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ULTRASOUND-ASSISTED SYNTHESIS AND BIOLOGICAL EVALUATION OF 1,4-BENZODIOXANE-2-CARBOXYL-AMINO ACIDS AND PEPTIDES

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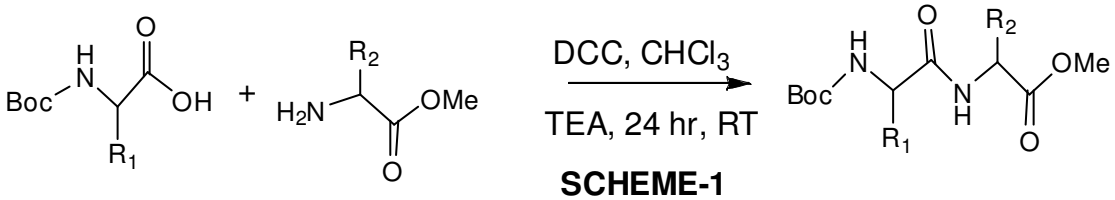
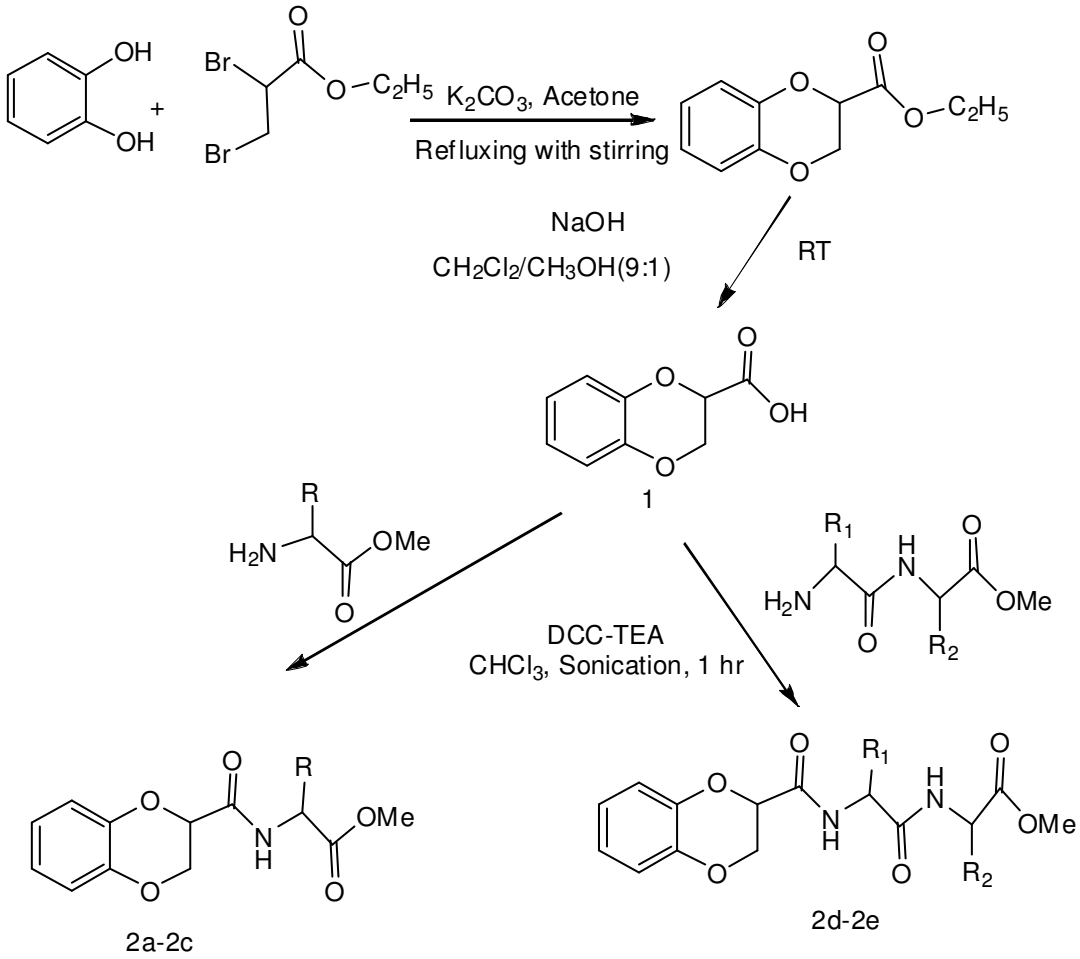
A series of peptides containing 1,4-benzodioxane nucleus were attempted to synthesize by conventional as well as green techniques like microwave-assisted and sonication, using dicyclohexylcarbodiimide (DCC) as a coupling reagent and triethylamine as a base. Sonication has proved to be the efficient method for the synthesis of the title compounds. All the compounds were characterized by FTIR, ^{1}H NMR and Mass spectral studies. They were evaluated for their insecticidal and anthelmintic activities. All the compounds showed moderate anthelmintic activity as compared to the standard drug mebendazole. However, the compounds exhibited potent insecticidal activity, the most potent being 1,4-benzodioxane-2-carboxyl-phenylalanine-tryptophan-methyl ester as compared to the standard, chloropyrifos.

The 1,4-benzodioxane moiety represent a series of compounds of considerable medicinal importance. It is present in many natural and synthetic compounds and is kown for its excellent pharmacological activity. Some, 1,4-benzodioxane derivatives are cardiovascular agents as adrenoreceptor antagonists[1-3], while other compounds with a high affinity for 5-HT receptor subtypes[4] have been shown to exhibit neuroleptic activity. It also shows antihepatotoxic activity[5], antiaggregator activity[6], glycogen phosphorylase inhibitory activity[7], antioxidant activity[8-9] and anti-inflammatory activity[9]. The 1,4-benzodioxane structure constitutes an ideal precursor of 1,4-benzodioxins, a relatively little studied class of compounds with only a few synthetic preparation methods available[10,14]. The 1,4-benzodioxane system is considered homologous to the 1,4-benzodioxepin, substructure of the known calcium antagonist HP-406[11]. Therefore the presence of the moiety gives the molecule calcium antagonistic activity also. It is also known to have insecticidal and anthelmintic properties. The benzodioxane moieties are also useful in increasing the aromaticity of any compound attached to it. As we know, aromaticity plays a large role in the absorption of substances in the stomach. Therefore, it can be used to increase the absorptivity of substances in the digestive system and therefore go a long way in making a drug orally active. Fusion of the amino acids and peptides with the benzodioxane moiety may result in increased biological activity due to their penetration enhancing property and hence



SCHEME-1



R=side chain of Phe (2a), Trp (2b), Gly (2c)
R_1, R_2=side chains of Phe, Trp (2d) and Phe, Gly (2e)

SCHEME-2

Table-1
Optimization of reaction conditions for synthesis of **2**a-e

Sl. No.	Reaction conditions	Time (hr)	Results	Yield (%)
1	Microwave (440 W)	0.5	Charred	-
2	Sonication	1	Reaction completed	92-95
3	Conventional (stirring, RT)	30	Reaction completed	85-89
4	Reflux	72	No reaction	-

increase of bioavailability of the bioactive molecule or drug.

For past two decades, green and accelerated techniques[12] have invaded the synthetic laboratories due to the advantages of drastically reduced time, energy and improved yields. Hence, an attempt has been made to synthesise the title compounds with various techniques like conventional as well as microwave-assisted and ultrasound-assisted (sonication) methods.

Anthelmintic activity

Anthelmintic activity studies were carried out against earthworms (*Eudrilus eugeniae*) by Garg *et al* method[15]. Suspensions of the samples were prepared by triturating the samples with 15% tween 80 and distilled water and the resultant mixtures were stirred using a mechanical stirrer for 30 mins. The resulting suspensions were used for the activity studies. The suspensions were diluted to contain 100mg in 5 mL of the test samples. Standard drug, Mebendazole was also prepared in the same concentration in a similar way.

Five earthworms of similar sizes were placed in a Petri plate of 4 inches diameter containing 50 mL of suspension, the test standard drug (Mebendazole) at RT. Another set of five earth worms was kept as control in 50 mL suspension of distilled water and 15% tween 80. 50 ml each of the suspensions of the test compounds were added into separate petri plates containing five earthworms in each. The time required for the paralysis and death of the worms was noted. The death time was ascertained by placing the earthworms in warm water at 50^0, which stimulated the movement if the worms were alive.

The synthesized compound have shown moderate anthelmintic activity against *Eudrilus eugeniae* at the concentration of 100 µg/5 mL of test samples, as compared to the standard drug Mebendazole.

Insecticidal activity

Insecticidal activity was carried out on termites (*Coptotermes formosanus*) by Morita *et al*[16] method. Suspensions of the samples were made in acetone such that the concentration was 70 mg/mL. Each of the samples was spread uniformly across a filter paper placed at the bottm of a petri plate. The top of the petri plate was packed with wet cotton. 5 termites were introduced in each of these petri plates. As control, a petri plate with only wet cotton packed on the top and filter paper below was used. Termites were placed in it. Standard drug (Chloropyrifos) with similar concentration as the sample was loaded onto another petri plate containing filter paper. The activity of the termites was continuously monitored.

Compounds **2**a, **2**c and **2**e showed better insecticidal activity against *Coptolermes formosanus* at the concentration of 70 mg/mL of test samples, as compared to the standard drug Chloropyrifos, while

the other two compounds (**2**b and **2**d) showed moderate insecticidal activity.

Experimental

The amino acids are purchased from Spectrochem Private Limited, Mumbai. Organic extracts were dried over anhydrus 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 Jasco FT-IR-5300 IR spectrometer using a thin film supported on KBr pellets for solids and chloroform as a solvent for semisolids (λ_{max} cm^{-1}). ^1H NMR spectra were recorded in $CDCl_3$ on Bruke-AC NMR 400 MHz spectrometer. Mass spectra were recorded on Shimadzu GC-MS (at 70eV) Mass spectrometer using xenon as the carrier gas. Boc-amino acids, amino acid methyl ester hydrochlorides were prepared by standard procedure[13].

Preparation of dipeptides[14]

Amino acid methyl ester hydrochloride (10 mmol) was dissolved in chloroform ($CHCl_3$) (20 ml). To this, triethylamine (TEA) (4 ml, 28.7 mmol) was added at 0^0C and the reaction mixture was stirred for 15 mins. Boc-amino acid (10 mmol) in $CHCl_3$ (20 mL) and DCC (10 mmol) were added with stirring. After 12 hrs, the reaction mixture was filtered and the residue was washed with $CHCl_3$ (30 mL) and added to the filtrate. The filtrate was washed with 5% $NaHCO_3$ (20 mL) and saturated NaCl (20 mL) solutions. The organic layer was filtered and evaporated in vacuum. To remove the traces of the dicyclohexylurea (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. Boc-Phe-Trp-OMe and Boc-Phe-Gly-OMe were prepared in this manner (Scheme-1).

1,4-Benzodioxane-2-carboxylic acid (1)

Anhydrous potassium carbonate (5g) was added in portions to a stirred solution of 5.5g of catechol in 20 mL of dry acetone followed by the drop wise addition of 3.4g of ethyl-2,3-dibromopropionate. Another 5g of potassium carbonate and 3.4g of the dibromoester were added similarly and this was repeated two times more using a total of 20g of potassium carbonate and 13.6g of ester. Stirring and refluxing was continued for another 15 hr. The reaction mixture was then filtered and the solid was washed several times with acetone. The filtrate was concentrated to about 10 mL and the residue was diluted with 10mL of cold water. The oily layer was separated from the aqueous layer and the latter was extracted repeatedly with ether. The combined oily layer and ether extracts were washed with water, dried over magnesium sulfate, and evaporated. The dark residue was distilled at $96\text{-}97^0$ to get the desired ester[17]. This was followed by ester hydrolysis to give 1,4-benzodioxane-2-carboxylic acid.

Coupling of 1,4-benzodioxane-2-carboxylic acid with amino acids and dipeptides

The dipeptides after appropriate deprotection and the amino acid methyl esters were coupled with 1,4-benzodioxane-2-carboxylic acid using DCC as a coupling reagent and TEA as a base in different reaction conditions like microwave, sonication, refluxing and conventional methods to give the desired products (**2**a-**2**e) (Scheme-2).

1,4-Benzodioxane-2-carboxyl-phenylalanine methyl ester (2a)

IR (cm^{-1}): 3325 (NH stretch), 2928 (CH stretch, asym), 2863 (CH stretch, sym), 1746, 1673 (C=O stretch), 3029 (Ar-CH stretch). ^1H NMR : δ 7.4-6.8 (9H, m, ArH), 6.1 (1H, b.s., NH), 4.9 (1H, m, C_3H), 4.7 (1H, m, C_2H), 4.5 (1H, m, C_3H), 4.25 (1H, m, C_3H), 3.7 (3H, s, OMe-H), 3.15 (2H, m, β C-H), m/z value: Molecular ion peak at 342, $C_{19}H_{19}NO_3$, MW-341.6, yellow semisolid.

1,4-Benzodioxane-2-carboxyl tryptophan methyl ester (2b)

IR : 3320 (NH stretch), 2929 (CH stretch, asym.), 2854 (CH stretch, sym), 1656 (C=O stretch), 3045

(Ar-CH stretch). ^{1}H NMR : 10.2 (1H, s, Indole N-H), 7.35 (5H, m, ArH), 6.9 (4H, m, ArH), 6.1 (1H, b.s., NH), 4.9 (1H, m, C_3H), 4.7 (1H, m, C_2H), 4.5 (1H, m, C_3H); 4.25 (1H, m, C_3H), 3.7 (3H, s, OMe-H), 3.15 (2H, m, β C-H), m/z value: Molecular ion peak-indole ring peak at 268, $C_{21}H_{20}N_2O_5$, MW-380.14, brown semisolid.

1,4-Benzodioxane-2-carboxyl-glycine methyl ester (3c)

IR : 3316 (NH stretch), 2930 (CH stretch, asym), 2855 (CH stretch, sym), 1657 (C=O stretch). ^{1}H NMR : 6.8 (4H, m, ArH), 6.1 (1H, b.s., NH), 4.9 (1H, m, C_3H), 4.7 (1H, m, C_2H), 4.5 (1H, m, C_3H), 4.25 (1H, m, C_3H), 3.7 (3H, s, OMe-H), m/z value: Molecular ion peak at 253, $C_{12}H_{13}NO_4$, MW-251.23, brown semisolid.

1,4-Benzodioxane-2-carboxyl-phenylalanine-tryptophan methyl ester (2d)

IR : 3308 (NH stretch), 2929 (CH stretch, asym.), 2856 (CH stretch, sym.), 1648, 1689 (C=O stretch). ^{1}H NMR : 10.2 (1H, s, indole N-H), 7.4-6.8 (13H, m, ArH), 6.1-5.9 (1H, b.s., NH), 4.9 (2H, m, C_3H), 4.7 (1H, m, C_2H), 4.5 (1H, m, C_3H), 4.25-4.1 (1H, m, C_3H), 3.7 (3H, s, OMe-H), 3.15-2.9 (2H, m, β C-H), m/z value : Molecular ion peak at 525, $C_{30}H_{29}N_3O_5$, MW-527.21, brown semisolid.

1,4-Benzodioxane-2-carboxyl-phenylalanine-glycine methyl ester (2e)

IR : 3305 (NH stretch), 2925 (CH stretch, asym.), 2856 (CH stretch, sym.), 1649, 1700 (C=O stretch), 3031 (Ar-CH stretch). ^{1}H NMR : 7.4-6.8 (9H, m, ArH), 6.1 (1H, b.s., NH), 4.9-4.7 (3H, m, C_3H), 4.6 (1H, m, C_2H); 4.5-4.4 (1H, m, C_3H), 4.25-4.1 (1H, m, C_3H), 3.5 (3H, s, OMe-H), 3.15-2.9 (2H, m, β C-H), m/z value : Molecular ion peak at 398, $C_{21}H_{22}N_2O_6$, MW-398.4, yellow semisoled.

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