Original Article

Insilico Design, Synthesis of Hybrid Taurine Amino Acid and Peptide Analogues for Studies on Antioxidant and Hepatoprotective Activity

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ABSTRACT

Introduction: The liver performs normal metabolic homeostasis of the body as well as biotransformation, extraction and detoxification of many compounds. Due to this it is more susceptible to disease. Near about 900 drug are withdraw from the market due to hepaptoxicity. The objective of the work to synthesis the series of hybrid taurine amino acid and peptide analogues in which the various combinations of taurine amino acid, Di-peptide and Tri- peptide were synthesized. **Aims**: In this study, we mainly focused on the hepatoprotective aspects of hybrid taurine amino acid peptides analogues before the synthesis we carry out the *insilico* designing of molecules and from the results of this *insilico* study we carry forward the synthesis of hybrid compound followed by their *in vitro* and *in vivo* studies. **Results:** the binding affinity of the designed compound towards CYP2E1 (3GPH) was selected on the basis docking score The compound SSSB-16 shows the maximum score having the docking score is -24.84 as compared with single taurine and other taurine hybrid compound. The compound SSSB 15 is second in the list of docking score with the docking score is -24.67. The reference ligand .having the docking score is -11.90. All the compounds were screened for their *in vitro* antioxidant activity by employing DPPH, Nitric oxide scavenging method. From the *in vitro* result of antioxidant activity those compound which had shown maximum activity till use for hepatoprotective activity. The compound SSSB 3 which is the combination of Taurine-Glycine-Glycine shows the maximum activity as compared to all other compounds. **Conclusion:** From result good activity was noted for **SSSB3 (Taurine-Gly-Gly)** compound. From this it can be concluded that the amino acid hybrid with future being proof to be novel compound as hepatoprotective activity. It may be use as a supplement with the drugs to reduced hepatotoxicity.

Key words: *Taurine, Peptides, Cyp2e1, Antioxidant, Hepatoprotection.*

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INTRODUCTION

Amino acids are attracting increasing attention as therapeutics due to their role as mediators of key biological functions associated with their low toxicity and high specificity. A large number of peptide –based drugs are now being marketed because new synthetic strategies have been developed in recent year. Many amino acid conjugate is known for reducing hepatotoxicity and improving the physicochemical properties of the various drugs. The amino acids are the dietary constituents and nontoxic as compared to the other carrier. The water soluble amino acids have the ability to increase the water solubility of poorly soluble drug.[1] Due to this property, amino acid helps to improve the bioavailability and the pharmacokinetics of drugs. In this paper we are trying to focus on the *insilico* design, synthesis and there antioxidant study of some novel taurine and its hybrid amino acid peptide analogues. Taurine has been mostly used as an antioxidant apart from this it is also used in the treatment of various disease conditions like

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cardiovascular diseases, epilepsy, and macular degeneration, Alzheimer's disease, hepatic disorders, cystic fibrosis and the treatment for alcoholism.[2]

Chemistry of Taurine

Taurine or 2 aminoethane sulfonic acid Figure 1 is an organic acid. It is also the major constituent of bile acid. It is the derivative of sulfur containing amino acid. The taurine is a small molecule and consist of carbon, hydrogen, nitrogen, sulfur, and oxygen.

Mostly amino acids have L or D – configuration which is having capacity to rotate the plane polarized light. But in case of taurine this property is absent because taurine does not have the L or D configurations. Taurine is water soluble and does not easily cross fatty membranes of the body. The physicochemical properties of Taurine [Table 1].[2,3]

In this we manly focus on *Insilico* drug design it includes Docking, homology modeling, virtual screening, structure activity relationship and scrutiny of data as the essential component. Thus, all components are very important for new drug designing, discovery of new molecules for the target specificity and superior affinity of a compound. Apart from this pharmacokinetic parameter like Absorption, Distribution, Metabolism and Excretion and Toxicity studies are being equally important for the screening of new drug molecules.[4]

In this study we carried out *insilico* modeling of all the proposed derivatives of taurine hybrid amino acid and peptide analogues using different computational software for the prediction of physico-chemical and physiological parameters. From this the best resulted compound are used to carry out synthesize and furthers for its antioxidant and hepatoprotective study.

MATERIALS AND METHODS

Docking study was carried out on HP Intel® core 2 duo ® processor E8300@2. 83Hz with 1GB RAMS, having 32-BIT operating system, windows vista using Bisolve IT (Lead IT 2.1.3), 1TB Hard disk. All the structures were drawn by using Chem. Office (version: 3.5) and there energy minimization was done. The Ligprep, were performed by using the Ligand Preparation wizard of Maestro8.5 of Schrodinger software installed on a Dell system (3.4GHzprocessor, 512RAM, 80GBHard disk with RedHat Linux Enterprise(version3.0) as the operating system. All chemicals which are used for synthesis were purchased from the following companies: Sigma-Aldrich corpora ration, Merck& CO., Inc., Rankem, Spectrochem Pvt. Ltd. or Merck India. Solvents were of reagent or HPLC grade and were purified and dried by usingstandard procedure. The melting points of compounds were determined using OPTIMELT (Stanford research systems, California). Reactions were monitored using thin layer chromatography (TLC) on silica gel plates. The spots were visualized either in iodine vapors or under UV light at 254nM in a suitable UV chamber (Z-Glass, India).Taurine is purchased from the CDH (Central Drug House) of 99% assay purity. Elemental analyses were performed. Mass spectra and ^{1}H NMR Spectra were recorded in DMSO.

Receptor and Enzymes

The co-crystallized structure of the target Protein, of CYP2E1 were obtained from the RSCSB Protein Data Bank (PDB) database; having PDB ID 3GPH (http://www.pdb.org.).

Ligand preparation

The proposed Taurine hybrid amino acid and peptide molecules were drawn by using Chem Office 10.0. There energy minimization was performed to recognize low energy conformers and saved in the mol2 file format. These molecules were imported into the project table of the Maestro 5.0, Schrodinger and ligand preparation was done from the application option and exported in the SDF format. Further, these molecules were imported into the docking library of FlexX docking software and used for docking. In this the all molecules were analyzed for their energy of tautomeric and for stereochemistry.[5]

Validation of docking protocol

In validation of docking the Reference ligand which is bind with receptor protein was removed from the active site of the receptors 3GPH and re-docked in same receptor and its conformation and orientation were compared with the conformation and orientation of the original X-ray crystallographic structure downloaded from Protein Data Bank.[13] The RMSD value of was below 2 A^{0}. That indicates that the docking method used has good ability to reproduce conformation. It was used for docking of Taurine hybrid amino acid and peptide.

Molecular docking

The structures of various conformations of the molecule and targeted receptor protein (3GPH) were loaded into the Biosolve IT Flex X. The Ligand Preparation of all the agonists were done using the application option of maestro 5.0 and loaded as the docking library and then Docking was done to generate best pose by analyzing the interactions and binding affinities and their score, it poses both 2D and 3D views generated and saved.[6,7]

General method for protection of amino acids

A mixture of purified phthalic anhydride (0.01mol,) and amino acid (0.01 mol,) was heated on sand bath, within 25 minutes the mixture melted; heating was continued for more 10 minutes. The reaction mixture was cooled, the residue was dissolved in ethanol and filtered off water was added to clear filtrate, till turbidity occurs and then the solution was kept at 0^0C for 24 hours fine crystals of Phthaloyl amino acid separated were filtered and dried and there melting point Rf value were found out for their characterization.[8,9]

Method for Preparation of Di-Peptides

Preparation of chlorophosphate ester (CPE) reagent

Phosphorous pentachloride (0.06mol) was taken in a clean and dry RBF, which was cooled at 0-5°C. To it anhydrous ethanol (0.06mol) was added drop wise over a period of 10 min with constant stirring first 10 ml of anhydrous alcohol was added with due precaution as phosphorous pentachloride gives highly corrosive fumes in presence of air or moisture. After the complete addition of ethanol, mixture was stirred for further half an hour. The CPE reagent thus prepared was stored in tightly closed container.

Caution: Special care was taken while handling phosphorus pentachloride and exposure to eyes and hands was avoided as far as possible.

Phthaloyl Glycine-Gly

The CPE reagent was added to Phthaloyl Glycine (0.01mol) and then stirred to a clear solution. Then Glycine (0.01mol) was added to the mixture and stirred to a clear solution. To this mixture triethylamine was added to pH 7 maintaining the reaction temperature below 5°C. This mixture was left for 6 hours at 0°C, crystalline product separated was filtered and washed with solvent ether and further deprotection of Phthaloyl group was done, dried and characterized. The procedure was repeated for other combinations of Di-peptides.

Preparation of Tri-Peptides

Gly-Gly-Glycine

The PhthaloylGly-Gly It was then dissolved in CPE using minimum amount. Glycine (0.01mol) was added and stirred to clear solution. Triethylamine was added to pH7 maintaining the temperature below 5°C. The mixture was left for 6 hrs at 0°C.The solid separated was filtered, dried and washed with solvent ether. The product obtained is Phthaloyl Gly- Gly-Glycine. It was dissolved in 20 ml ethanol. Hydrazine hydrate (70%) was added to the mixture and heated on steam bath for 2 hrs. The reaction mixture was cooled, acidified with conc. HCl and again heated at 50°C for 1 hr Phthaloyl-hydrazide was removed by filtering it out. The filtrate was neutralized with pyridine to liberate free peptide. Removal of solvent was done by vacuum distillation followed by crystallization from ethanol.

ANTIOXIDANT ACTIVITY

DPPH Scavenging activity

DPPH is stable radical that can accept an electron to form a stable diamagnetic molecule due to presences of odd number of electron. This DPPH Methanolic solution shows a strong absorption band at 517 nm by accepting the electron from the electron donating molecules. It forms colourless 2, 2'-diphenyl-1-picryl hydrazine reduction of the DPPH radicals can be estimated quantitatively by measuring the decreasing in absorbance at 517 nm.

Equal volumes of 100 µM DPPH in methanol was added to different concentration of synthesizes compounds from 10-250uM/ml in methanol ,mixed well and kept in dark for 20 min. the absorbance at 517 nm was measured using the spectrophotometer. Plotting the percentage DPPH scavenging against concentration gave the standard curve and the percentage scavenging was calculated from the following equation:

$$Scavenged\ (\%) = \frac{A\ cont - A\ test}{A\ cont} \times 100$$

Where A cont. is the absorbance of control (control containing all reagents except the test compound), A test is the absorbance of test compound at different concentrations.

IC_{50} was calculated from a plot between concentration of test compound and % scavenging .for DPPH ascorbic acid was used as a standard.[10]

Nitric Oxide Scavenging Activity

The nitric oxide will be generated by sodium nitropruside in presences of an antioxidant scavenger the amount of Nitric oxide generated will be less. The excess nitric oxide will be estimated by Griess reagent. Thenitric oxide will give pink colour complexes estimated at 540 nm.

Procedure: in a reaction mixture containing sodium nitropruside (4 ml).phosphate buffer saline solution (1 ml) and various concentration of synthesized testing compounds were incubated at 25°C for about 150 min

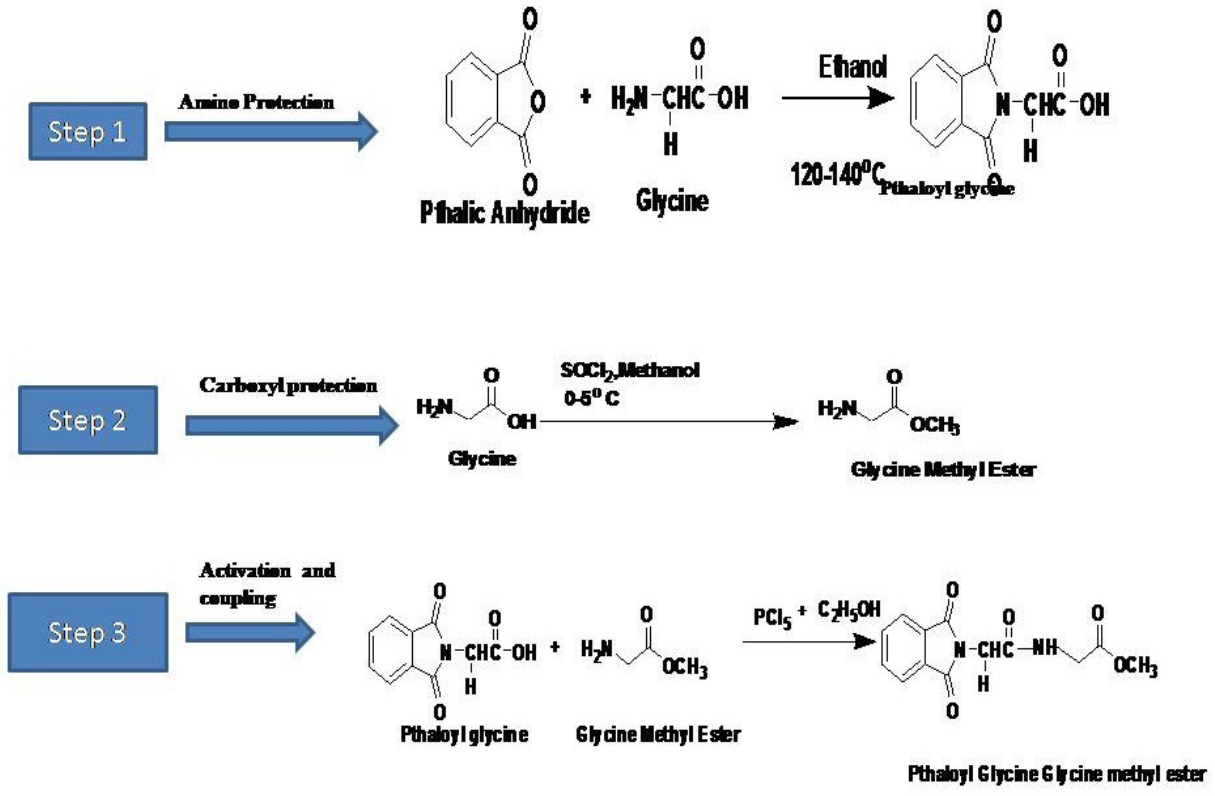


Figure 4:Scheme for synthesis of Taurine –amino acid, Dipeptides, Tripeptides compounds

after incubation, 0.5 ml of reaction mixture containing nitrate was removed and 1.0ml of sulphanilic acid was added mixed well and allowed to stand for 30 min in dark at room temperature the absorbance of these solution was measured at 540 nm against corresponding blank solution without sodium nitropruside. The scavenging and IC_{50} values were determined.[10]

EVALUATION OF HEPATOPROTECTIVE ACTIVITY

Those compounds which had shown potent antioxidant activity are further studied for its hepatoprotective activity. The compound includes **SSSB1, SSSB2, SSSB3, SSSB6, SSSB7, SSSB9, SSSB15, and SSSB16.** The studies were carried out onhealthy Wistar rats weighing from 200± **25**gm. The animals were collected randomly from animal house of PBRI, Bhopal. These animals werehoused in separate cages under controlled condition of temperature from 22 to 24 °C. All animal were given standard diet and water ad libitum. This animal also kept at 12: 12 light and dark cycle and finally it is dividing into group eleven containing three animals in each group. The study protocol was approved by the institutional animalethics committee of Pinnacle Biomedical research institute Bhopal. Registration Approval No:PBRI/IAEC/PN-16046dated 17th Jan 2017.

Acute oral Toxicity test

Acute oral toxicity test was carried out as per the protocol given in the OECD 423 guidelines for dose levels were selected for acute oral toxicity. 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg were used as dose range.

EXPERIMENTATION

All animals were divided randomly into eleven groups with three animals in each group. Group I(**T1**), receive normal saline p.o. only and served as control, Group II, III, IV, V and VI,VII,VIII.IX,X were treated with carbon tetrachloride (CCl_4). Group II**(T2)** received Carbon tetrachloride p. o., Group III**(T3)** received Silymarin100 mg/kg per day p.o, Group IV**(T4)** received SSSB-6(60 mg/kg/day),Group V(**T5**) received SSSB-7 (60 mg/kg/day), Group VI(**T6**)received SSSB-3 (60 mg/kg/day), Group VII(**T7**) received SSSB-15 (60 mg/kg/day), Group VIII(**T8**) received SSSB-2 (60 mg/kg/day), Group IX(**T9**) received SSSB-1 (60 mg/kg/day), Group X(**T10**) received SSSB-16

(60 mg/kg/day), Group XI(**T11**) received SSSB-9 (60 mg/kg/day). All dosing of test samples was done p.o. for 7 days.

Assessment of Hepatotoxicity: After overnight fasting (deprived of food for 16 h had been allowed free access to water),On the 1st day hepatotoxicity was induced in rats by CCl4 suspension in olive oil (1:1 v/v) at a dose of 1 ml/kg body weight orally by oral gavage tube.

On day 7, blood was collected by cardiac puncture under mild ether anesthesia. Serum was separated and analyzed for SGOT, SGPT, ALP, Total Bilirubin and Cholesterol using commercial kit of Aspen Lab, Span diagnostic LTD, and Merck India diagnostic respectively. Analysis was done using Rapid Bioautoanlayser (Star 21).

HISTOPATHOLOGICAL STUDIES

The animals were sacrificed and the abdomen was cut open to remove the liver. Then 5mm thick piece of the liver were fixed in Bouin's solution (mixture of 75 ml of saturated picric acid, 25 ml of 40% formaldehyde and 5ml of glacial aceticacid) for 12 h and then embedded in paraffin, using conventional methods andcut into 5 m microscope for histopathological changes in liver architecture, andtheir photomicrographs were taken.[11,12]

Statistical Analysis

The mean values ± SEM was calculated for each parameter. Percentage reduction against the hepatotoxins by the test sample was calculated byconsidering enzyme level difference between the hepatotoxin treated. Fordetermining the significant inter-group difference, each parameter wasanalyzed separately, and one way analysis of variance (ANOVA) was carriedout. Then the individual comparison of the group means values were done usingDennett's't' test procedure [29]. P values <0.05 were considered significant.

RESULT AND DISCUSSION

Validation of docking protocol

In this study, we mainly used flex X of Biosolve IT. Firstly we validate with the receptor 3GPH by carrying out the re-docking of reference ligand. In the redocking process ligand which is attached to the co-crystallized structure of protein was removed and re-docked to the active site and RSMD value is noted. The RMSD was below $2A^0$ justifying it means the receptor is having well for docking and reproduced good result. [Figure 2][13,14]

Protein and Ligand preparation

The co-crystallized structure of the target Protein, of CYP2E1 were obtained from the RSCSB Protein Data Bank (PDB) database; having PDB ID 3GPH (http://www.pdb.org.) it was prepared by using Flex X. In this modification are done by removing unwanted chains, removing water molecule and set of receptor generation and finally the energy minimization of the protein ligand complex is done. The proposed Taurine hybrid amino acid and peptide molecules were drawn by using Chem. Office 10.0. There energy minimization was performed to recognize low energy conformers and saved in the mol2 file format. These molecules were imported into the project table of the Maestro 5.0, Schrodinger and ligand preparation was done from the application option and exported in the SDF format. Further, these molecules were imported into the docking library of FlexX docking software and used for docking. In this the all molecules were analyzed for their energy of tautomeric and for stereochemistry.[18]

Molecular docking

All the compounds were docked into the active site of 3GPH using Flex X software .The docking scores for taurine hybrid amino acid and peptide analogues as shown in Table 2. By analyzing the scores and hydro-

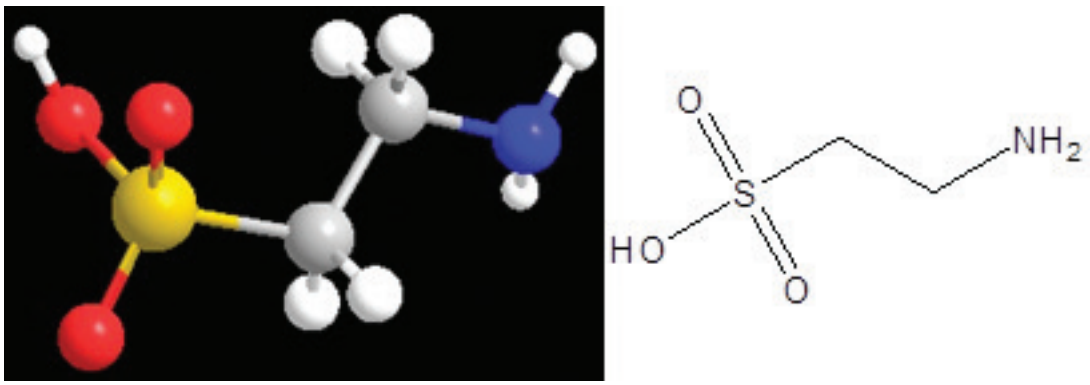


Figure 1: 2D and 3 D structure of Taurine

philic and hydrophobic interactions, it was found that all hybrid compounds show better binding with the receptor as compared with taurine. This is due to the number of hydrogen bonds and hydrophobic interactions made with the amino acids present at the active site. The compound SSSB-16 shows the maximum score having the docking score is -24.84 as compared with single taurine and other taurine hybrid compound. The compound SSSB 15 is second in the list of docking score with the docking score is -24.67. The compound SSSB16 [Figure 3] shows three hydrogen bonding with amino acid viz. Phe-203, Thr303and Asn 206 and hydrophobic interaction with amino acid viz. Phe 203,

Table 1: Physicochemical properties of Taurine

Molecular formula	$C_2H_7NO_3S$
Molecular weight	125.15
Physical state	Rod shaped like a crystal
Colour	White crystal
Odour	Odorless
Solubility	Soluble in water
Melting point	300 ^{0}C
Optical rotation	Nil

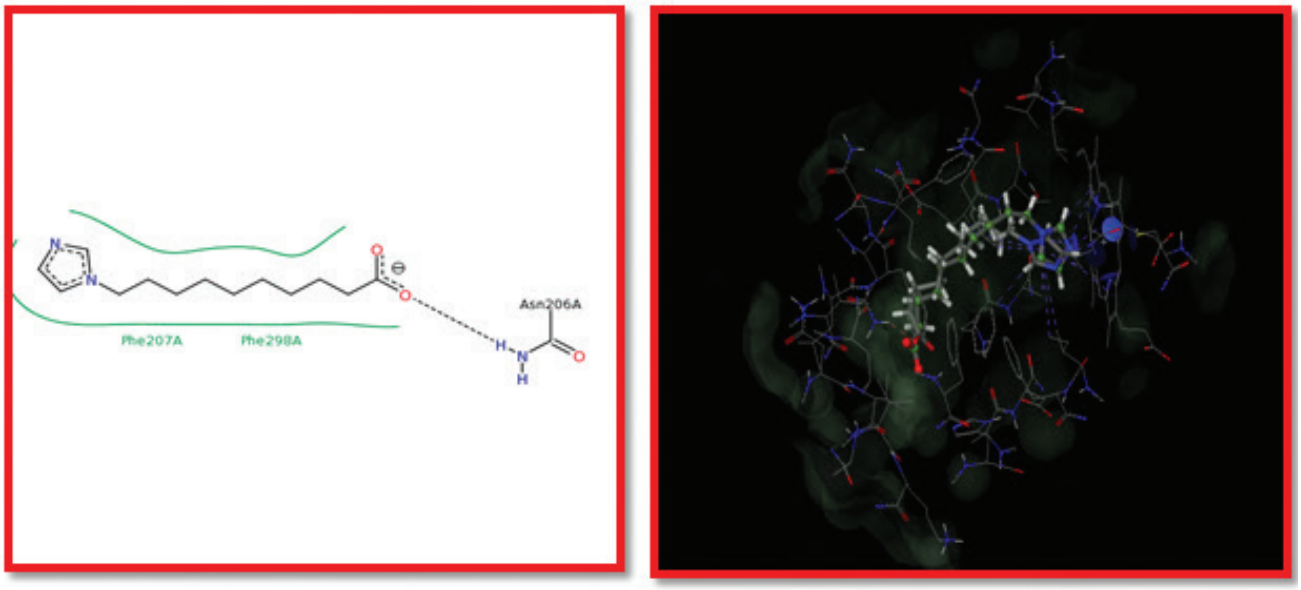


Figure 2: Re-docked poses of the Reference ligands in 3GPH

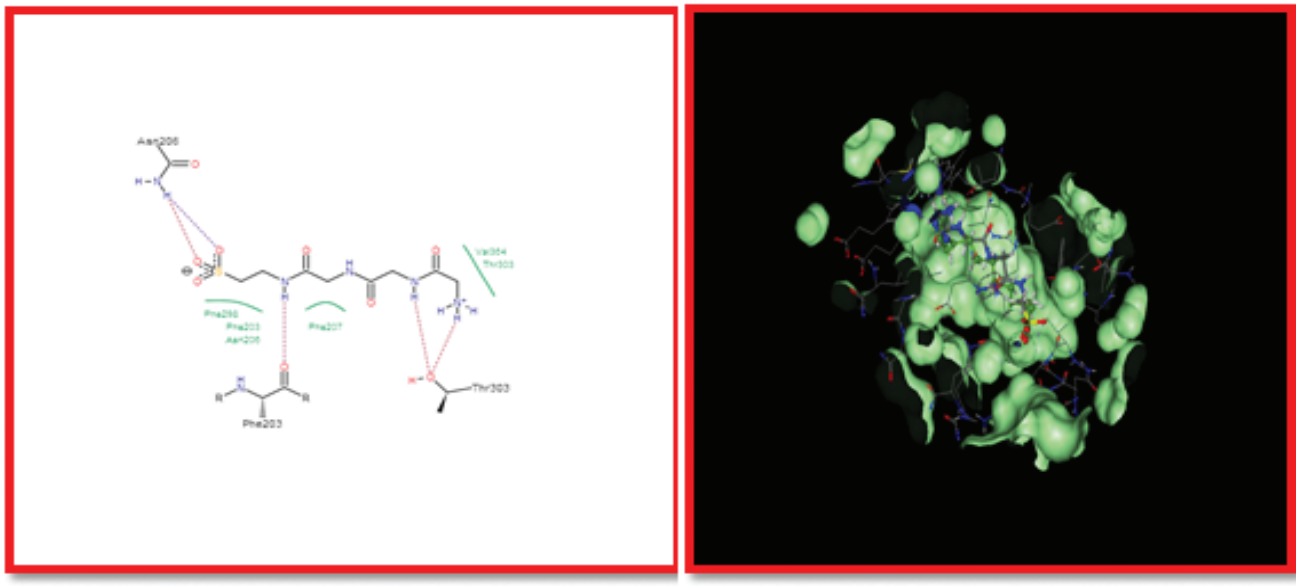


Figure 3: Interaction of SSSB16 with 3GPH 2 Dand 3 D view.

Asn206, Phe 207, Val 364 and Thr 303 as compared to the reference ligand which is having the hydrogen bonding with the Asn-206 and hydrophobic interaction with Phe-207, Phe-283.having the docking score is -11.90. From this result it concluded that all the designed hybrid molecules are having activity at 3GPH receptors. They are making sufficient number of hydrogen bonds and hydrophobic interaction with the active site.

General scheme of preparation hybrid Taurine-Amino acid compounds

Phthaloyl protecting amino acid (0.01mol) was taken in RBF in ice bath, to this 1:1 ratio of dioxan and water followed by 4 ml of aq. NaOH till it get dissolves. Add $SOCl_2$ to the solution drop wise allowed to stir for 30 min then remove the ice bath and continue string at room temperature for 4 hrs and monitored by TLC. Then taurine (0.01mol) dissolved in water and add to the reaction mixture and stir it continually till reaction proceeds monitored by TLC. Reflux the mixture for 10 min. Solvent is removed by distillation and compound was collected.DeprotectPhthaloyl group and crystalline compound was collected and characterization was done by melting point,Rf value, IR, NMR, and Mass spectra analysis.[8,9] The same procedure is followed for the preparation ofTaurine-single amino acid,Taurine – Dipeptides, Taurine –Tripeptides compound preparation. [Figure 4]

- SSSB2 (*Taurine-Glycine*): Yield: 80% ,**C**olour: white, Rf value(*butanol: acetic acid: water =4:1:1):* 0.72, Melting Point: -196-198 ^{0}C, ^{1}H NMR: δ 1.57 (s, 2H, N**H2**), 2.12 (s, 1H, O**H**-SO_2), 3.66–3.64 (t, 2H, C**H2**-SO_2), 3.74-3.72 (t, 2H, C**H2**-NH), 3.79(s, 2H, C**H2**-NH_2), 8.09 (s, 1H, N**H**). ESI-MS (m/z): 182.Calculated C: 26.37, H: 5.53, N: 15.38, S: 17.60 observed C: 27.34, H: 5.75, N: 16.32, S: 17.94.
- SSSB5 *(Taurine-Alanine):*Colour: white solid, Yield: 62%,Rf value: (*butanol: acetic acid: water=4:1:1)* 0.75, Melting Point: -198-200 ^{0}C^{1}H NMR: δ 1.29 (d, 3H, C**H3**), 2.12 (s, 1H, O**H**-SO_2), 3.65–3.63 (t, 2H, C**H2**-SO_2), 3.69-3.66(q, 1H, C**H**-NH_2) 3.72-3.70 (t, 2H, **CH2**-NH_2), 5.21(s, 2H, N**H2**), 8.09 (s, 1H, N**H**). ESI-MS (m/z): calculated :196.22 observed mass 197.30, Calculated C: 30.60, H: 6.16, N: 14.28, S: 16.34 observed C: 30.45, H: 6.82 , N: 14.59, S: 16.16
- SSSB11*(Taurine-Valine):*Colour: Cream white, Yield: 72%,Rf value: : (*butanol: acetic acid: water=4:1:1):* 0.68,Melting Point:222-230^{0}C,^{1}H NMR: **:** 0.9 (d, 6H C**H3**), 2.1(m, 1H, C**H**), 2.7(t, 1H, C**H**-NH_2), 3.00(d, 2H, C**H2**-NH), 3.15(t, 2H, N**H2**), 3.7(t, 2H, C**H2**-SO_2), 8.4(s, 3H, N**H**). ESI-MS (m/z): Calculated: 224.28 observed mass 225.30, Calculated C: 37.49, H: 7.19, N: 12.49, S: 14.30 observed C: 38.40, H: 7.28 , N: 12.50, S: 14.45.
- SSSB12 *(Taurine-Leucine):*Colour: white solid, Yield: 71%,Rf value : (*butanol: acetic acid: water=4:1:1):* 0.65,Melting Point:248-250^{0}C,^{1}H NMR: δ 0.91 (d, 6H, C**H3**), 1.35-1.33 (t,2H, C**H**$_2$), 1.82-1.80 (m, 1H, C**H**-$(CH_3)_2$), 2.12(s, 1H, O**H**-SO_2), 3.37-3.34(t, 1H, C**H**-NH_2) 3.69–3.67 (t, 2H, C**H2**-SO_2), 3.79-3.77 (t, 2H, C**H2**-NH), 5.11(s, 2H, N**H2**), 8.12 (s, 1H, N**H**). ESI-MS (m/z): Calculated: 238.30 observed mass 239.40, Calculated C: 40.32, H: 7.61, N: 11.76, S: 13.46 observed C: 40.44, H: 7.82, N: 11.59, S: 13.16.
- SSSB 13 *(Taurine-Glutamic acid)*: Colour: white solid, Yield: 79%, Rf value: (*butanol: acetic acid: water=4:1:1):* 0.7,Melting Point:200-201^{0}C^{1}H NMR: δ 2.06-2.03 (q, 2H, C**H2**), 2.15(s, 1H, O**H**-SO_2), 2.34-2.32(t, 2H, C**H2**-CO), 3.37-3.34(t, 1H, C**H**-NH_2) 3.69–3.67 (t, 2H, C**H2**-SO_2), 3.79-3.77 (t, 2H, C**H2**-NH), 5.11(s, 2H, N**H2**), 8.12 (s, 1H, N**H**), 11.57(s,1H, O**H**-CO). ESI-MS (m/z): Calculated: 254.26 observed mass 255.30, Calculated C: 33.07, H: 5.55, N: 11.02, S: 12.61 observed C: 33.77, H: 5.10, N: 11.59, S: 11.80.
- SSSB3*(Taurine- Gly –Gly):*Colour:white solid **,**Yield:64%, Rf Value: (*butanol: acetic acid: water=4:1:1)* 0.61, Melting Point: -239.25^{0}C, ^{1}H NMR: δ 1.57 (s, 2H, N**H2**), 2.12 (s, 1H, **OH**-SO_2), 3.65–3.63 (t, 2H, C**H2**-SO_2), 3.72-3.70 (t, 2H, C**H2**-NH), 3.79(s, 2H, C**H**$_2$-NH_2), 4.14 (s, 2H, **CH2**), 8.09 (s, 2H, NH). ESI-MS (m/z): 239. Observed 240.71, Calculated C: 31.06, H: 4.62, N: 12.91, S: 11.40 observed C: 31.12, H: 4.75, N: 13.32, S: 11.94.
- SSSB7 (*Taurine-Gly- Valine):*Colour: white solid, Yield:73%, Rf Value: : (*butanol: acetic acid: water=4:1:1):* 0.68,MeltingPoint:185-183^{0}C^{1}HNMR:δ 0.91 (d,6H, C**H3**), 2.17 (s, 1H, O**H**-SO_2), 2.26-2.23(m, 1H, C**H**-$(CH_3)_2$), 3.55-3(d, 1H, C**H**-NH_2) 3.69–3.67 (t, 2H, C**H2**-SO_2), 3.79-3.77 (t, 2H, C**H2**-NH), 4.09 (s, 2H, C**H2-** NH), 5.11(s, 2H, N**H2**), 8.12 (s, 3H, N**H**). ESI-MS (m/z): Calculated: 281.33 observed mass 282.46, Calculated C: 38.42, H: 6.81, N: 14.94, S: 11.40 observed C: 38.50, H: 6.88, N: 14.59, S: 11.54.
- SSSB 10 *(Taurine-Glycine-Alanine):*Colour: white solid, Yield : 78, Rf value: (*butanol: acetic acid: water=4:1:1):* 0.55,Melting Point:180-182^{0}C, ^{1}H NMR:1.4 (d, 3H C**H3**), 2.7(t, 1H, C**H**-NH_2), 3.00 (d, 2H, C**H2**-NH), 3.15(s, 2H, N**H2**), 3.6(t, 2H, C**H2**- SO_2), 3.8(t, 2H, C**H2**-CH_2-SO_2), 8.5(s, 2H, N**H**). ESI-MS (m/z): Calculated: 253.28 observed

mass 254.30, Calculated C: 33.19, H: 5.97, N: 16.59, S: 12.66 observed C: 34.00, H: 5.82, N: 16.59, S: 12.16.

- SSSB 15*(Taurine-Glycine-L Leucine)*:Colour: white, Yield: 70%, Rf value: : (*butanol: acetic acid: water=4:1:1):* 0.59,Melting Point:243-245^{0}C^{1}H NMR: 0.9(d, 6H C**H3**), 1.6(t, 2H, C**H2**), 1.8(m, 1H, C**H**), 2.3(s, 1H, O**H**), 2.7(t, 1H, C**H**-NH_2), 3.00(t, 2H, C**H2**-NH), 3.15(s, 2H, N**H2**), 3.7(t, 2H, C**H2**- SO_2), 3.8(t, 2H, C**H2**-CH_2-SO_2), 8.5(s, 2H, N**H**). ESI-MS (m/z): Calculated: 295.36 observed mass 296.30, Calculated C: 40.67, H: 7.17, N: 14.23, S: 10.86 observed C: 40.45, H: 7.82 , N: 14.59, S: 10.96.
- SSSB 14 *(Taurine-Gly-Glutamic acid)*: Colour: white solid, Yield: 79%,Rf value: (*butanol: acetic acid: water=4:1:1):* 0.65,Melting Point:244-246^{0}C, ^{1}H NMR: δ 2.06-2.03 (q, 2H, C**H2**), 2.15(s, 1H, O**H**-SO_2), 2.34-2.32(t, 2H, C**H2**-CO), 3.38-3.36 (t, 1H, C**H**-NH_2) 3.69–3.67 (t, 2H, C**H2**-SO_2), 3.79-3.77 (t, 2H, C**H2**-NH), 4.10(s, 2H, C**H2-**NH), 5.11(s, 2H, N**H2**), 8.12 (s, 1H, N**H**), 11.57(s,1H, O**H**-CO). ESI-MS (m/z): Calculated: 311.08 observed mass 312.12, Calculated C: 34.72, H: 5.50, N: 13.50, S: 10.30 observed C: 34.45, H: 5.09, N: 13.59, S: 10.16.
- SSSB16*(Taurine-Gly-Gly-Glycine)*:Colour: white, Yield: 76%, Rf value: : (*butanol: acetic acid: water=4:1:1)*:0.68, Melting Point: 240-242^{0}C ^{1}H NMR**:** 2.7(t, 2H, C**H**-NH_2), 3.00(t, 6H, C**H2**-NH), 3.6(t, 2H, C**H2**-SO_2), 4.3(s, 2H, N**H2**), 8.3(s, 3H, N**H**). ESI-MS (m/z): Calculated: 296.30 observed mass 297.45, Calculated C: 32.43, H:5.44, N:18.91, S: 10.82 observed C: 32.45, H: 5.82 , N: 18.59, S: 10.16.
- SSSB4 *(Taurine-Gly-Gly-Alanine)***:**Colour: white solid, Yield: 73%, Rf value: (*butanol: acetic acid: water=4:1:1)* 0.59, Melting Point: -235-238 ^{0}C ^{1}H NMR : 1.4 (d, 3H C**H3**), 2.7(t, 1H, C**H**-NH_2), 3.00(d, 4H, C**H2**-NH), 3.15(s, 2H, N**H2**), 3.6(t, 2H, C**H2**-SO_2), 3.8(t, 2H, C**H2**-CH_2-SO_2), 8.5(s, 3H, N**H**). ESI-MS (m/z): calculated 310.33 observed 311.40, Calculated C: 34.83, H: 5.85, N: 18.05, S: 10.33 observed C: 34.45, H: 5.820, N: 18.59, S: 10.16.
- SSSB9 (*Taurine-Gly-Gly-Valine*): Colour: White solid crystalline, Yield: 74%, Rf Value: : (*butanol: acetic acid: water=4:1:1)* : 0.75, Melting Point:210-215^{0}C, ^{1}HNMR : 0.9(d, 6H C**H3**), 2.1(s, 1H, O**H**), 2.7 (t, 1H, C**H**-NH_2), 3.00(d, 4H, C**H2**-NH), 3.3(d, 2H, N**H2**), 3.6(t, 2H, C**H2**- SO_2), 3.7(t, 2H, C**H2**-CH_2-SO_2), 8.4(s, 3H, N**H**). ESI-MS (m/z): calculated :281.33 observed mass 282.46, Calculated C:39.04, H: 6.55, N: 16.56, S: 9.48 observed C: 39.45, H: 6.82 , N: 16.65, S: 9.16
- SSSB 8 *(Taurine-Gly-Gly-l Leucine)***:**Colour:- White solid crystalline, Yield: 74%,Rf Value: : (*butanol: acetic acid: water=4:1:1):* 0.75, Melting Point:190-191^{0}C^{1}HNMR**:** 0.9(d, 6H C**H3**), 1.6(t, 2H, C**H2**), 1.8(m, 1H, C**H**), 2.7(t, 1H, C**H**-NH_2), 3.00(d, 4H, C**H2**-NH), 3.3(d, 2H, N**H2**), 3.6(t, 2H, C**H2**- SO_2),

Table 2: Docking score

Compound Code	Compound Combinations	FlexX Score
Reference Ligand	–	-11.90
SSSB 1	Taurine	-8.94
SSSB 2	Taurine-Glycine	-14.21
SSSB 3	Taurine-Glycine-Glycine	-17.34
SSSB 4	Taurine –Glycine-Glycine-Alanine	-23.42
SSSB 5	Taurine –Alanine	-16.81
SSSB 6	Taurine- Glycine-Glycine-Glutamic Acid	-19.20
SSSB 7	Taurine–Glycine-Valnine	19.25
SSSB 8	Taurine- Glycine-Glycine-l Leucine	-23.79
SSSB 9	Taurine- Glycine-Glycine-Valnine	-22.19
SSSB 10	Taurine- Glycine-Alanine	-20.31
SSSB 11	Taurine-Valnine	-17.58
SSSB 12	Taurine-l Leucine	-15.22
SSSB 13	Taurine-Glutamic Acid	-13.08
SSSB 14	Taurine-Glycine-Glutamic Acid	-16.10
SSSB 15	Taurine-Glycine-l Leucine	-23.42
SSSB 16	Taurine-Glycine-Glycine-Glycine	-24.84

Table 3:Antioxidant activity of synthesized compounds

DPPH SCAVENGING ACTVITY			NITRIC OXIDE SCAVENGING ACTVITY	
SN	**COMPOUND**	**IC50 value μM**	**COMPOUND**	**IC50 value μM**
1	Std	13.27	Std	7.41
2	SSSB1	93.01	SSSB1	99.12
3	SSSB2	64.01	SSSB2	93.24
4	SSSB3	43.34	SSSB3	65.86
5	SSSB4	190.96	SSSB4	279.72
6	SSSB5	447.1	SSSB5	264.57
7	SSSB6	113.49	SSSB6	122.47
8	SSSB7	57.34	SSSB7	90.12
9	SSSB8	237.15	SSSB8	155.89
10	SSSB9	127.66	SSSB9	128.25
11	SSSB10	148.95	SSSB10	154.37
12	SSSB11	146.28	SSSB11	146.27
13	SSSB12	169.71	SSSB12	143.14
14	SSSB13	199.54	SSSB13	226.58
15	SSSB14	257.24	SSSB14	207.49
16	SSSB15	121.66	SSSB15	122.45
17	SSSB16	143.82	SSSB16	138.68

3.8(t, 2H, C**H2**-CH_2-SO_2), 8.5(s, 3H, N**H**). ESI-MS (m/z): Calculated: 352.41 observed mass 353.40, Calculated C: 40.90, H: 6.86, N: 15.90, S: 9.10 observed C: 40.45, H: 6.82, N: 15.59, S: 10.16.

- SSSB6 (*Taurine-Gly-Gly-Glutamic acid*)**:** Colour: white, Yield: 75% ,Rf value: : (*butanol: acetic acid: water=4:1:1):* 0.71,Melting Point: 265 266^0C,^{1}H NMR: δ 2.15 (s, 1H, O**H**-SO_2), 2.21-2.20 (q, 2H, C**H**$_2$), 2.34-2.32 (t, 2H, C**H2**-CO), 3.38-3.36(t, 1H, C**H**-NH_2) 3.69–3.67 (t, 2H, C**H2**-SO_2), 3.79-3.77 (t, 2H, C**H2**-NH), 4.08 (s, 4H, C**H2**), 5.11(s, 2H, N**H2**), 8.12 (s, 3H, N**H**), 11.57(s,1H, O**H**-CO). ESI-MS (m/z): calculated : 386.36 observed mass 387.40, Calculated C: 35.87, H:5.47, N: 15.21, S: 8.70 observed C: 35.45, H: 5.82 , N: 15.59, S: 8.16

ANTIOXIDANT ACTIVITY

In order to investigate the free radical scavenging ability of all compounds, the methods like DPPH [di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium], Nitric Oxide scavenging were used. DPPH method is widely accepted that its reaction with compounds proceeds through two differentmechanisms, including the direct hydrogen atom transfer and the sequential proton loss electron transfer. Thus, the unpaired electron becomes paired in the presence of a free radical scavenging antioxidant or hydrogen donor, decreasing theabsorption.In the DPPH antioxidant assay method, we are tested 16 compounds from the concentration ranges from scavenging at concentrations as low as 10 and 250 μM. The results of antioxidant values expressed in the form of IC_{50} with different antioxidant markers used are shown in Table 3. out of 16 compounds tested the **SSSB3** showed most potent IC_{50} at **43.34** μM, when compared to that of the standard ascorbic acid at 13.27μM. followed by SSSB 3 compounds**SSSB 7(IC_{50} 57.34 μM), SSSB2(IC_{50} 64.01 μM)** also shows the potent activity as compared to **SSSB 1 (IC_{50} 93.01 μM).**

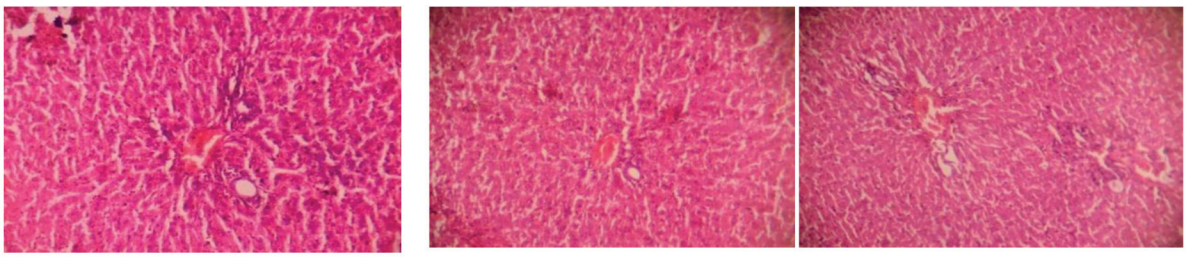
Further, from the antioxidant studies carried out using Nitric oxide scavenging assay for the 16 test compounds, **SSSB 3, SSSB 7,** and **SSSB 2** showed IC_{50} values at **65.86, 90.12, 93.24** μM respectively when compared to that of the standard ascorbic acid at 7.41 μM. and the **SSSB 1 (IC 50 , 99.12** μM).

HEPATOPROTECTIVE STUDY

After the treatment of animal with $CC1_4$, the SGOT, SGPT, ALP, Total Proteins, Bilirubin and Cholesterol-levels have been significantly increased. This significant increased in level has reduced markedly in the group of animal treated with T3 andT6 compounds when compared with $CC1_4$-treated rats. Followed by the T5, T7 as compared to this three compound the compound T9 shows less hepatoprotective activity. Table 4 The

Table 4: Biochemical analysis of treated groups

Treatment	SGPT (IU/dl)	SGOT (IU/dl)	ALP (IU/dl)	TOTAL BILIRUBIN (mg/dl)	CHOLESTEROL (mg/dl)
T1	33.45±4.89	50.67±4.23	100.34±6.49	0.46±0.067	61.29±5.92
T2	96.63±5.488	111.00±7.708	206.23±8.398	1.28±0.066	134.33±11.835
T3	56.60±3.242	62.83±3.252	111.97±3.808	0.56±0.045	72.60±6.188
T4	79.67±3.853	87.80±5.840	166.37±8.351	1.10±0.059	118.83±6.834
T5	67.17±3.855	75.07±4.574	140.33±6.841	0.69±0.035	103.43±6.459
T6	64.60±5.856	70.30±4.729	134.03±5.352	0.66±0.053	91.70±6.551
T7	69.43±3.803	81.87±4.905	153.57±7.856	0.74±0.059	111.33±5.326
T8	83.89±2.87	100.78±4.09	178.89±7.98	1.78±0.078	135.78±6.98
T9	70.56±2.90	78.67±4.12	155.76±7.90	0.80±0.061	112.34±5.36
T10	85.78±2.98	98.90±4.12	167.98±7.98	1.01±0.076	136.78±4.60
T11	84.76±2.18	99.20±4.18	178.09±6.89	1.12±0.07	126.98±4.98



T2 T3 T6

Figure 5: Images of Liver Section at 10X
T2: Photomicrograph of a section from Liver section of CCl_4 treated animal,
T3: Photomicrograph of a section from Liver section of Silymarin treated animal,
T6: Photomicrograph of a section from Liver section of compound SSSB3 treated animal

compound T6have shown a better recovery of the hepatocyte which clearly indicated the partial protection of liver cells. Also liver section showed partial disappearance of fatty deposit and necrosis comparable. (Figure 5).

CONCLUSION

The docking study revealed that hybrid compounds of taurine containing amino acid and peptides hadshows batter binding with all important amino acid residues of 3GPH receptor .Our study is mainly focused on to established hepatoprotective drug to syntheses of novel hepatoprotective compounds since many drugs causes liver toxicity and due to which they are withdraw from the market in different stages of clinical trial. In this study we found that the hybrid compounds had better docking score as compared to the simple taurine. The compound SSSB16 (Taurine-Gly-Gly-Gly), SSSB15 (taurine-Gly-lLeucine) and SSSB3(Taurine-Gly-Gly) is having docking score -24.84, -23. 42 and -17.34 while SSSB 1 (Taurine) is having docking score -11.90. After the satisfactory results of *insilico* study, we have successfully synthesized a taurine amino acid and peptide analogs, the compounds were purified by recrystallsation using appropriate solvent. The synthetic yields of the generated products ranged from 40to 60% and their structures were established by spectraldata. Finally, all of synthesized compoundshave been tested for their antioxidant activities using DPPH method, nitric oxide scavenging method and for hepatoprotective activity. From result good activity was noted for SSSB3 (Taurine-Gly-Gly) compound. From this it can be concluded that the amino acid hybrid with future being proof to be novel compound as hepatoprotective activity. It may be use as a supplement with the drugs to reduced hepatotoxicity.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

ABBREVIATIONS USED

CYP2E1:Cytochrome P450 2E1;**RMSD:** Root-Mean-Square Deviation; **CPE:** Chlorophosphate Ester;**TLC:** Thin layer chromatography; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **CCl_4:** Carbon tetrachloride; **SGOT:** serum glutamic oxaloacetic transaminase; **SGPT:** serum glutamic-pyruvic transaminase; **ALP:** Alkaline phosphatase; **IR:** infrared spectroscopy; **NMR:** Nuclear magnetic resonance.

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PICTORIAL ABSTRACT

SUMMARY

A novel taurine amino acid hybride analogues having dipeptide and tripeptides have been synthesized and final compounds were charactrised by FTIR,1HNMR and mass spectroscopy. Compound SSSB 3 significantly reduced the levels of liver enzyme markers like SGOT, SGPT, ALP, bilurubin and cholesterol which was elevated by CCL4 administration.
In present study it was found that the compound SSSB3 (Taurine-Gly-Gly) exhibited potent antioxidant and hepaptoportective activity.

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