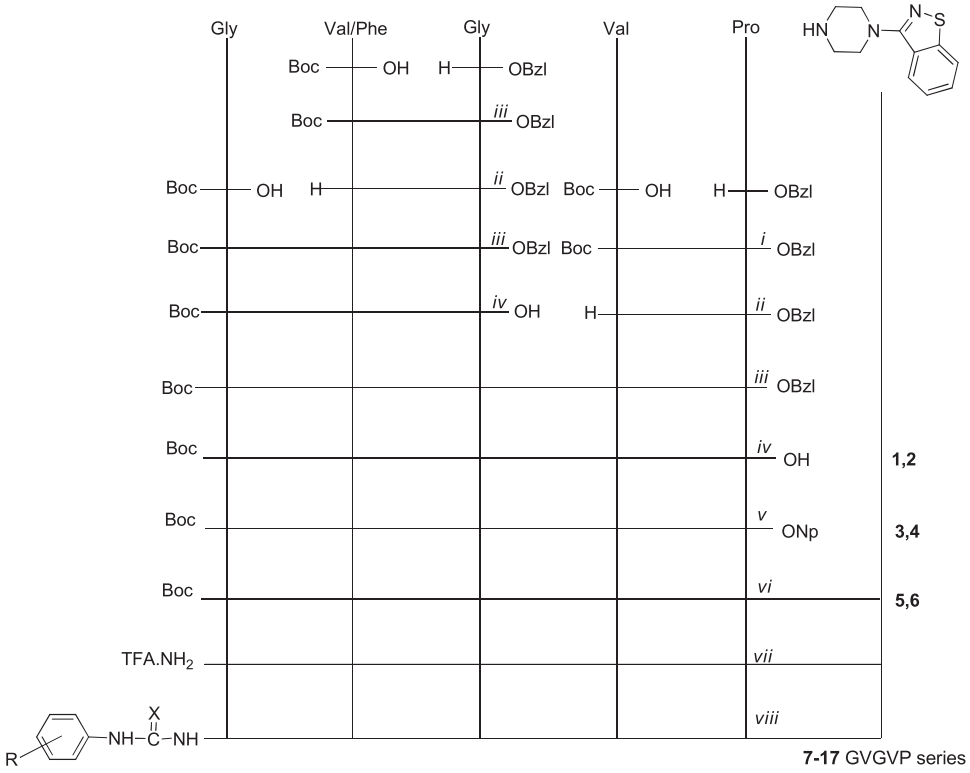
Aldrich (USA), Spectrochem Pvt. Ltd. (India) and Rankem Pvt. Ltd. (India) and were used without further purification. Progress of the reaction was monitored by TLC using silica gel coated on glass plates with solvent system comprising chloroform/methanol/acetic acid in the ratio 98:02:03 (R_f^a)/95:05:03 (R_f^b) and the compounds on TLC plates were detected by iodine vapours. Melting points were determined on a Superfit melting point apparatus (India) and are uncorrected. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on Bruker multinuclear FT-NMR Spectrometer (Japan) using DMSO (d_6) as solvent. The chemical shifts are reported as parts per million (ppm) using TMS as an internal standard. Mass spectra were obtained on Agilent LCMS (Ireland). Compounds were analysed on a Vario ELIII Elemental Analyser (Germany) for C, H, N and S. All the chemicals and reagents used for biological studies were purchased from Aldrich (USA).

4.2. Chemistry

PBT was synthesized according to the literature [32]. Peptides were synthesized by solution phase method using Boc chemistry. Boc group was removed with 4 N HCl in dioxane unless otherwise mentioned. All the coupling reactions for peptide synthesis were achieved by the mixed anhydride method. Procedure followed for the syntheses is outlined in Scheme 1.

4.2.1. Synthesis of Boc-GZGVP-ONp [where Z = Val for GVGVP and Phe for GFGVP]

Boc-GZGVP-OH were synthesized by (3 + 2) fragment coupling strategy. The tripeptide Boc-GZG-OH and dipeptide HCl.NH_2-VP-OBzl were synthesized separately and coupled to obtain the pentamer. In this approach, Boc-VP-OBzl was synthesized by mixed anhydride method in the presence of HOBt and deblocked to obtain HCl.NH_2-VP-OBzl. On the other hand, Boc-ZG-OBzl was synthesized by mixed anhydride method, deblocked and coupled to Boc-Gly-OH to obtain Boc-GZG-OBzl which was finally hydrogenolysed using $HCOONH_4$/10% Pd–C to get Boc-GZG-OH. Now the tripeptide and dipeptide fragments were coupled by mixed anhydride method to obtain Boc-GZGVP-OBzl. These were then treated with $HCOONH_4$/10% Pd–C to get the corresponding free acids **1** and **2**. Boc-GZGVP-OH were dissolved separately in



No.	R	X	No.	R	X
7	2-F	O	**18**	2-F	O
8	2-F	S	**19**	2-F	S
9	3-F	O	**20**	3-F	O
10	4-F	O	**21**	4-F	O
11	4-F	S	**22**	4-F	S
12	2-OCH_3	O	**23**	2-OCH_3	O
13	2-OCH_3	S	**24**	2-OCH_3	S
14	3-OCH_3	O	**25**	3-OCH_3	O
15	3-OCH_3	S	**26**	3-OCH_3	S
16	4-OCH_3	O	**27**	4-OCH_3	O
17	4-OCH_3	S	**28**	4-OCH_3	S

i. IBCF/HOBt, NMM; *ii.* HCl/dioxane; *iii.* IBCF, NMM; *iv.* $HCOONH_4$/10% Pd-C; *v.* Bis-PNPC/ Pyridine; *vi.* HOBt, NMM; *vii.* TFA; *viii.* R-C_6H_4-N=C=X (R: F, OMe; X: O, S)

Scheme 1. Synthetic pathway for the preparation of R–C_6H_4–NH–C(=X)–NH–Gly-Val/Phe-Gly-Val-Pro-PBT.

pyridine and bis-PNPC (1.5 eq) was added to obtain the respective active esters. The mixture was stirred until the reaction was complete (TLC). The solvent was removed under reduced pressure and the residue was taken up with chloroform and washed with water (30 × 1), 10% citric acid (30 × 2), water (30 × 1), 5% $NaHCO_3$ (30 × 2). Solvent was removed under reduced pressure and the residue was triturated with ether, filtered, washed with ether and dried to obtain **3** and **4**.

4.2.2. Synthesis of Boc-GZGVP-PBT

Boc-GZGVP-ONp (0.0034 mol, 1 eq.) and HOBt (0.521 g, 0.0034 mol) were dissolved in DCM and cooled to 0 °C. Reaction mixture was stirred for 10 min and was added a pre-cooled solution of PBT (0.869 g, 0.0034 mol) and NMM (0.8 mL, 0.0068 mol) in DMF. After 20 min, the pH of the solution was adjusted to 8 by the addition of NMM and the reaction mixture was stirred overnight at room temperature. Solvent was removed under reduced pressure and the residue was dissolved with chloroform. The organic layer was washed with 5% $NaHCO_3$ solution (3 × 100 mL), water (1 × 100 mL), 0.1 N cold HCl solution (2 × 100 mL) and brine (1 × 100 mL). The organic layer was dried over anhydrous sodium sulphate. Solvent was removed under reduced pressure and the residue was triturated with *n*-hexane, filtered and dried to get **5** and **6**.

4.2.3. Deblocking of Boc group

Boc-GZGVP-PBT (200 mg) was stirred with 2.0 mL of TFA for 45 min at room temperature. After completion of the reaction (TLC), the mixture was concentrated under high vacuum to get TFA.H-GZGVP-PBT which was triturated with dry ether, filtered and dried.

4.2.4. General procedure for the synthesis of urea/thiourea derivatives

To an ice-cooled solution of TFA.H-GZGVP-PBT (0.001 mol) in DMF (1 mL) and NMM (0.164 mL, 0.0015 mol) the required phenyl isocyanates/isothiocyanates (0.0012 mol) were added dropwise maintaining the temperature at 0 °C. The reaction mixture was stirred for 8 h slowly warming to room temperature. DMF was evaporated under high vacuum and the residue was poured into about 20 mL ice-cold 90% saturated $KHCO_3$ solution and stirred for 15 min. The precipitate was extracted with chloroform and washed sequentially with 5% $NaHCO_3$ solution (2 × 50 mL), water (2 × 50 mL), 0.1 N HCl (2 × 50 mL) and brine. Organic layer was dried over anhydrous sodium sulphate, evaporated under reduced pressure, triturated with *n*-hexane, filtered and dried to get **7**–**28**.

Experimental details for the biological studies are given as Supplementary Information.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.09.098.

References

[1] R.E.W. Hancock, H.G. Sahl, Nat. Biotechnol. 24 (2006) 1551–1557.
[2] S.M. Huang, K.N. Chen, Y.P. Chen, W.S. Hong, M.J. Chen, Int. J. Food Sci. Technol. 45 (2010) 1061–1067.
[3] S.K. Kim, I. Wijesekara, J. Funct. Foods 2 (2010) 1–9.
[4] H. Korhonen, A. Pihlanto, Int. Dairy J. 16 (2006) 945–960.
[5] D. Agyei, M.K. Danquah, Biotechnol. Adv. 29 (2011) 272–277.
[6] V. Marx, Chem. Eng. News 83 (2005) 17–24.
[7] I.L. Expósitoa, I. Recio, Int. Dairy J. 16 (2006) 1294–1305.
[8] S.F. Gauthier, Y. Pouliot, D. Saint-Sauveur, Int. Dairy J. 16 (2006) 1315–1323.
[9] K.D. Hargrave, F.K. Hess, J.T. Oliver, J. Med. Chem. 26 (1983) 1158–1163.
[10] W.C. Patt, H.W. Hamilton, M.D. Taylor, M.J. Ryan, D.G. Taylor Jr., C.J.C. Connolly, A.M. Doherty, S.R. Klutchko, I. Sircar, B.A. Steinbaugh, B.L. Batley, C.A. Painchaud, S.T. Rapundalo, B.M. Michniewicz, S.C. Olson, J. Med. Chem. 35 (1992) 2562–2572.
[11] B.S. Holla, K.V. Malini, B.S. Rao, B.K. Sarojini, N.S. Kumari, Eur. J. Med. Chem. 38 (2003) 313–318.
[12] K. Tsuji, H. Ishikawa, Bioorg. Med. Chem. Lett. 4 (1994) 1601–1606.
[13] F.W. Bell, A.S. Cantrell, M. Hogberg, S.R. Jaskunas, N.G. Johansson, C.L. Jordon, M.D. Kinnick, P. Lind, J.M. Morin Jr., R. Noreen, B. Oberg, J.A. Palkowitz, C.A. Parrish, P. Pranc, C. Sahlberg, R.J. Ternansky, R.T. Vasileff, L. Vrang, S.J. West, H. Zhang, J. Med. Chem. 38 (1995) 4929–4936.
[14] S. Oncu, M. Punar, H. Eraksoy, Chemotherapy 50 (2004) 98–100.
[15] Y.J. Kim, J.H. Ryu, Y.J. Cheon, H.J. Lim, R. Jeon, Bioorg. Med. Chem. Lett. 17 (2007) 3317–3321.
[16] O. Adeoye, A.A. Ayandele, O.A. Odunola, J. Agric. Biol. Sci. 2 (2007) 4–5.
[17] J.D. Bloom, R.G. Dushin, K.J. Curran, F. Donahue, E.B. Norton, E. Terefenko, T.R. Jonas, A.A. Ross, B. Feld, S.A. Lang, M.J. Di-Grandi, Bioorg. Med. Chem. Lett. 14 (2004) 3401–3406.
[18] J. Rojas, M. Paya, J.N. Domínguez, L. Ferrandiz, Bioorg. Med. Chem. Lett. 12 (2002) 1951–1954.
[19] R. Suhas, S. Chandrashekar, D.C. Gowda, Eur. J. Med. Chem. 46 (2011) 704–711.
[20] G.P. Suresha, R. Suhas, W. Kapfo, D.C. Gowda, Eur. J. Med. Chem. 46 (2011) 2530–2540.
[21] R. Suhas, D.C. Gowda, J. Pept. Sci. 18 (2012) 535–540.
[22] R. Suhas, S. Chandrashekar, D.C. Gowda, Int. J. Pept. Res. Ther. 18 (2012) 89–98.
[23] C.S. Shantharam, D.M. Suyoga Vardhan, R. Suhas, M.B. Shridhara, D.C. Gowda, Eur. J. Med. Chem. 60 (2013) 325–332.
[24] A. Sharma, R. Suhas, D.C. Gowda, Arch. Pharm. Chem. Life Sci. 346 (2013) 359–366.
[25] A. Sharma, R. Suhas, K.V. Chandana, S.H. Banu, D.C. Gowda, Bioorg. Med. Chem. Lett. 23 (2013) 4096–4098.
[26] G.A. Patani, E.J. LaVoie, Chem. Rev. 96 (1996) 3147–3176.
[27] H.J. Böhm, D. Banner, S. Bendels, M. Kansy, B. Kuhn, K. Müller, U. Obst-Sander, M. Stahl, ChemBioChem 5 (2004) 637–643.
[28] S.B. Upadhyay, Indian J. Biotech. 11 (2012) 381–388.
[29] L. Oble, E. Carlsson, P. Lindberg, Nat. Rev. Drug Discov. 2 (2003) 132–139.
[30] K-Fr Sewing, P. Harms, G. Schulz, H. Hannemann, Gut 24 (1983) 557–560.
[31] T.M. Devlin (Ed.), Textbook of Biochemistry with Clinical Correlations, seventh ed., John Wiley and Sons, Inc, 2011, pp. 1042–1043. Ch. 25.
[32] H. Goda, J. Sakamoto, S. Sakaue, S. Kajihara, M. Todo, US 5861511, 1997.