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Design, synthesis and pharmacological evaluation of flurbiprofen prodrugs for neurodegenerative disorders

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ABSTRACT

The aim of the present study is to synthesize the aminoacidprodrugs of flurbiprofen and perform the physico-chemical characterization as well as pharmacokinetic studies. The in-silico docking and ADME prediction were also conducted to attain the knowledge about the novel pordrugsand their action in the central nervous system. In the present research work, use of prodrug concept, temporarily mask the acidic group of Flurbiprofen has been reveal as an innovative and novel approach to enhance the transport property across blood brain barrier (BBB). In this study, amide prodrugs of flurbiprofen with various amino acids such as phenylalanine, glycine and valine were synthesized by using Dicyclo hexyl carbodiimide (DCC) to obtain corresponding prodrugs such as Flurbiprofen-Phenylalanine (FP), Flurbiprofen-Glycine(FG) and Flurbiprofen-Valine (FV). The anticipated structures were confirmed by spectral analysis like IR, ^{1}H NMR, ^{13}C NMR and Mass spectroscopy. The hydrolytic studies were conducted to know the percentage release of the drug in various P^{H} and that showed the considerable stability of the prodrug in the acidic environment. The in-silico docking study on beta-secretase enzyme as well as ADME prediction were done to understand the action of NSAID, flurbiprofen, in the activities of the brain. From the insilico studies, concluded that flurbiprofen having considerable BBB penetration ability and docking score in the beta secretase enzyme, which is having significant function in Alzheimer's disease. So the amino acid flurbiprofen prodrug approach is very beneficial andsuggested for the degenerative conditions in the brain.

INTRODUCTION

Prodrug based drug design leads to the development of targeted drug delivery. One of the principle advantages of this targeted drug delivery is reduction in dose and side effects of parent drug. Neurodegenerative diseases are increased day by day in CNS it is mainly due to neuroinflammation. Many of the epidemiological studies suggest that long term use of NSAIDs reduces the risk of developing Neurodegeneration. But the hydrophilic nature of NSAIDs limits the passage across blood brain barrier. In order to improve the transport properties of NSAIDs a prodrug approach could be done by using different carrier's specially amino acids.BBB penetration of the synthesized compounds is evaluated by Topological polar surface area [TPSA] value that can be found out by Swiss ADME. In silico studies were performed to find out the effectiveness of Flurbiprofen in Alzheimer's disease. Although the compound is an excellent anti inflammatory agent, decided to study the ability of Flurbiprofen to inhibit Beta-secretase enzyme, a key drug target in Alzheimer's disease using some *in silico* computational tools. Docking studies were done to determine the potential leads. The final evaluation is done with the docking score and the single best pose is generated as the output for a particular ligand. Most of the molecules fail in the drug discovery process due to poor pharmacokinetic parameters. QikProp is an *in silico* tool frequently used for ADME analysis of potential leads. In this study Flurbiprofen conjugated with amino acids such as phenylalanine, glycine and valine to produce corresponding prodrugs. The synthesized prodrugs are subjected to characterization, Hydrolytic studies are done to determine the release of prodrug under different PH conditions. Hydrolysis study indicates the acid stability and also sustained release of drug in intestine. In solico studies are done by using various

softwares[1,2,3].

MATERIALS AND METHODS

The amino acids were obtained from LobaChemie, Cochi, and drug Flurbiprofen were obtained from TCI chemicals. Other solvents and reagents were of analytical grade. The melting points were recorded by melting point determination apparatus BMQR892 (Sigma Instrument, Mumbai, India) and are uncorrected. The infrared spectra were recorded on IR spectrophotometer (Shimadzu 8201 PC) in KBr phase, Al-shifa College of pharmacy. Shimadzu model pharma spec-1800 UV visible double beam spectrophotometer with 1cm matched quartz cell was used for recording spectra and absorbance measurement. 1H NMR and 13C NMR spectra recorded in Bruckeravance II 400 NMR Spectrometer (Chandigarh). Mass spectra were recorded in mass spectrophotomerter (Q-TofMicrowaters-Mass Spectrometry). Deterrmination of physicochemical properties were carried out in Department of Pharmaceutical Chemistry Alshifa College of pharmacy.

GENERAL PROCEDURE (Synthesis of prodrugs)

The amide prodrugs of flurbiprofen ((RS)-2-(2-fluorobiphenyl-4-yl) propionic acid) with amino acids(Phenylalanine, Glycine, Valine) were synthesized by coupling reactions using dicyclohexylcarbodiimide (DCC).T). The weighed quantity of flurbiprofen (0.01M) was dissolved in 40 ml of dichloromethane (DCM) and to this solution DCC (0.01M) was added. It was then stirred for half an hour at room temperature. To this solution corresponding aminoacid (0.01M) in 20 ml DCM was added dropwise. This reaction mixture was stirred for 3hr at 0°c and left overnight as such at room temperature. The progress of the reaction was monitored by thin layer chromatography using hexane:ethylacetate (4:1) as mobile phase.

Next day after completion of reaction, reaction mixture was filtered off to remove precipitated dicyclohexyl urea. Recrystallise the product from ethanol and water.The schematic representation of the synthesis of Flurbiprofen prodrugs with phenylalanine, glycine and valine are shown in the figure 1.

CHARACTERIZATION OF DRUGS AND PRODRUGS

After the completion of synthesis, the synthesized compounds are subjected to different characterization techniques like Thin layer chromatography, Melting point determination, solubility studies and spectral analysis. Thin layer chromatography was done by suitable solvent system on pre coated silica G plates. Hexane: ethyl acetate (4:1) is used as a solvent system without producing tailing. Melting point was determined by capillary fusion method.

SYNTHESIS OF FLURBIPROFEN-AMINIACID PRODRUG

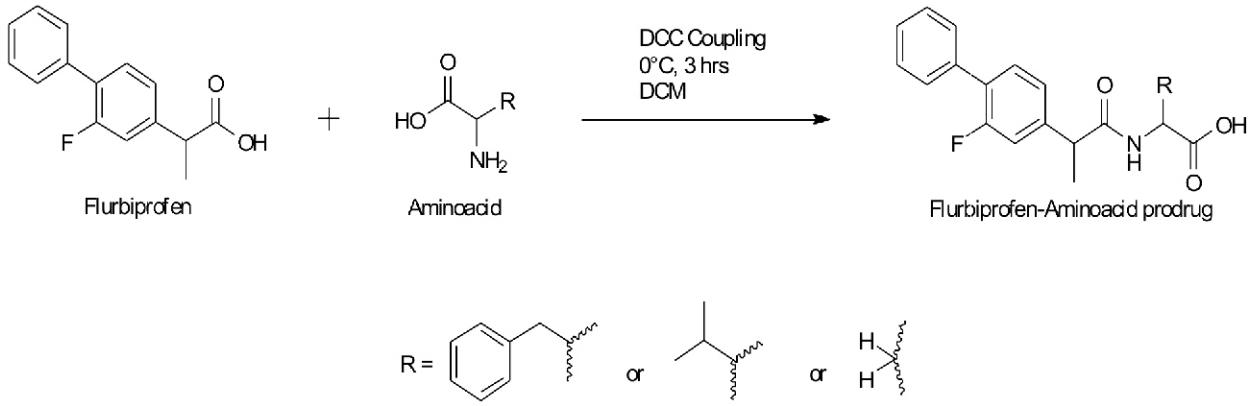

Fig. 1 : Scheme for the synthesis of Flurbiprofen-Aminoacidprodrug

Table 1 : Characterization of prodrugs

Prodrugs	Molecular weight (g/mol)	Colour	Melting point	Yield	R_f value	Log P
FP	391.43	White	128-132	79	0.80	0.92
FG	301.31	White	126-130	73	0.78	0.81
FV	343.39	White	118-122	75	0.68	0.86

Pharmacokinetic studies

Hydrolysis study

The pure drug and synthesized prodrugs are subjected to hydrolytic studies to determine the release of drug in hydrolytic conditions. Hydrolytic studies are carried out in simulated gastric fluid (SGF) at PH 1.2 and simulated intestinal fluid (SIF) at PH 7.4[5].

Partition coefficient

Partition coefficients of the synthesized prodrugs are determined by shake flask method. In a separating funnel accurawxc487tely weighed 100mg of drug was transferred and add 30 ml octanol. To this add 10ml phosphate buffer (PH 7.4). The contents of separating funnel were shaken for 1hr at room temperature. After that the amount of the drug and prodrug present in the solution was measured by the absorbance at 230nm and calculated Log p value. Log P value reveals the lipophilic character of drug and synthesized compounds[6].

Evaluation of BBB penetration

BBB penetration of the synthesized prodrugs can be evaluated by Topological polar surface area [TPSA] value, done by Swiss ADME [7].

- Calculation of important molecular properties (Log p, polar surface area, number hydrogen bond donors and acceptors)
- Prediction of bioactivity score for the most important drug targets (GPCR ligands, kinase inhibitors, ion channel modulators, nuclear receptors) and possible molecular toxicity.

In silico studies

In silico studies are done to find out the effectiveness of flurbiprofen in neurodegenerative disease. Docking studies were performed by grid-based ligand docking. Docking calculations were performed using XP mode. The final evaluation is done with the docking score and the single best pose is generated as the output for a particular ligand. QikProp is an *in silico* tool frequently used for ADME analysis of potential leads. All the synthesized molecules were evaluated for their drug-like behavior through analysis of pharmacokinetic parameters required for absorption, distribution, metabolism and excretion by use of QikProp [8,9,10].

RESULTS

Characterization of prodrugs

The synthesized compounds are subjected to characterization techniques.The results of TLC shows single spot, without tailing reveal that they are free from impurities. The higher R_f value of prodrugs as compared to that of parent drug (0.62) indicates the superior lipophilicity of prodrugs. Flurbiprofen showed a melting point range 114-117°C whereas the Prodrugs showed increased values. The increase in melting point confirms the product formation. High partition coefficient of synthesized Prodrug as compared to the parent drug indicates the increase in lipophilicity of the compound. This may contribute to the higher absorption of the compound through lipoidal cell membrane.

Hydrolysis study

The results of hydrolysis study shows that minimum reversion was observed at gastric pH, indicating the stability of synthesized prodrugs in acidic environment. Higher hydrolysis rate was shown in intestinal pH.The percentage release of FP, FV, FG in SIF and SGF 72.87, 64.65, 70.45 respectively.The percentage release of prodrugs are shown in figure 2,3,4.

Evaluation of BBB penetration

BBB penetration of drug and synthesized Prodrugs were evaluated by cheminformatics. Topological polar surface area and other properties were found out from Swiss ADME. The properties of Flurbiprofen, FP, FG and FV were given in the table 2. From the table explored that all the synthesized compounds showed better penetration than the parent drug. The increased Log p value indicates the higher lipophilicity of the synthesized Prodrugs. That also indicates the ability of the synthesized compounds to penetrate the BBB

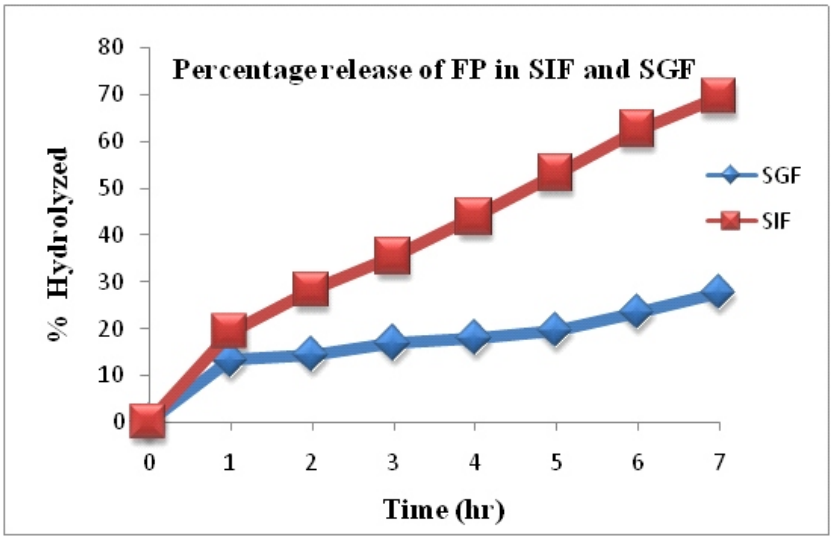

Fig. 2 : Percentage release of FP in SIF and SGF

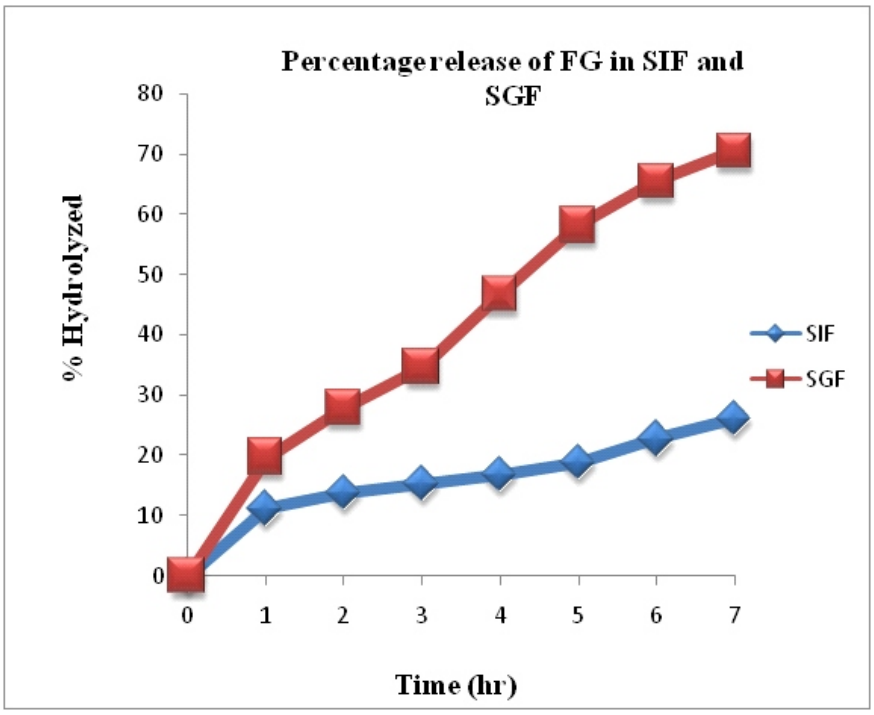

Fig. 3 : Percentage release of FG in SIF and SGF

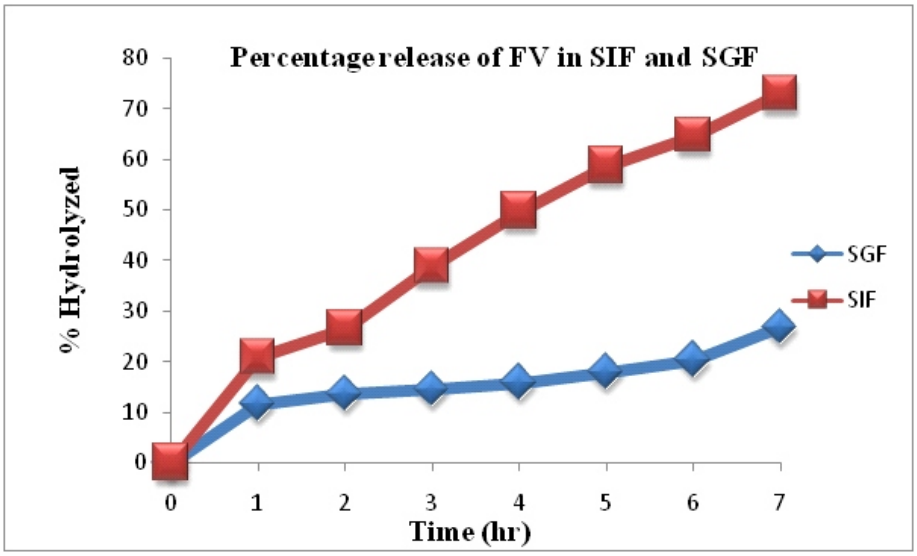

Fig. 4 : Percentage release of FV in SIF and SGF

IN SILICO DOCKING STUDIES

The low energy conformation of the ligands was selected and was docked into the grid generated from protein structures using a extra precision (XP) docking mode. The docking study reveals that Flurbiprofen is able to bind to the active site of the Beta-secretase enzyme, with a docking score of -5.044.The ligand interaction diagram of FBN as shown in figure 5.

In silico ADME prediction

The drug and prodrugs are subjected to in silico ADME prediction. The results showed that all these pharmacokinetic parameters are within the acceptablerange defined for human use, thereby indicating their potential as drug-like molecules. Table3 shows the various pharmacokinetic parameters of flurbiprofen and its prodrugs.

a- Predicted octanol/water partition co-efficient log p (acceptable range: −2.0 to 6.5).

b- Predicted aqueous solubility; S in mol/L (acceptable range: −6.5 to 0.5).

c- Predicted Caco-2 cell permeability in nm/s (acceptable range, <25 is poor and >500 is great).

d- Predicted apparent MDCK cell permeability for the

Table 2 : Properties of the compounds from Swiss ADME

Properties	FBN	FP	FG	FV
TPSA	37.30	66.40	66.40	66.40
Log P	3.61	4.49	3.64	3.89
No. of Heavy atom	18	29	22	25
No.of Rotatable bonds	3	8	6	7
No.of Violations	0	0	0	0
No. of H bond Acceptors	3	4	4	4
No.of H bond Donors	1	2	2	2
No. of Aromatic Heavy atom	12	18	12	12

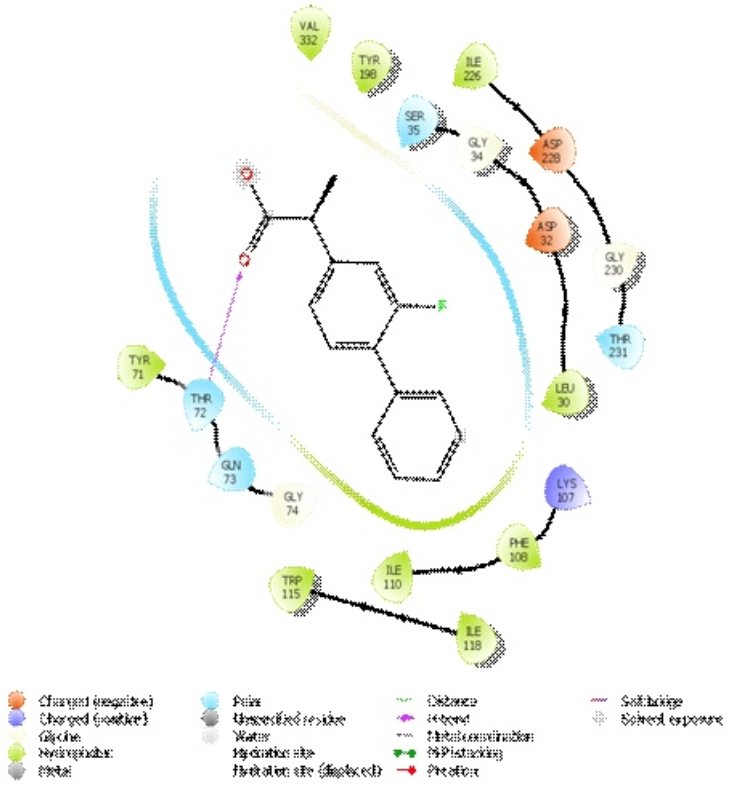

Fig. 5 : Ligand interaction diagram of Flurbiprofen with Beta- secreatase

blood-brain barrier, (acceptable range, < 25 is poor and >500 is great).

e- Predicted blood/brain barrier (-3.0-1.2).

f- Percentage of human oral absorption (< 25% is poor and >80% is high).

Out of the prodrugs studied, FV was found to have

Table 3 : Various pharmacokinetic parameters of flurbiprofen and its prodrugs

Molecule	QPlogPo/w[a]	QPlogS[b]	QPPCaco[c]	QPPMDCK[d]	QPlogBB[e]	%Human oral absorption[f]
FBN	6.179	-4.449	339.886	291.976	-0.355	96.598
FP	5.258	-5.922	176.406	193.144	-0.879	84.984
FG	3.227	-4.22	85.139	105.669	-0.968	80.385
FV	4.351	-5.076	191.096	218.752	-0.738	93.253

betterpharmacokinetic profile. The percentage of oral absorption and solubility was enhancedwhen compared to the parent drug.

DISCUSSION

The prodrugs of Flurbiprofen with amino acids were synthesized as per the scheme 1 and also perform the physicochemical characterization. The results obtained from physicochemical characterization reveal the structure and purity of synthesized compounds. In order to prove the pharmacokinetic profile of prodrugs pharmacokinetic studies are done. From these results, high partition coefficient of synthesized Prodrug as compared to the parent drug indicates the increase in lipophilicity of the compound. Hydrolysis study reveals that prodrugs are stable in gastric environment and release in intestine. The table 2 explains about properties of compounds from Swiss ADME table shows TPSA value in between 50-80A^0.The result showed that all the synthesized compounds showed better BBB penetration than the parent drug.The docking study reveals that Flurbiprofen is able to bind to the active site of the Beta- secretase enzyme, with a docking score of -5.044.*In silico* ADME prediction explains that, these pharmacokinetic parameters are within the acceptablerange defined for human use, thereby indicating their potential as drug-like molecules. From all these data concluded that Flurbiprofen prodrugs are better for Neurodegenerative disorders.

CONCLUSION

In the present research work, synthesis and evaluation of amide prodrugs, to temporarily conjugate the acidic group of NSAIDs such as Flurbiprofen with amino acids is synthesized and evaluated. The prodrugs were synthesized and characterized by modern analytical techniques.High partition coefficient of synthesized Prodrug as compared to the parent drug indicates the increase in lipophilicity of the compound. From the results of hydrolysis study concluded that prodrugs are stable in gastric environment and release in intestine. *In silico* studies were performed to find out the effectiveness of prodrugs in neurodegenerative disorders. The percentage of oral absorption and solubility was enhanced when compared to the parent drug Flurbiprofen.On the basis of the results, it can be concluded that prodrug approach could successfully attain the goal of improving thetransport properties through the BBB and neuroprotective effect in the brain.

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