Available online at www.ijpcr.com
International Journal of Pharmaceutical and Clinical Research 2017; 9(2): 123-130

DOI number: 10.25258/ijpcr.v9i1.8294

ISSN- 0975 1556

Research Article

Drug Development of Mefenamic Acid Derivatives as Analgesic by Molecular Approach

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Available Online:25th February, 2017

ABSTRACT

A new compound of Mefenamic Acid derivate, 4-nitrobenzoyl-mefenamic acid has been synthesized by benzoylation reaction between mefenamic acid and 4-nitrobenzoyl chloride after prediction by in silico study/molecular approach. A derivative of mefenamic acid (4-NO2-benzoyl-mefenamic acid) has been synthesized for increase its activity as candidate of analgesic drug/inhibitor COX-2 (Cyclooxigenase-2). This compound has been purified by Column Chromatography and analyzed using TLC-Densitometry to determine purity with Rf value 0,8. The spot has good purity and then it was identified this structure using H-NMR 400 MHz and FTIR-KBr. The result showed that this compound is 4-nitrobenzoyl-mefenamic acid (4NBMA). 4NBMA gives white yellow color with melting point 198-199^{o}C. Finally, 4NBMA was tested analgetic activity by hot plate method and it showed that 4-nitrobenzoyl-mefenamic acid has been higher activity than mefenamic acid.

Keyword: *4-nitrobenzoyl-mefenamic acid, analgesic, benzoylation, molecular approach.*

INTRODUCTION

Pain is a multidimentional sensory experience. International Association for the Study of Pain (IASP) defines that pain is a sensory and unpleasant emotional experience associated with tissue damage, both actual and potential[5,22]. Chronic pain becomes a serious problem if it increases rate of pain and gives chronic prevalence. Pain is one of the most frequently reported symptom occurs in one from six people in the population and it is estimated to occur in 2-40% of adult population[11]. Some studies estimate that the prevalence of chronic pain in Europe is up to 55.2%[4]. In Indonesia, the population of the elderly, reported that 25-50% of them experienced pain[7]. Chronic pain causes increasing health care costs[11]. The research in the United States showed that the cost yearly for chronic pain is estimated around 100 billion dollars. Mefenamic acid is a drug in the market as NSAIDs (Non-steroidal AntiInflammatory Drugs) which has long been used as an analgesic-inflammatory COX and widely used in the world for treatment of diseases to relieve pain/pain and inflammation such as rheumatoid arthritis, toothache, gout and peripheral muscle pain. In the effort to design and develop new drugs, the first step is modification of commercial drug that has been known its molecular structure and biological activity and was become guidance based on a systematic and rational research to reduce trial and error. Further guidance from lead compounds were developed and modified that become new compounds/derivatives and then was tested this biological activity[14,16]. Because of extensive use of mefenamic acid so we effort to develop new drugs and created derivatives. Based on previous research, mefenamic acid was substituted benzenesulfonic, bromo anthranilic, paracetamol, phenoxybenzoic, cyclocarboimide the coupling reaction, cyclourea, esters and amides, hydrazine and hidramin can enhance the analgesic effect of anti-inflammatory and reduce the side effects (ulcers)[1,9,10,15,17,19,21]. In this study conducted with the benzoyl derivative substitution by topliss theory and then was predicted by molecular approach. The substitution by benzoyl based on research Jayaselli et al and Susilowati through benzoylation reaction of NSAIDs such as paracetamol and piroxicam derivatives gives greater biological activity than the lead compound[6,18]. We used several substituents benzoyl derivative is based on the Topliss theory that predicted by lipophilic, electronic and steric parameters as substituents. Lipophilic parameters associated with penetration rate in biological membranes. Electronic parameters contributed in the process of drug interactions with receptor by ionization and polarization proccess thus increasing the biological effectiveness overall. Steric parameters related to the compatibility of the interaction of the compound with the receptor in the cell that it effected maximum binding orientation so increased activity[16]. Increased lipophilic properties can be done by inserting a non-polar groups such as aromatic rings, while the increase in electronic properties can be done by inserting electronegative substituents as steric halogen. Steric properties can be done by creating a more bulky structure that serves as a shield and encourages interaction between drug and active site of receptor. While we modified mefenamic acid by functional groups that increase in lipophilic, electronic and steric parameters that influenced the

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biological activity[16]. The synthesis of mefenamic acid derivative is reacted between benzoyl derivatives and mefenamic acid via nucleophilic addition. The mechanism can be seen Figure 1.

Mefenamic acid derivatives activity can be predicted before synthesized by molecular approach that used docking with Molegro Virtual Docker (MVD). The goal of docking estimates the interaction between mefenamic acid derivatives as ligands with COX-2 receptors that act as enzymes in inflammatory pain. Then from the molecular docking will be obtained compounds are the most potent predictions which will then be synthesized. The compounds synthesized were tested for purity by melting point and Thin Layer Chromatography (TLC). Further, the structure of compounds was identified by with the Infrared Spectrometry (FTIR) and Proton Nuclear Magnetic Resonance Spectrometry (1H-NMR) and then analgesic activity was tested by the hot plate method.

METHODS

Instrument

Glass beaker (Iwaki Pyrex), bulb pipettes, pipette, stirrer, micropipette (Blaubrand® IntraEnd), chamber, magnetic stirrer, oven, analytical balance (Sartorius), Ultraviolet Lamp (UV) dryer/hair dryer, Electrothermal melting point apparatus, 1H-NMR 400 MHz JEOL Resonance, FTIR-KBr Perkin Elmer-Spectrum One, TLC-Densitometry (Camag), digital cameras, ChemBioOffice 2008 trial version, Molegro Virtual Docker trial version.

Material

Mefenamic acid (Merck), 4-nitrobenzoylchloride (Sigma), acetone (Sigma), Silica gel 60 F 254, hexane (Merck), toluene (Merck), ethyl acetate (Merck), methanol (Merck), KBr (Merck), chloroform-d pro NMR (Sigma), tetramethylsilane (TMS) pro NMR (sigma), mice.

Molecular docking

Structure mefenamic acid and its derivatives as ligands drawn with Chemoffice program 2008 while 3D structure of the cyclooxygenase (COX-2) as the receptor is taken from the Protein Data Bank (PDB). Furthermore, docking ligand-protein using the program MVD 2008[3,20]. *Preparation Ligand and Receptor*. The structure of all compounds mefenamic acid derivative according to Topliss theory (4-OCH3, 4-Cl, 4-Br, 4-F, 4-NO2, 3-NO2, 4-CF3, 3-Cl, 2Cl, H, 3.4 -Cl benzoyl) drawn using Draw Ultra 11.0 ChemBio 2008. Further optimized by using 3D Ultra 11.0 ChemBio 2008 and the minimization of the energy of the most stable ligand with MM2 to produce a ligand conformation pose consistent. Prospective pose energy minimized in the active site using a grid-based method for evaluating the protein-ligand interaction energy stored in the docking receptor Mol2. We must remove water before preparation ligand and then determine active site via Detect Cavity[20]. *Validation Docking*. Validation is performed to calibrate the method of docking software. The parameter is used to assess of the validity that is RMSD value where RMSD value is less than 2 that means position of the ligand copy superimpose with native ligand so that the methods used would be more appropriate. RMSD value is also influenced by the resolution of the receptor protein and the receptor modeling methods. Validation is performed on ligand binding site pocket with 10 times replication for each receptor. Docking is operated by HP pavilion with a processor Intel (R) 2.2 GHz, 2.00 GB of RAM, and a 64 bit operating system. Docking software is done by MVD 2008 with a grid resolution of 0:30, iteration a maximum of 1500, maximum population size 50, a pose energy generation 100.00, evolution simplex used at step 300 and max distance scanning 1.00. The parameters of docking are MolDock Score, Rerank Score, RMSD (Root Mean Standard Deviation) and H Bond. MolDockScore value of ligands which has a lower energy so it is more stable in receptor binding and it can be choosen to synthesized[20].

Synthesis of mefenamic acid derivatives

The first stage mefenamic acid (2 mmol) was dissolved in 30 ml acetone and then added NaHCO3 0.19 g (2.2 mmol) and benzoyl chloride derivative which is optimum according to the prediction of molecular docking approach (4-NO2-benzoyl chloride) (2.2 mmol) in 5 ml acetone from the funnel into the flask drop by drop over 30 minutes thus stirring in ice bath. After the completion reagents are added, the mixture is heated at 40° C and stirer for 4 hours. When the reaction is complete, the reaction proceeds until the solvent is evaporated by the evaporator runs out then the precipitate is added with water (10 ml) and ethyl acetate (30 ml) resulting in two phases, organic and water phase. Further, 2 phase of solution was washed with HCl pH 3-4 (10 ml) and separated with a separating funnel where collected by ethyl asetat. Ethyl acetate phase was washed with NaHCO3 pH 7-8 (10 ml) and separated with a separating funnel and collected. Thus, Ethyl acetat phase was dried with sodium sulfate anhidrat and evaporated. The residue is purified by column chromatography with methanol:toluene (2:8). The crystals were collected and saved in exicator[12].

Purity test of mefenamic acid derivatives

Purity Test of mefenamic acid derivative

Thin Layer Chromatography (TLC). Purification using TLC (Thin Layer Chromatography) need to mobile phase methanol: toluene (2: 8). After eluation finished and observed with 254 nm. *b. Melting point range*. The range of melting point requirement to determine of purity of compounds is $\leq 2^oC$[6]

Identification of mefenamic acid derivatives

a. Infrared spectrophotometry. A number of mixed homogeneous powder sample with KBr and made the form of pellets with hydraulic presses and the spectrum observed at wave numbers 4000-400 cm^{-1} by FTIR. *b. Spectro 1H-Nuclear Magnetic Resonance*. Samples were prepared by dissolved in chloroform-d1 (CDCl3), with the internal standard tetramethylsilane (TMS) and irradiated with radio wave. Spectrum wave of H NMR is a graph of the amount of energy absorbed (I or intensity) against strong magnetic field.

Analgesic Acivity Test

Mice were divided into 6 groups, each consisting of 4

Table 1: Molecular Docking Scoring of mefenamic acid and its derivatives into (COX-2).

No	Compounds	Moldockscore	Rerank score	RMSD	H Bond
1	Native ligan	-105.321	-84.4739	0.822142	0.35223
2	Asam mefenamat	-107.7271	-87.27073	4.653694	-3.406527
3	4Cl-benzoil Asam mefenamat	-119.3606	-53.30389	3.119501	-5.826691
4	4Br-benzoil Asam mefenamat	-108.14942	-3.870076	4.080552	-0.3546422
5	4F-benzoil Asam mefenamat	-118.41	-49.06499	3.218392	-5.691136
6	4CF3-benzoil Asam mefenamat	-124.8416	-55.21401	3.733331	-5.601465
7	**4NO2-benzoil Asam mefenamat**	**-131.7728**	**-19.617856**	**3.013529**	**-5.2265942**
8	2Cl-benzoil Asam mefenamat	-109.707	64.167485	4.423048	2.7628988
9	4OCH3-benzoil Asam mefenamat	-123.5064	-54.77227	3.061372	-5.698905
10	3Cl-benzoil Asam mefenamat	-112.0296	-21.040703	2.862561	0.2114293
11	3NO2-benzoil Asam mefenamat	-127.2584	-78.79086	3.738665	-5.04409
12	Benzoil Asam mefenamat	-113.3884	-41.10053	3.058158	-4.8811931
13	3,4Cl-benzoil Asam mefenamat	-128.4548	-54.73877	3.245705	-6.471567

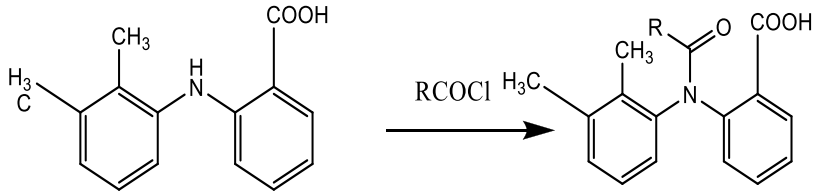

Figure 1: Reaction of Mefenamic acid derivative.

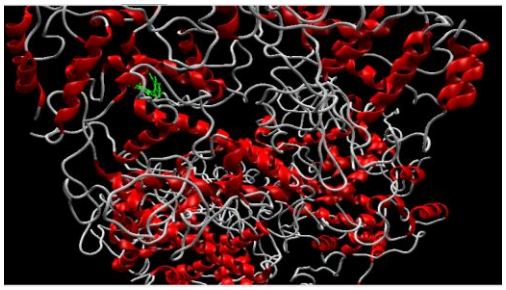

Figure 2: Molecular Modelling mefenamic acid by secondary sheet in COX-2 receptor.

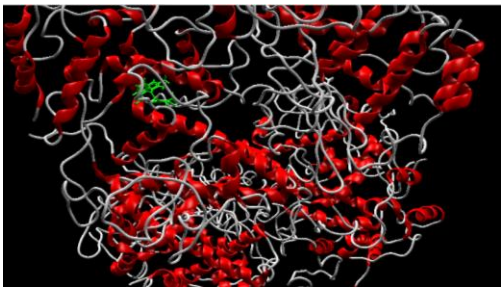

Figure 3: Molecular Modelling 4-NO2-benzoyl mefenamic acid (4NBMA) by secondary sheet in COX-2 receptor.

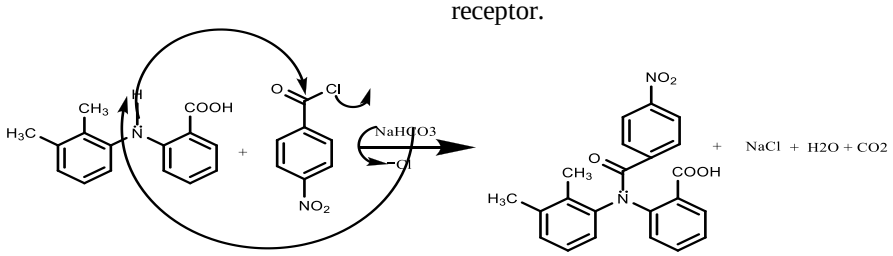

Figure 4: Benzoylation reaction of 4-NO2-benzoyl mefenamic acid (4NBMA) between mefenamic acid and 4-NO2-benzoyl chloride.

Table 2: Spectrum Characteristic Infra Red of mefenamic acid.

Wavelength (cm-1)	Function Groups
3311	N-H (secondary)
1511-1651	C=O Carboxyl salt
2924	C=C aromatic
893	O-substitution

mice. Groups I was negative control (CMC Na 1%). Groups II is the positive control group that treated mefenamic acid. Groups III-VI is treated group given mefenamic acid derivative synthesized with 4 concentration. Administration of mefenamic acid derivative was given one times one day for 7 to 14 days.

Method of Hot Plate Analgesic

Pain stimuli used in hot plate (55-56ºC). Hot pain in the legs of mice led to responses raised front legs and licked. The average pet mice will provide a response to this method within 3-6 seconds. Mice weighed (25-35 g) is noted and given symbol. 30 minutes later the mice were placed on a hot plate that had been set the temperature at 50 ± 0.5ºC. Observe the time the mice began to put on a

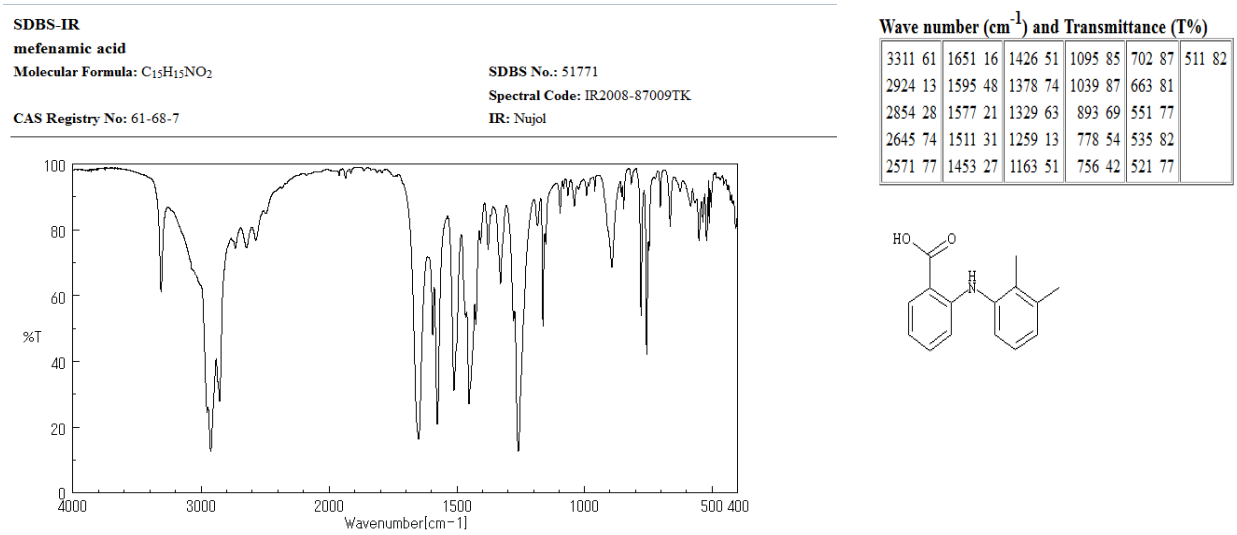

Figure 5: IR Spectrum of Mefenamic Acid (Anonymous, 2014).

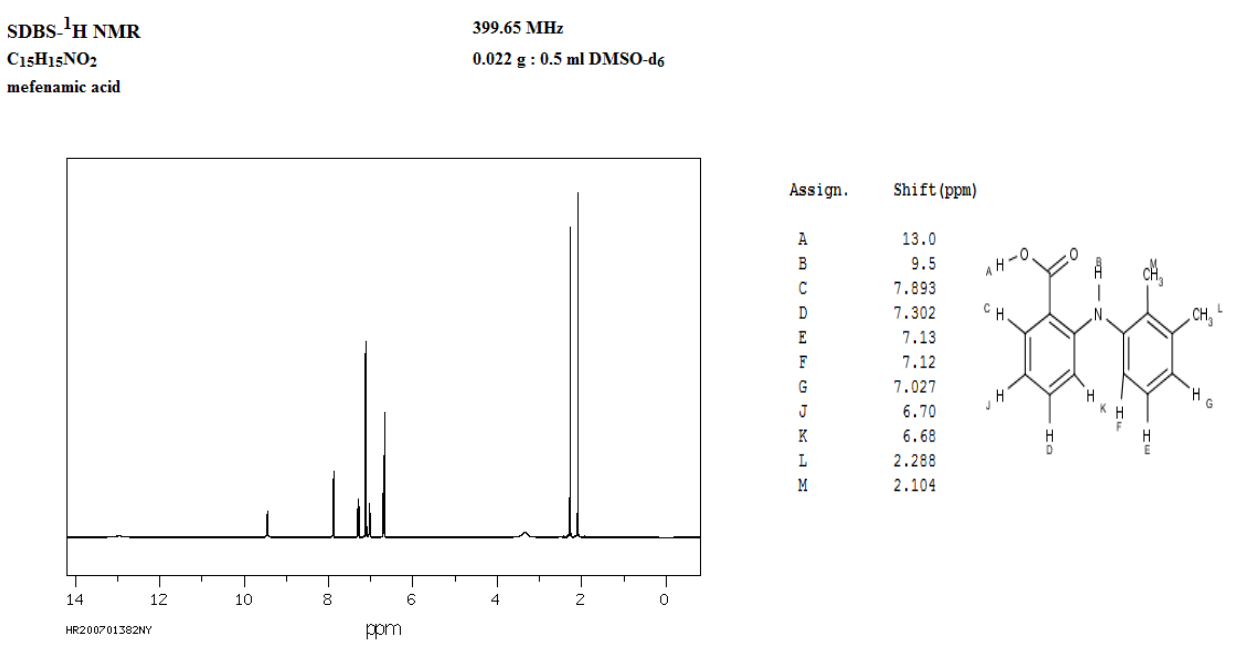

Figure 6: H NMR Spectrum of Mefenamic Acid (starting material) (Anonymous, 2014).

Table 3: Spectrum Characteristic H NMR of mefenamic acid.

Chemical Shift (ppm)	Integration	Multiplicity	Proton from groups
13	1	singlet	COOH
9.5	1	singet	NH
6,68-7,893	7	multiplet	H aromatis
2.288	1	Singlet	CH3
2.104	1	Singlet	CH3
Total			11

hot plate until the onset of signs of pain are visually like to lick the front legs, lifting the front legs, jumping or twisting the body, bringing both legs forward, licking legs forward, trying to jump out the area warm plate, and stomping the hind limbs. This time is called the latent period. Prior to the study, basal latency time of each mice was observed before. Mice with latent period greater than 15 seconds will be eliminated from this research. The longest exposure time of 60 minutes. This study was observed at 0, 30, 60, 90, 120, 150 minutes after administrated of compounds. Observation was performed by table and curves that showed relationship between dose and mice's response to a painful stimulus[12].

Data analysis

Time heat resistance of mice stimulation data is displayed and described.

RESULTS AND DISCUSSION

Molecular Docking

The interaction between mefenamic acid and their derivatives as ligands with COX-2 (1PXX) in protein A can be predicted using MVD. From the table showed that

Table 4: Spectrum Characteristic Infra Red of 4-NO2 benzoyl Mefenamic Acid

Vibration type	Wave number (cm^{-1}) from Literature	Wave number (cm^{-1}) from Experiment
N tertier	3500-3100	3330
O-H carboxyl	3400-2400	3078-3112
C=C aromatic	1600-1475	1442
Para-substitution benzene (Cl)	850-800	877
orto-substitution benzene	800-700	858
C=O Amide	1680-1630	1679
C=O carboxyl	1725-1700	1718

Table 5: Spectrum Characteristic H NMR of 4-NO2 benzoyl Mefenamic Acid

Chemical Shift (ppm)	Integration	Multiplicity	Proton from groups
9.2	1	singlet	H aromatic
8.2	1	doublet	H aromatic
8.0	1	doublet	H aromatic
7.3	1	Multiplet	H aromatic
7.2	2.3	Multiplet	H aromatic
6.7	2	Multiplet	H aromatic
4	1	Singlet	CH
2.3	3.23	Singlet	CH3
2.1	3.1	Singlet	CH3
Total			16

docking of 4-NO2-benzoyl-mefenamic acid (4NBMA) into COX-2 was MoldockScore -131.7728 ± 4.7304 where this score lower than mefenamic acid score. This is supported by RMSD value of mefenamic acid derivatives where were lower than mefenamic acid which means that the distance between the ligand (mefenamic acid derivatives) and the receptor is more stable than the lead compound (mefenamic acid). Results of Molecular Docking Scoring by Molegro Virtual Docker in Table 1 can be seen below. Molecular Modelling interaction of mefenamic acid and derivatives can be seen Figure 2 and 3.

Based on figures and tables molecular modeling indicate that mefenamic acid derivatives (4-NO2-benzoyl mefenamic acid) has better binding affinity than mefenamic acid. Besides MolDockScore value of stability bond between the ligand and receptor is also supported by RerankScore, H.Bond, and RMSD where the smaller the value, the more stable bond.

Synthesis of mefenamic acid derivatives

Synthesis and modification of mefenamic acid derivative compounds is done by adding the N aryl mefenamic acid and then treated with 4-nitrobenzoil chloride which after predicted by molecular docking where the ligand binding to the receptor substituent 4-NO2-benzoyl provides the most stable bond results of the others. 4-Nitrobenzoyl chloride reacted with mefenamic acid to form amide 4-NO2-benzoyl mefenamic acid via nucleophilic substitution reaction where carbonyl carbon of the 4-NO2-benzoyl chloride that has partial positive charge reacts with N of mefenamic acid as nucleophilic with acetone as solvent. Benzoylation reaction mechanism with 4-NO2-benzoyl chloride through nucleophilic addition and elimination of chloride ions. Acetone is used as a polar aprotic solvent that will not interfere with the reaction that releases HCl. 4-NO2-Benzoyl chloride is a derivative of benzoyl chloride which is highly reactive reagents that do not need to add a catalyst to accelerate the reaction. 4-NO2-benzoyl chloride dissolved in acetone and dripped slowly into mefenamic acid dissolved in acetone. Benzoylation reaction will be eliminated Cl that may disturb the reaction, but added NaHCO3 that will be bonding between Cl and Na of NaHCO3 become NaCl, H2O and CO2 which would be lost if washed with water. The synthesis result was collected then washed with water and the last stage is purification and recrystallization. The mechanism reaction between mefenamic acid and 4-NO2-benzoyl chloride by benzoylation can be seen in Figure 4.

Synthesis yield was 88% for 4-nitro benzoyl mefenamic acid which was powder with yellowish color. The reaction results showed that the presence of electron withdrawing groups (nitro) with big enough electronegativity so help the polarization of C atoms that are electropositive thus more readily react with the N atom of mefenamic acid. Percentage synthesized yield was significant value because has been more than 50%.

Purity test of mefenamic acid derivatives

Purity test of the compounds has been synthesized by thin layer chromatography-densitometry (TLC) and melting point determination. Eluated of the mefenamic acid derivatives by TLC uses toluene: methanol (8:2) and compares with starting material. The mobile phase used in TLC method of mefenamic acid was based on the literature[8]. The existence of spot different Rf with starting material on the plates of TLC after illuminated by UV 254 nm show that the result is a single compound that is different from the starting materials. Test purity by TLC with a purity test showed the compound is pure (ok) with r = 0.999643. The purity test showed that the compounds was pure and different from the starting materials. Optimization of reaction time of 4-NO2-benzoyl mefenamic acid was 4 hours where was the highest chromatograms area of the others.

Identification of mefenamic acid and its derivatives

After synthesis and purity test, the compounds were identified by infrared spectrophotometer, and proton nuclear magnetic resonance. Sample preparation in infrared spectroscopy using KBr pellets that can be used in the range of wave numbers 4000-400 cm^{-1}.

H NMR and FTIR Mefenamic acid (Starting Material)

IR spectrum of mefenamic acid according to literature SDBS[2], there is a peak in wave number 1511-1651 cm^{-1} which indicates presence of carbonyl groups in carboxylate salt form (COONa). In addition to vibration of the spectrum appears a secondary amine stretching at wave number 3311 cm^{-1} and double bond C = C aromatic was shown in the area around 2924 cm-1. IR spectra for

Table 6: Analgesic Activity Test between Mefenamic acid and 4-NO2 benzoyl Mefenamic acid by Hot Plate Methods

Time of study (minute)	Time Respon of Mice										
	Control	Dosis of 4-NO2 benzoyl Mefenamic acid (mg/kgWB)					Dosis of Mefenamic acid (mg/kgWB)				
	CMC Na	25	50	100	250	500	25	50	100	250	500
0	9.1	7.9	7.1	3.8	4.8	4.8	7.7	5.7	5.3	7.1	4.7
30	6.7	3.9	4.6	4.6	5.7	7.1	7.9	4.9	6.1	5.7	4.6
60	8.3	4.6	4.7	4.6	6.7	6.4	11.3	5.7	7.7	5.4	8.5
90	8.2	6.2	6.8	8.2	10.3	8.5	11.5	5.0	5.6	9.1	9.0
120	7.3	5.5	4.6	6.0	10.9	6.8	9.3	4.3	8.2	9.0	7.4
150	6.0	4.4	5.3	3.1	10.4	5.8	9.7	3.2	3.8	6.5	5.2
Total	45.5	32.5	33.1	32.3	48.7	39.4	57.4	28.8	36.8	42.7	39.4

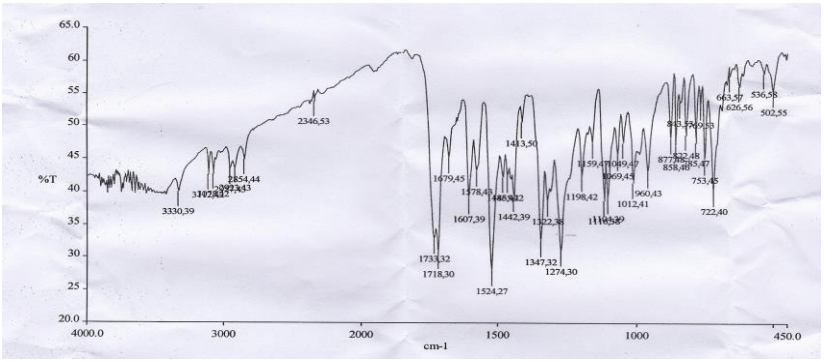

Figure 7: IR Spectrum 4-NO2 benzoyl Mefenamic Acid.

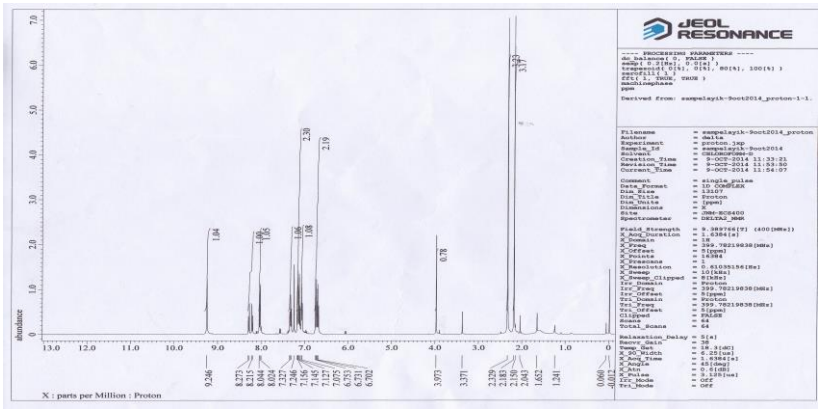

Figure 8: H NMR Spectrum of 4-NO2 benzoyl Mefenamic Acid.

bonding O-substituted aromatic exists at wave number 893 cm^{-1}. H NMR and FTIR spectra of mefenamic acid (starting material) can be seen in Figure 5 and 6.

H NMR and FTIR Mefenamic acid derivative (4-NO2-benzoyl-mefenamic acid/4NBMA)

In the IR spectrum mefenamic acid derivative (4-NO2-benzoyl-mefenamic acid) according to experiment, there were a peaks in the area of wave number 1718 cm^{-1} which indicates a carboxylic carbonyl group (COOH), OH of carboxylate 3078-3112 cm^{-1} and amide carbonyl bond (NC = O) at wave number 1679 cm^{-1}. In addition to vibration of the spectrum appears tertiary amine stretching at wave number 3330 cm^{-1} and double bond C = C aromatic indicated in the area around 2923 cm^{-1} and 1442 cm^{-1}. IR spectra for bonding O-substituted aromatic exists at wave number 858 cm^{-1}. Spectrum at wave number 877 cm-1 indicated the bond of benzene and nitrous groups was observed at wave number 1347 cm^{-1}[13]. H NMR and FTIR spectra of 4-NO2-mefenamic acid can be seen in Figure 7 and 8.

Analgesic Activity Test of 4-NO2 benzoyl Mefenamic acid by Hot Plate Methods

DISCUSSION

The development of drugs to modify the structure of commercial drugs has benefit cause more efficient in terms of screening new drugs. Pain diseases gives research opportunities in the development of pain medications / analgesic drugs. In the future, drug development will become more sophisticated NSAIDs especially with the increasing prevalence of pain. Modification of drugs, particularly NSAIDs, has been done, but not as much as other drugs. Mefenamic acid is one of the analgesic drug that has been widely used in the

market. This drug inhibits the enzyme cyclooxygenase (COX) 1 and 2. We try to modify the structure of mefenamic acid to obtain greater activity through molecular approaches. Molecular approach resulted that 4-nitrobenzoil-mefenamic acid gives the best interaction in receptor and then 4-nitrobenzoil-mefenamic acid was tested analgesic activity by hot method plate. Modifications of mefenamic acid derivative is 4-nitrobenzoil mefenamic acid that corresponding test by in silico Thus, mefenamic acid reacted by benzoylation reaction and the mechanism can be seen in Figure 4. The synthesis is done obtained yield as much as 88%. Furthermore, the TLC-Densitometry and meting point to obtain purity of compounds showed pure and the last was analyzed the structure using FTIR, and H-NMR.

Identification of the structure using FTIR can demonstrate functional groups for each specific wave number. IR spectrum of mefenamic acid derivatives according to the results of research, there are peaks in the area of specific wave number that indicated 4-NO2-benzoyl-mefenamic acid[13]. Identification of the structure was analyzed by H NMR using chloroform-d1 as solvent and TMS (tetramethylsilane) as internal standard. Based on theory, 4-NO2-benzoyl-mefenamic acid (4NBMA) must have 18 protons. But the results of 1H-NMR experiments, it was found 17 protons, one atom of hydrogen/proton does not appear/weak because COOH groups was downshield in chemical shift of H NMR[13]. Comparison of integration 4NBMA between litarature and experiment were shown in Table 5. From the analysis using FTIR and H-NMR can be ascertained that compound was 4NBMA (Figures 5,6,7 and 8). Melting point range of compounds is 198-199 ℃, where range of melting point requirement of 4NBMA was ≤2ºC that showed pure compounds[6]. 4NBMA was studied analgesic activity compared to mefenamic acid. Based on experiments 4NBMA has greater activity than lead compounds/mefenamic acid. Increased greater activity is likely due to structure modifications of mefenamic acid into 4-nitrobenzoil mefenamic acid/ 4NBMA which 4NBMA has larger lipophilic, electronegative, and steric properties than mefenamic acid that affect the biological activity of a drug.

CONCLUSIONS

Based on the results of this study was concluded that mefenamic acid derivatives (4-nitrobenzoyl mefenamic acid/4NBMA) can be synthesized and can be proved by the identification of the structure using FTIR and H NMR. 4NBMA was synthesized by benzoylation reaction between mefenamic acid and 4-nitrobenzoyl chloride after prediction by in silico study/molecular approach using COX-2 receptor. 4NBMA has greater activity than lead compound (mefenamic acid) in analgesic test using Hot Plate Methods.

ACKNOWLEDGEMENTS

Thanks to the University of Jember through to BOPTN 2014 for funded this research.

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