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A facile synthesis of *N*-Z/Boc-protected 1,3,4-oxadiazole-based peptidomimetics employing peptidyl thiosemicarbazides

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ABSTRACT

Synthesis of 1,3,4-oxadiazole containing peptidomimetics is described by a *p*-TsCl/pyridine-mediated cyclization of the corresponding dipeptidyl thiosemicarbazides, which are readily prepared by coupling N-protected amino acid hydrazides with amino acid-derived isothiocyanato esters. Further, the protocol has also been extended for the synthesis of orthogonally protected 1,3,4-oxadiazole tethered mimetics as well. The synthetic route is simple and mild conditions are used so that the chirality of the starting amino acids is retained.

1. Introduction

Peptides are essential components of living organisms functioning as signal molecules such as hormones, neurotransmitters, and neuromodulators. They exert an essential influence on basic biological functions including metabolism, reproduction, respiration, and immune defense.[1] However, their clinical applications have been limited due to their rapid hydrolysis and low bioavailability.[2] To circumvent some of these problems, peptide bonds are replaced with a wide variety of structural functionalities such as retro-amide, urea,[3] peptoid,[4] carbamate,[5] sulfonamide,[6] and heterocycles,[7] which contribute to the hydrolytic resistance and enhanced bioavailability of the resulting peptidomimetics.

1,3,4-Oxadiazole is an important bioactive class of heterocycle with a broad spectrum of pharmaceutical applications.[8] In particular, marketed antihypertensive agents such as tiodazosin[9] and nesapidil[10] as well as antibiotics such as furamizole[11] contain the oxadiazole nucleus. They have also been utilized as bioisosteres of the carboxamide moiety in benzodiazepine receptor agonists,[12] muscarinic receptor agonists,[13] NK1 receptor antagonists,[14] and in the design of dipeptidomimetics[15] as peptide building blocks. In addition, 1,3,4-oxadiazoles are also useful intermediates in organic synthesis, particularly as electron-deficient azadienes in the inverse electron demand Diels–Alder reactions.[16]

As a part of our continued interest in the synthesis of heterocycles tethered peptidomimetics,[17] we report herein the synthesis of 1,3,4-oxadiazole-derived peptidomimetics. Several approaches have been reported to assemble 1,3,4-oxadiazole containing molecules,[18] in particular, 2-amino-1,3,4-oxadiazoles are prepared by cyclization of the corresponding acyclic semicarbazide or thiosemicarbazide derivatives both in solution as well as in solid phase. When thiosemicarbazides are used as oxadiazole precursors, a variety of reagents have been employed for their cyclization including I_2/NaOH,[19] carbodiimide,[20] tosyl chloride (*p*-TsCl),[21] and stoichiometric mercury salts.[22] Selective activation of the sulfur moiety followed by cyclization has also been achieved by coupling reagents such as dicyclohexylcarbodiimide (DCC),[23] 1-ethyl-3-(3-dimethylaminoprophyl)carbodiimide (EDC),[24] and highly reactive alkylating agents such as methyl iodide[25] and ethyl bromoacetate.[26]

Though 1,2,4-oxadiazole bearing peptidomimetics are known in the literature,[17,18,27] the corresponding 1,3,4-oxadiazoles are scanty. Luthman and co-workers,[15] employed 1,3,4-oxadiazole to synthesize enantiomerically pure Boc-Phe-Gly dipeptidomimetic with a methylene spacer (Fig. 1 A). For this, initially Boc-L-Phe hydrazide was treated with methyl malonyl chloride in the presence of $SOCl_2$/pyridine to afford 1,2,3,4-oxathiadiazole S-oxide intermediate which on thermal elimination of sulfur dioxide yielded the target mimetic. They also reported several Xaa-Gly mimetics containing 1,3,4-oxadiazoles (Fig. 1 B).[28] Batey and co-workers,[29] described a one-pot procedure for the synthesis of 1,3,4-oxadiazoles from Boc-protected amino acid hydrazides and arylisothiocyanates in the presence of $HgCl_2$ (Fig. 1 C). Kudelko

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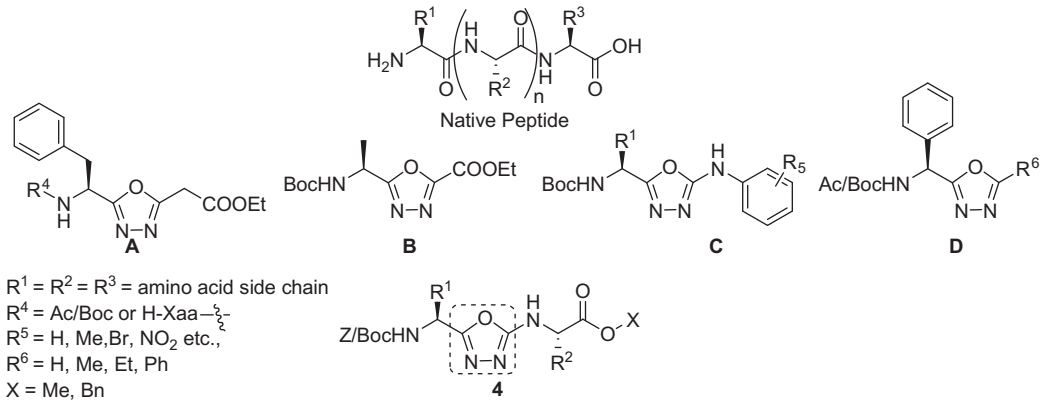
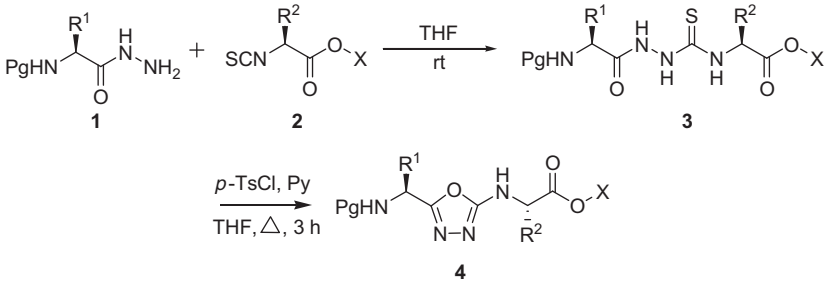


Figure 1.

and Zielinski[30] group reported the synthesis of 2-aminomethyl-1,3,4-oxadiazoles from N^{α}-Ac and N^{α}-Boc-protected phenylglycine hydrazide and triethyl orthoesters under reflux in acetic acid (Fig. 1 D). To the best of our knowledge, 1,3,4-oxadiazole bearing dipeptidomimetics of the type **4** are yet to be reported. This letter describes the facile synthesis of 1,3,4-oxadiazole linked peptidomimetics via the corresponding thiosemicarbazides which possess both amino and carboxy functionalities, which can be employed for the elongation of the peptide chain or to increase the molecular diversity.

2. Results and discussion

In the present work, the precursor, peptidyl thiosemicarbazides which themselves are *hitherto* unreported class of intermediates, were prepared by the reaction of N-protected amino acid-derived hydrazides and isothiocyanato esters. Initially, several *N*-Boc/Z-protected amino acids were converted to the corresponding hydrazides **1** following the literature procedure.[31] The isothiocyanates **2** derived from amino acid esters are well known entities in peptide chemistry.[32] We have recently reported the synthesis of N^{β}-urethane-protected amino alkyl isothiocyanates and demonstrated their utility in the synthesis of dithioureidopeptides.[33] Following the similar protocol, in the current study, isothiocyanato esters were prepared by treating the amino acid esters with CS_2 and triethylamine (TEA) in THF at 0 °C for 20 min and subsequent decomposition of the in situ generated dithiocarbamic acid salt with *p*-TsCl at the same temperature.[34] In the next step, the reaction of **1** with the isothiocyanato esters **2** in THF afforded the corresponding Boc/Z-peptidyl thiosemicarbazides **3** (Scheme 1, Table



Scheme 1. Synthesis of oxadiazoles **4**.

Table 1
List of dipeptidyl thiosemicarbazides **3** prepared

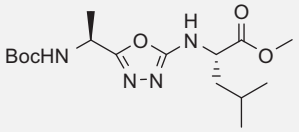
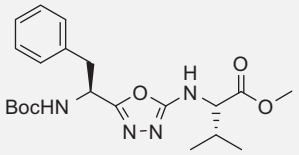
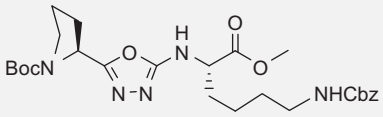
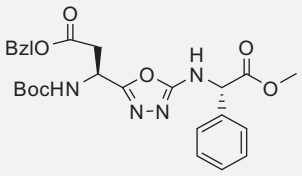
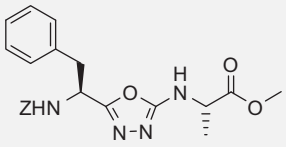
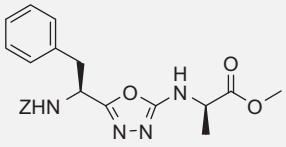
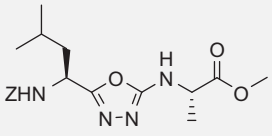
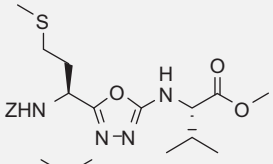
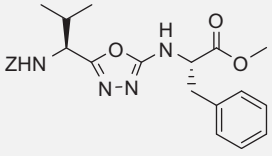
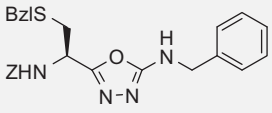
Entry	Hydrazide **1**		Isothiocyanate **2**	Time (h)	Mp (°C)	Yield (%)	Mass calcd/obsd
	Pg	Amino acid					
3a	Boc	Ala	Leu-OMe	2.0	Gum	75	413.2/413.1[a]
3b	Boc	Phe	Val-OMe	0.5	68	68	475.1991/475.1996[b]
3c	Boc	Pro	Lys(Z)-OMe	1.0	Gum	64	566.2/566.5[c]
3d	Boc	Asp(Bzl)	Phg-OMe	2.0	72	74	567.1889/567.1883[b]
3e	Z	Phe	Ala-OMe	0.5	108	88	459.1702/459.1708[d]
3f	Z	Phe	D-Ala-OMe	0.5	112	82	459.1702/459.1707[d]
3g	Z	Leu	Ala-OMe	1.0	Gum	80	447.1678/447.1686[b]
3h	Z	Met	Val-OMe	1.5	69	76	471.2/471.0[c]
3i	Z	Val	Phe-OMe	1.0	140	69	487.2/487.1[c]
3j	Z	Cys(Bzl)	Benzyl	0.5	139	92	531.1501/531.1508[b]

[a] ESI-MS [M+Na$^+$].
[b] HRMS [M+Na$^+$].
[c] ESI-MS [M+H$^+$].
[d] HRMS [M+H$^+$].

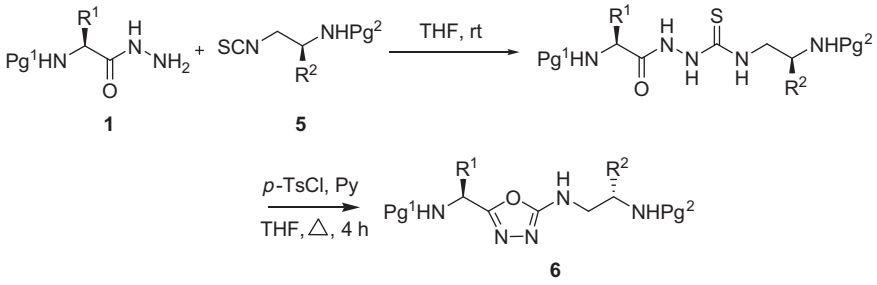
1). The reaction proceeded smoothly at room temperature and the chromatographic purification yielded analytically pure peptidyl thiosemicarbazides in good yields.[35]

In the next step, the cyclization reaction of the thiosemicarbazides **3** was undertaken. Various aforementioned reagents like DCC, *p*-TsCl, and $HgCl_2$ were explored for cyclodesulfurisation. However, during the optimization of reaction conditions, *p*-TsCl/pyridine[21] was found to be attractive in terms of furnishing good yield and mild reaction condition. In a typical procedure, Boc-Phe-NH-NH-CS-Val-OMe **3b** on treating with 1.5 equiv of *p*-TsCl and 2.5 equiv of pyridine in THF under reflux for 3 h resulted in the formation of 1,3,4-oxadiazole derivative **4b** which was isolated by a simple work-up followed by column chromatography in good yield[36] (Scheme 1, Table 2). Extending this protocol, a series of Boc and

Table 2
List of oxadiazoles **4**

Entry	Compound	Mp (° C)	Yield (%)	Mass calcd/obsd
4a		Gum	80	357.2/357.6[a]
4b		78	74	441.2114/441.2118[b]
4c		Gum	73	532.3/532.2[a]
4d		Gum	68	533.2012/533.2017[b]
4e		171	84	447.1644/447.1649[b]
4f		178	76	425.2/425.2[a]
4g		175	71	413.1801/413.1798[b]
4h		120	68	459.1678/459.1684[b]
4i		175	78	475.1957/475.1962[b]
4j		101	85	475.2/475.0[a]

[a] ESI-MS [M+H$^+$].
[b] HRMS [M+Na$^+$].



Scheme 2. Synthesis of orthogonally protected 1,3,4-oxadiazoles **6**.

Table 3
List of orthogonally protected oxadiazoles **6**

Entry	Hydrazide **1**	Isothiocyanate **5**	Yield (%)
6a	Boc-Ala	Z-Val	72
6b	Boc-Leu	Z-Phe	69
6c	Z-Phe	Boc-Val	74

Z-protected 1,3,4-oxadiazole containing dipeptidomimetics **4a–j** were prepared. All the synthesized dipeptidyl 1,3,4-oxadiazole derivatives were characterized by ^{1}H NMR, ^{13}C NMR, and mass spectroscopic analyses. Also the course of the reaction was found to be recemization free as was evident by both ^{1}H NMR[37] and HPLC studies.[38]

The present approach was then extended to prepare the orthogonally protected oxadiazoles **6** as well (Scheme 2). In this approach, both the starting materials, that is, hydrazides and isothiocyanates were prepared through the carboxy modification of N-protected amino acid. Orthogonality of the N-protecting group of participating reactants was maintained so as to enable selective chain extension. For this, Boc/Z-protected amino alkyl isothiocyanates were prepared from the corresponding vicinal diamines,[32] purified, and subsequently coupled with Z/Boc-amino acid hydrazides **1** in THF to obtain the key thiosemicarbazide intermediates which upon desulfurative cyclization with *p*-TsCl/pyridine under reflux conditions furnished the oxadiazoles **6** in good yields.[39] The crude 1,3,4-oxadiazoles were purified through column chromatography and were fully characterized (Table 3). HPLC analysis carried out on these derivatives proved that all the products are free from racemization.[37]

In conclusion, a simple and convenient method for the synthesis of 1,3,4-oxadiazole-linked dipeptidomimetics from corresponding peptidyl thiosemicarbazides has been described. The protocol has also been extended to prepare few *N,N′*-orthogonally protected dipeptidomimetics. All the products were found to be chemically homogeneous as analyzed by spectroscopic techniques. These dipeptidomimetics can be utilized for the preparation of 1,3,4-oxadiazole containing oligopeptidomimetics through N- and C-terminal chain extensions.

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34. *General procedure for the synthesis of isothiocyanato amino acid ester* **2**: To a solution of amino acid ester salt (10 mmol) in CH_2Cl_2 was added CS_2 (0.66 mL, 11 mmol) followed by TEA (5.58 mL, 40 mmol) at 0 °C, and stirred for 30 min at

the same temperature and then for 30 min at room temperature. The reaction mixture was again cooled to 0 °C and *p*-TsCl (2.48 g, 13 mmol) was added. The stirring was continued for 3 h or until the completion of the reaction (TLC analysis). An excess of DCM (5 mL × 2) was added and the organic layer was washed twice with 10% citric acid solution (15 mL), followed by water (10 mL × 2) and brine (10 mL × 2). It was then dried over anhydrous sodium sulfate and concentrated under reduced pressure. The resulting crude product was subjected to column chromatography (10% EtOAc in hexane) to afford the product as pure one.

35. *General procedure for the synthesis of thiosemicarbazide* **3**: To a solution of hydrazide **1** (10 mmol) in THF (10 mL) was added the isothiocyanate **2** (10 mmol), and the reaction mixture was stirred at room temperature till the completion of the reaction (TLC analysis). The solvent was removed under reduced pressure and the crude compound was purified by column chromatography (20% EtOAc in hexane). Data for compound **3e**: yield 88%; R_f 0.20 (4:6 EtOAc/*n*-hexane); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.43 (d, J = 6.8 Hz, 3H), 3.08–3.14 (m, 1H), 3.33–3.39 (m, 1H), 3.72 (s, 3H), 4.12–4.18 (m, 1H), 4.22 (t, J = 7.2 Hz, 1H), 4.97 (br, 1H), 5.07 (s, 2H), 5.97 (br, 1H), 7.20–7.33 (m, 10H), 8.85 (br, 1H), 9.52 (br, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 16.35, 37.79, 54.19, 54.31, 60.37, 67.11, 127.81, 127.70, 127.97, 128.35, 128.49, 128.57, 128.63, 129.11, 129.34, 129.42, 133.56, 135.85, 156.48, 170.82, 171.71, 181.13; HRMS calcd for $C_{22}H_{26}N_4O_5S$ *m/z* 459.1702 [M+H$^+$], found 459.1708 [M+H$^+$].
36. *General procedure for the synthesis of oxadiazoles* **4**: To a solution of thiosemicarbazide **3** (10 mmol) in THF (10 mL), *p*-TsCl (2.85 g, 15 mmol) and pyridine (2 mL, 25 mmol) were added and it was refluxed for 3 h. The solvent was removed in vacuo and the crude was dissolved in EtOAc and it was washed successively with 10% citric acid (10 mL × 2), 5% Na_2CO_3 (10 mL), water (2 × 10 mL), brine (10 mL), and dried over anhydrous sodium sulfate. The solvent was removed under vacuum and purified by column chromatography (20% EtOAc in hexane) to afford analytically pure product. Data for compound **4b**: yield 74%; R_f 0.33 (4:6 EtOAc/*n*-hexane); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.98 (d, J = 5.2 Hz, 6H), 1.36 (s, 9H), 2.18–2.24 (m, 1H), 2.83–2.89 (m, 2H), 3.36 (d, J = 4.8 Hz, 1H), 3.71 (s, 3H), 3.97–4.04 (m, 1H), 5.21 (br, 1H), 7.03–7.18 (m, 5H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 18.52, 28.31, 33.67, 47.5, 49.55, 52.30, 63.38, 79.11, 126.26, 127.44, 127.80, 128.83, 130.88, 136.42, 155.36, 170.62, 171.51, 171.60; HRMS calcd for $C_{21}H_{30}N_4O_5$ *m/z* 441.2114 [M+Na$^+$], found 441.2118 [M+Na$^+$].
37. Two epimeric thiosemicarbazides Z-Phe-ψ-[CONHNHCSNH]-L-Ala-OMe **3e** and Z-Phe-ψ-[CONHNHCSNH]-D-Ala-OMe **3f** were prepared. In the ^{1}H NMR spectrum, the alanyl methyl group of **3e** appeared as doublet at 1.40 and 1.42 ppm, while **3f** had distinct doublets at 1.43 and 1.45 ppm confirming the presence of single epimer in each sample. In a similar way, the oxadiazole samples Z-Phe-ψ-[C_2N_2O]-L-Ala-OMe**, 4e** and Z-Phe-ψ-[C_2N_2O]-D-Ala-OMe**, 4f** were studied and in this case also the alanyl methyl group was observed as distinct doublets at δ 1.39 and 1.41; 1.41, and 1.43, respectively, thus confirming the absence of racemization.
38. HPLC particulars: Agilent 1100 series having G1311A VWD at λ = 254 nm, flow 0.5 mL/min, Column: Agilent Eclipse XDB-C18, pore size-5 μm, diameter × length = 4.6 × 150 mm; Method: gradient 0.1% TFA water–acetonitrile; acetonitrile 30–100% in 30 min. HPLC profile of thiosemicarbazide, **3e** and its epimer, **3f** had peaks at R_t values of 14.98 and 15.02 min, respectively. Similarly, the oxadiazole samples **4e** and its epimer **4f** appeared at R_t 19.68 and 18.56 min, respectively. Thus from the above observations it is evident that the dipeptide derivatives are optically pure and free from racemization. Similarly for the compound **6a**, the R_t value is 12.28 min and for its epimer prepared from Boc-D-Ala is 12.06 min.
39. *Compound* **6a**: yield 72%; R_f 0.35 (4:6 EtOAc/*n*-hexane); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.98 (d, J = 6.2 Hz, 6H), 1.38 (s, 9H), 1.28 (d, J = 4.8 Hz, 3H), 2.32–2.38 (m, 1H), 3.28–3.34 (m, 1H), 3.48–3.54 (m, 1H), 3.83–3.88 (m, 1H), 4.60–4.65 (m, 1H), 4.90 (br, 1H), 5.06 (s, 2H), 5.61 (br, 1H), 7.03–7.24 (m, 5H), 8.81 (br, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 15.11, 19.17, 29.21, 30.74, 45.67, 47.70, 53.82, 66.39, 80.02, 127.03, 127.87, 128.14, 128.49, 129.49, 135.87, 153.12, 156.34, 169.66, 171.28; HRMS calcd for $C_{22}H_{33}N_5O_5$ *m/z* 470.2379 [M+Na$^+$], found 470.2383 [M+Na$^+$].