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Synthesis of palmitic acid derivatives of *p*-aminophenol and *p*-amino benzoic acid with improved pharmacodynamic profiles

Asma Saqib[1], Mohammed Afzal Pasha[2], Chandrakant Karigar[1*] and Metticule Shankar Narayana Rao Harish[3]

[1*]Department of Biochemistry, Central College Campus, Bangalore University, Bangalore, 560001, India.
[2] Department of Chemistry, Central College Campus, Bangalore University, Bangalore, 560001, India.
[3] Naragund College of Pharmacy, Department of Pharmacology, Bangalore 560078, India.

Abstract

In the present study, *p*-amino phenol (PAP) and *p*-amino benzoic acid (PABA) have been modified as lipids palmitoyl *p*-amino phenol (PPAP) and palmitoyl *N* - *p*-amino benzoic acid (*N*-PPABA) by a simple amide formation reaction of the respective compounds with palmitoyl chloride. On screening PPAP and *N*-PPABA for pharmacological activity, PPAP (an analogue of paracetamol) has been found to exhibit potent and persistent analgesic and inflammatory activity than paracetamol. Whereas *N*-PPABA, an amide of *p*-amino benzoic acid demonstrated novel analgesic, antiinflammatory and antipyretic effects.

Keywords: Paracetamol, PPAP, *N*-PPABA, Cyclooxygenase, antinoniceptive, NSAID's.

Introduction

The pharmacological action of NSAID's like (paracetamol, acetylsalicylic acid, indomethacin, diclofenac, ibuprofen, phenylbutazone, sulfasalazine) is related to their inhibitory action upon cyclooxygenase and lipoxygenase enzyme systems involved in the biosynthesis of prostaglandins and leukotrienes.The use of NSAID's is accompanied by a number of side effects. The problem associated with paracetamol is that excessive dose cause liver damage leading to jaundice, confusion, unconsciousness and liver failure leading to death. The potentially deleterious effects of the acidic NSAID's on the stomach are well known (Beck et al. 1990, Rainsford 1998, Jones 1985). The direct contact mechanism was considered to play a determinant role in the formation of gastrointestinal lesions (Cioli et al. 1979). It is probably attributed to the free carboxylic group of the NSAIDs and to local inhibition of the cytoprotective action of prostaglandins on gastric mucosa (Shanbhag et al. 1992). On the other hand, acetaminophen is a commonly used analgesic, which is remarkably safe at therapeutic doses.

***Corresponding author**: karigar@bub.ernet.in

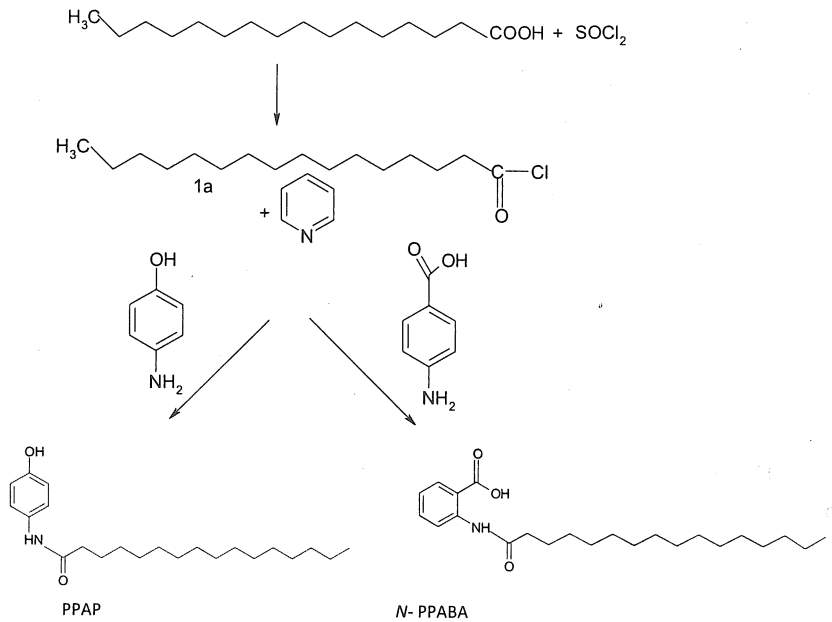
However several reports stated that large overdoses of paracetamol can produce a fulminant hepatic and renal tubular necrosis due to formation of the toxic metabolite, *N*-acetyl-*p*-benzoquinoneimine, NAPQI, 2 (Boyer et al. 1971 and Vermeulen et al. 1990).

It is also reported that the conversion of these carboxylic acid containing NSAIDs into ester or amide derivatives generates derivatives that are potent and highly selective COX-2 inhibitors (Amit Kalgutkar et al. 2000). Amide derivatives of the NSAIDs with cysteamine were almost non-toxic in the GI tract, under experimental conditions, in contrast to parent NSAIDs (Kourounakis et al. 2000). By preparing certain amide derivatives, it is possible to achieve more potent antiinflammatory compounds which inhibit COX enzymes (Kalgutkar et al. 2002 and 2005) and attachment of lipid moiety to acetyl salicylic acid allows the drug to pass through the gastrointestinal tract without significant damage (Ivey et al. 1988)

Thus, a considerable decrease in the solubility of drugs in the aqueous phase, including acid medium, can be achieved through chemical modification of these drugs by conjugating with lipid molecules. This communication is aimed at designing the palmitoyl amide derivatives like PPAP and *N*-PPABA. We also report that upon conversion into a lipid bound amide derivatives of PAP and PABA, resulted in enhanced anti-inflammatory, analgesic and antipyretic activities.

Experimental

The synthetic pathway for PPAP and *N*-PPABA is given in scheme 1. Melting points of the reaction products were determined with a Buchi melting point apparatus. IR, ^{1}H NMR, ^{13}C NMR were recorded on Nicolet 400D FT-IR spectrophotometer, 400 MHz Brucker spectrometer respectively. Purity of the compounds was ascertained by Thin Layer Chromatography on silica gel plates using iodine as visualizing agent. All reagents were of analytical grade commercial products and used without further purification.



Scheme 1. Chemical synthesis of PPAP and N-PPABA

Synthesis of Palmitoyl chloride

Palmitoyl chloride (1a, Scheme.1) was prepared by gently refluxing a mixture of palmitic acid (25.6 g, 100m mole) and thionyl chloride (23.7 g, 14.3 ml, 200 m mole) on a steam bath for about 30 min. The excess of thionyl chloride was removed by washing successively with water and the residue was dried over anhydrous Na_2SO_4 to get palmitoyl chloride (20.4 g, 74.45 %).

Synthesis of Palmitoyl p-amino phenol

To a mixture of palmitoyl chloride (2.74 g, 10 m mole) and *p*-amino phenol (2.54 g, 10 mole). Pyridine (0.79 g, 9.7 ml, 10 m mole) was added to this solution slowly and stirred at room temperature (25 °C) for 10 min, after the completion of reaction (TLC) neutralized with ice cold dilute HCl. The solid thus precipitated was filtered, washed with water, dried and recrystallised with ethanol to get pure palmitoyl *p*-amino phenol (PPAP)(2.2 g, 86.6 %).

Black colored solid, Melting point- 250 ^{0}C – 251.5 ^{0}C

IR spectrum (V_{max} cm $^{-1}$): υ 1265, 1560, 1750, 3320, 3580

^{1}H NMR ($CDCl_3$): δ 0.96 (t, 3H, CH_3), 1.29 (qn, 22H, $(CH_2)_{11}$), 1.33 (m, 2H, CH_2), 1.67 (q, 2H, CH_2), 2.23 (t, 2H, CH_2), 5.0 (s, 1H, OH) 6.71- 7.47 (m, 4H, aromatic CH), 8.0 (s, 1H, OH).

^{13}C NMR ($CDCl_3$): δ 14.0 (aliphatic CH_3), 22.1 (aliphatic CH_2), 23.6 (aliphatic CH_2), 25.7 (aliphatic CH_2), 29.8 (aliphatic CH_2), 29.0 (aliphatic CH_2), 29.3 (seven aliphatic CH_2), 36.3 (aliphatic CH_2), 115.0-123.5 (four aromatic CH), 131.5 (aromatic C), 144.4 (-C (=O)-C), 154.5 (-N-C(= O)-C), 173.1 (carbonyl C for amide bond).

Synthesis of palmitoyl p-amino benzoic acid

Palmitoyl chloride (2.74 g, 10 m mole and para amino benzoic acid (2.4 g, 10 m mole) solution was condensed in presence of pyridine (0.79 g, 9.7 ml, 10 m mole) was added slowly to this mixture and stirred at 25 °C for 10 min, neutralized with ice cold dilute HCl to get a solid. Filtration followed by washing with water, dried and recrystallised from hot ethanol, afforded pure palmitoyl para amino benzoic acid (*N*- PPABA) (2.0 g, 80 %).

Brown colored solid, Melting point- 250 ^{0}C – 251.5 ^{0}C

IR spectrum (V_{max} cm $^{-1}$): υ 1265, 1560, 1750, 3320, 3580

^{1}H NMR ($CDCl_3$): δ 0.96 (t, 3H, CH_3), 1.29 (qn, 22H, $(CH_2)_{11}$), 1.33 (m, 2H, CH_2), 1.67 (q, 2H, CH_2), 2.23 (t, 2H, CH_2), 7.85- 8.11 (m, 4H, aromatic CH), 8.0 (s, 1H, OH), 11.0 (s, 1H, OH)

^{13}C NMR ($CDCl_3$): δ 14.0 (aliphatic CH_3), 22.1 (aliphatic CH_2), 23.6 (aliphatic CH_2), 25.7 (aliphatic CH_2), 29.8 (aliphatic CH_2), 29.0 (aliphatic CH_2), 29.3 (seven aliphatic CH_2), 32.5 (aliphatic CH_2), 36.3 (aliphatic CH_2), 115.0- 123.5 (four aromatic CH), 131.5 (aromatic C), 144.4 (-C (=O)-C), 154.5 (-N-C(= O)-C), 173.5 (carbonyl C for amide bond).

Pharmacological analysis

Wistar rats (180-230 gms) and albino mice of either sex chosen as experimental models were maintained at Government Pharmacy College, Bangalore. The animals were maintained in an animal house under standard environmental conditions. Ethical approval was obtained from Animal Ethical Committee, Government Pharmacy College, Bangalore, India.

All the compounds (500 mg kg^{-1} body mass) and reference NSAID; paracetamol (200 mg kg^{-1} body mass) were suspended in 1ml soya oil and administered orally using an animal feeding needle. The control group received appropriate volume of vehicle (1 ml soya oil, oral).

Antiinflammatory activity

Each group of six animals were injected with 0.1 ml of freshly prepared suspension of carageenan (1.0 % w/v) in the plantar region of right hind paw. Test animal groups were pretreated with test drug suspended in soya oil one hour before carageenan treatment. Control group animals received soya oil. The quantity of edema formed was measured for three hours after every hour using plethysmograph (Amir et al. 2005).

Antipyretic activity

Rats were given (*s. c.*) 20 ml/kg of 20 % aqueous suspension of sterilized bacterial LPS. After 18 h animals showing an increase of rectal temperature>0.5 ^{0}C were selected. Control group received vehicle 1ml soya oil, treated groups received PPAP and *N*-PPABA respectively. Rectal temperature was determined by digital thermometer before (pre treatment) 30 min and at 30 min, 1, 2, 3 and 4h after administration (Woolfe et al. 1994).

Analgesic activity

Tests were carried by hot plate, tail immersion or acetic acid induced writhing tests as described below.

Hot plate test

Male and female albino mice showing reaction time of 10 sec to 55 ± 1 °C thermal stimulus were selected. The time for hind paw licking or jumping on heated plate of analgesiometer was taken as reaction time for every 30, 60 and 90 min intervals. Aspirin was used as standard (Fadl et al. 1998).

Tail immersion test

Mice were placed in a restrainer cage after oral dose of drug. Animal was placed in such a way that its tail hangs out. In this position the tail (5 cm) dipped into the water bath maintained at 55 ± °1C. The reaction times (time to withdraw tail) were recorded after every one hour (Ark et al. 2004).

Acetic acid induced writhing test

The mice were divided into four groups. The first two groups of 16h fasted mice were administered with PPAP and *N*-PPABA orally. Mice from 3rd group received paracetamol (200 mg kg^{-1} body mass) and 4th group received 0.3 ml of carrier soya oil and they served controls. One hour after introduction of the drug all the mice received 0.1 ml of 3 % acetic acid to induce characteristic writhing response. The number of writhing occurring in 30 min was recorded. Analgesic activity was calculated as percentage inhibition of abdominal constrictions compared to control group (Ark et al. 2004).

$$\% \text{ Analgesic activity} = (n - n^{1} / n) \times 100$$

Where n = mean no of writhes of control group and n^{1} = mean no of writhes of test group.

Statistics analysis

The statistical significance of treatments between groups was determined by one way ANOVA followed by student's unpaired t test to find correlation between the control group and groups of animals treated with the test compound. Results shown are mean ± S.E mean, n=6, ***p<0.01

Results and Discussion

Chemistry

The palmitic acid derivatives of para amino phenol and para amino benzoic acid were prepared by amide bond formation reactions of PPAP and *N*-PPABA with palmitoyl chloride in the

presence of pyridine as a base. The reaction of para amino phenol and para amino benzoic acid in basic medium afforded palmitoyl Para amino phenol (PPAP) and *N*-palmitoyl *p*-amino benzoic acid (*N*-PPABA). The IR spectra of the these compounds revealed presence of absorption band at 1600 cm^{-1} for amide bond; also ^{1}H NMR of PPAP is in agreement with it's structure, displayed multiplet at δ 6.71- 7.47 for four ArH proton and triplet at δ 0.96 indicating terminal three protons of long chain fatty acid .whereas ^{13}C NMR spectra show signal δ 173.1 for carbonyl carbon of amide bond confirming the formation of long chain amide. *N*-PPABA ^{1}H NMR showed singlet at δ 8.0 for NH proton and triplet at δ 0.96, also ^{13}C NMR displayed signal at δ 173.5 for carbonyl carbon confirming the formation of amide bond.

Antiinflammatory studies

The anti-inflammatory potential of PPAP and *N*-PPABA (500 mg/kg body weight) against various animal models exhibited significant activity. The effect of PPAP, *N*-PPABA and paracetamol on inflammation are summarized in (Figure 1).

As shown in (Figure 1) pretreatment with PPAP showed maximum inhibition of 4.5 % after 3h of treatment and standard paracetamol showed 24.27 % whereas *N*-PPABA reduce edema only by 36.36 %. From these results it is suggested that antiinflamatory effect of PPABA is more potent than standard paracetamol, the anti-inflammatory effect was started from 1st h and retained till 3rd hr for *N*-PPABA and standard, whereas for PPAP it was persistent till 4th h and activity may be related to inhibition of inflammation mediators.

Antipyretic activity

The subcutaneous injection of sterilized bacterial LPS suspension markedly elevated the rectal temperature after 24 h of administration, treatment with *N*-PPABA decreased the rectal temperature. The antipyretic effect of *N*-PPABA maintained till 4t h whereas standard paracetamol showed significant activity only in 1st h (Figure 2).

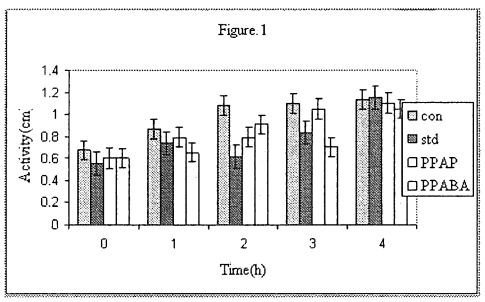


Figure 1. Rats were administered vehicle, Std, PPAP and *N*-PPABA 15 m before inter plantar carrageenan. Hind paw edema was measured by plethysmograph.

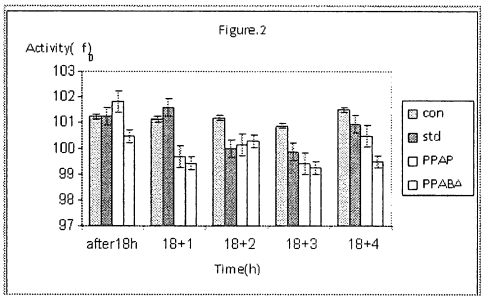


Figure 2. Rats were given (*s. c*) sterilized bacterial LPS. After 18 h animals showing an increase of rectal temperature >0.5 °C were selected. Control group received 1ml soya oil; treated groups received PPAP and *N*-PPABA respectively. Rectal temperature was determined by digital thermometer at 1, 2, 3 and 4 h after administration

Analgesic activity

In order to distinguish between the central and peripheral action of PPAP and *N*-PPABA acetic acid induced writhing response in mice were carried out. Writhing test is not only simple and reliable but also affords rapid evaluation of peripheral type of analgesic action. In this test animal reacts with characteristic stretching behavior called writhing was found. PPAP and *N*-PPABA inhibited writhing response by 75.95 % and 75.26 %. *N*-PPABA potentiated the analgesic activity of paracetamol by 59.92 %. The abdominal constriction is related to sensitization of nociceptive receptors to prostaglandins. It is therefore possible that PPAP and PPABA produce analgesic effect may be due to inhibition of prostaglandin synthesis.

The mechanical hyperalgesia by hot plate method was found to be suitable for evaluation of centrally but not peripherally acting analgesics (Martins Do Monte et al. 2004). The validity of this test has been shown even in presence of substantial impairment of motor performance.

The thermal hyperalgesia as measured by tail immersion test showed, persistent activity initiating from 1^{st} -3^{rd} h. The results showed that PPAP is more efficient than paracetamol. *N*-PPABA also confers significantly enhanced and persistent analgesic activity. Therefore, these compounds may be producing their effect both peripherally (writhing test) and centrally (tail immersion and hot plate tests).

Maximum tolerance studies

Acute toxicity studies were performed with rats of either sex by administration of single oral dose of 2000 mg/kg body weight for three different groups of animals (n = 6). The 1^{st} group (control) was treated with soya oil while other two groups received drug to be tested and observed for 48h. All the test animals survived without any side effect; hence dosage till 2000 mg/kg body weight is safe. Hence, $1/10^{th}$ of maximum dose was considered safe for further studies (Health effects test guidelines; OPPTS 870.1100).

Conclusions

At physiological pH the gastric tolerability of NSAIDs may be markedly improved by decreasing their solubility. Hence conversion of palmitoyl para amino phenol and palmitoyl para amino benzoic acid into palmitoyl derivatives through an amide bond to the NH_2 residue of the latter may reduce their acidic nature. Thus the results of this study suggest that PPAP possesses promising anti-inflammatory and analgesic properties compared to paracetamol. The *N*-PPABA exhibited potent pharmacological properties after derivatisation. Thus, the conjugation of palmitic acid to NSAID leads to new compounds that posses improved and efficient pharmacological properties. Hence, conjugation reaction products with lipids can attribute to eliminate side effects inherent in the initial compounds. The mechanisms involved in the cell membrane penetration of these compounds are underway. Such a study may identify their interaction with cell receptors and their metabolic roles.

Table 1. Comparison of analgesic activities of PPAP and PPABA.

Groups	Treatment	Dose mg/kg body weight	Writhing test (% inhibition)	Hot plate method			Tail immersion test			
				Time in minutes			Time in hour			
				30	60	90	0	1	2	3
Group-I (Control)	Soya oil	1 ml		14.667±1.229	16.00±1.528	12.667±1.202	3.3±0.2108	3.66±0.5578	3.33±0.6146	2.33±0.2108
Group-II (Standard)	Paracetamol	200	59.92	Ns 20.167±2.994	*** 66.667±4.595	*** 232.333±1.081	Ns 3667±0.7149	* 5.33±0.5578	Ns 5.0±0.3651	Ns 3.0±0.6325
Group-III (PPAP)	Palmitoyl Para amino phenol	500	75.95	Ns 20.333±4.302	*** 121.67±15.420	*** 106.67±20.602	Ns 3.167±0.3073	*** 7.083±0.4549	*** 11.083±0.8604	Ns 3.500±0.6055
Group-IV (N-PPABA)	Palmitoyl Para amino benzoic acid	500	75.26	* 27.33±1.687	*** 95.00±16.021	*** 93.33±17.591	Ns 2.667±0.3333	Ns 3.750±0.1708	*** 9.333±0.4410	* 3.083±0.2007

References

Amir, M. and Kumar, S. (2005). Anti-inflammatory and gastro sparing activity of some new indomethacin derivatives. *Arch. Pharm. Chem. Life Sci.* 338: 24-31.

Amit K. S., Alan, M. B., Brenda, C. C., Rory, R. P. and Lawrence, M. J. (2000). Ester and amide derivatives of the nonsteroidal antiinflammatory drug, indomethacin, as selective Cyclooxygenase-2 inhibitors. *J. Med. Chem.* 43: 2860-2870.

Beck, W. S., Schneider, H. T., Dietzel, K., Nuernberg, B. and Brune, K. (1990). Gastrointestinal ulceration induced by antiinflammatory drugs in rats. *Arch. Toxicol.* 64: 210-217.

Boyer, T. D. and Rouff, S.C. (1971). Acetaminophen induced hepatic necrosis and renal failure. *J. Am. Med. Assoc.* 218: 440-441.

Cioli, V., Putzolu, S., Barcellona, P.S. and Corradino, C. (1979). The role of direct tissue contact in the production of GI ulcers by NSAIDs in rats. *Toxicol. Appl. Pharmacol.* 50: 283- 289.

Fabrício Hoffmann Martins Do Monte, Jair Guilherme dos Santos, Jr., Michael Russi,Vanusa Maria Nascimento Bispo Lanziotti, Luzia Kalyne Almeida Moreira Leal, Geanne Matos de Andrade Cunha. (2004). Antinociceptive and anti-inflammatory properties of the hydroalcoholic extract of stems from *Equisetum arvense L* in mice. *Pharmacol. Res.* 49: 239 -243.

Fadl, T. A. and. Omar, F. A. (1998). Paracetamol esters of some NSAID carboxylic acids as mutual prodrugs with improved theraupetic index. *Inflammo Pharmacology* 6: 143- 157.

Health effects test guidelines; OPPTS 870.1100.Acute oral toxicity published by United States Environmental protection Agency.

Ivey, K. J. (1988). Mechanisms of non steroidal anti-inflammatory drug-induced gastric damage. *Am. J. Med.* 84: 41-48.

Jones, G. (1985). Decreased toxicity and adverse reactions via prodrugs. In: Bundgaard H, ed. Design of Prodrugs. Amsterdam: Elsevier. 199- 241.

Kalgutkar, A. S., Crews, B. C., Saleh, S., Prudhomme, D. and Marnett, L. J. (2005). Indolyl esters and amides related to indomethacin are selective COX-2 inhibitors. *Bioorg. Med. Chem.* 13: 6810–6822.

Kourounakis, P. N., Tsiakitzis, K., Kourounakis, A. P. and Galanakis, D. (2000). Reduction of gastrointestinal toxicity of NSAIDs via molecular modifications leading to antioxidant anti-inflammatory drugs. *Toxicology* 144: 205-210.

Ark, M., Üstün, O. and Yeşilada, E. (2004). Analgesic activity of *Cistus laurifolius* in mice. *Pharmaceutical Biology.* 42: 176-178.

Rainsford, K. D. (1988). Gastrointestinal damage from NSAIDs. *Toxicol Pathol.* 16: 251- 259.

Shanbhag, V. R., Crider, A. M., Gokhale, R., Harplani, A. and Dick, R. M. (1992). Ester and amide prodrugs of ibuprofen and naproxen: Synthesis and antiinflammatory activity and gastrointestinal toxicity. *J. Pharm. Sci.* 81: 149-154.

Vermeulen, N. P. E., Straat, R. V., TeKoppele, J. M. *et al.* (1990). Molecular mechanisms in toxicology and drug design. *Trends in Drug Research* 13: 71.

Woolfe, G. and Mac Donald, A. D. (1994). The evaluation of analgesic action of pethidine hydrochloride. *J. Pharmacol. Exp. Ther.* 80: 300.

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