

Dipeptide Conjugates: An Important Class of Therapeutic Agents

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ABSTRACT

Dipeptide conjugates and conjugates of amino acids with heterocycles or other chemical entity are significant class of medicinal compounds with a wide range of biological actions including antibacterial, antiviral, anticancer, antimalarial, and immunomodulatory activity. Such conjugates demonstrated a wide range of applications in biology and are utilized in a number of fields including medicinal chemistry, material science etc. The several biological activities shown by these conjugates are encouraging the chemists and researchers in this field to synthesize and screen them for various biological activities. This review highlights the research work carried out in this area in relation to the synthesis of conjugates for various biological activities.

Keywords: Anticancer, Immunoactivators, Antivirals, Antimicrobials, Antimalarials.

INTRODUCTION

Peptides are very resourceful biological molecules and most bio-compatible class of medicinal compounds with potent antimicrobial, anti-inflammatory, antiviral, insecticidal and anticancer properties. Peptide conjugated derivatives with smaller peptide units like dipeptides have sparked a lot of interest in recent years due to their broad biological activities, biocompatibility and the possibility of revealing structural diversity in them. Synthesis of such novel dipeptide conjugates with specific chemical entity, amino acid conjugates with heterocycle or other chemical entity has become an effective strategy for obtaining small bioactive molecules for specific target. The chemical synthesis of peptide conjugates and amino acid conjugates proved fruitful in obtaining new derivatives with potent biological activities.

e.g. Thiourea derivatives showed enhanced anticancer activities when thioureas are conjugated with amino acids.

e.g. The combination of amino acid and aminophosphonate was used to create novel thiourea derivatives known as

pseudopeptide thioureas, which have the potential to be used as anticancer agents.¹

Over the years, conjugated molecules, as novel chemotherapeutics, have made significant progress due to the accessibility of combining the structural features of two or more small bioactive themes to achieve novel molecules with enhanced bioactivities. It has been reported that 20-(S)-Camptothecin (CPT) conjugated dipeptides can be preassembled into nanotubes with diameters ranging from 80 to 120 nm, have enhanced resistance to hydrolytic deactivation, and have high *in vitro* potency against several human cancer cell types.² It is possible to use dipeptides or amino acids as promoiety to associate with pharmacologically active elements and bring prodrugs to target tissues. Conjugating dipeptide structures with existing pesticides is expected to be another potential strategy to obtain new candidates for phloem-mobile pesticides, which could enhance the efficiency and reduce the consumption of pesticides.³ Such conjugates of peptides or amino acids display wide structural heterogeneity due to variable amino

Submission Date: 05-07-2021;
Revision Date: 08-04-2022;
Accepted Date: 07-09-2022.

DOI: 10.5530/001954640093

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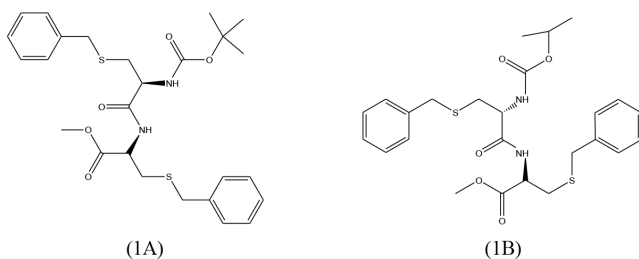


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acid sequences and are also effective against a broad spectrum of infections, ranging from Gram-positive to Gram-negative bacteria and fungal to protozoal and viral infections. The structural reliability of such conjugates allows them to take on different amphiphilic conformations responsible for the particular activity.⁴ The synthesis of such dipeptide conjugates for various biological activities is concised here as:

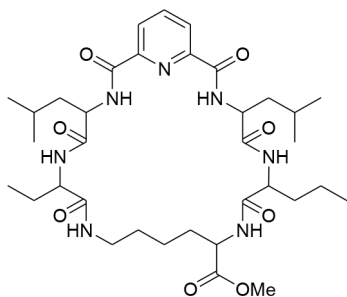
DIPEPTIDE CONJUGATES AS ANTICANCER LEADS

A suitably safe cystine-based dipeptide as well as its unprotected form has been synthesised by Banerji B *et al.* MTT assay, a laboratory test and a normal colorimetric assay for measuring cellular proliferation and phase-contrast images confirmed potent anticancer activities. The IC_{50} value is an indicator of a compound's efficacy in inhibiting biological processes. All the synthesised molecules showed very strong binding at sub-micromolar concentration.⁵ Compound (1A) and (1B) were found to be the most effective conjugates.



Cystine-derived Dipeptide Compounds (1)

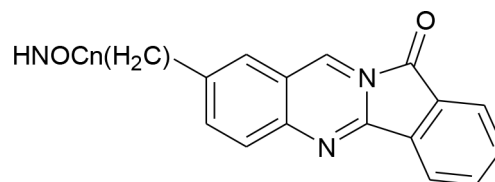
Macrocytic peptides have recently attracted a lot of attention as effective cancer therapeutic agents, owing to their synthetic accessibility and lower toxicity to normal cells. Development of new macrocytic pyridoheptapeptide derivatives was carried out by Abo-Ghaila MH *et al.* in this study. Normal chemical and spectroscopic analytical techniques were used to classify the synthesised derivatives² and compound (2) showed potent activity.



Macrocytic pyridoheptapeptide derivative (2)

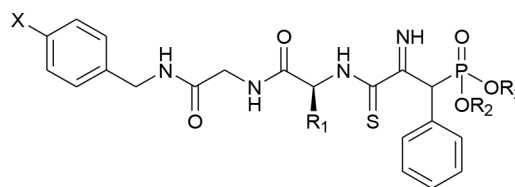
Dzierzbicka K *et al.* synthesised MDP (muramyl dipeptide) or nor-MDP (normuramyl dipeptide) conjugates with batracyclin (BAT) or batracyclin derivatives

modified at the peptide part is identified. A modified process was used to make batracyclin. The multigram scale synthesis of BAT through this modified route now appears to be possible. Data from preliminary screening at the National Cancer Institute revealed that even at 10^{-4} to 10^{-8} M or g/mL, the conjugates had no cytotoxic activity. Two analogues (3) were found to minimise the proliferation of Ab melanoma cells *in vitro* when compared to batracycline alone in studies conducted at the Medical University of Gdansk in Poland.⁶



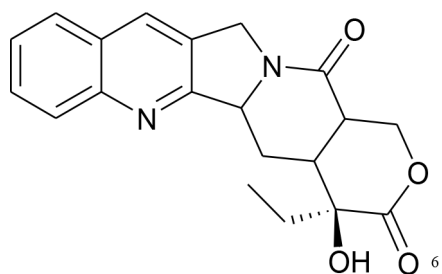
Batracyclin Derivative (3)

Thiourea derivatives have been shown to be effective against a variety of leukemias and tumour cell lines. The combination of thiourea and phosphonate was shown to be an effective strategy for developing antitumor agents. Synthesis and testing of a series of novel chiral dipeptide thioureas containing α -aminophosphonate moiety was carried out by Liu J *et al.* The final novel dipeptide thioureas were found to inhibit BGC-823 and A-549 cells similarly to Cisplatin.¹

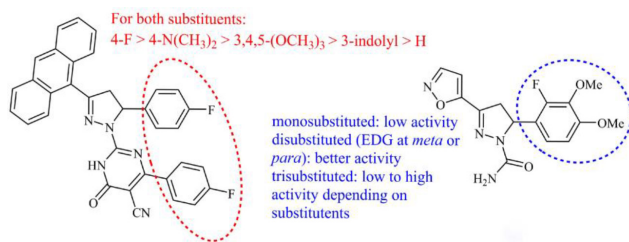


Thiourea dipeptide conjugates (4)

Kim SH *et al.* discovered that Camptothecin (5)-conjugated dipeptides preassemble into nanotubes with diameters ranging from 80 to 120 nm. *In vitro*, these nanoassemblies sustain a high drug loading (47%) and demonstrate greater drug stability (i.e., resistance to lactone hydrolysis), resulting in greater efficacy against many human cancer cells (HT-29, A549, H460, and H23). The use of the CPT conjugated dipeptide as both the drug and the precursor to the nanostructured carrier, which simplifies the overall fabrication process, is a central and defining feature of this method.²

**Camptothecin (5)**

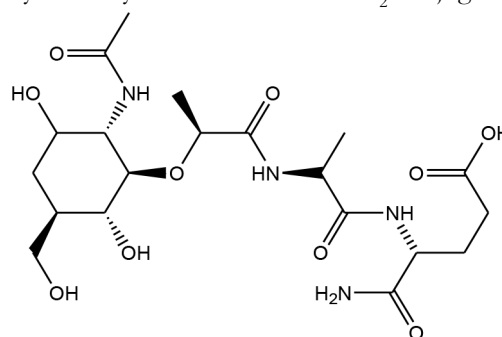
Three new dipeptide conjugates with monosubstituted anthracenyl-peptides have been synthesised by Meikle *et al.* and tested as topoisomerase (topo) I and II inhibitors.⁷ Each of the three conjugates (designated NU/ICRF 600-602) was shown to inhibit both topoisomerase I and II catalytic activity, with NU/ICRF 602 being the most active [100% inhibition of both enzymes at 5 micrograms/ml (approximately)]. None of the compounds bound to DNA in a topo I/DNA unwinding assay, indicating genuine inhibition of catalytic activity. Although none of the compounds induced topo II-mediated DNA cleavage, NU/ICRF 600 stabilised topo I cleavable complexes.⁸

**Monosubstituted anthracenyl-dipeptide pyrazoline Hybrid as potent Anticancer Agent (6)**

DIPEPTIDE CONJUGATES AS IMMUNOACTIVATOR

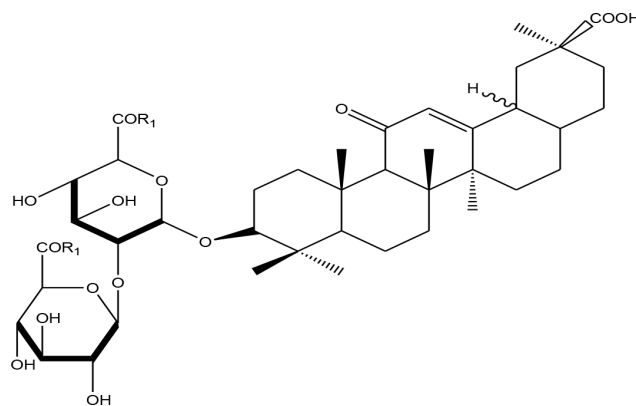
Chedid L and Parant F synthesised Muramyl dipeptides such as N-Acetylmuramyl-L-Ala-D-Glu-NH₂ (muramyl dipeptide) and their derivatives are potent immunoactivators that can improve nonspecific resistance to infection while also inducing fever. One of its stereoisomers, N-acetylmuramyl-D-Ala-D-Glu-NH₂, on the other hand, lacks both of these activities. The macromolecularization of muramyl dipeptide by attaching several units to a multi-poly (DL-Ala)-poly (L-Lys) carrier enhances both its pyrogenic and immunostimulant function, (7) as demonstrated in this study. This branched polymer is widely used as a hapten carrier. Surprisingly, after conjugation under the same conditions, inactive N-acetylmuramyl-D-Ala-D-Glu-NH₂, despite its lack of pyrogenicity, is capable of increasing nonspecific immunity. The N-acetylmuramyl-D-Ala-D-Glu-NH₂ conjugate also lacks any adjuvant,

sensitising, or eliciting properties. Furthermore, there is no adjuvant, sensitising, or eliciting operation in the N-acetylmuramyl-D-Ala-D-Glu-NH₂ conjugate.⁹

**N-Acetylmuramyl-L-Ala-D-Glu-NH₂ Muramyl Dipeptide Conjugate (7)**

DIPEPTIDE CONJUGATES AS IMMUNOSTIMULANT AND ANTIVIRAL AGENTS

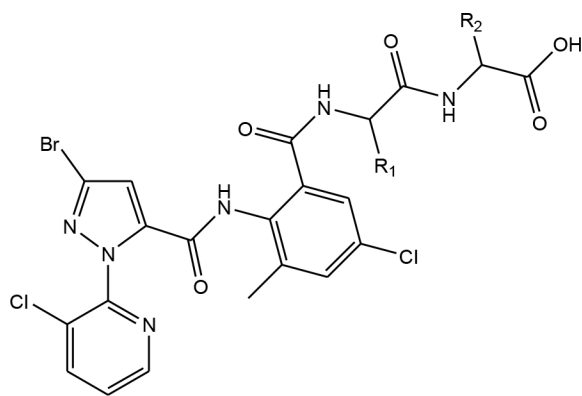
Synthesis and Anti-HIV-I Activity of new conjugates of Glycerrhizic acid with aspartic acid esters was carried out by Baltina LA *et al.* It was found that the conjugate of 18β-GA with Asp (OMe) (8) at a concentration of 250 μg/mL inhibited effectively RT of HIV-1 and the accumulation of virus antigen p24 in MT-4 cell culture (95–97%) and protected cells from the cytopathogenic action of the virus.¹⁰

**18β- and 18α-Glycerrhizic acid with aspartic acid esters (8)**

Phloem uptake bioactivities of dipeptide-Chlorantraniliprole derivatives

For insecticides to combat sucking insects, phloem systemicity is a desirable property. The production of phloem systemic insecticides, on the other hand, is difficult. One technique is to combine existing insecticides with endogenous compounds, resulting in conjugates that can be transported into the phloem by specialised transporters. In this report, chlorantraniliprole (9) was given new dipeptide promoters and is an effective and

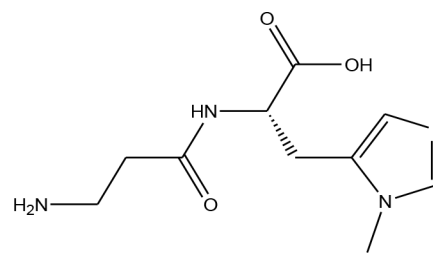
broad-spectrum anthranilic diamide insecticide with no phloem mobility. There have been 22 new dipeptide-chlorantraniliprole conjugates synthesised. In *Ricinus communis*, systemic tests revealed that all conjugates had phloem mobility. In comparison to its parent compound chlorantraniliprole, conjugates were able to demonstrate comparable insecticidal activity against *Plutella xylostella* L. and *Spodoptera exigua* in a bioassay. Zheng S *et al.* found that dipeptide structures can help boost chlorantraniliprole absorption and phloem mobility, and two phloem mobile conjugates with satisfactory *in vivo* insecticidal effect were discovered as new candidates for high-efficiency insecticides.¹¹



Chlorantraniliprole (9)

DIPEPTIDE CONJUGATES FOR SKELETAL MUSCLE METABOLISM

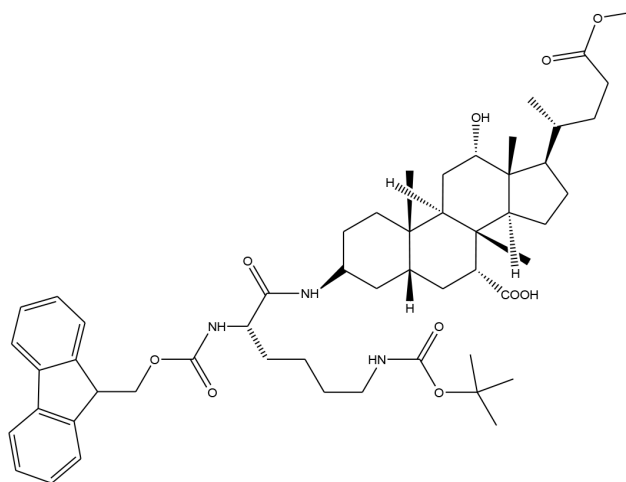
HCDs (histidine-containing dipeptides) have a variety of ergogenic and therapeutic effects, but their primary function in skeletal muscle is unknown. pH regulation, defence against reactive oxygen/nitrogen species, and Ca^{2+} regulation are all possible roles. A comparative physiology method was used to investigate physiological processes *in vivo*, for which two avian species, i.e., hummingbirds and chickens, were selected. The findings of Dolan E *et al.* indicate that HCDs are non-essential for the development of highly oxidative and contractile muscle. In contrast, their abundance in the glycolytic chicken muscle, indicates that they are important in anaerobic bioenergetics as pH regulators. This evidence provides new insights on the HCD role in skeletal muscle, which could inform widespread interventions, from health to elite performance.¹²



Anserine (10)

DIPEPTIDE CONJUGATES AS ANTIMICROBIALS

The existence of multidrug-resistant microbes is a major public health issue, necessitating the development of new antimicrobials on a regular basis. Antibiotics that target the membrane are promising candidates because they reduce the ability of microbes to establish resistance. The main reason for using cholic acid as the critical scaffold in this study is that it has a facially amphiphilic nature, which allows for plenty of opportunities to refine the amphiphilicity by connecting the amino acid lysine. By Singla P *et al.* sequentially linking lysine to C3-amino cholic acid methyl ester, a total of 16 novel amphipathic cholic acid derivatives were synthesised to preserve the hydrophobic/hydrophilic balance, which could be an important pre-requisite for antimicrobial activity. Among the synthesized conjugates, a series with fluorenyl-9-methoxycarbonyl moiety attached to cholic acid via lysine linker showed promising antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*.⁴

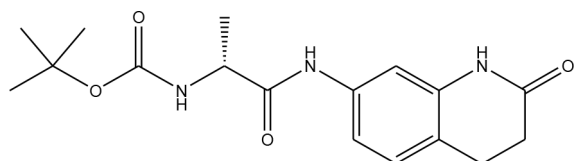


Monolysine-Derived Cholic Acid Conjugate (11)

DIPEPTIDE CONJUGATES AS ANTIMICROBIALS AND ANTIOXIDANT

New dipeptide-dihydroquinolinone derivatives were successfully synthesised by benzotriazole mediated nucleophilic acyl substitution reaction and their

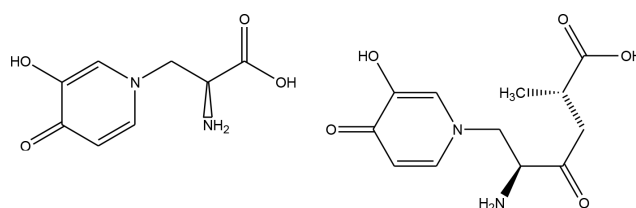
structures were elucidated by spectroscopic and analytic techniques carried out by Küçükbay H *et al.*⁴ The carbonic anhydrase (CA, EC 4.2.1.1) inhibitory activity of the new compounds was determined against four human (h) isoforms, hCA I, hCA II, hCA IX and hCA XII. While all compounds showed moderate to good *in vitro* CA inhibitory properties against hCA IX and hCA XII with inhibition constants at the micromolar level (37.7–86.8 and 2.0–8.6 μM , respectively), they did not show inhibitory activity against hCA I and hCA II up to 100 μM concentration. The antioxidant capacity of the peptide–dihydroquinolinone conjugates was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method. Most of the synthesised compounds showed low antioxidant activities compared to the control antioxidant compounds BHA and α -tocopherol.¹³



Dihydroquinolinone derivative (12)

DIPEPTIDE CONJUGATES AS MELANOGENESIS INHIBITORS

Melanogenesis and Cyclooxygenase Inhibitors have been improved. Melanogenesis (13) is responsible for the development of melanin pigments, which protect the skin from UV damage. However, an excessive accumulation of this pigment causes unsightly hyperpigmentation. In an attempt to develop effective depigmenting agents, a small library of mimosine dipeptide enantiomers (Mi-L/D-amino acid) that inhibit the melanogenesis in B16F10 melanoma cells by down-regulating the cellular tyrosinase with little effect on their growth or viability was developed. In B16F10 melanoma cells, two of them, Mi-D-Trp and Mi-D-Val, proved to be the most effective inhibitors of melanin content and cellular tyrosinase. Furthermore, most mimosine dipeptides were more effective than mimosine at inhibiting cyclooxygenase 1 (COX-1) with IC_{50} values ranging from 18 to 26 M. With IC_{50} values of 22 and 19 M, respectively, Mi-L-Val and Mi-L-Trp inhibited cyclooxygenase 2 (COX-2) more potently than indomethacin. The findings of Nguyen BCQ and Tawata S indicate that mimosine dipeptides may be better candidates (than mimosine) for anti-melanogenic (skin hyperpigmentation treatment) and cyclooxygenase (COX) inhibition than mimosine.¹⁴

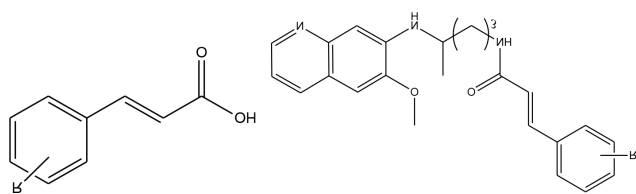


Mimosine (13)

Mi-D-Ala (14)

DIPEPTIDE CONJUGATES AS ANTIMALARIAL DRUGS

Cinnamic acids are natural compounds found in several different parts of a wide variety of plants, where they play a wide range of biological functions, mostly in conjugated form. These efforts by Zhao J *et al.* have been gradually revealing promising drug leads, but there is still a large chemical room that needs to be explored further. The combination or conjugation of cinnamic acids with known drugs has been approached in an attempt to achieve either synergistic or multi-target action, according to various published approaches.¹⁵



Cinnamic acid (15) Primaquine-cinnamic acid conjugate (16)

CONCLUSION


Dipeptide conjugates and amino acid conjugates synthesized using various heterocycles or active moiety found to show various biological activities against specific target. In the current advances of medicinal chemistry, dipeptide conjugates and amino acid conjugates are of utmost importance due to good specificity and stability, indicating them as good alternative to older therapeutic agents. In future, better indulgent about actions of these molecules will lead to more rational selection of targeting therapeutics for various activities against specific targets.

CONFLICT OF INTEREST

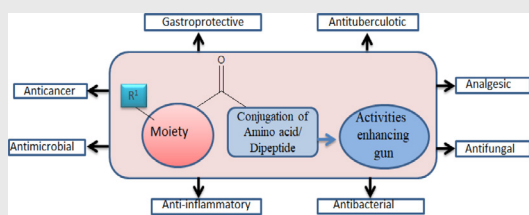
The authors declare no conflict of interest.

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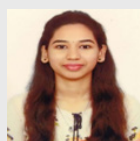
PICTORIAL ABSTRACT



SUMMARY

Overall, this systematic review emphasized the huge variety of dipeptide conjugates and or amino acid conjugates being studied as therapeutic agents. Such dipeptide-conjugates will proceed to hold an important place in pharmaceuticals and healthcare. We are hopeful that this review will expedite the future translational research in the field of peptide chemistry and medicinal chemistry.

About Authors



Ms. Ankita Borse, completed M. Pharmacy in Pharmaceutical Chemistry under the esteemed guidance of Dr. Nirmala V. Shinde from S.M.B.T. College of Pharmacy, Dhamangaon, Nashik. She has worked on research project "Synthesis and Characterization of some aldehyde-amino acid ester conjugates of medicinal interest". Presently she is working as QA Officer at KESAR PHARMA PVT.LTD. Gujrat



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Cite this article: Borse A, Shinde N. Dipeptide Conjugates: An Important Class of Therapeutic agents. *Indian J of Pharmaceutical Education and Research.* 2023;57(1):15-21.