

Synthesis, biological evaluation and kinetic studies of glyceride prodrugs of biphenyl acetic acid

M S Y Khan, Mymoona Akhter* & A Husain

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard,
New Delhi 110 062, India
E mail: myzoon@rediffmail.com

Received 16 February 2005; accepted (revised) 5 May 2005

Glyceride derivatives (**3a** and **3b**) of biphenyl acetic acid have been synthesized to reduce the gastrointestinal toxicity associated with it and are tested for their ulcerogenicity, anti-inflammatory and analgesic activity and hydrolytic behaviour. The prodrugs have been prepared by reacting 1,2,3-trihydroxypropane-1,3-dipalmitate/stearate **1** with the acid chloride of biphenyl acetic acid. The prodrugs are significantly less irritating to the mucosa as indicated by scores of 0.75 and 0.82 by **3a** and **3b** compared to 2.3 of biphenyl acetic acid. The prodrugs show better anti-inflammatory and analgesic activity than the parent drug. The hydrolysis studies of prodrugs show that the prodrugs are resistant to hydrolysis at pH 3, 4, 5 than at pH 7.4. The peak plasma concentration (C_{max}) 28.63 $\mu\text{g/mL}$ of biphenyl acetic acid is attained in 2 hr whereas in case of **3a** and **3b** treated animals C_{max} 33.5 and 34.6 $\mu\text{g/mL}$ is attained in 3 hr respectively. All these studies indicate that the glyceride prodrugs of biphenyl acetic acid might be considered as potential biolabile prodrugs of biphenyl acetic acid.

Keywords: Glyceride derivatives, biphenyl acetic acid, gastrointestinal toxicity, biological evaluation, kinetic study

IPC: Int.Cl.⁷ C 07 C

4-Biphenyl acetic acid is the active metabolite of fenbufen which is twice as active as the parent drug^{1,2}. This compound has been introduced as an independent drug in the form of gel for local applications in the symptomatic relief of articular inflammation and pain³. However, it has been observed that biphenyl acetic acid has severe gastrointestinal side effects on oral administration due to its free carboxylic group and therefore restricts its use⁴. The aim of our study has been to mask the free carboxylic acid group of biphenyl acetic acid temporarily by synthesizing their glyceride prodrugs, which can pass through the stomach without releasing active drug in stomach in significant quantity and it shall also increase their absorption pertaining to the absorption of natural triglycerides. Triglycerides being the major constituent of the dietary fat, their absorption involves simple hydrolysis mainly by pancreatic *lipase* to monoglycerides and free fatty acids. These prodrugs therefore do not involve risk of unwanted effects after the prodrug is hydrolyzed. The synthesized compounds were evaluated for their

ulcerogenic, anti-inflammatory and analgesic effect. The *in vitro* and *in vivo* kinetics of the prodrugs were also studied by reverse phase HPLC. The prodrugs were obtained by condensing 1,2,3-trihydroxypropane-1,3-dipalmitate/stearate⁵ **1** with the acid chlorides (ref. 6), **2** of the biphenyl acetic acid in the presence of dry pyridine (**Scheme I**).

Results and Discussion

Pharmacological studies

The anti-inflammatory activity of the compounds was carried out by the method of Winter *et al.*⁷. The synthesized compounds **3a** and **3b** showed good anti-inflammatory activity at 3 hr with a percentage inhibition of 79.82, 76.01 compared to 75.2 of biphenyl acetic acid.

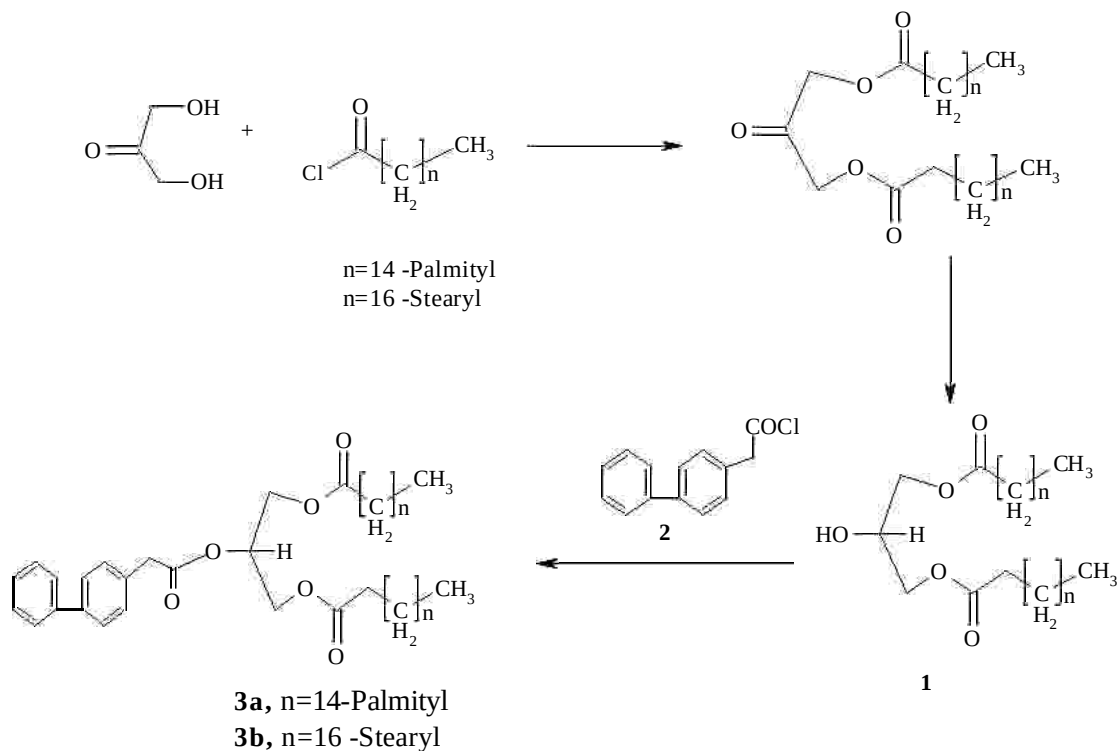
Analgesic activity was carried out by the method of Seigmund *et al.*⁸. The activity was observed as decrease in number of writhings produced by acetic acid injection after treatment with the drugs. The percentage

protection observed was 54.06 and 56.52% by **3a** and **3b** compared to 52.6% by biphenyl acetic acid.

The ulcerogenic potential of the drugs was studied by the method of Cioli *et al.*⁹. The prodrugs were significantly less ulcerogenic than the parent drug with severity index of 0.75 and 0.82 by **3a** and **3b** compared to 2.3 by biphenyl acetic acid. The percentage of animals with ulcers was more than double in biphenyl acetic acid treated animals than prodrugs treated animals (**Table I**).

Hydrolysis studies

The hydrolysis studies of the prodrugs were studied with reverse phase HPLC. Hydrolysis profile of the synthesized glyceride prodrugs revealed that the prodrugs hydrolyze readily at pH 7.4 corresponding to the pH of blood (**Figure 1**). A graph of log concentration of residual drug vs time was plotted for **3a** and **3b** respectively; a linear plot was obtained suggesting pseudo-first order rate kinetics. The rate constant (k_{obs}) and the corresponding half-lives ($t_{1/2}$)



Scheme I

Table I — Comparative chart of biphenyl acetic acid, **3a** and **3b** with respect to their dose and biological activity

Parameters		Biphenyl acetic acid	3a	3b
Dose (mg/kg)		20	71.88 [#]	77.16 [#]
Anti-inflammatory activity	2 hr	54.35*	56.15*	58.35*
	3 hr	75.2*	79.82*	76.01*
Analgesic activity		52.66*	54.06*	56.52*
Gastric ulcers;(% of animals with ulcers)		58	20	25
Severity index		2.3**	0.75**	0.82**

[#]The dose quantities are molar equivalent of biphenyl acetic acid

* $P < 0.001$ compared to control, ** $P < 0.0001$ compared to control

‘Severity index’ mean score of treated group minus mean score of control group

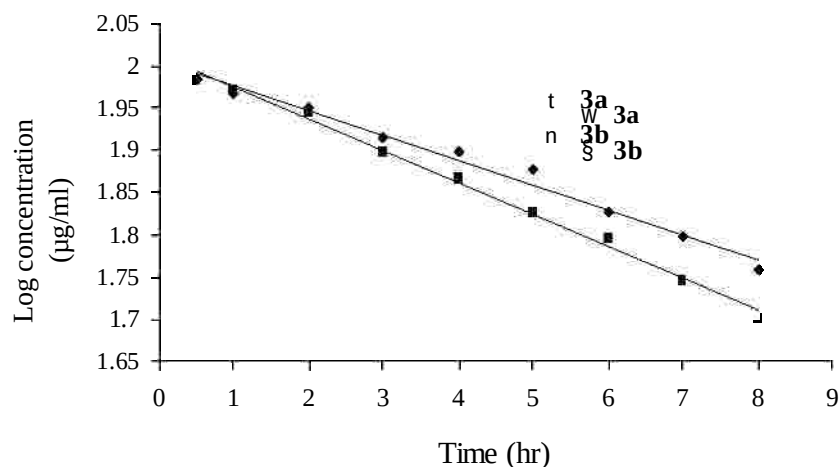


Figure 1—First order hydrolysis plot for **3a** and **3b** at pH 7.4

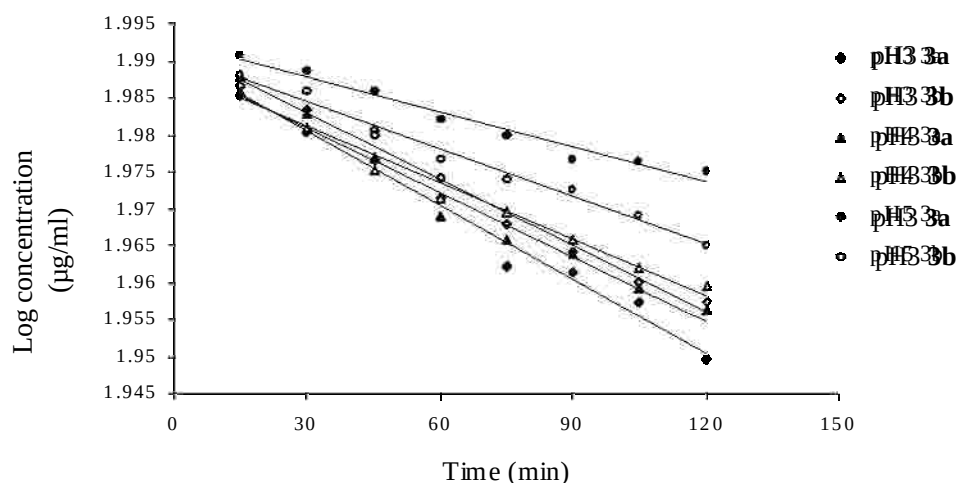


Figure 2—Hydrolysis of **3a** and **3b** at pH 3, 4 and 5

for the respective prodrugs were calculated from the linear regression equation correlating the log concentration of the residual prodrug vs time.

It was observed that hydrolysis at pH 3, 4, and 5 corresponding to the pH of stomach was very less whereas the drug was readily released at pH 7.4 indicating that the prodrugs are resistant to acidic environment (Figure 2).

A comparison of the plasma concentration vs time curves of diclofenac and biphenyl acetic acid indicate that the esters were rapidly hydrolyzed to the parent drug in rats. Maximum plasma concentration of the liberated parent drug in animals treated with glyceride prodrugs was attained in 3 hr whereas C_{max} in biphenyl acetic acid was attained in 2 hr. At all times the

concentration of biphenyl acetic acid in the animals treated with **3a** and **3b**, were higher in comparison to the animals receiving the parent drug. The peak plasma concentrations of biphenyl acetic acid obtained after treatment with biphenyl acetic acid, **3a** and **3b** were 28.63, 33.5 and 34.6 µg/mL, respectively (Figure 3).

Conclusion

The glyceride derivatives of biphenyl acetic acid were successfully synthesized and evaluated for gastrointestinal toxicity, anti-inflammatory activity, analgesic activity, ulcerogenicity and hydrolysis profile. The prodrugs were considerably less ulcerogenic compared to the parent drugs indicating that the gastrointestinal toxicity due to direct contact of the carboxylic function of the NSAIDs have been

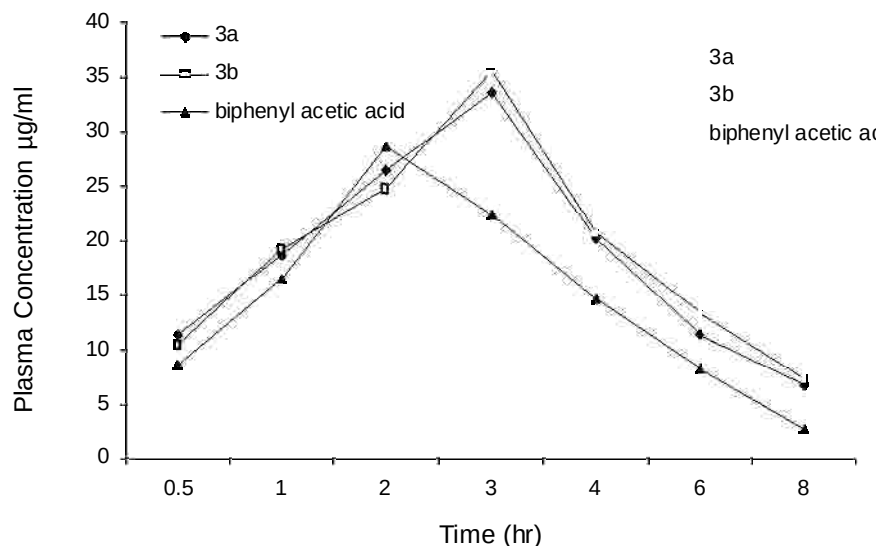


Figure 3—Plasma concentration of biphenyl acetic acid after oral administration of the **3a**, **3b** and biphenyl acetic acid

reduced with good anti-inflammatory and analgesic activity with sustained release and high plasma levels.

Experimental Section

All the reagents were obtained from E. Merck (India) Ltd. except dihydroxy acetone which was purchased from E. Merck KGa A, Germany. TLC of the synthesized compounds was carried out in petroleum benzene-petroleum ether (5:1; v/v) solvent system. The TLC spots were located by exposing to iodine vapours. Melting points were recorded in liquid paraffin-bath using open-end capillaries and are uncorrected. ^1H NMR spectra were recorded on a Bruker spectropin Avance DP $\times$ 200, 300 MHz in CDCl_3 ; mass spectra on a Jeol 5 $\times$ 102/DA-6000 Mass spectrometer; and FT-IR spectra on a Perkin-Elmer spectrometer. HPLC analysis was recorded on a Shimadzu Model LC-10ATVP (Japan) system containing a quaternary pump, UV detector and c 18 reverse phase column. Dissolution was carried out by SR8 Plus dissolution test apparatus, Hanson Research, USA. Samples were filtered with 0.45 μm Millipore filter and eluted with methanol and 0.05% phosphoric acid (70:30; v/v) at 2 mL/min. The effluent was monitored at 232 nm by UV detector, Shimadzu Model SCL-10AVP, Japan.

Synthesis

The glyceride prodrugs of biphenyl acetic acid were successfully synthesized. The yields of the compounds were good. The structures of the synthesized

compounds were established by elemental analysis, ^1H NMR, mass and FT-IR spectral methods. All the compounds gave satisfactory elemental analysis within $\pm 0.4\%$ of the theoretical values. The purity was determined by TLC.

Preparation of glyceride prodrug 3a. Compound **3a** was prepared by the reported method¹⁰, yield 67%; m.p. 48-50°C; R_f 0.65; IR (KBr): 2921.3, 2852.2 (C-H), 1717.4 (C=O), 1606.1 (aromatic), 1258.9 (C-O-O), 754.38, 726.3 cm^{-1} ; ^1H NMR (CDCl_3): 0.84 (6H, t, $2\times\text{CH}_3$), 1.22 (m, $28\times\text{CH}_2$), 1.61 (4H, m, $2\times\text{CH}_2$, β to CO), 2.25 (4H, t, $2\times\text{CH}_2$ α to CO), 4.05 (4H, m, $2\times\text{CH}_2$), 3.6 (2H, s, CH_2), 5.3 (1H, m, CH), 6.98-7.5 (9H, m, diphenyl system); Mass: m/z 762 (M^+) (molecular ion peak was not observed), 551, 507, 313, 283, 281.

Preparation of glyceride prodrug 3b. Compound **3b** was prepared by the reported method¹⁰, yield 52%; m.p. 128-30°C; R_f 0.71; IR (KBr): 2923.47, 2857.82 (C-H), 1724.64 (C=O), 1611.2 (aromatic), 1261.88 (C-O-O), 756.13, 727.33 cm^{-1} ; ^1H NMR (CDCl_3): 0.86 (6H, t, $2\times\text{CH}_3$), 1.23 (m, $28\times\text{CH}_2$), 1.63 (4H, m, $2\times\text{CH}_2$, β to CO), 2.25 (4H, t, $2\times\text{CH}_2$, α to CO), 4.1 (4H, m, $2\times\text{CH}_2$), 3.7 (3H, s, CH_2), 5.24 (1H, m, CH), 6.92-7.5 (9H, m, diphenyl system); Mass: m/z 812 (M^+) (molecular ion peak was not observed), 607, 535, 341, 283, 281.

Pharmacological Activity

Anti-inflammatory activity

The anti-inflammatory activity was evaluated using carrageenan induced paw edema on rat method⁸. Wistar

rats of either sex (150-200 g) were divided into groups of 6 animals each. The prodrugs were suspended in 0.5% (w/v) sodium CMC in distilled water. The prodrugs were administered orally at dose 71.88 mg/kg and 77.16 mg/kg of **3a** and **3b**, respectively where the dose was molecularly equivalent to the parent drug. Biphenyl acetic acid was used as standard (20 mg/kg). For control 0.5% (w/v) sodium CMC in distilled water was given orally (5 ml/kg). Thirty minutes after the drug administration 0.2 mL of 1% (w/v) carrageenan solution was injected in the subplanter region of the left hind paw of the animals. The paw volume was measured at 0 hr, 2 and 3 hr after the drug administration. The data was analyzed using students "t" test and the level of significance was defined as $P < 0.001$.

Analgesic activity

Analgesic activity was carried out by using acetic acid induced writhing method⁸ in Swiss albino mice (25-30 g) of either sex. A 1% (v/v) solution of acetic acid was used as writhing inducing agent. Test compounds were administered orally 3 hr prior to acetic acid injection. Number of writhings for 10 min in control and test compounds were counted and compared. Analgesic activity was measured as percent decrease in writhings in comparison to control. Mice were divided into 4 groups of 6 animals each. Group I served as a control group, while group II received biphenyl acetic acid (20 mg/kg), groups III and IV received prodrugs 71.88 mg/kg and 77.16 mg/kg of **3a** and **3b**, respectively, where the dose of prodrugs was molecularly equivalent to the biphenyl acetic acid. Drug solutions were prepared as a homogeneous suspension in aqueous solution of sodium CMC (0.5%w/v). Acetic acid was administered intraperitoneally 1 mL/100 g body weight of the animal. The data was analyzed using students "t" test and the level of significance was defined as $P < 0.001$.

Ulcerogenicity

Wistar rats of either sex (150-200 g) were taken into groups of six each. Group I served as control and received vehicle only. Group II received biphenyl acetic acid (50 mg/kg) as a suspension in 0.5% (w/v) of sodium CMC, group III and IV received test compounds **3a** and **3b** in dose molecularly equivalent to the parent drug. The animals were fasted 8 hr prior to a single dose of each of the standard and test

compounds and sacrificed 17 hr later during which time food and water remained available. The stomach was opened along with the greater curvature and mounted on a board. It was examined for the severity of ulcers and score was ascertained by Cioli *et al.*⁹ method. The mean score of each treated group minus the mean score of the control group was considered the 'severity index' of gastric damage (Table I).

In vitro Pharmacokinetics studies¹¹

The hydrolysis studies of the prodrugs were studied with reverse phase HPLC. The mean fasting stomach pH of adult is approximately 2 and increases to 4-5 following ingestion of food¹², consequently pH 3, 4 and 5 were selected to mimic the appropriate clinical range and pH 7.4 corresponding to pH of blood was also selected for the *in vitro* studies. Buffers of required pH were prepared and equilibrated at 37°C for 1 hr and 100 mg of **3a** and **3b** were added. The mixtures were agitated by an overhead stirrer. An assay time of 2 hr was selected as the stomach emptying would normally be effectively complete by the time. At selected time intervals 15, 30, 45, 60, 75, 90, 105 and 120 min, 0.1 mL of the mixture was withdrawn, diluted upto 10 mL with mobile phase and 20 µL of this solution was injected for HPLC analysis. At pH 7.4 samples were withdrawn at 0.5, 1, 2, 3, 4, 5, 6, 7 and 8 hr. Calibration curves for biphenyl acetic acid were prepared in the concentration range of 1-100 µg/mL. The samples were eluted by using methanol-0.05% phosphoric acid (70:30; v/v) at a flow rate of 2 mL/min. The effluent was monitored at 232 nm.

In vivo Pharmacokinetics studies

The *in vivo* hydrolysis studies of the prodrugs were studied by using the reverse phase HPLC. Rats were divided into 3 groups of 6 animals each, group I received biphenyl acetic acid orally (20 mg/kg). Groups II and III received **3a** (71.88 mg/kg) and **3b** (77.16 mg/kg), respectively where the dose was molecularly equivalent to the parent drug. The animals were fasted overnight prior to experiments. Blood samples were collected at different time intervals 0.5, 1, 2, 3, 4, 6 and 8 hr. Plasma was separated by centrifugation at 4000 rpm and refrigerated (-20 °C) till analysis. Calibration curve of biphenyl acetic acid was prepared in rat plasma in concentration range of 1-40 µg/mL. In a 5 mL centrifuge tube 0.05 mL of plasma was taken and to it was added 1.5 mL

acetonitrile and vortexed for 2 min. The precipitated proteins were separated by centrifugation at 4000 rpm for 15 min. Mobile phase used was methanol-phosphoric acid (0.05%) (70:30; v/v) at a flow rate of 2 mL/min. The effluent was monitored at 232 nm. The injecting volume was 20 µL.

References

- 1 Chiccarelli F S, Eisner H J & Van Lean G E, *Arzneim-Forsch/Drug Res*, 30, **1980**, 707.
- 2 Sloboda A E, Tolman E L & Osterberg A C, *Arzneim-Forsch/Drug Res*, 30, **1980**, 713.
- 3 Prous J R, *Drug News Perspect*, 3, **1990**, 19.
- 4 Sloboda A E, Tolman E L & Osterberg A C, *Inflammation*, 1, **1976**, 415.
- 5 Bentley P H & McCrae W, *J Org Chem*, 35, **1970**, 2082.
- 6 Vogel A I, *Textbook of practical organic chemistry*, 4th Edn, (ELBS/Longman, London) **1987**, 455.
- 7 Winter C, Risley E & Nuss G, *Proc Soc Exp Biol Med*, 111, **1962**, 544.
- 8 Seigmund E, Cadmus R & Lu G, *Proc Soc Exp Biol Med*, 95, **1957**, 729.
- 9 Cioli V, Putzolu, S, Rossi V, Corza B P & Corradino C, *Toxicol Appl Pharmacol*, 50, **1979**, 283.
- 10 Khan M S Y & Akhtar M, *Indian J Exptl Biol*, 42, **2004**, 1066.
- 11 Lalande M, Wilson D L & McGilveray I J, *J Chromatogr*, 337, **1986**, 410.
- 12 Hunt J N & Spurrell W R, *J Physiol*, 113, **1951**, 157.