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Research Article

SYNTHESIS, DOCKING STUDIES AND ANTIOXIDANT ACTIVITY OF 1, 3-BENZODIOXOLE-5-CARBOXYL AMINO ACIDS AND DIPEPTIDES

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ABSTRACT
A novel series of Benzodioxole-5-carboxyl-amino acids and dipeptide methyl esters were designed and synthesized by solution phase peptide technique using DCC and EDC as the coupling reagents. The synthesized compounds were characterized by FTIR, ^{1}H & ^{13}C NMR and Mass spectral techniques. Molecular docking was carried out to predict the anticancer activity of the designed molecules. The synthesized compounds were evaluated for their antioxidant activity as a preliminary study for anticancer activity. The compounds exhibited significant antioxidant activity as compared to the standard antioxidant BHT.
KEYWORDS: Benzodioxole-5-carboxylic acid, amino acid/dipeptide, antioxidant activity.

INTRODUCTION

Benzodioxole moiety can be identified in several bioactive natural anticancer agents, such as etoposide, teniposide, podophyllotoxin, steganacin and combretastatin.[1] Derivatives of these natural products are used as inhibitors of mono-oxygenase enzymes, pesticides or pesticide intermediates, herbicides, antioxidants, antimicrobials, and medicines.[2] Micale *et al.*[3] reported that 1, 3-benzodioxole derivatives[4-6] exhibited *in vitro* tumour growth inhibition activity.

In view of the diverse biological activities associated with benzodioxoles, we wish to report the synthesis and antioxidant activity of amino acids and dipeptides ester incorporated with 1,3-Benzodioxole-5-carboxylic acid. The molecule with Benzodioxole-5-carboxylic acid derivatives of amino acids and dipeptides were synthesized by using DCC/EDC mediated solution phase technique[7-9] of peptide synthesis. The acid group was protected by esterification process.

The Boc-amino acids were coupled with amino acid methyl ester hydrochlorides by using 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) as a coupling agent and triethylamine (Et_3N) as a base to get protected dipeptides esters. The Boc group was removed using trifluroacetic acid. The Benzodioxole-5-carboxylic acid was coupled with amino acid methyl ester hydrochlorides and dipeptides esters using DCC/EDC to get Benzodioxole-5-carboxylic acid derivatives of amino acid and dipeptides esters.

MATERIALS AND METHODS

All the reactions requiring anhydrous conditions were conducted in flame dried apparatus. The amino acids used are L-amino acid, purchased from Spectrochem Private Limited, Mumbai, India. Solvents and reagents were purified by standard methods. Boc-amino acids, amino acid methyl ester hydrochlorides and dipeptides were prepared by standard procedures. Organic extracts were dried over anhydrous sodium sulphate. Melting points were determined by an open capillary method and are uncorrected. The completion of the reaction and purity of the compounds were checked by thin layer chromatography. IR spectra were recorded on Nicolet impact 400 FT/IR using KBr pressed pellet technique. ^{1}H NMR spectra were recorded on GEOL-JMS D-400 (MHz) NMR spectrometer. Mass spectra were recorded on Shimandzu mass spectrometer. Molecular docking was performed using Hex 4.5 software.

Docking

Docking is the method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. It plays a key role in rational design of drugs; the results of docking can be used to predict whether the drug can bind to the protein with good complementarity. In the present work Hex 4.5 software is used to dock the ligands with the target protein. The five designed ligands were docked against target protein cellular tumor antigen 2IOI (Fig 1), which was collected from protein data bank (PDB). In standard virtual docking studies, ligands were docked into the

binding site of a receptor where the receptor was held rigid and the ligand was free to move (Fig 2). The docking score of five designed ligands were compared with 1,3-Benzodioxole-5-carboxylic acid and is mentioned in Table -1.

Preparation of the Dipeptides

Amino acid methyl ester HCl (10mmol) was dissolved in chloroform ($CHCl_3$) (20ml). To this triethylamine (Et_3N) (4 ml, 28.7mmol) was added at 0^0C and the reaction mixture was stirred for 15 minutes. Boc amino acid (10mmol) in chloroform (20ml) and EDC (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide) (2.2gm, 10mmol) were added with stirring. After 24hrs, the reaction was filtered and the residue was washed with $CHCl_3$ (30ml) and added to the filtrate. The filtrate was washed with 5% $NaHCO_3$ (20ml) and plain water (20ml).The organic layer was dried over anhydrous sodium sulphate (Na_2SO_4), filtered and evaporated in vacuum.

Synthesis of Benzodioxole-5-carboxyl-amino acids/peptides

Amino acid methyl ester HCl (10mmol) was dissolved in chloroform ($CHCl_3$) (20mmol). To this triethylamine (Et_3N) (4 ml, 28.7mmol) was added at 0^0C and the reaction mixture was stirred for 15 minutes. 1, 3-Benzodioxole-5-carboxylic acid (10mmol) in chloroform (20ml) and DCC (Dicyclohexylcarbodiimide) or EDC (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide) (2.2gm, 10mmol) were added with stirring. After 24hrs, the reaction was filtered and the residue was washed with $CHCl_3$ (30ml) and added to the filtrate. The filtrate was washed with 5% $NaHCO_3$ (20 ml) and plain water (20ml).The organic layer was dried over anhydrous sodium sulphate (Na_2SO_4), filtered and evaporated in vacuum.

To remove the traces of Dicyclohexyl urea (DCU), the product was dissolved in minimum amount of $CHCl_3$ and cooled to 0^0C. The crystallized DCU was removed by filtration. Petroleum ether was added to the filtrate at 0^0C to recrystallize the pure product.

The carboxyl group of 1, 3-Benzodioxole-5-carboxylic acid is coupled with carboxyl protected amino acids or dipeptides using DCC/ EDC and Et_3N to get the Heterocyclic-coupled compounds. The physical data of the synthesized compounds is mentioned in the tabular column-2.

Antioxidant activity

The free radical scavenging activity of synthesized compounds was measured by DPPH (1, 1-diphenyl-2-picrylhydrazyl) by modifying Blois method, where in the bleaching rate of a stable free radical; (DPPH) is monitored at a characteristic wavelength in the presence of sample. In its radical form, DPPH absorbs at 517nm, but upon reduction by an oxidant or a radical species its absorption decreases. All the synthesized compounds were evaluated in vitro for their antioxidant activity. The compounds were tested at the concentration of 25, 50 and 75 µg/ml. Butylated hydroxyl toluene (BHT) was taken as standard[10-12]. The results are presented in table-3.

Spectral Data

Compound-1: 1, 3-Benzodioxole-5- carboxyl~Val-OH: Pale yellow solid (64%), m.p- 129-131°C. **I.R. ($^{-1}$):** 3313.71 (N-H stretch), 3064.89 (Ar C-H stretch), 2931.80, 2852.72 (aliphatic C-H stretch), 1697.36 (C=O stretch (acid)), 1647.21 (C=O stretch amide linkage). **^{1}H NMR ($CDCl_3$):** δ1.291 (d, 1H, CH_3 δ1.991-2.031(m, IH, CH), δ3.54 (d, 1H, CH), δ6.737 (s, 2H, CH_2), δ7.07-7.514 (m, Ar-H, 3H) ; **^{13}C NMR:** δ170.530 (C=O of acid),165.935 (C=O of amide), δ154.537-121.292 (6C, Ar-C), δ101.616, 57.532, 30.785 (aliphatic-C) . **MS m/z** (rel. intensity): 264.1 (M^-, 100).

Compound-2: 1, 3-Benzodioxole -5-carboxyl~Phe-OMe: Yellow solid (82%), m.p- 81-83°C. **I.R. (cm^{-1}):** 3323.35(N-H stretch), 3022.45 (Ar C-H stretch), 2924.09 (aliphatic C-H stretch), 1747.51 (C=O stretch (ester)), 1641.42 (C=O stretch (amide linkage)). **^{1}H NMR ($CDCl_3$):** δ3.251 (m, 2H, CH_2), δ3.780 (s, 3H, -OCH_3), δ5.064 (m, 1H,CH), δ6.029 (s, 2H, CH_2), δ6.503-7.333 (m, 8H, Ar-H), δ7.520 (br, 1H, NH) . **^{13}C NMR:** δ172.164 (C=O of ester), 166.127 (C=O of amide), δ150.606 -107.652 (12C, Ar-C), δ101.731, 53.568, 37.906 (aliphatic-C), δ52.431 (C-O). **MS m/z** (rel. intensity): 327.9 (100).

RESULTS AND DISCUSSION

Structural modification of 1, 3-Benzodioxole-5-carboxylic acid was carried out by coupling amino acid / dipeptides methyl ester with the carboxyl group of 1, 3-Benzodioxole-5-carboxylic acid and the synthesized compounds were characterized by FTIR, ^{1}H NMR, ^{13}C NMR and Mass spectral analysis. Docking studies have been carried out for all the synthesized compounds with target protein (2IOI), which showed moderate anticancer activity. The compounds were subjected to antioxidant evaluation by DPPH method. All the compounds showed moderate antioxidant activity when compared to the standard antioxidant butylated hydroxyl toluene (BHT). The dipeptide methyl ester coupled to benzodioxole-5-carboxylic acid showed less antioxidant activity compared to the standard antioxidant. The aliphatic amino acid ester coupled to benzodioxole-5-carboxylic acid showed very good antioxidant activity compared to

the standard antioxidant. The antioxidant activity of aliphatic amino acid esters was found to be greater than aliphatic amino acids.

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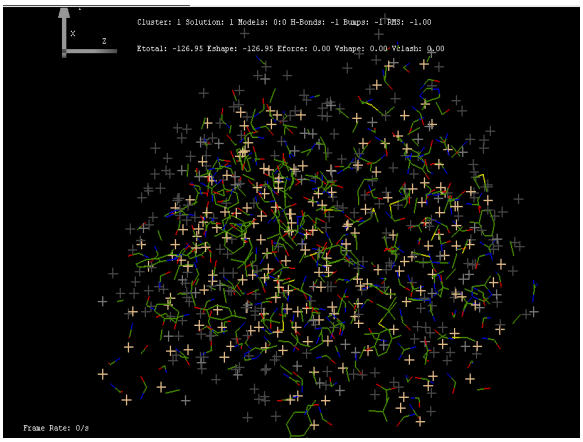


Fig: 1- 3D View of 2IOI Protein

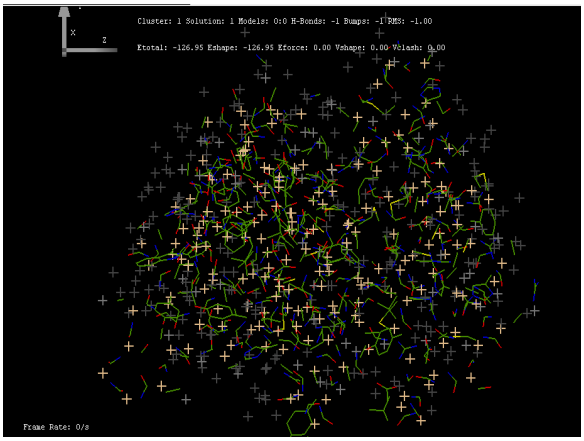


Fig:2- Binding interaction of 2IOI protein with compound 3.3

Table 1: Molecular Docking Score of Designed Ligands

S.NO.	SYNTHESISED COMPOUNDS	PDB CODE	Etotal(KJ/Mol)
1.	1, 3-Benzodioxole-5-carboxylic acid	2IOI	-111.27
2.	1,3-Benzodioxole-5- carboxyl~Ala-OMe	2IOI	-120.23
3.	1,3-Benzodioxole-5- carboxyl~Val-OH	2IOI	-122.18
4.	1,3-Benzodioxole-5- carboxyl~Val-OMe	2IOI	-122.53
5.	1,3-Benzodioxole -5-carboxyl~Phe-OMe	2IOI	-123.97
6.	1,3-Benzodioxole-5- carboxyl~Ile-Ile-OMe	2IOI	-126.95

Table- 2: Physical data of synthesized amino acid/ dipeptide Benzodioxole-5-carboxylic acid

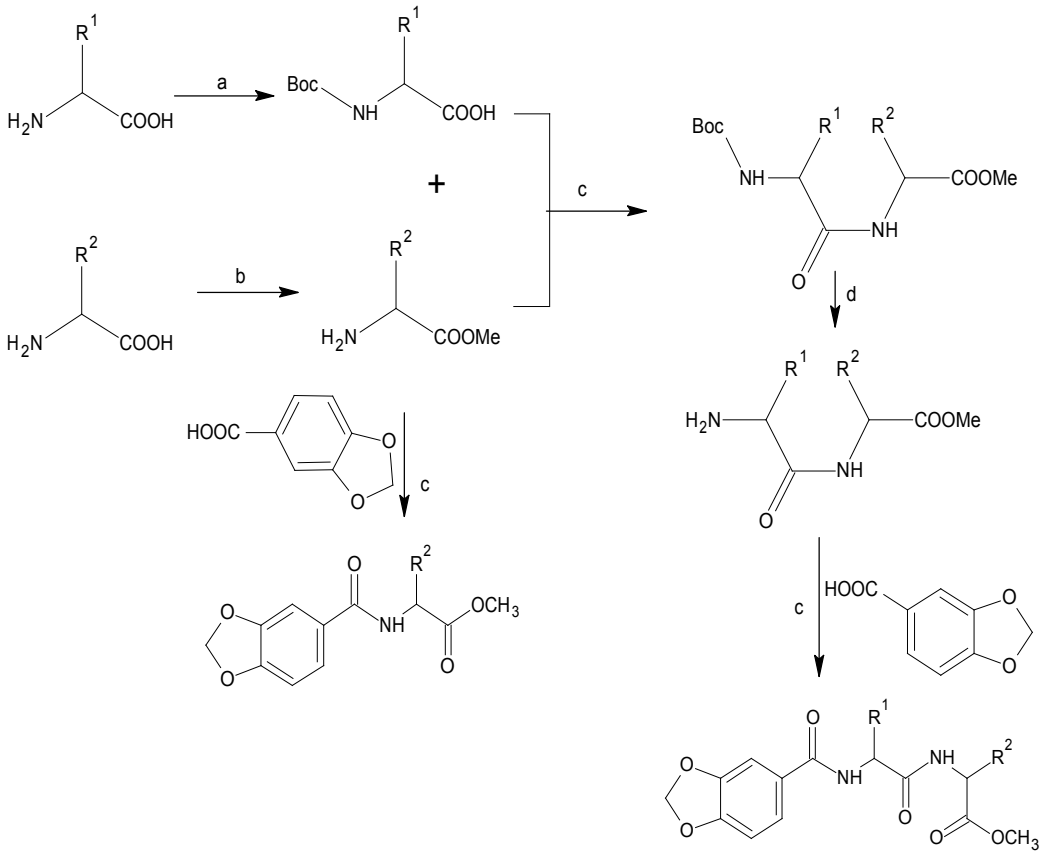
S. No	Benzodioxole carboxyl~amino acid/OMe	Physical state	M.P(^{o}C)	Yield (%)
1.	Benzodioxole-5- carboxyl~Val-OMe	Pale yellow solid	142-144	79
2.	Benzodioxole-5- carboxyl~Ala-OMe	Pale yellow solid	94-96	71
3.	Benzodioxole-5- carboxyl~Phe-OMe	Yellow solid	81-83	82
4.	Benzodioxole-5- carboxyl-valine	Pale yellow solid	129-131	64
5.	Benzodioxole-5- carboxyl~Ile-Ile-OMe	Semi solid mass	-	56

Table- 3: Results of Antioxidant activity

S.No.	SYNTHESIZED COMPOUNDS	%INHIBITION		
		25µg/ml	50µg/ml	75µg/ml
1.	Benzodioxole-5-carboxyl~ Val-OMe	57.25	83.06	91.12
2.	Benzodioxole-5-carboxyl~ Val-OH	56.51	66.12	75.80
3.	Benzodioxole -5-carboxyl~Phe-OMe	43.54	80.64	82.64
4.	Benzodioxole-5- carboxyl~Ile-Ile-OMe	41.93	43.54	46.77
5.	Benzodioxole-5- carboxyl~Ala-OMe	58.06	65.32	89.51
6.	BHT (Standard)	45.96	70.16	91.93

R1 and R2 = Side chain of amino acids
a = (Boc)2O, 1N NaOH, 2h, RT
b = SOCl2, CH3OH, 10hrs, reflux
c = EDC/DCC, Et3N, CHCl3, 24h, RT
d = TFA, CHCl3 , 2h, RT
e = THF:H2O, LiOH, 1h, RT

Scheme-1



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