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Synthesis of 3,5-disubstituted 1,2,4-oxadiazoles as peptidomimetic building blocks

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Abstract—Twelve new 1,2,4-oxadiazole based compounds have been synthesized. Their structures contain a protected amine and a carboxyl or an ester group, and thus serve as potential peptidomimetic building blocks. The synthetic route is simple and mild conditions are used so that the chirality of the starting amino acids is retained.

Recently many bioactive peptides have been discovered that play diverse roles, functioning as hormones, enzyme inhibitors or substrates, growth promoters and inhibitors, neurotransmitters, etc. However, their clinical application has been somewhat limited due to their rapid hydrolysis by peptidase enzymes. To circumvent this problem, the native peptide has been modified to afford a peptide-mimicking compound, that is, a peptidomimetic. Numerous peptidomimetic analogues of native peptides have exhibited improved pharmacological and pharmacokinetic properties.[1,2] Bioisosteric replacements for the amide bond constitute an important aspect of peptide chemistry because of its implications in the design of peptidomimetics.[3]

The 1,2,4-oxadiazole[4–6] nucleus is often used as a classical bioisosteric replacement for an amide or ester functionality,[7,8] because of its electronic properties. Its derivatives can be found in a vast number of compounds exerting biological activity such as ligands of benzodiazepine receptors,[8,9] anti-inflammatory agents,[10–12] antiviral agents,[13] inhibitors of protein tyrosine phosphatases,[14] agonists of muscarinic receptors,[15,16] inhibitors of Src SH2,[17] antagonists of histamine H_3-receptors,[18] integrin receptor antagonists,[19] angiotensin II receptor antagonists[20,21] and HIV-1 reverse transcriptase inhibitors.[22] 1,2,4-Oxadiazole moieties have been used in the design of dipeptidomimetics as peptide building blocks.[23,24] The compounds described have an ester functionality attached directly to the heterocycle at the 3-position, rendering them relatively stable. However, when used in following reactions, they had to be converted to free acids, which caused spontaneous decarboxylation.[23–25] It is a well known fact that 1,2,4-oxadiazole-3-carboxylic acids are readily decarboxylated.[25]

In an attempt to overcome this problem, we describe the synthesis of 12 new 3,5-disubstituted 1,2,4-oxadiazoles, bearing in their structures a Boc-protected amine and a carboxylic acid or an ester functionality, which makes them useful as peptidomimetic building blocks. One, two and three methylene groups have been incorporated between the heterocycle and the carboxylic acid group to increase the stability of the final compounds. The synthetic steps are simple and require only readily accessible chemicals (the amidoxime precursors are derived from α-amino acids[26]). The mild reaction conditions allow the chiral configuration of the starting amino acids to be retained, thus affording enantiopure products.

The starting amino acids were first N-protected by reaction with Boc-anhydride[27] (87–97% yields). Their respective amides were then synthesized in a one-pot reaction,[28] in which the carboxylic acid group of the *N*-Boc-amino acids was converted to the desired amide in good yields (70–88%) by treatment with ethyl chloroformate in the presence of triethylamine and gaseous ammonia (Scheme 1). Dehydration then followed, using trifluoroacetic acid anhydride in the presence of pyridine

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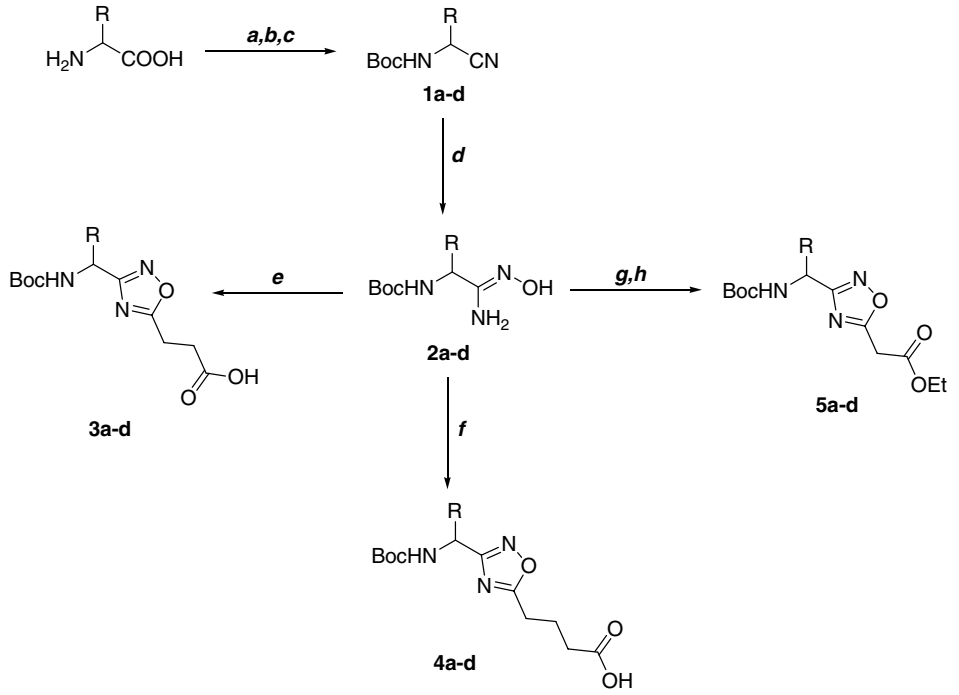

Scheme 1. Reagents and conditions: (a) $(Boc)_2O$, NaOH, dioxane/water, 0 °C; (b) ethyl chloroformate, Et_3N, CH_2Cl_2, −10 °C then $NH_{3(g)}$; (c) cyanuric chloride, DMF, rt; (d) $NH_2OH \times HCl$, Et_3N, EtOH, reflux, 20 h; (e) succinic acid anhydride, DMF, pyridine, 110 °C, 4 h; (f) glutaric acid anhydride, DMF, pyridine, 110 °C, 4 h; (g) ethyl malonyl chloride, Et_3N, CH_2Cl_2, 0 °C; (h) toluene, 120 °C, 72 h.

in dioxane, to give the corresponding nitriles.[29] This proved incomplete and lengthy, and prompted us to consider a different route. Cyanuric chloride was used as a dehydrating agent[30] in dimethylformamide at room temperature, and proved an excellent alternative, since the reaction was complete in only half an hour in good yields (65–89%). The corresponding amidoximes were obtained from the nitriles by treatment with hydroxylamine hydrochloride in the presence of triethylamine in refluxing ethanol[31] (63–97% yields). 1,2,4-Oxadiazoles are commonly synthesized from amidoximes and carboxylic acid derivatives.[17,19,23,24,32–43] Our synthesis was performed in a one-pot reaction in which formation of the heterocyclic ring was accomplished with concomitant introduction of different length chains bearing a carboxylic group. Using simple anhydrides[19,34] as activated acid derivatives in DMF/pyridine, *O*-acyl amidoximes were formed which were subsequently cyclized.[44] Cyclodehydration to 1,2,4-oxadiazoles **3a–d** and **4a–d** was carried out in a weakly basic solvent at a high temperature of 110 °C to avoid racemization which usually occurs after the use of strong bases in the cyclization procedure.[37] Alternative procedures which avoid racemization have also been described.[36] Heating the mixture to 110 °C gave rise to the formation of minor side products and consequently low yields.[34]

Four further oxadiazole derivatives **5a–d** were synthesized from amidoximes **2a–d** which were first acylated with ethyl malonyl chloride in the presence of triethylamine in dichloromethane, and then converted into the respective oxadiazoles by heating in dry toluene at 120 °C for three days.[45]

Structures **3a–d**, **4a–d** and **5a–d** were fully characterized by spectroscopic data.[46] The enantiomeric purity of the

Table 1. 3,5-Disubstituted 1,2,4-oxadiazole derivatives produced via Scheme 1

Compound	R	$[\alpha]_D$[a]	t_R (min)	Overall yield[f] (%)
3a[g]	Methyl	−40.0 (*c* 2.55)	2.87[b]	6
3b	Isopropyl	−56.8 (*c* 3.10)	8.87[c]	17
3c	Benzyl	−13.7 (*c* 3.15)	5.86[d]	31
3d[h]	Methyl	+50.6 (*c* 2.35)	2.50[b]	13
4a[g]	Methyl	−42.1 (*c* 2.80)	3.69[b]	18
4b	Isopropyl	−55.7 (*c* 2.10)	7.34[c]	23
4c	Benzyl	−9.8 (*c* 2.45)	8.61[d]	32
4d[h]	Methyl	+51.7 (*c* 3.15)	3.44[b]	16
5a[g]	Methyl	−54.3 (*c* 4.20)	8.44[b]	9
5b	Isopropyl	−61.0 (*c* 3.00)	6.72[d]	17
5c	Benzyl	−20.0 (*c* 2.65)	4.24[e]	24
5d[h]	Methyl	+46.0 (*c* 2.50)	8.10[b]	8

[a] The compounds were dissolved in MeOH.
[b] Reverse phase HPLC analyses were run on a Chiral-AGP column (150 × 3.0 mm, 5 μm; ChromTech). 10 mM $CH_3COO^-NH_4^+$/isopropanol (96:4), pH = 5.5, at a flow rate of 0.5 ml/min was used as the mobile phase. The detection wavelength was 210 nm.
[c] Same conditions as in b, except for the mobile phase which consisted of 10 mM $CH_3COO^-NH_4^+$/isopropanol (92:8).
[d] Same conditions as in b, except for the mobile phase which consisted of 10 mM $CH_3COO^-NH_4^+$/isopropanol (90:10).
[e] Same conditions as in b, except that the mobile phase used for HPLC was 10 mM $CH_3COO^-NH_4^+$/isopropanol (80:20).
[f] Overall yield of the total synthesis from the starting amino acids to the final compounds (5 steps or 6 in the case of compounds **5a–d**).
[g] Derivatives of L-alanine.
[h] Derivatives of D-alanine.

products was evaluated by HPLC using a chiral stationary phase (Chiral-AGP column 150 × 3.00 mm, 5 μm; ChromTech). Enantiomeric purity was estimated for compound pairs **3a**–**d**, **4a**–**d** and **5a**–**d**, derivatives of L-alanine (**3a**, **4a** and **5a**) and D-alanine (**3d**, **4d** and **5d**), respectively. In cases where the retention time was determined only for the L-enantiomer, enantiomeric purity was estimated by assuming a similar separation factor and detection limit as in the chromatograms of the above mentioned compounds.

The 12 new compounds synthesized (Table 1) represent a set of potentially useful peptidomimetic building blocks. The methylene groups separating the heterocycle and the carboxyl functionality are employed as a spacer to allow for increased conformational flexibility and also to render the compounds more stable. The amidoxime precursors are derived from α-amino acids, using a simple synthetic route affording the products in good yields. In addition, the chirality of the starting amino acids is retained during the whole process due to the mild reaction conditions.

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44. *A typical procedure*: Amidoxime (4 mmol) and succinic anhydride (4 mmol) were dissolved in dry dimethylformamide (5 ml) to which pyridine (3 ml) was added. The reaction was heated at 100 °C for 3 h, then a catalytic amount of KF was added and the mixture heated at the same temperature for half an hour. On completion of the reaction, the mixture was cooled to room temperature, water (20 ml) was added and the solution acidified to pH 3 with 4 M aqueous HCl. The solution was extracted with EtOAc (3×20 ml) and the combined organic layers washed with water (3×10 ml) and brine (10 ml), then dried (Na_2SO_4) and concentrated in vacuo to afford the crude product. The residue was purified by chromatography on a silica gel column (dichloromethane/methanol 10:1) to afford the corresponding products as solid compounds (**3a**–**d**).
45. *A typical procedure*: Amidoxime (4 mmol) was dissolved in dry dichloromethane (20 ml), cooled to 0 °C, then triethylamine (8 mmol) was added followed by a slow dropwise addition of ethyl malonyl chloride (8 mmol). The mixture was allowed to warm to room temperature and stirred for 20 min. On completion of the reaction, the solvent was removed under reduced pressure, the residue dissolved in dichloromethane (20 ml) and washed with water (3×10 ml) and brine (10 ml), then dried (Na_2SO_4) and concentrated in vacuo to afford a crude product. The crude product was used without purification in the next step in which it was suspended in dry toluene (5 ml) and heated at 120 °C for three days. On completion of the reaction, the solvent was removed under reduced pressure and the residue dissolved in EtOAc (20 ml), washed with water (3×10 ml) and brine (10 ml), then dried (Na_2SO_4) and concentrated in vacuo to give an oil. The oil was purified by chromatography on a silica gel column (dichloromethane/methanol 15:1) to afford the corresponding products (**5a**–**d**).
46. Representative examples. Compound **3a**: white solid; mp 67–70 °C; IR (KBr, cm^{-1}): 3350, 2986, 1728, 1691, 1523, 1163, 1061, 896, 614; ^1H NMR (DMSO-d_6, 300 MHz): δ (ppm) = 1.36 (d, 3H, $J = 7.5$ Hz, CH_3), 1.37 (s, 9H, $C(CH_3)_3$), 2.73 (t, 2H, $J = 6.9$ Hz, CH_2CO), 3.09 (t, 2H, $J = 7.2$ Hz, CH_2), 4.67–4.74 (m, 1H, CH), 7.42 (d, 1H, $J = 8.1$ Hz, CONH), 12.37 (s, 1H, COOH); MS (FAB) m/z: 286 (MH^+); Anal. Calcd for $C_{12}H_{19}N_3O_5$: C, 50.52; H, 6.71; N, 14.73. Found: C, 50.69; H, 6.89; N, 14.83. Compound **5a**: brown oil; IR (NaCl, cm^{-1}): 3853, 3742, 2981, 1702, 1519, 1167; ^1H NMR (DMSO-d_6, 300 MHz): δ (ppm) = 1.20 (t, 3H, $J = 7.2$ Hz, CH_3CH_2), 1.38 (d, 3H, $J = 6.6$ Hz, CH_3CH), 1.38 (s, 9H, $C(CH_3)_3$), 4.15 (q, 2H, $J = 7.2$ Hz, CH_2CH_3), 4.24 (s, 2H, CH_2COO), 4.73–4.78 (m, 1H, CH), 7.47 (d, 1H, $J = 7.8$ Hz, CONH); MS (EI) m/z: 300 (MH^+); MS (FAB) m/z: 300 (MH^+); HRMS calcd for $C_{13}H_{22}N_3O_5$ m/z: (MH^+) 300.15686, found 300.15595.