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Development, Characterization and Pharmacological Investigation of Umbelliferone Conjugates of NSAIDs

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Abstract

The present investigation developed the ester prodrugs of Non-steroidal anti-inflammatory drugs (NSAIDs), Mefenamic acid(MA) and Flurbiprofen(FBN) by conjugating with the natural antioxidant, 4-methyl umbelliferone that resulted in the formation of Mefenamic acid-umbelliferone ester prodrug (MU) and Flurbiprofen-umbelliferone ester prodrug(FU) .The principal objective of this study is the synthesis of the ester prodrugs of NSAIDs with the enhanced therapeutic activity and minimized side effects. Prodrugs were synthesized by coupling method using N,N'- dicyclohexylcarbodiimide/4-dimethylaminopyrimidine, and the resulted prodrugs were subjected to physical, chemical characterization, spectral characterization (IR, ^{1}H NMR, ^{13}C NMR and Mass spectra), hydrolysis-kinetic study and pharmacological evaluation such as anti-inflammatory, ulcerogenecity as well as the effect of the NSAIDs in the central nervous system against degenerative mechanisms. The current study revealed that the umbelliferone conjugates of NSAIDs upon administration would release the parent drug by hydrolysis in the desired site with enhanced anti-inflammatory activity and reduction in the gastro intestinal toxicity. Also, the synthesized pordrugs showed enhanced brain targeting efficiency with protective action against the degenerative processes.

Keywords: Mefenamic acid, Flurbiprofen, Anti-inflammatory activity, Prodrug, Umbelliferone, Gastrointestinal toxicity.

Introduction

NSAIDs, well accepted for the therapeutic activities such as Analgesic, Anti–inflammatory and Anti-pyretic activities based on the mechanism of cyclooxygenase(COX-1 and COX-2) enzyme inhibition and formation of prostaglandins [(1)] .The various pharmacological activities produced by the NSAIDs can be explained through different basic mechanisms such as nitric oxide system and transcriptional factors that showed direct relationship with cytokine expression which is having a significant role in the anti-inflammatory process [(2)]. Mefenamic acid, MA(2-(2, 3-dimethylphenyl)amino benzoic acid) and Flurbiprofen, FBN(2 -(2 –fluorobiphenyl – 4 –yl) propanoic acid) are NSAIDs widely used for the treatment of arthritic pain, inflammatory condition and dysmenorrhea etc [(3)]. The major side effect produced by the NSAIDs is the gastric-duodenal ulceration due to the free carboxylic acid functional group in the structure also several mechanisms was put forward such as the inhibition of prostaglandin synthesis, irritant effect on the epithelial tissue, effect in the gastric-mucosal blood flow [(4)].The structure activity relationship of NSAIDs was proved that the free carboxylic acid functional group in the molecular structure of the NSAIDs is necessary for the binding with COX receptors to elicit the pharmacological action and the hydrolysis of the prodrugs produced the anti-inflammatory activity. So the novel strategies are appreciated for designing and developing the ester and amide compounds by derivatization of the – COOH functional group produced considerable therapeutic activity with the reduction in side effect due to the free carboxylic acid group in the structure [(5)].The modification of carboxylic acid functional group can improve the transport properties across blood brain barrier and provide enhanced distribution thus therapeutic activity [(6)].

Several studies have been conducted for the reduction of the side effects and improve the transport profile of NSAIDs. Among that one of the most relevant approaches is prodrug based drug design. Prodrug based scheme can overcome the limitations and side effects produced by the NSAIDs. The prodrugs based approaches such as carries linked and bioprecursor approaches have significant role in the drug research and development [(7)].The drug molecules after the development process may be failed in the required therapeutic activity because of the pharmacokinetic profile, transport profile and solubility etc and prodrug approach successfully overcome the above limitations [(8)].In the worldwide pharmaceutical market, about ten percentages of the drugs are considered as prodrugs that can effectively overcome the limitations of the NSAIDs. The gastric lesions produced by the long term use of NSAIDs are regulated by the release of reactive oxygen species. So this study expected to decrease the gastric ulceration by the synthesis of the prodrugs using the antioxidant conjugate [(9)].

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The NSAIDs-Umbelliferone conjugates are developed with improved physical as well as chemical characteristics thus therapeutic profile. Different NSAID derived compounds showed better activity, reduction in the side effect, transport profile and effective therapeutic activity [(10)]. The natural antioxidant used in this study is the coumarin derivative, 4-methyl umbelliferone and studies showed that this natural compound has enough biological activities such as antioxidant, anti-inflammatory, anti-diabetic, anti tumor and neuroprotective effect [(11)].

This research work aim to develop the two ester prodrugs of NSAID, mefenamic acid and flurbiprofen, by conjugating with the antioxidant such as umbelliferone and identify the physical, chemical, hydrolytic test and pharmacological activities like anti-inflammatory activity, anti-ulcerogenic activity by using various methods expected to bring about improved characteristics.

Materials and Methods

Materials and measurements

The drug MA and FBN was obtained from TCI chemicals (India)Pvt.Ltd., Chennai, Tamilnadu. The antioxidant 4-methyl umbelliferone was obtained from Sigma Aldrich Chemicals Pvt .Ltd. Mumbai. The FTIR spectra of the compounds were recorded on IR spectrometer (bruker, software: Opus), Al shifa college of pharmacy, kerala. The elemental analysis by using elemental analyzer (Thermo finnigan, Italy, FLASH EA 1112 series) was done in Sophisticated Analytical Instrumentation Facility (SAIF), Lucknow. ^{1}H NMR (Cryo-magnet spectrometer, Bruker), [(13)] CNMR spectra (Cryo-magnet spectrometer, Bruker) and MASS spectra (Micromass Q-Tof Micro) were performed in SAIF Panjab University, Chandigarh. The melting points of the prodrugs were recorded using melting point apparatus (Sigma scientific products, Tamilnadu), Al Shifa College of pharmacy, Kerala. The absorbance was measured in the UV spectrophotometer (Shimadzu, Japan).Determination of physicochemical properties and the pharmacological evaluations were carried out in Department of Pharmaceutical Chemistry and Department of Pharmacology, Al Shifa College of Pharmacy. The histo-pathological studies were carried out in department of Pathology, KIMS and Al Shifa hospital, Kerala.

Synthesis of NSAIDs -Antioxidant prodrug:

This research work developed the ester prodrug of NSAIDs, MA and FBN by conjugating with the antioxidant, 4-methyl umbelliferone thus mefenamic acid-umbellierone prodrug[MU] and Flurbiprofen - umbelliferone prodrug[FU].

To a stirred solution of 10 mmol of carboxylic acid in 15 ml of anhydrous dichloromethane (DCM) 110mg of 4-dimethylaminopyrimidine (DMAP) and 10mmol of 4-methyl umbelliferone was added. Then 10mmol of N,N'- dicyclohexylcarbodiimide (DCC) were added to the reaction mixture at 0-8^{0}C, which is then stirred for 5 minutes at 0-8^{0}C and 3hr at 20-25^{0}C.After completing the reaction, the precipitated urea was filtered off and the filtrate was evaporated. The residue was taken in 10ml DCM and washed with saturated sodium bicarbonate solution and then dried over magnesium sulphate. The solvent is removed by evaporation and the ester was purified by recrystallisation. Before the recrystallisation the product was washed with alcohol to remove the excess of umbelliferone. If the product after synthesis was sticky, it was washed with petroleum ether two or three times. This procedure was used for the synthesis of both MU and FU and that was shown in the Figure 1 and 2 [(12)].

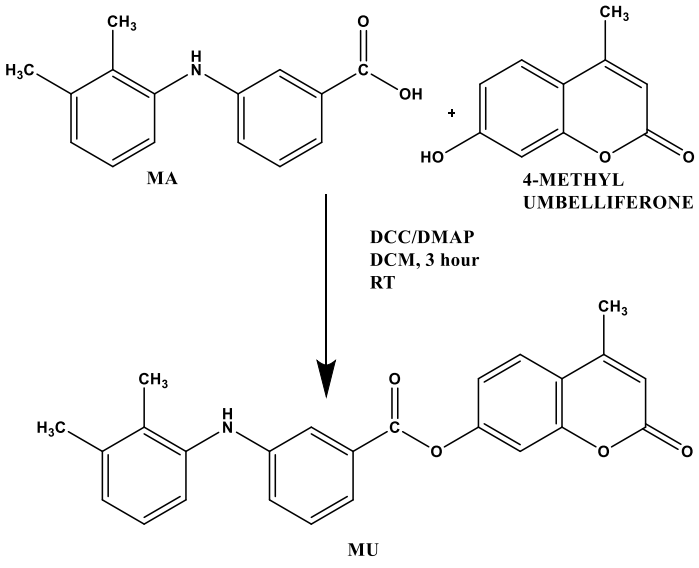


Figure 1. Scheme for the synthesis of MA-antioxidant prodrug

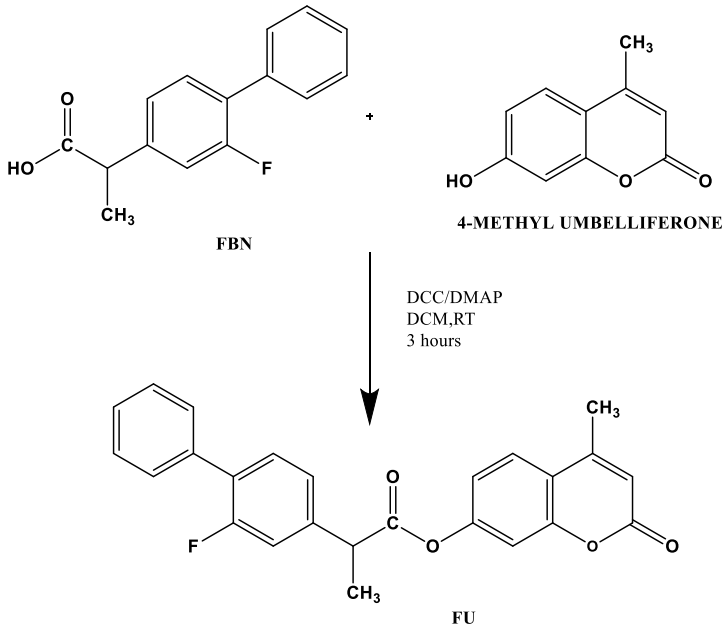


Figure 2. Schematic representation of the synthesis of FBN-antioxidant prodrug

Physical and chemical characterization of the prodrugs

The physical as well as chemical properties of the synthesized prodrugs were done by different methods such as solubility, determination of partition coefficient, melting point determination, thin layer chromatography, elemental and spectral analysis etc and the data obtained was compared with that of the parent NSAIDs, FBN and MA. The solubility of the prodrugs were evaluated in organic solvents such as chloroform, methanol, acetone, dimethylsulfoxide and the aqueous solvents such as water, sodium hydroxide (0.1N), hydrochloric acid (0.1N).This study is used to prove the hydrophilic and lipophilic nature of the drugs and prodrugs [(13)].Thin layer chromatography was done to check the progression of the reaction and also confirm the purity of the synthesized compounds that was done on the pre coated silica G plates. The detection of the spots visually done by using UV chamber. The solvent system used here was ethyl acetate:hexane 1:2. The determination of melting point of the synthesized compounds and the results were compared with that of the reactants to confirm the formation of the product and also assure the purity of the synthesized compounds. The determination of partition coefficient aim to attain the knowledge of the lipophilic profile of the synthesized compounds and that was done by shake flask method in which n-octanol saturated with phosphate buffer (pH 7.4). The concentration of the drug and each produg was monitored by measuring the absence by UV-VISIBLE spectrometer [(14)]. The quantification of the elements present in the synthesized compounds and the data was compared with that of the theoretical value was very much relevant and that determine the percentage of carbon, nitrogen, oxygen and hydrogen present in the synthesized ester prodrugs .The structure of the developed prodrugs were confirmed by IR spectra, ^{1}H NMR spectra, ^{13}C NMR and Mass spectra. The spectral data of the prodrugs were compared with that of the standard NSAIDs, MA and FBN affirm the formation of the compounds.

Hydrolytic study

Simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were used for the in vitro hydrolytic study having the P^{H} of 1.2 and 7.4 respectively.10 mg of the prodrug was in 90ml of the SGF and SIF and 15 ml of the solution was withdrawn and centrifuge and make up the volume with methanol up to 8 hours. After centrifugation 5ml of the supernatant was taken and monitored the free concentration of MA and FBN by UV-Vis spectrometer at 288nm, 247 respectively. The rate of hydrolysis was calculated by **K= (2.303/t) log [b/ (b − y)]:** where K represents hydrolysis constant, t is the time in minutes, b is the initial concentration of prodrug, y is the amount of prodrug hydrolyzed and (b − y) is the amount of prodrug remaining [(15)].

Pharmacological evaluation

The wistar albino rat was used for the study and all the animal experiments were conducted after obtaining the institutional ethical committee approval (Reg.No:1195/PO/Re/S/08/CPCEA), Al Shifa College of pharmacy, Kerala.

Ant-inflamamtory activity

The screening of anti-inflammatory activity was done by using the egg albumin induced inflammatory model. In this inflammation was induced by 0.1ml egg albumin in 1% normal saline and it was administered in to the sub plantar tissue of the right hind paw. The linear circumference of the injected paw was monitored before and after the administration of the agent by using vernier calipers (0.5, 1, 2, 3, 4 and 5 hours). Inflammation is the

difference in the paw circumferences between the control and other treated groups before and after the treatment of phlogistic agent [16].

Anti-ulcerogenic study

Gastro intestinal toxicity expressed as lesions produced by the drugs and prodrugs and the mucosal damage was examined by using of an electron microscope. The severity of the gastric toxicity was measured by the parameter mean ulcer index as per the procedure explained by Arun Rasheed et al., 2017 [17].

Activity in brain

The distributed NSAIDs showed the activity in the brain can be evaluated by using behavioral test, antioxidant test and histopathology of the brain cortex valuation. The albino mice was used for the study and all the animal experiments were conducted after obtaining the institutional ethical committee approval (Reg.No:1195/PO/Re/S/08/CPCEA), Al Shifa College of pharmacy,Kerala. The model used for pharmacological screening was aluminium chloride induced neurotoxicity model [18].The animals were divided in to six groups and each contain six animals. The Group I received normal saline which acts as control, Group II received aluminium chloride (50mg/kg) that acts as a negative control, Group III,IV,V and VI received MA(3.08mg), FBN(1.92mg), FU(3.2mg) and MU(4.3mg) respectively. This chronic neurotoxicity model was conducted and evaluated for 90 days.

Behavioral tests

Open field tests

The rats were placed in the open field apparatus and after placing the animals, allowed to move them without any disturbance for 5 minutes and number of head dips, line crossing and rearing were counted

Marble burying assay

The marble burying test is a simple behavioral test conducted in rodents, especially rats and mice are exposed to glass marbles placed on thick bedding materials. Thirty clean glass marbles were arranged evenly on the bedding. After 30 minutes exposure to the marbles, mice were removed and unburied marbles were counted. A marble was considered buried if its two-third size was covered with saw dust and the total number of marbles buried was considered as an index of locomotion

Water maze test

The rats were placed in the apparatus and escape latency was monitored. The apparatus consists of a large circular pool including a wooden material below [19].

Antioxidant parameters

For the in vivo test of antioxidant parameters, after behavioral study the mice were sacrificed and brain tissue homogenate was prepared with normal saline and centrifuged. The supernatant was used for the tests.

Superoxide dismutase

Assay mixture contain 0.1ml supernatant,1.2 ml sodium pyrophosphate buffer (pH 8.3,0.025 M), 0.1ml phenazine methosulphate ,0.3ml nitroblue tetrazolium, 0.2 ml NADH (780µm) and make up the volume to 3ml by adding water.Incubated at 30^0C for 90 s and 0.1 mL glacial acetic acid added, stirred with 4 ml n-butanol. It was allowed to stand for 10 min. The separated butanol layer having colour and it was measured by UV-visible spectrometer at 560nm. The SOD activity was tabulated.

Catalase

0.1ml of the brain tissue homogenate was treated with 0.9 ml of phosphate buffer and 0.4 ml of hydrogen peroxide. After 60seconds 2 ml dichromate acetic acid mixture was added. The color developed and it was measured at the wavelength 620nm. The activity was expressed as units / g tissue[20].

Histopathology of brain cortex

Histopathology of the brain cortex of the different treated groups were examined by hemotoxylin-eosin stain and monitored under electron microscope [21].

Statistical analysis

Statistical significance was done by ANOVA and the values were expressed as mean ± SD.

Results and discussion

Physical and chemical characterization

The two ester prodrugs of NSAIDs, FBN and MA were synthesized and physical as well as chemical characterization was done. The ester prodrugs of FBN and MA were synthesized by conjugating with the natural antioxidant 4-methyl umbelliferone by DCC/DMAP coupling method and the two synthesized compounds were FU and MU. FU is 4-methyl-2-oxo-2H-chromen-7-yl-2-(2-flouro-[1, 1'-biphenyl]-4-yl) propanoate and MU is 4-methyl-2-oxo-2H-chromen-7-yl-2-((2,3-dimethyl phenyl)amino)benzoate. The thin layer chromatography was used to determine the progression, formation and check the purity of the synthesized ester prodrugs. The single spot obtained from the thin layer chromatography and the R_f values were 0.69 and 0.51 for FU and MU respectively. The molecular weight FU and MU was found to be 402 and 391 respectively. The remarkable difference in the melting point confirmed the completion of the synthesis of the compounds. The solubility studies proved the high solubility of the prodrugs in the organic solvents and that sowed the enhanced lipophilic behavior.

Also the enhancement of the lipophilic profile was proved by partition coefficient studies. The elemental analysis was done to find out the percentage of C, N, O and H present in the synthesized compounds and that is compared with that of the calculated values. The calculated and found values were comparable. The results of physical and chemical characterization were given in the table 1.

Table 1. Physical and chemical characterization of FU and MU

Prodrug	Molecular weight	Colour	Melting point (^{0}C)	Log P	Percentage yield	R_f Value	Elemental analytical data		
							Calculated percentage		**Found percentage**
FU [$C_{25}H_{19}FO_4$]	402	Pure white crystals	90-92	1.35	70	0.69	C	74.62	74.65
							H	4.76	4.72
							F	4.72	4.75
							O	15.90	15.92
MU [$C_{25}H_{21}NO_4$]	391	yellowish white crystals	140-143	1.43	76	0.51	C	78.57	78.60
							H	5.83	5.80
							N	3.53	3.58
							O	12.08	12.05

FU(4-methyl-2-oxo-2H-chromen-7-yl-2-(2-flouro-[1,1'-biphenyl]-4-yl)propanoate):**FTIR**(cm^{-1},KBr):2,980(C–H),1,713(C=O,ester)and1,071(CO,ester);**^{1}HNMR**($CDCl_3$,): 7.56(m,J=7.75,3H)7.45(d,J=10.62,3H),7.37(m,J=5.7,1H),7.23(dd,J=6.3,1H),7.06(d,J=2.15,1H), 7.01(d,J=8.65,1H), 6.26(s,1H), 4.028(q,J=7.1,1H), 2.415(d,J=7.1,3H),1.67(d,J=7.15,3H,); **^{1}HNMR**(D_2O):7.56(m,J=7.75,3H)7.37(d,J=10.62,1H),7.45(m,J=5.7,3H),7.23(dd,J=6.3,2H),7.06(d,J=2.15,1H), 6.99(d,J=8.65,1H), 6.25(s,1H), 4.028(q,J=7.1,1H), 2.415(d,J=7.1,3H),1.67(d,J=7.15,3H,);**Mass(m/z):**403;**^{13}CNMR(CDCl$_3$,500MHz)** 18.35(CH_3),18.72(CH_3),45.15 (CH, Aliphatic), 110.29(CH, Benzene),114.69(**C**H-COO, lactone), 115.25(C, Benzene), 117.85(CH, Benzene), 117.95(CH, Benzene), 123.54(CH, Benzene) , 125.38(C-**C**), 127.84(CH, Benzene), 128.33(CH, Benzene), 128.44(CH, Benzene), 128.51(2C, CH Benzene), 128.98(CH, Benzene), 131.17(CH, Benzene), 135.28(C, Benzene), 140.68(**C**-C), 153.11(C-O, Benzene), 154.17(CH, Benzene), 158.85(C, Benzene), 160.42(C-F), 160.83 (carboxyl, lactone -C=O), 171.9(Carbonyl -**C**=O).

MU (4-methyl-2-oxo-2H-chromen-7-yl-2-((2, 3-dimethyl phenyl) amino) benzoate): **FTIR** (cm^{-1} KBr): 3364 (N-H stretching), 2,980 (C–H), 1,718 (C=O ester), and 1,071 (C–O, ester); ^{1}H NMR ($CDCl_3$,δppm) 8.17(d,J=6.55,1H,), 7.67(d,J=8.6,1H,), 7.33(t,J=5.45,1H,),7.28(s,1H), 7.22(d,J=6.3,1H), 7.15(d,J=7.25,1H,), 7.12(t,J=7.75,1H,), 7.05(d,J=7.2,1H,), 6.77(d,J=10.06,1H,), 6.75(t,J=7.05,1H,), 6.29(1H), 2.46(s,3H), 2.32(s,3H), 2.18 (s,3H); ^{1}H NMR (D2O,δppm) 8.17(d,J=6.55,1H,), 7.67(d,J=8.6,1H,), 7.33(t,J=5.45,1H,),7.28(s,1H), 7.22(d,J=6.3,1H), 7.15(d,J=7.25,1H,), 7.12(t,J=7.75,1H,), 7.05(d,J=7.2,1H,), 6.77(d,J=10.06,1H,), 6.75(t,J=7.05,1H,), 6.29(1H), 2.46(s,3H), 2.32(s,3H), 2.18 (s,3H);**Mass(m/z):**400;**^{13}CNMR(CDCl$_3$, 500 MHz)**14.01(CH_3),18.77(CH_3),20.60(CH_3),108.79(**C**H-COO, lactone) ,110.99 (C, Benzene),113.38 (CH, Benzene),114.54 (CH, Benzene),,116.29 (C fused),117.89 (CH, Benzene), 118.6 (CH, Benzene),, 123.46 (CH, Benzene), 125.4 (C-**C**), 126.05 (CH, Benzene), 127.31(CH, Benzene), 131.89(CH, Benzene), 132.74 (C, Benzene), 135.46 (CH, Benzene), 138.12 (C, Benzene), 138.38 (C, Benzene), 150.64 (C, Benzene), 151.98(C-O, Benzene), 153.40(**C**-N), 154.32(C-fused), 160.58(carboxyl, -C=O), 166.69(Carbonyl -**C**=O).

In vitro hydrolysis study

In vitro hydrolytic study of the synthesized ester prodrugs was conducted in the enzyme free simulated intestinal fluid (p^H 7.4) and simulated gastric fluid (p^H 1.2) and the results proved the enhanced stability of the prodrugs in gastric p^H 1.2 and enhanced percentage hydrolysis of the synthesized ester prodrugs in the intestinal p^H 7.4. The percentage hydrolysis of the synthesized ester prodrugs were graphically represented in the Figure 2 and 3. The data showed that the synthesized ester prodrugs FU and MU showed the percentage of hydrolysis after 8 hrs in SGF was 29 and 29.50 respectively. The percentage hydrolysis of the FU and MU in SIF was 76.50 and 75.32 respectively. This result established, the fact that the natural antioxidant conjugated prodrug showed considerable stability in SGF and considerable hydrolysis in SIF. The kinetic study FU and MU follow first order kinetics that was understood from the correlation coefficient data with the half-life of 318 and 301 minutes respectively in SIF. The results of pharmacokinetic study in SIF were given in the table 2 and first order kinetic graph of the prodrugs in SIF was given in Figure 4.

Table 2. Pharmacokinetic data in SIF

Prodrug	SIF [Correlation Coefficient]		First order kinetic data	
	Zero order	**First order**	**Rate constant**	Half life($t_{1/2}$)[min]
FU	**0.9817**	**0.9928**	**0.00218**	318
MU	0.9812	0.9956	0.00230	301

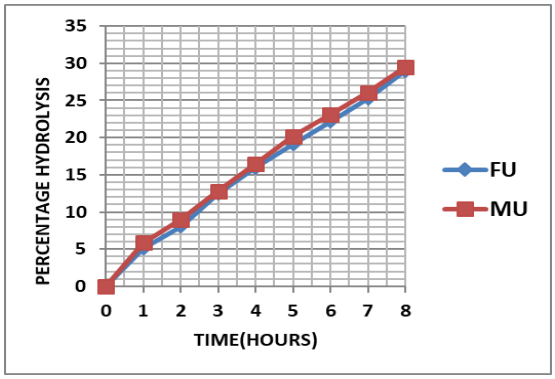


Figure 3. Percentage hydrolysis of the FU and MU in SGF

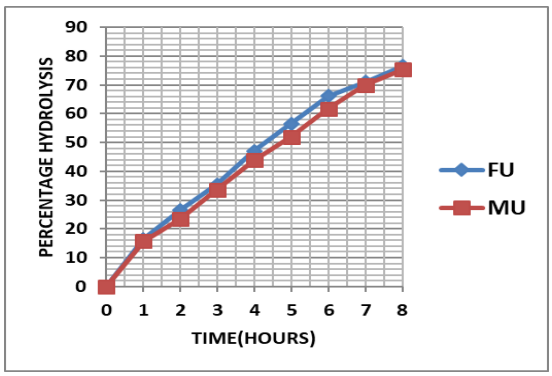


Figure 4. Percentage hydrolysis of the FU and MU in SIF

Anti inflammatory activity

The drugs MA and FBN are good anti-inflammatory agents but the synthesized umbelliferone conjugated prodrugs showed enhanced anti-inflammatory activity. The comparative study was shown graphically in figure 5.The data showed the enhanced activity from the six hours observations and the prodrugs showed the values 44.1 to 75.5, 46.0 to 78.4 in the case of MU and FU respectively. The activity profile of the prodrugs showed enhanced pharmacological activity. The statistical significance ($p<0.05$) was done by one-way ANOVA and Dunnet's t test.

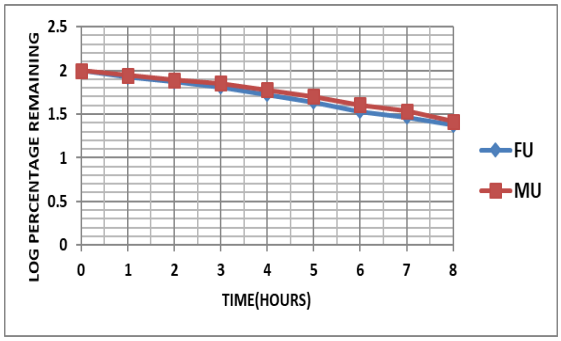


Figure 5. First order kinetic graph in SIF

Ulcerogenic activity

The side effect produced by the NSAID is the ulcer production in the gastric mucosa due to the free carboxylic acid functional group in the structure of NSAIDs. The ulcer formation in the different treated rodents was visually monitored and the parameter to access the ulcer formation is mean ulcer index. The photographs of the standard and test groups were given in the Figure 6 and the mean ulcer index was graphically represented in the Figure 7.

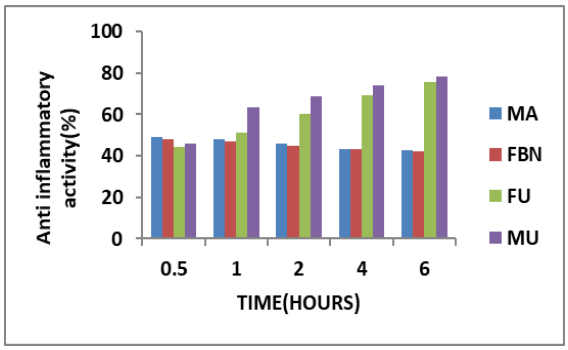


Figure 6. Anti inflammatory activity

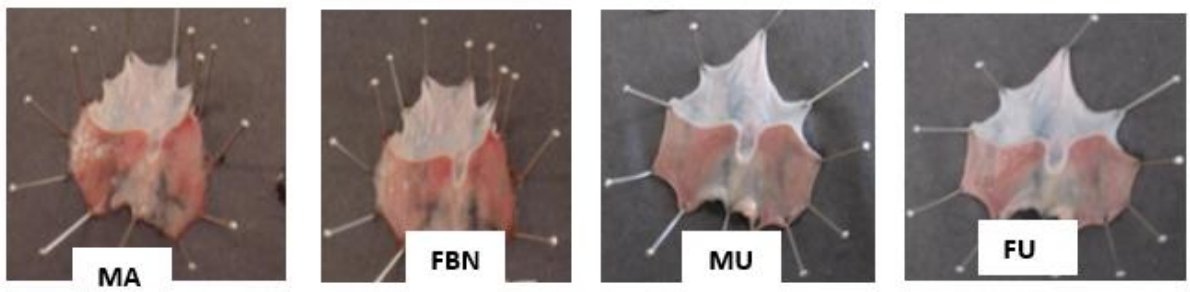


Figure 7. Ulcer formation in different groups: i. MA, ii.FBN, iii. MU and iv. FU

Activity in brain

Pharmacological evaluation was done by monitoring the activity in the brain by behavioral studies, anti-oxidant test and microscopically examined the histopathology of the brain cortex and the obtained data analysis confirmed the protective nature of the NSAIDs against degenerated conditions in the brain. The neurotoxicity was induced by aluminum chloride (50 mg/kg) explained in the methodology.

The behavioral tests assess the general behavior, memory, spatial learning, locomotor activity and anxiety etc. Behavioral parameters were monitored by using the tests Open field test, Marble burying test and water maze test.

The open field test explained graphically in figure 8. This behavioral evaluation proved that the number of head dipping, number of line crossing and rearing significantly ($p<0.001$) decrease in the neurotoxicity induced group II compared to the normal saline treated control group. But the treatment of the animals with prodrugs showed remarkable increase in the number of head dipping, rearing and line crossing significantly ($p<0.05$ and $p<0.01$) compared to the FBN and MU treated groups. But the behavioral activity of the FBN and MU showed similarity to the Group II that may due to the limited distribution of NSAIDs in the brain.

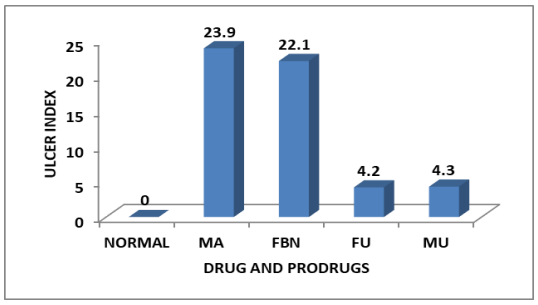


Figure 8. Ulcer index of the drugs and prodrugs

The results of the marble burying study graphically represented in figure 9. The results indicated that the number of marbles buried by the Group II was significantly decreased ($p<0.001$) compared to that of the Group I control. The NSAIDs treated groups showed similarity in the Group II significantly ($p<0.001$) and that proved the limited activity of the hydrophilic drugs in the brain. But the prodrug (FU and MU) treated groups showed significant ($p<0.05$ and $p<0.01$) increase in the number of marbles buried that proved the prodrug based synthesis provide the sufficient enhancement in the transport properties through the BBB and also the distribution, efficiency and protective effect.

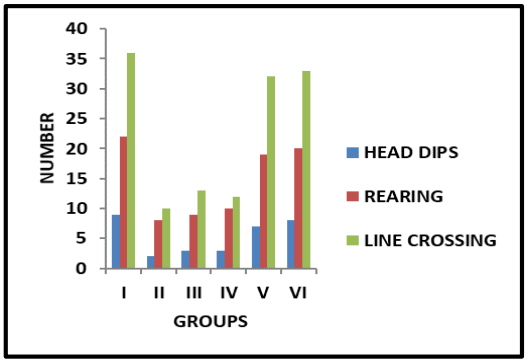


Figure 9. Open field test

The water maze test evaluated the spatial learning and memory and the results were graphically represented in figure 10. The results showed that the negative control group has increased escape latency compared to the control group ($p<0.01$). The time taken to escape is decreased in the case of prodrug treated groups compared to that of NSAIDs treated groups. The time taken to escape by the FBN and MA treated groups is comparable with that of the negative control group with a significance of $p<0.001$.The comparison in the data between the drug treated groups and prodrug treated groups revealed that after reaching the brain NSAID can perform an important role in the case of neuro-degenerative conditions.

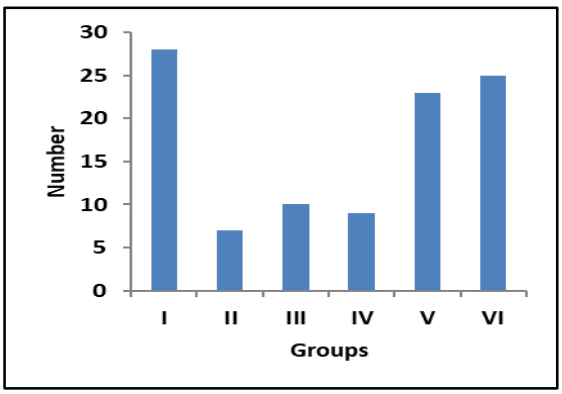


Figure 10. Marble burying test

The evaluation of the antioxidant activity (SOD and Catalase) indicated the protective effect of the compounds in the brain. The amount of SOD and catalase showed decreased in the negative control group compared with that of the normal control group ($p<0.001$). FU and MU treated groups showed significant increase ($^{c}P< 0.05$, $^{d}P<0.01$) in the SOD and catalase activity when compared to that of the FBN and MA treated groups and the data was given in figure 11. This proved the protective effect of the NSAIDs in the central nervous system.

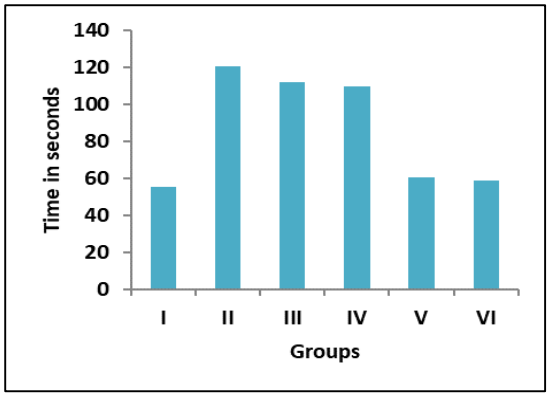


Figure 11. Water maze test

Histopathology of mice brain were from the normal saline group, negative control group, MA,FBN,MU and FU treated groups and the results were analyzed. In the prodrug treated group, all the two different ester prodrugs showed normal cells of cortex without any spongiform cells, indicates the protective effect of synthesized natural compound conjugating ester prodrugs of MA and FBN that was shown in the figure 12.

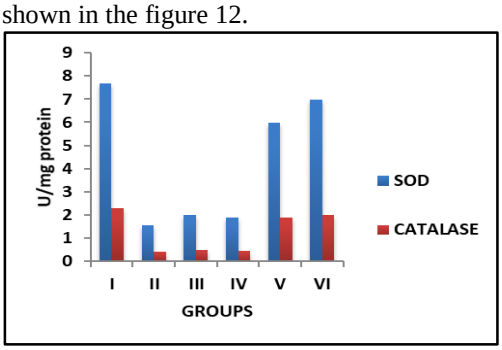
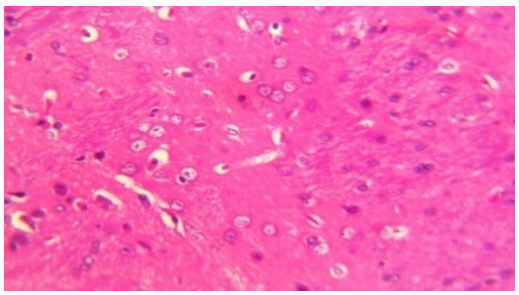
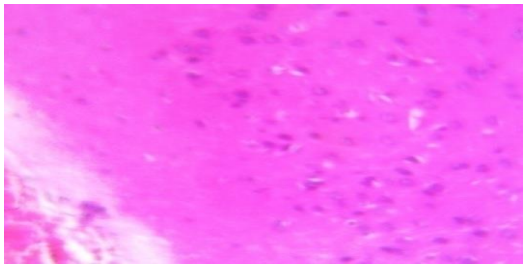


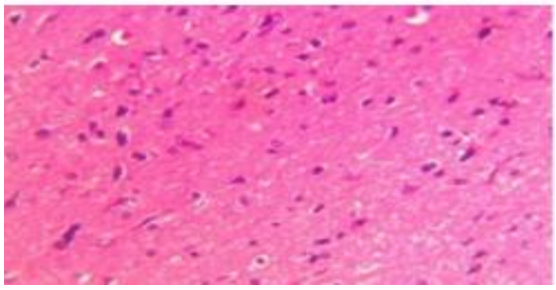
Figure 12. Antioxidant activity



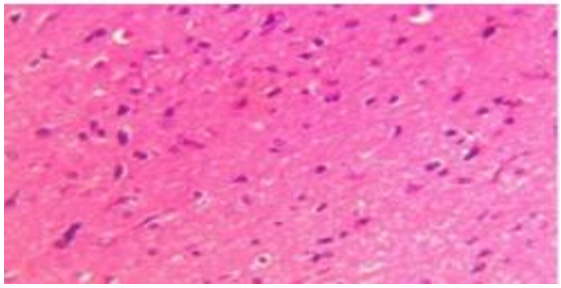
Normal



Aluminium chloride



FU



MU

Figure 13. Histopathology of brain cortex

References

1. Gunaydin C, Bilge SS. Effects of Nonsteroidal Anti-Inflammatory Drugs at the Molecular Level. Eurasian J Med. 2018; 50(2):116-121.
2. Asanuma M, Miyazaki I, Ogawa N. Neuroprotective effects of nonsteroidal anti-inflammatory drugs on neurodegenerative diseases. Curr Pharm Des. 2004;10(6):695-700.
3. Feng X, Wang X. Comparison of the efficacy and safety of non-steroidal anti-inflammatory drugs for patients with primary dysmenorrhea: A network meta-analysis. Mol Pain. 2018;14:1744806918770320. doi:10.1177/1744806918770320
4. Wallace JL. How do NSAIDs cause ulcer disease? Baillieres Best Pract Res Clin Gastroenterol. 2000; 14(1):147-59.
5. Qandil AM. Prodrugs of nonsteroidal anti-inflammatory drugs (NSAIDs), more than meets the eye: a critical review. Int J Mol Sci. 2012;13(12):17244-17274.
6. Qiming Yue, Yao Peng, Yi Zhao, Runxin Lu, Qiuyi Fu, Yang Chen, Yang Yang, Li Hai, Li Guo & Yong Wu, Dual-targeting for brain-specific drug delivery: synthesis and biological evaluation, Drug Delivery,2018, 25:1, 426-434.
7. Rautio, J., Meanwell, N., Di, L. et al. The expanding role of prodrugs in contemporary drug design and development. Nat Rev Drug Discov 2017;**17,** 559–587.
8. Najjar A, Najjar A, Karaman R. Newly Developed Prodrugs and Prodrugs in Development; an Insight of the Recent Years. Molecules. 2020;25(4):884. Published 2020 Feb 17. doi:10.3390/molecules25040884
9. Abet V, Filace F, Recio J, Alvarez-Builla J, Burgos C. Prodrug approach: An overview of

recent cases. Eur J Med Chem. 2017 Feb 15; 127:810-827.

10. Cacciatore I, Marinelli L, Fornasari E, et al. Novel NSAID-derived drugs for the potential treatment of Alzheimer's Disease. Int J Mol Sci. 2016;17(7):1035.

11. Ostrowska K. Coumarin-piperazine derivatives as biologically active compounds. Saudi Pharm J.2020;28(2):220-232.

12. GILLES, Vitor et al. A New, Simple and efficient method of steglich esterification of juglone with long-chain fatty acids: synthesis of a new class of non-polymeric wax deposition inhibitors for crude oil. J. Braz. Chem. Soc. [online]. 2015; 26(1), 74-83

13. Rasheed A, Kumar CK, Mishra A. Synthesis, hydrolysis studies and phamacodynamic profiles of amide prodrugs of dexibuprofen with amino acids. J Enzyme Inhib Med Chem. 2011;26(5):688-695.

14. Andrés A, Rosés M, Ràfols C, et al. Setup and validation of shake-flask procedures for the determination of partition coefficients (logD) from low drug amounts. Eur J Pharm Sci. 2015;76:181-191. doi: 10. 1016 /j. ejps. 2015 .05 .008.

15. A. Rasheed and C. K. Ashok Kumar: Design, synthesis, hydrolysis kinetics and phamacodynamic profiles of histidine and alanine conjugates of aceclofenac, Acta Pharm. 2010;60, 99–109.

16. Okokon, J. E., Udoh, A. E., Frank, S. G., & Amazu, L. U. Anti-inflammatory and analgesic activities of Melanthera scandens. Asian Pac J Trop Biomed, 2012;2(2), 144–148.

17. Jaya P. Peesa, Lakshmana R. Atmakuri, Prasanna R. Yalavarthi, Basaveswara R. Mandava Venkata, Arun Rasheed, Vengalrao Pachava, Oxaprozin prodrug as safer nonsteroidal anti-inflammatory drug: Synthesis and pharmacological evaluation, Archiv der Pharmazie, 10.1002/ardp.201700256, 2017;**35 1** (2)

18. Nija B, Arun Rasheed, Kottaimuthu A. Synthesis, characterization and pharmacological investigation of lysine and glycine conjugated amide prodrugs of (+)-ibuprofen. ijrps [Internet].2020;11(3):5022-30.

19. Smith G, Ahmed N, Arbuckle E, Lugo JN. Early-life status epilepticus induces long-term deficits in anxiety and spatial learning in mice. Int J Epilepsy. 2017;4(1):36-45. doi:10.1016/j.ijep.2016.12.005

20. Nija B, Arun Rasheed, Kottaimuthu A. Development, characterization and pharmacological evaluation of amino acid prodrugs of (+)-Ibuprofen. ijrps [Internet]. 2020Aug.17 [cited 2020Sep.25];11(3):4998-007.

21. Toxicologic Pathology, 39: 22-35, 2011, Histology of the Central Nervous System ROBERT H. GARMAN