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Synthesis, molecular docking and anticancer studies of peptides and *iso*-peptides

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ABSTRACT

Chiral peptides and *iso*-peptides were synthesized in excellent yield by using benzotriazole mediated solution phase synthesis. Benzotriazole acted both as activating and leaving group, eliminating frequent use of protection and subsequent deprotection. The procedure was based on the hypothesis that epimerization should be suppressed in solution due to a faster coupling rate than SPPS. All the synthesized peptides complied with Lipinski's Ro5 except for the rotatable bonds. Inhibition of cell proliferation of cancer cell lines is one of the most commonly used methods to study the effectiveness of any anticancer agents. Synthesized peptides and *iso*-peptides were tested against three cancer cell lines (MCF-7, MDA-MB 231) to determine their anti-proliferative potential. NFkB was also determined. Molecular docking studies were also carried out to complement the experimental results.

A diverse arsenal of peptide based drugs have been developed for the treatment of cancer, viral infections, pain management, and other diseases.[1,2] The ubiquity of biologically active peptides and peptide derivatives has attracted attention of the synthetic community. In this context the 1923 Nobel Prize was awarded to Banting and Macleod for the discovery and extraction of insulin.[2] du Vigneaud, a Nobel laureate in chemistry, presented the first total solution phase synthesis of a naturally occurring bioactive octapeptide, oxytocin.[3–5] Peptides are now routinely prepared by chemical synthesis in solution or solid phase and are being used as therapeutic agents such as leuprolide acetate (Lupron™), octreodide acetate (Sandostatin™) and goserelin acetate (Zoladex™).[2,5]

Peptides are intrinsically able to interact with biological systems and are therefore potent therapeutics,[6–8] but their conformational flexibility, low ability to cross physiological barriers and metabolic instability, represent major hurdles for the successful development of peptide-based drugs.[9]

Advantages for purely synthetic peptides include, large scale preparation, incorporation of unnatural amino acid residues to improve their absorption–distribution–metabolism profile, limitless sequence variations and well-defined homogeneity.[10–12] New and improved strategies lead to more efficient synthesis of complex peptide targets, opening avenues to both new drug candidates and a deeper understanding of the intimate relation between sequence, conformation and properties.

Despite recent progress and the arsenal of reagents available, peptide synthesis remains challenging.

Benzotriazole emerged as a powerful synthetic tool in 1987.[13–16] Since then tremendous progress has been achieved in this field.[17] This solution phase benzotriazole mediated peptide coupling avoids both epimerization and hydrolysis due to fast coupling rate.

Peptides containing *iso*-peptide bonds are called *iso*-peptides. *iso*-Peptides are useful for the synthesis of large peptides and proteins. The *iso*-peptide method led to the efficient preparation and purification of large peptides, which are known to aggregate in solution. Significantly, the combination of both techniques, peptide and *iso*-peptide synthesis has advanced the frontiers of synthetic peptide chemistry. '*O*-Acyl *iso*-peptides' are more hydrophilic and easier to purify by HPLC than the corresponding native peptides.[18]

This study reports the synthesis of chiral peptides and *iso*-peptides by benzotriazole chemistry and their anticancer activity. Molecular docking studies against kinases completes the study.

N-(Pg-Aminoacyl)-benzotriazoles **3a–d** were readily prepared from commercial *N*-protected-amino acids **1a–e** following

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established procedures.[19,20] *N*-Protected dipeptides **5a–h** were synthesized in 87–90% yields by peptide coupling reactions of *N*-(Pg-aminoacyl)benzotriazoles **3a–b** with free amino acid in the presence of triethylamine in aqueous acetonitrile at 20 °C (Scheme 1, Table 1).

Protected dipeptides **5a–b** were treated with benzotriazole **2** in the presence of thionyl chloride in DCM at −20 °C for 5 h to obtain *N*-(Pg-dipeptidioyl)benzotriazoles **6a–b** in good yields (Scheme 2, Table 2).

Isotripeptides **7a–e** were synthesized by peptide coupling reactions between protected dipeptides **5c–f** and *N*-(Pg-aminoacyl)benzotriazoles **3c–d** in dry acetone in the presence of two equivalents of triethylamine for one hour at −10 °C in 70–82% yields (Scheme 3, Table 3).

Deprotection of the Cbz group of **7a–c** with C/Pd in ethanol under hydrogen gave unprotected isotripeptides **8a–c** in good yields (Scheme 4, Table 4).

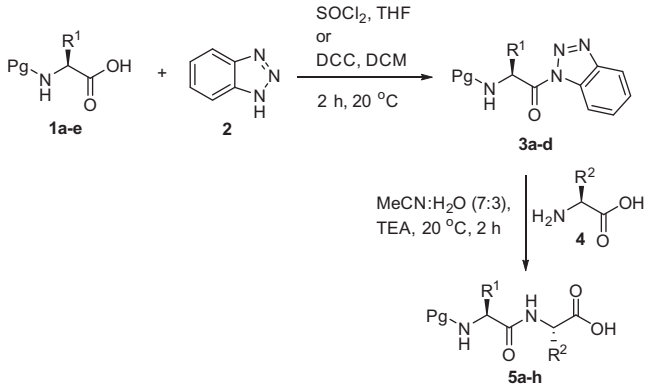
Peptides **5d**, **7d** and **8a** showed QR1 greater than 2 (Table SI 1). The cytotoxic potential of the synthesized peptides toward hormone responsive breast cancer cell line MCF-7 ATCC (HTB-22), and estrogen receptor negative breast cancer cell line MDA-MB-231 (ATCC, HTB-26) was determined and the results are shown in Table SI 1.

Inhibition of cell proliferation of cancer cell lines is one of the most commonly used methods to study the effectiveness of any anticancer agents. In the present study, three cancer cell lines (MCF-7, MDA-MB 231) were used to determine antiproliferative potential of selected samples. Both MDA-MB-231 and MCF-7 are cytotoxicity assays performed on breast cancer cell line. All assays were carried out at 20 μg/mL. In case of NFkB **5d**, and **6a** showed better inhibition.

Nuclear factor kappa-B (NFkB) is an inducible transcription factor that plays an important role in the regulation of apoptosis, cell differentiation, and cell migration. NFkB is commonly involved as a regulator of genes that control cell proliferation and cell survival. Many different types of human tumors have miss-regulated NFkB that is constitutively active. Thus, inhibition of NFkB signaling has a potential application for the prevention or treatment of cancer.[21,22] As NFkB is an important regulator in cell fate decisions, such as programmed cell death, proliferation control, and cell invasion, it is critical in tumor-genesis. Inhibition of NFkB signaling has potential applications for the prevention or treatment of cancer.[22,23]

In the quinoline reductase (QRI) assay,[24,25] after the initial testing of 22 samples, 3 samples were selected to determine CD values (concentration required to double the QRI activity). These samples showed either an induction ratio (IR) > 2 or cytotoxic activity with cell survival ⩽50% at 20 μg/mL.[24–26] In this study, 22 peptides showed various levels of inhibition of NFkB. Among them, the most potent **5d** and **6a** had ⩽50% inhibition at 20 μg/mL. Results are tabulated in Table SI 2.

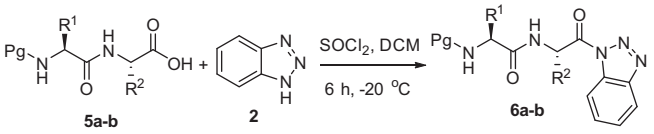
According to Lipinski's Ro5,[27] most drug like molecules have molecular weight ⩽500, logarithm of the octanol/water partition coefficient (log*P*) ⩽ 5, total polar surface area (TPSA) < 140 Å^2, number of hydrogen bond donors (HBD) ⩽ 5 and hydrogen bond acceptor (HBA) ⩽ 10.[27] Further modifications in the Ro5 were made by Veber et al. who suggested the number of rotatable bond (NOR) of a drug like molecule must be fewer or equal to 10.[28] Molecules violating more than one of these rules may have less bioavailability. These molecular descriptors were calculated for all the synthesized peptides, *iso* peptides and benzotriazolides by the ligand property calculation function of MOE (Table SI 3) and all of them were found to obey Lipinski's Ro5 cut-off limits, with the exception of number of rotatable bonds, revealing potent druglike compounds.



Scheme 1. Synthesis of dipeptides **5a–h**.

Table 1
Preparation of dipeptides **5a–h**

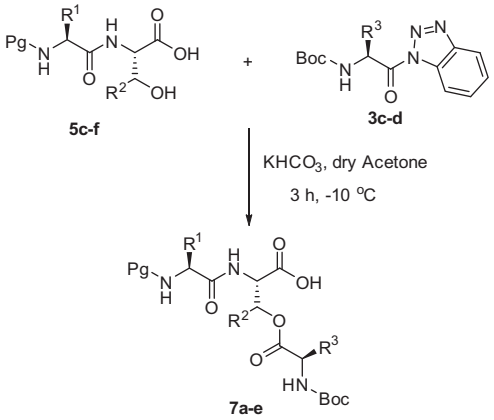
Entry	Compound **5**	Yield (%)	Mp (°C)	Lit mp (°C)
1	Cbz-L-Phe-Bt, **3a**	98	150–152	151–152[19]
2	Cbz-L-Ala-Bt, **3b**	95	109–110	114–115[19]
3	Boc-Gly-Bt, **3c**	94	69–70	68–69[20]
4	Boc-L-Ala-Bt, **3d**	94	84–86	85–86[20]
5	Cbz-L-Phe-Gly-OH, **5a**	89	164–165	163–165[20]
6	15-Cbz-L-Ala-L-Phe-OH, **5b**	88	142–144	141–143[20]
7	Cbz-L-Phe-L-Ser-OH, **5c**	90	140–141	140–141[19]
8	Cbz-L-Phe-L-Thr-OH, **5d**	89	146–148	
9	Cbz-L-Ala-L-Ser-OH, **5e**	80	192–194	192-194[19]
10	Cbz-L-Phe-D-Ser-OH, **5f**	87	191–193	
11	Cbz-L-Ala-D-Ser-OH, **5g**	89	200–202	
12	Cbz-L-Ala-L-Thr-OH, **5h**	88	203–204	



Scheme 2. Synthesis of *N*-(Pg-dipeptidioyl)benzotriazoles **6a–b**.

Table 2
Preparation of *N*-(Pg-dipeptidioyl)benzotriazoles **6a–b**

Entry	Compound **5**	Yield (%)	Mp (°C)	Lit mp (°C)
1	Cbz-L-Phe-Gly-Bt, **6a**	85	166–167	165–167[16]
2	Cbz-L-Ala-L-Phe-Bt, **6b**	86	148–149	148–149[19]



Scheme 3. Synthesis of *iso*-peptides **7a–e**.