Convenient Synthesis of novel amino acid coupled benzanilides

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Summary:

A facile and convenient synthesis of a variety of Methyl 2-(2-Benzoylamino-benzoylamino) alkanoate 4a-d has been developed by the DCC coupling of 2-Benzoylamino-benzoic acid with amino acid methyl ester. Compounds 4a-c were alternatively prepared by the reaction of aminoacid ester with 3,1-benzoxazinone 2 in pyridine. Dipeptides 8a-j were subsequently prepared following the azide coupling method. Compounds 4 and 8 were expected to possess antimicrobial activity.

Key Words: antibiotics, TCS, amino acids and dipeptide, DCC and azide coupling, benzanilides, 3,1-benzoxazinone.

Introduction

Pathogenic bacterial resistance to all existing classes of antibiotics has been recently detected. New classes of antibacterial are needed to overcome drug-resistant bacteria following a new mechanism of action. The new generation antibiotics are compounds that inhibit the two-component system (TCS) and are expected to block transcription of several genes in the bacteria. [1]. substituted salicylanilides are well known for their antimicrobial [2] and antifungal [2,3] action. Recently, salicylanilides have showed antimycobacterial activity against classical mycobacterium tuberculosis H37Rv [4]. They were proved to inhibit the bacterial TCS, which is also found in mycobacteria [5, 6]. Imramovský prepared a series of amino acids esters with highly active salicylanilides as a new group of prodrugs with high activity, improved solubility and low toxicity [7].

N-substituted carboxamide series of the isothiazole derivatives produced a remarkable activity: 5-benzoylamilno-N-(4-ethoxyphenyl)-3-methyl-4-isothiazolecarboxamide (ITF) I exhibited significant anti-inflammatory and broad antiviral activity [8], while 5-benzoylamine-N-(4-chlorophenyl)-3-methyl-4-isothiazole-carboxamide II, i.e. denotivir (ITCL, vratizolin) [9] exhibited antiviral drug, anti-inflammatory and immunotropic activities.

The pharmacophoric structure of all these compounds involve the presence of two phenyl rings linked to a spacer unit and two hydrogen bond donor sites. The spacer unit, when present, can be represented by a heterocyclic ring structure I and II, or by an acyclic moiety structure III and other salicylanilide derivatives.

This paper describes our development of a novel series of benzanilide derivatives IV whose chemical modifications include N-terminal coupled amino acid and dipeptide derivatives to 2-Benzoylamino-benzoic acid as active antibiotics agents. The amidic NH
could also represent one of the required H-bond-donor sites, while the other is the NH group of the amino acid residue.

![Chemical Structures]

**Results and Discussion:**
During the course of our continued interest in the development of new general synthetic routes for the synthesis of biologically active amino acid coupled derivatives we decided to synthesize methyl 2-(2-Benzoylamino-benzoylamino) alkanoate 4a-d. Treatment of 2-phenyl-4H-benzo[d][1,3]oxazin-4-one (2) with amino acid ester.HCl 3 in pyridine at 120°C for 24 h gave the acyclic amino acid ester derivative 4a-c in low yield [10]. However, a change in the reaction conditions (more reaction time at elevated temperature) and in the workup procedure (evaporation of pyridine under reduced pressure rather than aqueous workup) did not improve the yield of this reaction. This low yield could be explained by the reversibility of the reaction induced by the oxygen attack at the peptide bond with elimination of amino acid. This was confirmed by an endless amount of benzoxazine isolated from the reaction mixture.

![Chemical Reaction Scheme]

**Scheme 1**
The structure of acyclic amino acid ester 4a-d was chemically confirmed by an equivocal synthesis from the acid derivative 1 via DCC coupling method with an improvement in the yield of the reaction. The DCC coupling is one of the major tools employed in literature to introduce peptide bonds by the reaction of acid with amino acid methyl ester. Hydroxybenzotriazole (HOBT) is widely used as an additive to decrease racemization in the carbodiimide peptide coupling [11-13]. Thus, treatment of acid 1 with the amino acid esters hydrochloride 3 in presence of coupling reagents DCC and HOBT at 0 - 25 °C in acetonitrile afforded amino acid ester 4a-d, in 40-52 % yield. Benzoxazinone 2 was also isolated from the reaction mixture as a by product, which passively affected the yield of the reaction. This method has the advantage of a multi-component reaction with an overall moderate to good yield from easily available acid derivative 1 at low temperature to minimize the degree of racemization in amino acid coupling. The alternative reaction of benzoxazine in pyridine requires higher temperature and longer reaction time (120 °C, 24 h) to produce 4a-c. Both reactions reported herein are useful, due to their operational simplicity. In addition, the starting materials are readily available. One of our approaches involves formation of quinazoline 5 via intramolecular cyclocondensation of 4a-d. This approach usually involves similar conditions as employed for pyridine method [10]. However we failed to isolate quinazoline 5 using a variety of conditions (acetic anhydride, AcONa fused 90°C 24 h., DMF NEt3, NaOMe, MeOH, 60 °C , overnight).

The synthesis of the target dipeptide derivatives 8a-j were efficiently formed from key intermediate ester 4a-c via the azide coupling method [12, 13], which was reported to minimize the degree of racemization in amino acid coupling. The synthesis of dipeptide 8a-j via azide-coupling method is shown in Schemes 1, 2. The ester 4a-c was boiled with hydrazine hydrate in methyl alcohol to afford the hydrazide 6. The hydrazide 6 was subsequently converted into azide 7 by treatment with NaNO2 and HCl mixture Scheme 1, 2. The in situ generated azide 7 solution in ethyl acetate reacted with amino acid methyl ester hydrochloride 3 in the presence of triethyl amine to afford the the dipeptide derivative 8a-j in good yield, scheme 2.
The structure assignment of the amino acid ester 4a-d, hydrazide 6 and dipeptide 8 is based on spectral and physicochemical analysis, Figure 1.

Figure 1. Selected $^1$H NMR of compounds 4, 6 and 8
All isolated products exhibited a rather interesting conformation as represented in fig 1 and indicated from all 1H NMR spectra. Thus, the 1H NMR spectrum of 4a showed an interesting exchangeable singlet signal at δ 12.0 ppm corresponding to one NH group. This implies that the NH group participate in an intramolecular hydrogen bond interaction of the type N–H···O=C [9]. Further more, 1H NMR spectrum exhibited an aromatic proton at δ 8.84 ppm attributed to proton H3 of the phenyl ring, which implies an anisotropy caused by the adjacent carbonyl group. Both the hydrogen bond interaction and this anisotropy are present in all isolated products which indicate the fixed conformation of the basic acyclic skeleton. The 1H NMR spectrum of 4a also exhibits three signals at δ 6.85, 4.26 and 3.82 ppm corresponding to NH, CH2 and OCH3 of the glycine residue.

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\text{NH}_2 \quad \xrightarrow{\text{O}} \quad \text{NHNNH}_2 \quad \xrightarrow{\text{O}} \quad \text{NHCH}_2\text{COOCH}_3
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