Synthesis of Dihydropyridine Prodrugs with Expected Enhancement of brain Delivery

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Abstract

Recently non-steroidal anti-inflammatory drugs (NSAIDs) have been proposed to prevent or to cure Alzheimer's disease. In this respect, we synthesized new potential prodrugs of several NSAIDs in order to increase their access to the brain. The carboxylic group of NSAIDs was attached to the 1,4-dihydro-1-methylpyridine-3-carboxylate moiety, which acts as a carrier, via an amino acid bridge, according to the chemical delivery approach developed by Bodor.In this study new analogues were synthesized by insertion D- Threonine moiety using esterification method then linked to nicotinic acid by conventional solution method using DCC as coupling reagent all prodrugs were more lipophilic when compared to their corresponding parent compounds and consequently a better blood brain barrier (BBB) penetration is hypothesised the analogues were purified and monitered by TLC , melting point , and IR spectrophotometer FT.IR 8400 was achieved and CHNS analysis.

الخلاصة

حديثا تم اقتراح مضادات الالتهاب الغير ستيروديه لمنع او معالجة مرض الخرف المبكر (النسيان) في هذا المجال، تـم تخليـق مقدمات محتمله للعديد من مضادات الالتهاب الغير ستيروديه لزيادةعبورها إلى الدماغ. مجموعة الكاربوكسيل لهـذه الادويـه تـم ربطها لجزيئة – ١،٤ -دايهايدرو-١-مثيل بيريدين-٣-كاربوكسيل ، الذي يعمل كناقل ، عن طريق جسرٍ الاحمـاض ألامينيـة، حسب نظرية الادخال الكيميائيه التي طورتْ من قبل العالم Bodor . في هذه الدراسة تم تخليق العديد من النظائر الجديدة بادخال جزيئة الثريونين لهذه المركبات باستخدام طريقة الاستره من ثم تم ربطها إلى جزيئة حامض النيكوتين بإستعمال طريقة الطور السائل التقليدي باستخدام مادة ال .DCC كعامل اقتران . كُلّ المقدمات المحتمله كانتْ أكثردهنيه عندما قورنَت مع مثيلاتها مـن المركبات الأصل وبالتالي من المتوقع عبورافضل لحاجز الدماغ -الدم .

ان النظائر النهائية تم تصنيعها وتنقيتها بنجاح وكانت النتائج النهائية مركبات ذات طبيعة بلوريه. كما تم التثبت من البنية التركيبية والنقاوة لهذه المركبات باستخدام: درجة الأنصهار، كروماتوكرافيا الطبقة الرقيقة، مطياف الأشعة تحت الحمراء و التحليل الـدقيق للعناصر (C,H,N).

Introduction

The blood–brain barrier (BBB) presents an efficient structural and functional barrier for the delivery of therapeutic agents to the central nervous system (CNS). Due to its unique properties, passage across the BBB often becomes the main limiting factor for the delivery of potential CNS drugs into the brain parenchyma. In fact, it is estimated that more than 98% of small-molecular weight drugs and practically100% of large-molecular weight drugs developed for the CNS diseases do not readily cross the BBB (Pardridge , (2005)) Many of the pharmacologically active drugs tend to fail early in their development phase because these molecules lack the structural features that are essential for passing the BBB(Begley *et-al*., 2004) specific transporters exist at the BBB that permit nutrients to enter the brain and toxicant /waste products to exit(Smith and Clark,(2004)) Prodrugs are an established concept to overcome barriers to a drug usefulness(It is the term referred to a pharmacologically inactive compound that is transformed by the mammalian system in to an active substance (or substances) by either chemical or metabolic means (Ghosh and Mitra ,2008) .Also we can use a more general term, drug latentiation, to refer to drugs that were specifically designed to require bioactivation (Lambert *et-al.,2005)* .Prodrugs contain a pharmacologically active moiety that is either conjugated to a molecule with a known

transporter or to a lipophilicity enhancer, which is cleaved at or near the site of action, allowing drug to induce its effect. The rationale for prodrug design is that the structural requirements necessary to elicit a desired pharmacological action and those necessary to provide optimal delivery to the target receptor site may not be the same. The ideal prodrug is enzymatically stable in the blood, but rapidly degraded to the active parent compound once it is within the target tissue. Esters have shown particular promise in the area of prodrug design for brain delivery, owing to the abundance of endogenous esterases in the CNS. Esterification or amidation of amino, hydroxyl, or carboxylic acid-containing drugs may greatly enhance lipid solubility, and thus brain entry (Greene *et-al*.,2006). Once in the CNS, hydrolysis of the modifying group releases the active compound. Both aromatic benzoyl esters (Mishra *et-al*., 2008), and branched chain tertiary butyl esters (Anderson *et-al*.,2005) have shown stability in plasma, while still remaining adequately cleaved within the CNS. Lipophilic amino acids, such as phenylalanine (Phe), can be used as the cleavable unit.Alzheimer's disease (AD) is the most common form of dementia (Diaz *et - al.*,1998) Although at present the aetiology of AD is not well understood, many neurobiological and environmental factors contributing to the pathogenesis of AD (Eikelenboom *et- al*.,2001) have been described. Recently, it has been revealed that there is apresence of an inflammatory component in the pathogenesis of AD. In fact, immunochemistry completed on post mortem AD brains revealed that numerous inflammatory components are associated with neuritic plaques (Estes *et-al*,2007) and epidemiological studies have shown that therapy with anti-inflammatory drugs reduces the risk of developing AD.(Jarko *et-al*.,2008) In order to understand the mechanisms by which NSAIDs can protect the nervous system from the ravages caused by AD. All these data indicate that an anti-inflammatory therapy (Paradge ,2005) used in subjects without genetic predisposition. Chemical modification may be essential in order to improve stability by minimizing enzymatic cleavage . (Jarko *et-al*.,2008) demonstrated that by substitution of D –Thr for L– Gly at the N – terminal position and amidation of its C – terminal produce more stable analogue . Chemical modification has been proven successful for small peptides rather than for larger peptides , due to complexity of their structures .In addition , small peptides may be derivatized to produce prodrugs which possess favourable physicochemical properties in comparison to the parent compound by rendering the peptide more lipophilic thus facilitating its absorption. The aim of this study is to synthesize and predict the permeation profiles of a few NSAID derivatives designed to increase their access to the brain.Some potential prodrugs of several NSAIDs, such as diclofenac (DIK), ibuprofen (IBU) and naproxen (NAP) were synthesized using as a carrier, the 1,4-dihydro-1-methylpyridine-3-carboxylate, which was attached to the drug via an amino acid threonine. A successful prodrug approach that utilizes improved lipophilicity and also requires a sequential bioactivation steps for conversion to an active drug and a brain tissue trapped intermediate is often referred in the literature as a chemical drug delivery system (CDS) The CDS term was originally coined by Bodor and coworkers to distinguish this approach from prodrugs that typically require only a single activation step. However, many sophisticated prodrugs are now adays activated in multiple steps. The CDS has been explored with a wide variety of hydroxy- and amino-containing drugs (Bodor and Plorazki, 2007), and considerably increased brain targeting has been achieved, for example, for zidovudine (AZT) (Brewster and Webb ,2000) , ganciclovir.(Brewster ,, 1999) , benzylbenicillin (Perioli , 2004) and estradiol

Aim of the work

Synthesis of prodrugs that can pass blood brain Barrier these hydrophilic drugs molecules that cannot pass or pass in low concentration to CNS are combind with dihydropyridine through amino acid molecule. This model increase lipophilicity of drugs and it is the most interesting procedure to deliver drugs in a sustained and specific manner to the CNS. The release of active species from a lipophilic prodrug through a multi step conversion, based on a dihydropyridine- pyridinium salt equilibrium type redox molecular carrier, similar to the endogenous NADH/NAD+ coenzyme system. The dihydropyridine form of the drug is rapidly distributed throughout the body, including CNS. Next, the dihydropyridine moiety is oxidized to the pyridinium salt. This hydrophilic form is sequestrated in the CNS, whereas it is rapidly eliminated from the periphery, by enzymatic hydrolysis, the active drug is released into the brain and can exert its action as shown in the following figure.

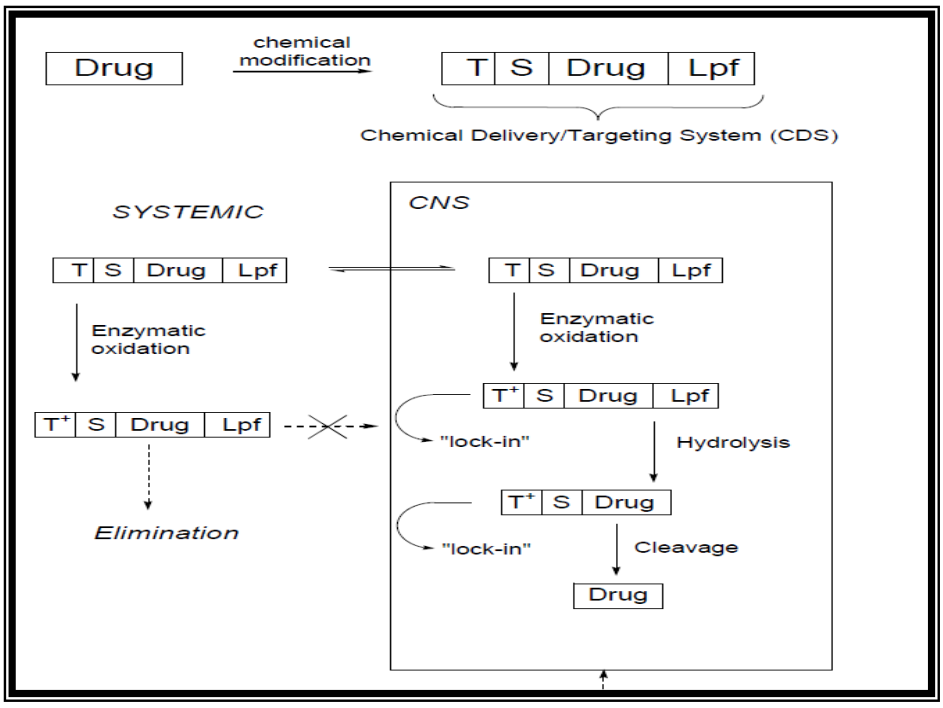


Figure : Drug delivery into CNS as CDS.

Materials and methods

D – amino **a**cid and Boc – protected -D – amino acids are purchased from Fluka AG/Switzerland and all solvents are of analar type without need further purification ; Diclofenac and naproxen from al muthana factory and nicotinic acid from sigma, germany Melting points determined by Thomas Hoover apparatus England by capillary method , and ascending thin layer chromatography TLC was run on Keisl gel GF 254 type 60 Merck , W . Germany, to check the progression of reaction and purity . IR spectra were recorded in KBr film ,FTIR at the college of science , university of Baghdad ,CHN analysis of intermediate and final analogues were made in France .

Table 1

EQUIPMENTS	COMPANY	COUNTRY
Electric melting point apparatus	Thomas Hoover	UK
Rotary evaporator	Heidolph	Germany
Electrical balance	Shimadzu	Japan
magnetic stirrer with hot plate	Lab. Companion MS-HP 3000	Germany
IR spectrophotometer FT.IR 8400	Shimadzu	Japan
Chiller	GMBH	Germany
Elemental analyzer	EA-Analyzer	Italy
Oven	Al Araby	Egypt

Acetonitril	BDH	England
Activated charcoal	Fluka	China
Anhydrous magnesium sulfate	Reachim	USSR
Benzene	BDH(Annular)	England
BOC-D-threonine	Fluka	Germany
Chloroform	Riedel de Haen	Germany
Concentrated Hydrochloric acid(35.5%)	Analytical Rasayan	India
Diethyl ether	BDH(Annular)	England
Diclofenac	Gulphar	Dubai
N,N-Dimethylformamide(DM)	Riedel de Haen	Germany
Ethyl acetate	Riedel de Haen	Germany
Ethanol(absolute)	Riedel de Haen	Germany
N-hydroxysuccinamide (HOSu)	Fluka	China
n-hexane	Searle	England
Methyl iodide	Riedel de Haen	Germany
4-Methylmorpholin (NMM)	Fluka	Switzerland
N2 gas	Alqasim	Iraq
Naproxen	SDI	Iraq
Nicotinic acid	sigma	Germany
Petroleum ether 40-60	BDH(Annular)	England
Sodium dithionate	BDH(Annular)	England
Sodium Chloride	Riedel de Haen	Germany
Sodium Hydroxide	BDH(Annular)	England
Sodium bicarbonate	Riedel de Haen	Germany

Thionyl chloride	Fluka	USA
Trifluoroacetic acid	Riedel de Haen	Germany

Table (2) : Solvent systems used in the TLC:

A	Chloroform	Methanol
	8	2
B	Ethyl acetate	Ethanol
	7	3
C	Petroleum ether	dichloroethane
	3	1

condensation method via solution method , where dicyclohexylcarbodiimide (DCC) was used as coupling agent and N-hydroxysuccinamide (HOSu) was used as coupling agent and prevents racemization in the peptide formation . It is desirable to have a range of protecting groups available that can be selectively removed , two protecting groups are said to be orthogonal protecting when they are removed by totally different classes of reagents (e.g. one by acid and the other by base) , the butyloxycarbonyl (BOC) group a widely used protective group that is generally removed from N – terminal by using moderate – strong acids e.g. trifluro acetic acid (TFA) and is stable under the basic conditions , while the C – terminal protected by methyl ester which is removed by using strong base e.g. NAOH and is stable under moderate strong acidic conditions .C – terminal protective peptide was saponified and acidified by using 1 N NAOH to give the goal analogue .

I. synthesis of nicotinyl alanine naproxen dihydropyridine .

1. **Extraction of pure naproxen from tablets**:

Twenty tablets of naproxen (naprox*,500mg) was grinded in amortar and added into 500 ml of methanol, and shacked vigorously for about 10 min then the suspension was filtered under vacuum. To the filterate, charcoal was added ,followed by filteration , then the solvent was evaporated under vacuum .White precipitate was obtained which was washed by water several times. The residue was then dissolved in 20 ml of 1N NaOH ,filtration ,and followed by the addition of 25 ml of 1N HCl to precipitate naproxen , which was washed several times with D.w. and dried in oven at 150 ˚C. After drying ,the obtained naproxen was added into 15 ml of benzene with shaking for 15 minutes ,filteration ,and wash the precipitate with excess benzene (naproxen is insoluble in benzene), then drying at 40˚C.Then it is re-crystals from acetone- hexane(1:10) mixture.The purity of naproxen was checked by TLC ,m.p. IR spectra of extracted naproxen compared with standerd sample of naproxen , The yield was 71%, m.p. was measured for standard naproxen ,for extracted naproxen and for a mixture of them R_f value and m.p. 0.79, (144-145)˚C respectively (Hull and feibech ,2008).

2. **Synthesis of BOC –D-threonine methyl ester** ;

A suspension containing BOC-D-threonine (2.85 gm ,13 mmol) in 30 ml methanol was cooled to -15 ˚C and (1ml ,13 mmol) thionyl chloride was added to the suspension with continuous stirring and keeping the temperature below (-10 ◦C) The reaction mixture was kept at (40°C) for (3 hrs.) followed by refluxing for (3 hrs.) and then left at ambient temperature (42°C) for overnight. Methanol was evaporated to dryness in vacuum, clear oily liquid obtained, which then re-dissolved in methanol

and evaporated. This process was repeated several times to ensure complete removal of thionyl chloride. The residue was then dried well to ensure complete solvent removal to obtain 90% yield. Physical appearance , melting point and R_f value are listed in table 4. (Hull and feibech ,2008).

3.Synthesis of Naproxen- N-*tert*-butyloxycarbonyl-Threonyl ester (NAP-Boc-Thr-O-CH3)COMP(3D) :

Suspension of BOC-D-threonine ester (2.33gm,10 mmol) and (2.3 gm , 10 mmole) of naproxen in 20 ml anhydrous chloroform was cooled to -15 C (0.8 ml , 10 mmole) of thionyl chloride was employed to get 90% yield. Another procedure is by coupling to a stirred solution of(0.23 gm , 1mmole) naproxen in 5 ml DMF , 0.11 ml NMM added and stirring for 10 min , solution of BOC-D- threonine (0.233gm, 1 mmole) added and stirring continue for 3 days at (0°C) and two days at room temp. A precipitate of N,N – dicyclohexyl urea (DCU) formed during the reaction was then , filtered and washed with ethyl acetate, and then with D.W . , 0.1 N HCl and 5% sodium bicarbonate respectively . The ethyl acetate layer was dried with anhydrous sodium sulphate and evaporated under vacuum and the product was re – crystallized from ethyl acetate – ether (3 : 7) to obtain 75% yield physical appearance , melting point and R_f value are listed in table 4.(Mishra, 2008).

4.Synthesis of Naproxen Threonyl ester (NAP Thr-OCH 3)comp (4D);

To a stirred solution of compound 3D (0.445 gm,1 mmole) in minimum amount of DMF (3ml) , 1ml of 90% triflouroacetic acid solution in dichloromethane was added dropwise over aperoid of 30 min . solvent was evaporated in vacuum , and the trifluoroacetic acid was coevaporated twice with ether . Residue was collected with 45% yield, physical appearance , melting point and R_f value are listed in table 4.

5. Synthesis of Naproxy-Threonyl Nicotinate comp(5D):

To a stirred solution of nicotinic acid (0.123 gm,1 mmole) in 5 ml DMF a (0.11 ml NMM , 1mmole) was added followed by stirring for 10 min , solution of threonine naproxen ester(compound 4D) (0.345 gm , 1 mmole) in 5 ml DMF was added to the reaction mixture. The mixture was then cooled to (-15°C), HOSu (0.23 gm, 2 mmole) was added followed by DCC (0.206gm, 1 mmole) with stirring which was continued for 72 hrs at (0°C) and for 48 hrs. at ambient temperature (18°C). Ethyl acetate (20 ml) was added to the reaction mixture which was then filtered to get rid of N,N-dicyclohexylurea (DCU). The filtrate was evaporated to dryness under vacuum, and the residue was re-dissolved in ethyl acetate (20 ml), the excess DCU which was still adhesive on the peptide residue was precipitated out and filtered. The clear filtrate was washed twice with (5 ml) HCl (0.1 N) solution, once with (10 ml) D.W., and with (10 ml) saturated NaCl solution using the sepertaory funnel, to ensure complete removal of impurities that dissolve in dilute aqueous acid solution . The ethyl acetate layer was dried over anhydrous magnesium sulfate, then evaporated to dryness. The dipeptide was re-crystallized from ethyl acetate: petroleum ether (40-60) mixture to get a yield of 65 %, physical appearance , melting point and R_f value are listed in table 4. (Lambert , 2005).

6. Synthesis of Naproxy- Threonyl -N-methyl Nicotinate iodide(6D):

compound 4B(0.468 gm,1mmole)and CH_3I(3.3mmole) was dissolved in 10 ml mixture of CH_2Cl_2/MeCN (3:1) were stirred in a closed system at room temperature for 1 day and The reaction mixtures were brought down to dryness in vacuo. White powder was obtained and recrystalized from methanol :ethyl diether .(1:10). get percent yield of 70% ,physical appearance , melting point and R_f value are listed in table 4 (Perioli, ,2004).

7. Synthesis of Naproxen –threonyl dihydropyridine(7D) :

$NaHCO_3$ (10 mmol) and $Na_2S_2O_4$ (2.2 mmol) in an aqueous solution (150 ml) and ethyl acetate (30 ml) were added in separatory funnel fitting to qiuck fit three secter round flask and an ice-cooled solution of methyl iodide pyridinium derivative of nicotinyl threonine naproxen (compound 6D) (0.302gm,1mmole) in degassed methanol (150) ml in the round flask under N_2 atmosphere that employed from one end of the flask maintaing the third opening free to exclude oxygen.the reagent in separatory funnel then added drop wise over the methyl iodide pyridinium derivative in the round with continuous stirring and maintaing flow of N_2 gas at 0°C ,maintaining the pH at approximately 7 by addition of $NaHCO_3$. After that the reaction mixture was transferred to separatory funnel the organic phase was washed with water several times, dried over Na_2SO_4, filtered and brought to dryness in vacuo to afford 1,4 derivative. physical appearance , melting point and R_f value are listed in table 4. (Perioli ,2004).

II) Synthesis of diclofenac threonine Dihydrpyridine :

1.chemical conversion to diclofenac acid from sodium salt (**1E)**:

Dissolve (3.2 gm, 10 mmole) of diclofenac sodium in ethanol and stirring for 10 min then followed by addition of 1 ml of (2 N) HCl drop wise addition, a precipitate of diclofenac formed then addition of ice water for termination of reaction , filter and wash the precipitate several times with water and dry in oven at 120 ˚C for two hrs then recrystalized from(ethyl acetate , ether), (1: 10) 80 % yield , physical appearance , melting point and R_f value are listed in table 4.

2. Synthesis of Diclofenac-Boc threonine ester,(Dic-Boc-thr-OCH3) (3E):

Suspension of BOC-D-threonine ester (2.33gm ,10 mmol) and(3 gm , 10 mmole) of diclofenac in 20 ml anhydrous chloroform was cooled to -15C , (0.8 ml, 10 mmole) thionyl chloride was added to get 92% yield a white crystalline precipitate obtained . Another procedure is by coupling to a stirred solution of(0.3 gm , 1mmole) diclofenac in 5 ml DMF, 0.11 ml NMM added and stirring for 10 min , solution of BOC-L- threonine ester (0.233gm, 1 mmole) added and stirring continue for 3 days at (0˚C) and two days at room temp a precipitate of N,N – dicyclohexyl urea (DCU) formed during the reaction was then , filtered and washed with ethyl acetate and then with D.W . , 0.1 N HCl and 5% sodium bicarbonate respectively . The ethyl acetate layer was dried with anhydrous sodium sulphate and evaporated under vacuum and the product was re–crystallized from ethyl acetate – ether (1: 10) to obtain 75% yield , physical appearance , melting point and R_f value are listed in table 4.(Mishra, 2008).

3.Synthesis of diclofenac-threonine ester (Dic-Thr-OCH_3) (4E):

To a stirred solution of compound (0.511 gm,1 mmole) in minimum amount of DMF , 1ml of 90% triflouroacetic acid solution in dichloromethane was added dropwise over a peroid of 30 min . solvent was evaporated in vacuum , and the trifluoroacetic acid was coevaporated twice with ether . Residue was collected with 45% yield , physical appearance , melting point and R_f value are listed in table 4.

4.Synthesis of diclofenac- threonine nicotinate , (Dic-Thr-NA) (5E):

To a stirred solution of nicotinic acid (0.123 gm,1 mmole) in 5 ml DMF a (0.11 ml NMM , 1mmole) was added followed by stirring for 10 min ,solution of Dic -Thr ester (0.34 gm , 1 mmole) in 5 ml DMF was added to the reaction mixture. The mixture was then cooled to (-15°C), HOSu (0.23 gm, 2 mmole) was added followed by DCC (0.206gm, 1 mmole) with stirring which was continued for 72 hrs at (0°C) and for 48 hrs. at ambient temperature (18°C). the same procedure was carried out as

in compound (4 D) with a yield 70 % ,physical appearance , melting point and R_f value are listed in table 4.

5.Synthesis of Diclofenac-Threonyl-N-methyl Nicotinate iodide(Dic-thr-NA-CH3I) comp(6E):

compound 5E(0.34gm ,1mmole)and CH3I(2.4 ml ,3.3mmole) was dissolved in 10 ml mixture of CH_2Cl_2/MeCN (3:1) were stirred in a closed system at room temperature for 2 days and The reaction mixtures were brought down to dryness in vacuo, the residue was induced to crystallize with methanol : diethyl ether (1:10) was carried out to get a percent yield of 70% ,physical appearance , melting point ,R_f value are listed in table 4.

6.Synthesis of Diclofenac –Threonyl –dihydronicotinate (comp 6E):

Sodium bicarbonate $NaHCO_3$ (0.85 gm ,10 mmol) and sodium dithionate $Na_2S_2O_4$ (0.38 gm ,2.2 mmol) in an aqueous solution (150 ml) and ethyl acetate (30 ml) were added to an ice-cooled solution of methyl iodide pyridinium derivative compound (**5E**)(0. gm,1mmole) in 150 ml of degassed water under nitrogen atmosphere,the reaction proceed as in comp(7D) to get 60% yield, physical appearance , melting point and R_f value are listed in table 4.

Result and Discussion

Infra – Red Spectrometry :

From the infra red spectra of the synthesized analogues as well as those of the intermediates showed a characteristic bands of absorption by which they were identified .IR data help us not only to identify the final compounds , but also they have advantage in follow up the reactions depending on appearance or disappearance of specific group frequencies .We can identify from the IR data of the free analogues , the following characteristic absorption bands :

Analogue Naproxy threonyl N-methyl dihydropyridine : 3395 cm^{-1},3100 cm^{-1},2928,2850,1626,1573,1535,1772,1550,1580,1420 cm^{-1}, 1690 cm^{-1},1540 cm^{-1},1420 cm^{-1},1280 cm^{-1},1100 cm^{-1},780 cm^{-1}, and 740 cm^{-1} .

The appeared absorption bands can be explained in the following manner :

1 – The appearance of a band near 3395 – cm^{-1}, resulting from asymetric N-H stretching vibration of amide.

2 – N – H symmetrical stretching vibration of amide appeared arround 3100 - 3000cm^{-1} .

3 –Asymmetrical and symmetrical C – H stretching vibration absorbed near 2928– 2850cm^{-1} .

4.C=O Stretching of amide and bending of 2 amide and C=C stretching included in this region(1626-1573-1535).

5 – Carbonyl stretching vibration of ester group absorbed near 1772 cm^{-1} .

6– The carboxylate anion COO^-absorbed near 1550 – 1580 cm^{-1} and at 1420 cm^{-1} .

7 - The band at 1220 – 1190 cm^{-1} arising from C – C (= O) – O of the carboxylate group and 1244 aromatic C-H stretching in plane bending.

8– Bands at 740 cm^{-1} and 780 cm^{-1} indicated the presence of the aromatic ring (C=C) stretching ,the same band also observed for Diclofenac-threonyl N-methyl dihydropyridine .

On the other hand , the infrared spectra of the protected compounds showed the following absorption bands (Intermediate):

1 – The N – protected group was characterized by the appearance of urethane group (tert-butyloxy carbonyl)((CH3)3-OOC -NH-), near 1740 – 1690 cm^{-1} .
2 – Appearance of 1640 - 1620 cm^{-1} for amide I and 1550 - 1510 cm^{-1} were an indication of amide II NH – bending .
3 – The methyl ester of the analogues characterized by a band near 1750 – 1710 cm^{-1} and C-O stretching band at 1210 – 1170 cm^{-1} .

Results of Elemental Analysis :

Results indicated that the values of the calculated and found elemental analysis , were approximately the same , i.e , the prepared compounds were valid and pure CHNS analysis made in analytical labrotary in france .

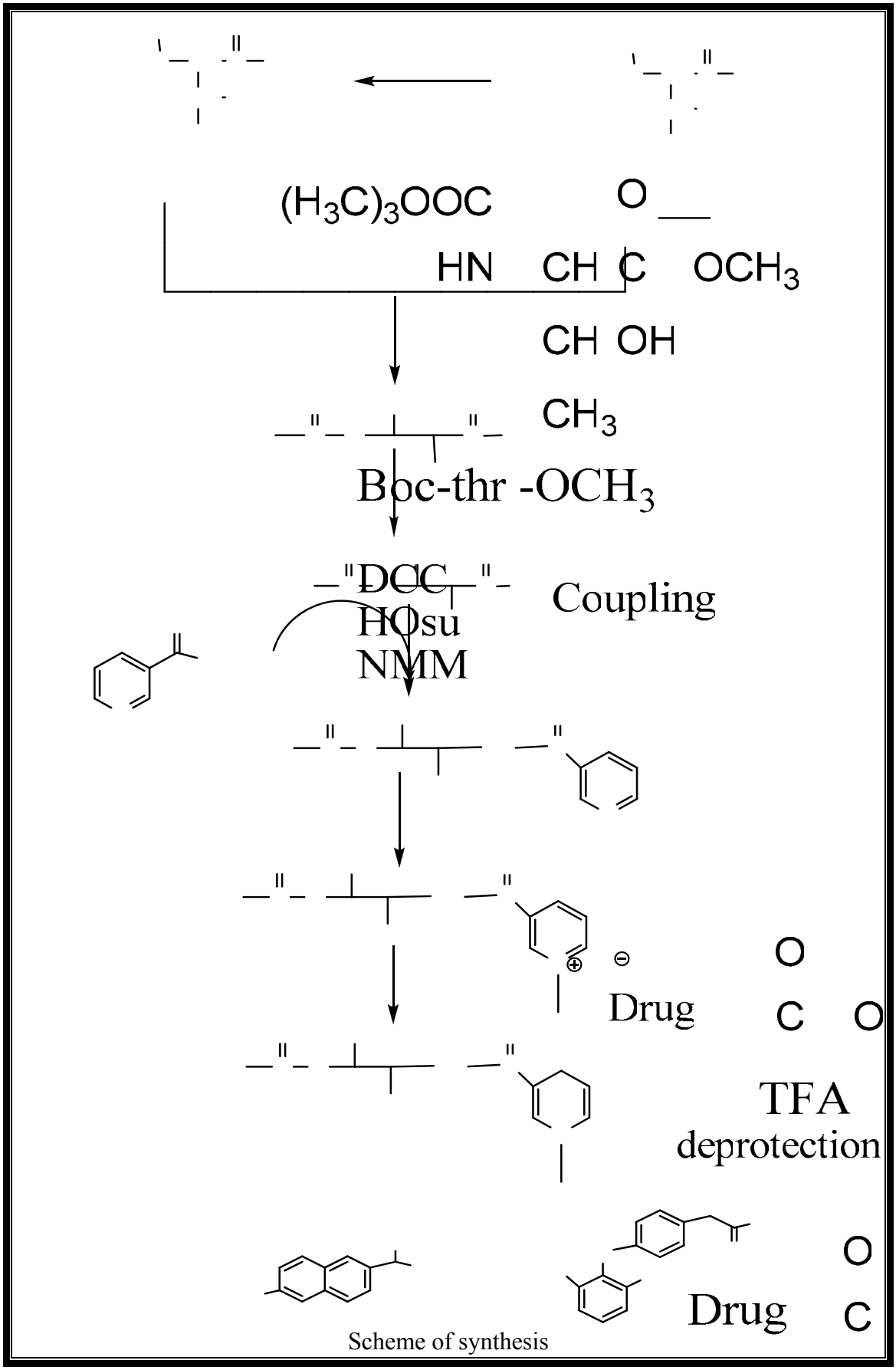
table 3

Comp. No.	Comp.	Chemical Formula	Calculated Found					
			C %	H %	N %	O%	CL%	I%
1	Naproxen – Threonyl-N – methylnicotinate iodide	$C_{26}H_{29}IN_2O_6$	52.71 52.60	4.93 4.90	4.73 4.65	16.20 16.31		21.42 21.20
2	Naproxen-Threonyl-N-methyl dihydropyridine	$C_{26}H_{30}N_2O_6$	66.94 66.91	6.48 6.50	6.00 5.95	20.58 20.32		
3	Diclofenac-Threonyl –N-methyl nicotinate iodide	$C_{29}H_{30}N_3O_7CL_2I$	47.69 47.58	4.140 4.12	5.754 5.69	15.33 15.11	9.71 9.66	17.37 17.29
4	Diclofenac-Threonyl-N-methyl dihydropyridine	$C_{29}H_{31}N_3O_7CL_2$	57.05 57.35	5.24 5.20	7.04 7.01	18.78 18.71	5.94 5.89	

Peptide synthesis is usually a sophisticated research project , the wide choice of protecting groups and coupling methods tests chemists ingenuitg in designing a synthetic scheme that offer the best chance for success with reasonable efforts . protecting groups can be chosen so that reactive side chains of amino acid may remain covered while protecting groups on other amino acid carboxyl group , may be the difference between a good yield and a poor yield or perhaps no yield at all .The choice of solvents and other reaction conditions often determines the yield obtained and nature and extent of undesired side reactions. Coupling reaction may take an undesired course such as the formation of acyl ureas during the activation of the carboxyl group .Racemization was avoided by use of HOSu .

Table (4):

Compound No.	*Physical appearance*	*Melting points (°C)*		R_f *values*	
		Observed	*Reported*	*A*	*B*
6D	***White crystal***	***145-146***	-	**0.40**	**0.426**
7D	***Clear oily liguid***	-	-	**0.2**	**0.15**
8D	***White crystal***	***110-112***	-	**0.27**	**0.41**
9D	***White crystal***	***219-220***	-	**0.26**	**0.36**
10D	***White crystal***	***200-202***	-	**0.63**	**0.34**
11D	***White crystal***	***129-130***	-	**0.52**	**0.64**
8E	***White crystal***	***211-213***	-	**0.46**	**0.52**
9E	***White crystal***	***218-220***	-	**0.37**	**0.63**
10E	***White crystal***	***230-233***	-	**0.92**	**0.84**
11E	***White crystal***	***210-213***	-	**0.45**	**0.56**
12E	***White crystal***	***228-230***	-	**0.94**	**0.88**



Scheme of synthesis

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