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Synthesis and stability of two indomethacin prodrugs

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Abstract—The purpose of this study was to synthesize and study the in vitro enzymatic and non-enzymatic hydrolysis of indomethacin–TEG ester and amide prodrugs. It was found that the ester conjugate **10** was comparatively stable between pH 3 and 6 (half-life >90 h), with a half-life equal to 5.2 h in 80% buffered plasma. In contrast, the amide conjugate **12** appeared to be stable over the entire pH range studied with the only observed degradation being cleavage of the indolic *N*-4-chlorobenzoyl moiety.

Indomethacin (1-[4-chlorobenzoyl]-5-methoxy-2-methyl indole-3-acetic acid) is a potent non-steroidal anti-inflammatory agent used primarily in the treatment of rheumatoid arthritis.[1] The compound also has potential for use in uveitis, a common disease afflicting 0.5% of the population[2] and responsible for over 30,000 cases of legal blindness in the U.S.[3] Indomethacin may also be used in the management of cystoid macular edema (CME), a disease characterized by a build up of serous fluid extracellular space in the retina caused by a disruption of the blood–retinal barrier. CME is a common occurrence after cataract and vitreo–retinal surgeries[4] and is frequently associated with diabetes.[5] In 1979 Klein et al.[6] showed that systemic indomethacin decreased the incidence of post surgical CME at 4–6 weeks, however, other investigators[7,8] failed to substantiate these findings. The blood–eye barrier restricts intraocular penetration of drugs requiring high systemic dosing to achieve therapeutic levels within the eye.[9] In the case of indomethacin, systemic toxicities including dyspepsia, headache, and dizziness[10] limit the dose that can be administered, and in many studies therapeutic concentrations may not have been achieved. The topical application of non-steroidal anti-inflammatory agents would avoid many of these problems, but is limited by the low concentrations that can be tolerated in the eye, and by poor ocular absorption.[11,12] In the soluble form, indomethacin is an ionic surface active compound, and such compounds have long been known to be damaging to biological membranes.[13] However, a non-ionic prodrug of indomethacin could be expected to be less damaging. Walters et al.[14] studied the effect of surfactants composed of a linear dodecyl chain linked to various polyoxyethylene glycol (PEG) chains (Brij surfactants). They found that surfactants composed of PEGs containing less than 8 ethylene oxide units caused little damage to rat GI tissues, while those with PEGs containing more than 10 units both decreased the barrier function and stripped proteins from the surface of the gastric mucosal membrane. Nishima and co-investigators[15] examined the transdermal absorption of Brij type surfactants in the mouse model and found that maximal absorption occurred with short ethylene oxide chains. These findings suggest that a PEG prodrug of indomethacin may have increased absorption and decreased irritancy compared to the parent compound. A further advantage of PEG as a potential prodrug moiety is that upon enzymatic cleavage, parent drug plus PEG would be regenerated, and PEG is essentially biologically inert, and is used as a marker substance to monitor GI absorption in human.[16,17]

The present research work focuses on the design and strategy involved in the synthesis and in vitro enzymatic and non-enzymatic hydrolysis of two prodrugs of indomethacin conjugated with triethylene glycol (TEG) at the carboxylic acid moiety via either an ester or an amide linkage. The two prodrugs were the indomethacin–TEG ester and amide conjugates.

Solvents were obtained from Fischer Scientific Co. (Fairlawn, NJ, USA). Indomethacin, borax, and

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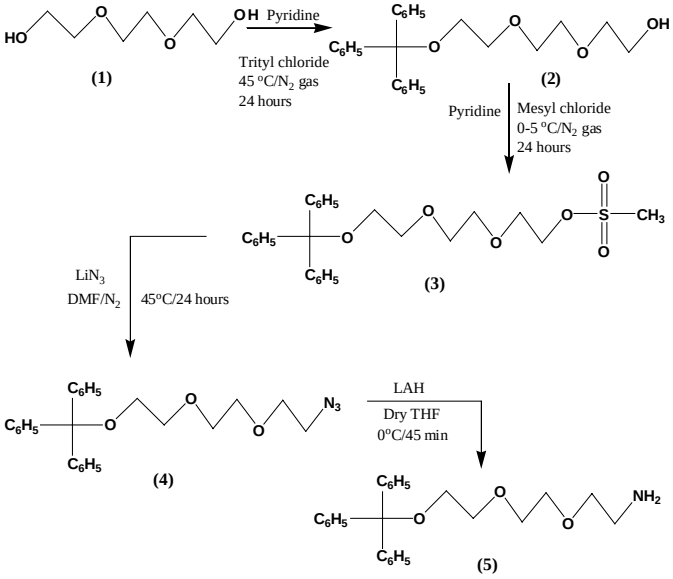
chemicals used in synthetic procedures were obtained from Sigma–Aldrich Chemicals (St. Louis, MO, USA). Lithium azide and palladium on 5% carbon were obtained from Eastman-Kodak (Rochester, NY, USA). Chromatographic silica (grade 62) was purchased from Davison Chemical, Baltimore, MD. PkF_6 silica gel preparative TLC plates (1000 μm) with fluorescent indicator were obtained from Whatman Labsales Inc. (Hillsoboro, OR, USA).

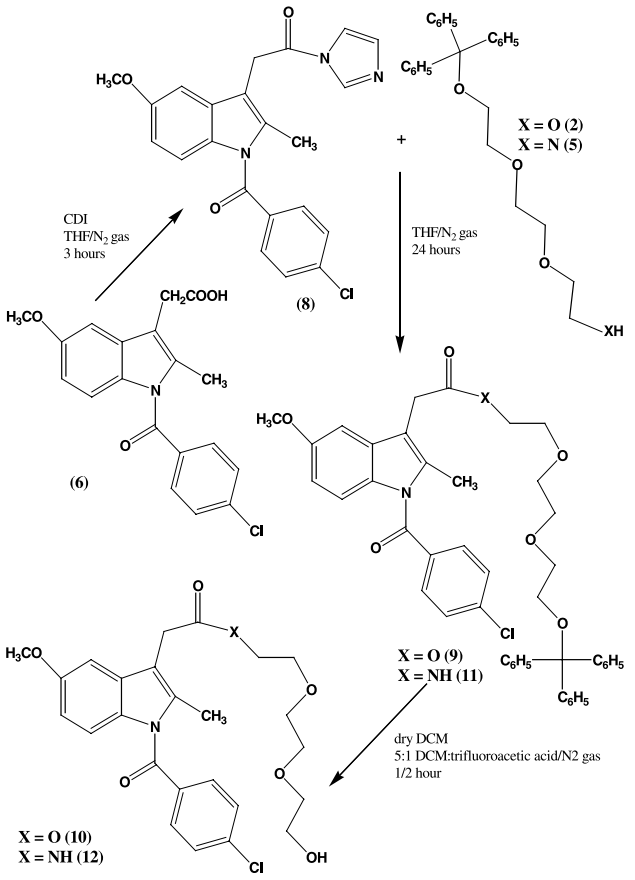
High-performance liquid chromatographic (HPLC) analyses were carried out on a Hitachi System consisting of an L-4000 UV detector, L-6200 intelligent pump, and AS-2000 Autosampler (Hitachi, Tokyo, Japan). The chromatographic column used was C_{18} reverse-phase column (250 mm × 4.5 mm × 5 μm) fitted with a precolumn (Hamilton, Reno, Nevada). Chromatograms were recorded on a D-2500 Chromatointegrator. The flow rate for the entire part of the analytical study was maintained at 1 mL/min, and detection was by UV absorption at 270 nm. All nuclear magnetic resonance (NMR) spectra were carried out in deuterated chloroform, unless otherwise stated, on a Varian VXR-300 spectrometer operating at 300 MHz (Varian Associates, Palo Alto, CA). Chemical shifts are recorded as δ values (ppm) downfield from TMS as an internal standard; all signals are classified as singlet (s), broad singlet (br s), doublet (d), triplet (t), multiplet (m), and apparent doublet (app d). Ultraviolet (UV) spectra were recorded on a Hewlett Packard 9153C spectrophotometer, connected to a Hewlet Packard 8452A diode array detector and a Hewlet Packard Thick Jet Printer.

For the non-enzymatic hydrolysis studies; a stock methanolic solution of each prodrug was prepared. In triplicate, 0.1 mL of this solution was added to 5 mL of 0.1 M buffer (pH 1–11) in a culture tube to give a final concentration of 1.28×10^{-5} M. The buffers used were hydrochloric acid, sodium acetate, potassium phosphate, and sodium borate. Ionic strength (μ) for each buffer was maintained at a constant value of 0.5, by addition of an appropriate amount of potassium chloride. Tubes were sealed and immersed in a water bath at 37 ± 2 °C. A sample of 200 μL was withdrawn periodically, quenched in an ice bath and assayed by HPLC. Both the disappearance of the prodrug and the appearance of indomethacin were measured. For the enzymatic hydrolysis studies; fresh human blood was withdrawn and allowed to stand for about 15 min. Fresh plasma was generated by centrifugation at 3000 rpm for 15 min. The supernatant plasma was carefully withdrawn and an 80% solution was prepared by addition of 20% of 0.1 M phosphate buffer (pH 7.4). Standard solutions of indomethacin prodrugs were prepared by dissolving the appropriate prodrug in methanol. The methanolic solution of the prodrug (50 μl) was added to 5 mL of fresh human buffered plasma to give a final concentration of 4.85×10^{-4} M. Tubes were sealed and immersed in a water bath at 37 ± 2 °C. In triplicate, 100 μl of the sample was periodically removed and added to 100 μl acetonitrile to precipitate plasma proteins in a microcentrifuge tube. Tubes were vortexed and centrifuged at 14,000 rpm for 8 min. Hundred microliter of the supernatant solution was analyzed by the HPLC for both the prodrug and indomethacin content.

The indomethacin prodrugs **10** and **12** were successfully synthesized according to Schemes 1 and 2 and carefully characterized. In summary, monotritylation of triethylene glycol (TEG) to give **2** was best achieved by utilizing a 1:1 molar ratio of trityl chloride and TEG (Scheme 1). Only a small amount of ditritylated TEG contaminated the final product and was efficiently removed by silica gel column chromatography. Activation of indomethacin by reaction with carbonyl diimidazole, and coupling of the resulting reactive intermediate **8** with the monotrityl TEG afforded the protected conjugate **9** in low yield (8–10%). O-detritylation of **9** was carried out using 20 % TFA in dichloromethane to afford the indomethacin–TEG ester prodrug **10** in 64% yield (Scheme 2).



Scheme 1. Synthesis of 1-hydroxy-8-trityloxy-3,6-dioxaoctane (**2**) and 1-amino-8-trityloxy-3,6-dioxaoctane (**5**).



Scheme 2. Activation of indomethacin by reaction with carbonyl diimidazole (CDI), and its use in the synthesis of indomethacin–TEG ester (**10**) and indomethacin–TEG amide (**12**) prodrugs.

The indomethacin–TEG amide prodrug **12** was synthesized utilizing a strategy similar to that employed in the preparation of **10** (Scheme 2), starting from the *O*-trityl protected precursor **5**, which was synthesized from TEG via the four-step procedure illustrated in Scheme 1. This procedure involved initial monotritylation of TEG followed by O-mesylation to give **3**, displacement of the *O*-mesyl group with azide ion to give **4**. Azide reduction was carried out using lithium aluminum hydride in anhydrous THF, affording compound **5** in 35% yield. Coupling of **5** with **8** afforded the protected conjugate **11** in 61% yield. Finally, O-detritylation with TFA afforded a good yield (74%) of the indomethacin–TEG amide prodrug **12.**

*10,10,10-Triphenyl-3,6,9-trioxadecan-1-ol (**2**).* Using a modification of the procedure reported by Blickenstaf,[18] triethylene glycol (TEG) **1** (1.5 g, 0.01 mol) was stirred into pyridine containing trityl chloride (2.7 g, 0.0096 mol). The resultant mixture was heated to 45 °C and allowed to stir overnight under nitrogen gas. The mixture was added to ice-cold water (200 mL) and partitioned between water and CH_2Cl_2 (4×20 mL). The organic layer was separated and dried over $MgSO_4$, and the solvent was removed azeotropically with toluene under vacuum on a rotary evaporator. The resultant syrupy mass was purified by column chromatography on silica gel, eluting with 2% methanol in chloroform. The eluent fractions containing the desired compound were combined and stripped to yield an oily mass (2.47 g, 63%)

*1-Mesyloxy-8-trityloxy-3,6-dioxaoctane (**3**).* Using a Modification of the procedure reported by Harris et al.[19] compound **2** was dissolved in pyridine (25 mL) and cooled to 0 °C under nitrogen gas. A solution of mesyl chloride (1.97 mL, 0.018 mol) was added slowly, and the reaction was allowed to proceed overnight at 4 °C. The resultant dark liquid was added to ice-cold water (200 mL) and partitioned between water and CH_2Cl_2 (4×20 mL). The organic layer was dried over anhydrous $MgSO_4$ and the solvent was removed azeotropically at low pressure on a rotary evaporator. The resulting syrup was eluted through a silica gel column with 2% MeOH in $CHCl_3$, to afford pale yellow crystals of compound **3** with a yield of 1.8 g (60%).

*1-Azido-8-trityloxy-3,6-dioxaoctane (**4**).* In an adaptation of the method employed by Reynolds et al.,[20] compound **3** (1.77 g, 0.0037 mol) was stirred into dimethylformamide (DMF) under nitrogen gas. Lithium azide (0.91 g, 0.019 mol) was added, and the resultant mixture was heated to 45 °C for 24 h, after which time the reaction was essentially complete. The DMF was removed in vacuo and the resulting syrup was added to water (15 mL) and partitioned between water and CH_2Cl_2 (4×20 mL). The organic layer was dried over $MgSO_4$ and evaporated to yield an oily liquid. The crude product **4** was used for the next synthetic reaction without further purification with a yield of 1.46 g (93%).

*1-Amino-8-trityloxy-3,6-dioxaoctane (**5**).* Compound **4** (0.300 g, 0.000718 mol) was added to lithium aluminum hydride (LAH) (0.120 g, 0.0032 mol) that had been dissolved in dry tetrahydrofuran (THF) (10 mL). The reaction mixture was cooled to 0 °C and allowed to stir for 45 min. The reaction was quenched by addition of a water–THF mixture (0.5 mL water in 10 mL THF) and filtered through a Celite filter pad using petroleum ether. The ethereal solution was evaporated to dryness and compound **5** was obtained as a syrup with a yield of 0.098 g (35%).

*1-{3-[1-(p-Chlorobenzoyl)-5-methoxy-2-methyl)-indolacetyl]}-imidazole (**8**).* Carbonyl diimidazole (CDI) (0.038 g, 0.000234 mol) was added to a solution of indomethacin **6** (0.077 g, 0.000215 mol) in dry THF (0.5 mL) and the solution was allowed to stir over 3 h. After this time, the reaction was found to be essentially complete, as indicated by TLC monitoring. The product from this reaction was immediately used for the next synthetic step without further manipulation.

*1-{3-[1-(p-Chlorobenzoyl)-5-methoxy-2-methylindol-acetoxy]}-8-trityloxy-3,6-dioxaoctane. [1-Indomethacin-8-trityl TEG (ester) conjugate] (**9**).* Compound **2** (0.2 g, 0.000509 mol) was dissolved in dry THF (1 mL) and the solution was added to the indomethacin–CDI intermediate **8**. The reaction was allowed to proceed over 24 h. The solvent was evaporated in vacuo, the residue was dissolved in chloroform, and silica gel column

chromatography was performed with 95:5 $CHCl_3$/MeOH as eluting solvent. The fractions were combined and evaporated to yield compound **9** as an oily liquid with a yield of 0.035 g (9%).

*1-{1-3-[(p-Chlorobenzoyl)-5-methoxy-2-methylindol-acetoxy]}-3,6-dioxaoctane. [Indomethacin–TEG ester conjugate] (**10**).* Using the synthetic method reported by Hanessian et al.[21] compound **9** was dissolved in CH_2Cl_2 (1 mL) and a 5:1 CH_2Cl_2/trifluoroacetic acid mixture was slowly added to the solution under a nitrogen atmosphere. After 30 min, the reaction was found to be essentially complete and was quenched by addition of MeOH. Purification of the product was carried out by elution through a silica gel pad with $CHCl_3$, followed by 95:5 $CHCl_3$/MeOH. After evaporation of solvent, a yield of compound **10** was obtained (0.015 g, 64%).

*8-Trityloxy-1-{1-3-[(p-chlorobenzoyl)-5-methoxy-2-methylindol-acetamido]}-3,6-dioxaoctane (**11**).* Compound **5** (0.08 g, 0.002 mol) was dissolved in THF (1 mL) and the resulting solution was added to a prepared mixture of the indomethacin–CDI intermediate (0.00027 mol). The mixture was warmed to 40 °C and left to stand for 24 h. The solvent was evaporated in vacuo, the residue was dissolved in chloroform and silica gel column chromatography was performed with 95:5 $CHCl_3$/MeOH as eluting solvent. The fractions were combined and evaporated to yield compound **11** (0.1 g, 61%).

*1-{1-3-[(p-Chlorobenzoyl)-5-methoxy-2-methylindol-acetamido]}-3,6-dioxaoctane. [Indomethacin–TEG amide conjugate] (**12**).* Compound **11** (0.08 g, 0.000106 mol) was dissolved in CH_2Cl_2 (1.5 mL) and a mixture of 5:1 CH_2Cl_2/trifluoroacetic acid (2 mL) was added to the solution. The mixture was stirred for 30 min, and the reaction was quenched by addition of MeOH, after confirming the completion of the reaction by TLC analysis (9:1 chloroform/methanol). The crude product was purified by preparative silica gel TLC (9:1 $CHCl_3$/MeOH) to obtain compound **12** as a crystalline, pale yellow solid with a yield of 0.04 g (74%).

The non-enzymatic kinetics of the two prodrugs **10** and **12** were studied in 0.1 M buffer solutions (hydrochloric acid, acetate, phosphate, and alkaline borate buffers), at pH 1–11 (1, 3, 5, 6, 7.4, 8.5, 9.5, 10, and 11), 37 ± 2 °C; pH rate profiles were obtained by plotting the logarithm of the observed first-order rate constant (k) versus pH (Figs. 1 and 2). The hydrolysis of the prodrugs followed apparent first-order kinetics, and the rate constants (k) were obtained as slopes from the semi-logarithmic plots of the unchanged prodrug concentration versus time.

Chemical stability was assessed by determining the decomposition half-life and the pH rate profile curve of indomethacin–TEG ester prodrug **10**, which indicates that this prodrug is subject to specific acid- and base-catalyzed hydrolysis. The calculated half-life ($t_{1/2}$) values indicate that the prodrug is most stable between pH 3 and 6. This is in agreement with earlier reports by Kahns et al.[22] who reported that indomethacin ester prodrugs

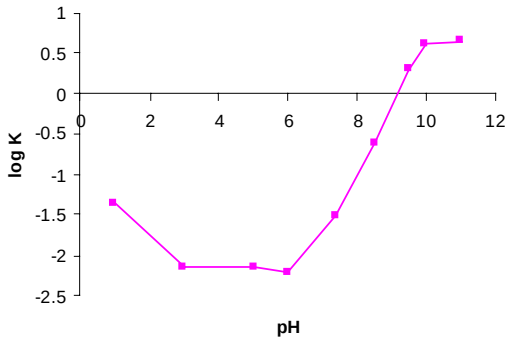


Figure 1. pH rate profile of indomethacin–TEG ester prodrug.

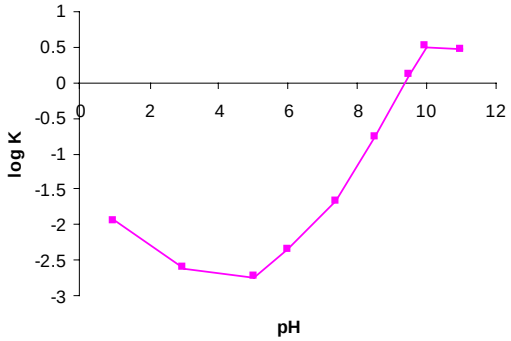


Figure 2. pH rate profile of indomethacin–TEG amide prodrug.

are most stable at pH 4.7. The sharp decline in the half-life value as the pH exceeds 7 indicates that the prodrug is more susceptible to hydrolysis by hydroxyl ion.

Earlier experiments have shown that amide prodrugs of indomethacin do not normally hydrolyze to yield the parent drug during the course of the experiment. The major hydrolytic product produced is from cleavage of the indole *N*-4-chlorobenzoyl group of indomethacin.[22] Degradation of the indomethacin–TEG amide prodrug **12** followed first-order kinetics. The $t_{1/2}$ values are fairly stable over the pH range tested and are most stable at pH 5. At this pH, a half-life of almost 15 days was observed. As the pH is increased, however, the stability of the prodrug drops, and the prodrug appears to be more susceptible to hydrolysis.

Hydroxyl ion is hypothesized to increase the susceptibility of the indomethacin–TEG amide bond to cleavage. However, under these conditions the indole *N*-amide bond may also be equally susceptible to cleavage, and 4-chloro-benzoic acid and the *N*-debenzylated indomethacin–TEG amid prodrug may be the major hydrolytic product. Thus, the prodrug could decompose either by hydrolysis of the indomethacin–TEG amide bond or by cleavage of the indole *N*-amide bond. The half-life of indomethacin in buffer and plasma is about 135 h at physiological pH and 37 °C, while the half-life of the amide prodrug **12** is about 35 h under the same conditions. This indicates that the prodrug is most likely being lost by indole *N*-amide bond cleavage, while it is still attached to the TEG chain, since no indomethacin was observed to be released from the amide prodrug).

Enzymatic hydrolysis of the above-mentioned prodrugs **10** and **12** in fresh buffered human plasma followed first order kinetics with some variation in the rate of hydrolysis among the different prodrugs (Figs. 3 and 4).

The average half-life of the ester prodrug **10** was 5.24 h, which is in accordance with the reported half lives of various indomethacin–PEG esters.[22] The amide prodrug **12** exhibited a half-life of about 74 h, indicating that it is fairly stable but not completely resistant to enzymatic hydrolysis. The prodrug **12** can theoretically be cleaved at the indomethacin–TEG amide bond to release indomethacin, which is subsequently degraded to the *N*-debenzoylated form, or the indomethacin moiety could be degraded via *N*-de-4-chlorobenzylation while it is still attached to the TEG backbone.

HPLC analysis of the product resulting from the amide prodrug hydrolysis did not show significant release of indomethacin, which suggests that the prodrug is broken down primarily by cleavage of the indole *N*-amide bond, affording *N*-debenzoylated indomethacin still linked to the TEG backbone, rather than by cleavage of the indomethacin–TEG amide linkage.

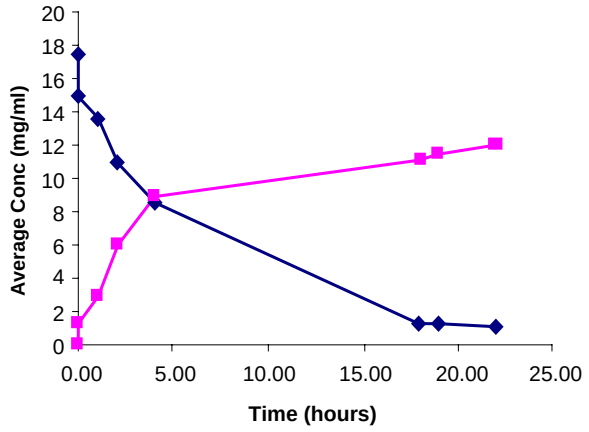


Figure 3. The stability of indomethacin–TEG ester prodrug **10** in 80% buffered human plasma. The data show the time course for the disappearance of the prodrug **10** (♦) and the appearance of the indomethacin parent drug (■).

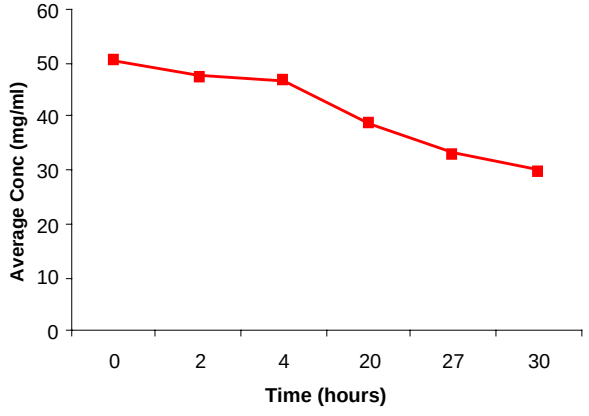


Figure 4. The stability of indomethacin–TEG amide prodrug **12** in 80% buffered human plasma. The data show the time course for the disappearance of the prodrug **12** (■). No significant release of indomethacin was observed.

The values of octanol–water partition coefficient (log *P*) were calculated using Daylight Toolkit from Daylight Chemical Information system (Mission Viejo, CA, USA). The log *P* values for the indomethacin ester and amide prodrugs were 3.8 and 3.1, respectively. These values are slightly higher than the log *P* value of 3.0 for indomethacin. The enhancement in partition coefficient of the ester prodrug compared to that of indomethacin is due to the non-ionic nature of this prodrug moiety compared to the ionic nature of the surface active indomethacin molecule. Ester prodrugs of this type should improve drug delivery of indomethacin and also render formulations of the indomethacin prodrug less damaging to biological membranes.

In conclusion, we have synthesized indomethacin prodrugs **10** and **12**, consisting of indomethacin conjugated to a TEG moiety via an ester and amide linkage, respectively.[23] As can be seen from the data, the esterase-sensitive prodrug **10** can be converted to indomethacin through a fairly rapid hydrolytic cleavage. However, this cleavage in buffered solutions at pH 3–6 is relatively slow, demonstrating that it may be possible to design and formulate stable and effective ester prodrugs of indomethacin in aqueous solution formulations.

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23. Compound **2**: ^{1}H NMR; δ 2.36 (1H, m, O*H*); 3.25 (2H, m, 8-CH_2); 3.61 (2H, m, 7-CH_2); 3.64–3.78 (8H, m, 1,2,3,4,5-CH_2's); 7.22–7.49 [15H, 2× m at 7.21–7.34 (*meta* and *para* aromatic protons, 9H) and 7.42–7.49 (*ortho* aromatic protons, 6H)]. Elemental analysis: theoretical C, 76.50; H, 7.19; found: C, 76.35; H 7.25%. Compound **3**: ^{1}H NMR: δ 2.92 (3H, s, CH_3SO$_2$); 3.22 (2H, t, 8-CH_2); 3.62 (2H, t, 7-CH_2); 3.70 (4H, s, 4,5 CH_2's); 3.80 (2H, t, 2-CH_2); 4.38 (2H, t, 1-CH_2); 7.22–7.49 [15H, 2× m at 7.21–7.34 (*meta* and *para* aromatic protons, 9H) and 7.42–7.49 (*ortho* aromatic protons, 6H]. Elemental analysis: theoretical C, 66.36; H, 6.43; found: C, 66.27; H, 6.40%. Compound **4**: ^{1}H NMR: δ 3.41 (2H, m, 8-CH_2); 3.56–3.61 (2H, m, 7-CH_2); 3.82 (8H, m, 1,2,4,5-CH_2's); 7.39–7.72 [15H, 2× m at 7.39–7.56 (*meta* and *para* aromatic protons, 9H) and 7.64–7.72 (*ortho* aromatic protons, 6H)]; ^{13}C NMR: 50.62 (1-*C*H$_2$); 63.25 (2-*C*H$_2$); 70.01 (4-*C*H$_2$); 70.69–70.79 (5,7,8-*C*H$_2$'s); 86.45 [(Ph)$_3$*C*-O]; 126.85–128.62 (*meta* and *para* carbons, trityl C's); 144.01 (*ortho* carbons, trityl C's); Elemental analysis: theoretical C, 71.92; H, 6.52; N, 10.06; found: C, 72.00; H, 6.53, N, 10.00%. Compound **5**: ^{1}H NMR: δ 1.82–2.21 (2H, br s, NH_2) 2.86–2.94 (2H, t, 1-CH_2); 3.51–3.59 (2H, t, 7-CH_2); 3.62–3.78 (6H, m, 2,4,5-CH_2's); 7.22–7.48 [15H, 2× m at 7.22–7.36 (*meta* and *para* aromatic protons, 9H) and 7.41–7.48 (*ortho* aromatic protons, 6H)]. Elemental analysis: theoretical C, 76.69; H, 7.47; N, 3.58; found: C, 76.69; H, 7.53; N, 3.49%. Compound **9**: ^{1}H NMR: δ 2.46 (3H, s, 2′-CH_3); 3.64–3.77 (12H, m, 1,2,4,5,7,8-CH_2's); 3.85 (3H, s, 5′-OCH_3); 6.64 (1H, m, 6′-C*H*); 6.87 (1H, d, 7′-C*H*); 6.98 (1H, d, 4′-C*H*) 7.20–7.41 [15H, 2× m at 7.20–7.35 (*meta* and *para* aromatic protons, 9H) and 7.38–7.41 (*ortho* aromatic protons, 6H)]; 7.44–7.52 (2H, apparent d, 2″, 6″-C*H*'s); 7.66–7.69 (2H, apparent d, 3″, 5″-C*H*'s). Compound **10**: ^{1}H NMR; δ 1.26–1.34 (1H, br s, O*H*); 2.40 (3H, s, 2′-CH_3); 3.62–3.74 (12H, m, 1,2,4,5,7,8-CH_2's); 7.42–7.50 (2H, apparent d, 2″, 6″-C*H*'s); 7,64–7.68 (2H, apparent d, 3″, 5″-C*H*'s). Elemental analysis: theoretical C, 61.29: H, 5.76; N, 2.86; found: C, 61.86; H, 5.94; N, 2.93%. Compound **11**: ^{1}H NMR: δ 2.32 (3H, s, 2′-CH_3); 3.18–3.21 (2H, m, 8-CH_2); 3.4–3.42 (2H, m, 7-CH_2);3.51–3.56 (2H, s, 1-CH_2); 3.58–3.62 (8H, m, 1,2,4,5-CH_2s); 3.8(3H, s, 5-OCH_3); 6.12 (1H, t, N*H*); 6.62–6.68 (1H, m, 6-C*H*); 6.81–6.83 (1H, apparent d, 7-C*H*); 6.86–6.91 (1H, apparent d, 4-C*H*); 7.19–7.42 [15H, 2× m at 7.19–7.34 (*meta* and *para* aromatic protons, 9H) and 7.40–7.42 (*ortho* aromatic protons, 6H)]; 7.42–7.46 (2H, apparent d, 2″, 6″-C*H*'s); 7.62–7.64 (2H, apparent d, 3″, 5″-C*H*'s). ^{13}C NMR: δ 11.80 (2′-*C*H$_2$); 32.50 (8-*C*H$_2$); 38.00 (7-*C*H$_2$); 56.00 (5′-O*C*H$_3$); 62.00 (*C*H$_2$-CONH); 69.80–70.50 (2,4,5-*C*H$_2$'s); 72.20 (1-*C*H$_2$); 101.00 (4′-*C*H); 112.0 (6′-*C*H); 113.20 (4″-*C*Cl); 115.00 (7′-*C*H) ; 129.50 (2″, 6″-*C*H's); 130.50 (1″-*C*H); 131.00 (2′-*C*H); 131.50 (3″, 5″-*C*H's); 133.80 (3′a-*C*H); 136.20 (7′a-*C*H); 139.60 (3′-*C*H); 156.00 (5′-*C*H); 168.80 (1′-N*C*=O); 170.00 (*C*ONH). Compound **12**: ^{1}H NMR: δ 1.24 (1H, s, O*H*); 2.40 (3H, s, 2′-CH_3); 3.38–3.44 (2H, m, 1-CH_2); 3.45–3.51 (8H, m, 2,4,5,7-CH_2's); 3.61–3.62 (2H, m, 8-CH_2); 3.62–3.66 (2H, s, 1-CH_2); 3.80 (3H, s, 5′-OCH_3); 6.18 (1H, t, N*H*); 6.64–6.68 (1H, m, 6′-C*H*); 6.82–6.85 (1H, apparent d, 7′-C*H*); 6.86–6.88 (1H, apparent d, 4′-C*H*); 7.42–7.51 (2H, apparent d, 2″, 6″-C*H*'s); 7.62–7.64 (2H, apparent d, 3′, 5′-C*H*'s). Elemental analysis: theoretical C, 61.41; H, 5.98; N, 5.73; found: C, 61.66; H, 6.04; N, 5.66 %.