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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and evaluation of novel prodrugs of naproxen

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Abstract A series of novel prodrugs of naproxen has been synthesized. Naproxen (**1**) was reacted with thionyl chloride to yield acid chloride (**2**) which was further reacted with glucose to form the glucosyl naproxen (**3**). Tetra-acetate of glucosyl naproxen was prepared and finally reacted with different amino acids to yield the title compounds. These compounds were evaluated for analgesic, anti-inflammatory activities, and for possible GI toxicity. Compound **5b** depicted most potent analgesic activity with percentage inhibition of 98.15%. Compound **5a** was found to be most potent anti-inflammatory agent with 76% inhibition. Compound **5n** was second most active analgesic (92.26%) and anti-inflammatory (73%) agent. In vitro hydrolysis pattern of synthesized prodrugs was studied in phosphate buffer of pH 7.4 and acetate buffers of pH 3.0, 4.0, and 5.0, respectively. Selected compounds were evaluated for their ulcerogenic potential and all the tested derivatives were significantly less irritating to gastric mucosa than the parent drug.

Keywords Naproxen · Prodrugs · Analgesic activity · Anti-inflammatory activity · In vitro hydrolytic studies

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Introduction

Naproxen [(+)-6-methoxy-α-methyl-2-naphthaleneacetic acid] is a nonsteroidal anti-inflammatory drug (NSAID) with analgesic, antipyretic properties, and is frequently used for treatment of rheumatoid arthritis and osteoarthritis (Rautio *et al*., 2000; Todd and Clissold, 1990). Oral administration of this drug leads to formation of crystals that coat the digestive mucus. Due to acidity and low solubility, crystals dissolve slowly causing irritation and damage to the stomach walls which on prolonged use can lead to the formation of ulcerations in the mucus (Otero Espinar *et al*., 1991). Generally, undesired GI irritation limits clinical utility of most of the conventional NSAIDs. Moreover, the main causes of NSAID-induced gastropathy are reduced mucosal cytoprotective prostaglandin (PG) levels, increased gastric acidity and motility. This enhanced gastric motility leads to reduction in mucosal blood flow, hypoxia, and destruction of mucous bicarbonate barrier, which prevents back diffusion of pepsin and hydrogen ions from lumen into the mucosal layer (Takeuchi, 1989). The GI side effects produced by NSAIDs are principally caused by two different mechanisms: a direct contact mechanism on the GI mucosa through oral dose and a generalized systemic action appearing after intravenous dosing (Bundgaard and Nielsen, 1988).

To overcome these undesired characteristics of available NSAIDs, several prodrugs have been developed and evaluated worldwide. Prodrug concept involves chemical modification of a drug into some bioreversible form in order to change its pharmaceutical and pharmacokinetic properties, thus enhancing its delivery (Bonina *et al*., 2001). In recent years, much attention has been focused on the development of prodrugs of NSAIDs so as to reduce

their GI adversities. The esterification of NSAIDs, however, results in reduced GI toxicities. The esters being pharmacologically inactive get readily hydrolyzed followed by their absorption to release parent drug in the blood. These esters should also possess desirable physicochemical properties such as aqueous solubility and lipophilicity (Bundgaard and Nielsen, 1988).

Various prodrugs of naproxen synthesized in the form of esters, amides, and glycolamides are reported. (Bundgaard and Nielsen, 1988; Giammona *et al*., 1989; Otero Espinar *et al*., 1991; Chang and Tsai, 1997; Chang *et al*., 1998; Rautio *et al*., 2000; Bonina *et al*., 2001; Sheha *et al*., 2002; Khan and Khan, 2002; Chang *et al*., 2004; Swart *et al*., 2005; Ranatunge *et al*., 2006; Bhushan and Tanwar, 2008; Stefanko *et al*., 2008). Moreover, conjugation with amino acid such as L-aspartic acid has also been studied (El-Kamel *et al*., 2008). Although considerable research has been carried out on designing prodrugs of naproxen with reduced GI toxicity, yet no one seems to be an ideal and hence, there is a need for the development of newer prodrugs with better pharmaceutical and pharmacological profile. Previous studies of β-D-glucopyranosyl derivative of ibuprofen have demonstrated that the prodrugs possess increased anti-inflammatory and analgesic activity with reduction in GI toxicity.

Hence in the light of these findings, the present study was undertaken in order to minimize the ulcerogenic potential of naproxen and to render it water soluble properties through glucosidation and Schiff's base formation for better absorption and sustained release characteristics. Specially designed prodrug was with expected advantages of affording gastric protection by (1) temporarily masking the acidic carboxylic group until absorption and (2) inhibition of gastric secretion.

Experimental

Chemicals and all solvents used in this study were procured from Merck AG, SD Fines, Rankem labs, and Qualigens. Distilled water was used in preparation of buffer solutions. Melting points were determined on a Labindia MR-VIS visual melting range apparatus and are uncorrected. The absorbance against wavelength was taken on a Systronic double beam UV spectrophotometer. IR spectra (KBr disc) were obtained with a Perkin Elmer IR spectrophotometer. NMR spectra were recorded using Bruker 400 spectrometer and chemical shifts are expressed as δ (ppm) with tetramethylsilane as internal standard for solutions in $CDCl_3$. Drug sample was procured as a gift sample from M/s M. J. Biopharma Private Limited, Navi Mumbai, India. Mass spectra were recorded on Waters Q-TOF micro mass spectrometer using electron spray ionization method.

Synthesis of glucosyl naproxen

Naproxen (2 g) **1** was dissolved in benzene (20 ml) in a 100 ml round-bottomed flask. Redistilled thionyl chloride (2.5 ml) was added drop wise. The reaction was carried out by heating under reflux for 3 h maintaining anhydrous conditions to get 2-(6-methoxy-2-naphthyl)-propionyl chloride **2**. Then, anhydrous glucose and pyridine were added to compound **2** and stirred over night to get glucosyl naproxen (**3**).

Synthesis of tetracetate

Further, anhydrous zinc chloride (0.5 g) and acetic anhydride (13.5 g; 12.5 ml; 0.13 mol) were placed in a 200 ml round-bottomed flask. A double surface condenser was attached and mixture was heated on a water bath until a clear solution was obtained and to this, compound **3** was added, shaking the mixture from time to time. Heating was further continued for around 2 h till a clear solution was obtained and then the reaction mixture was poured onto 250 ml of crushed ice. It was allowed to stand for 1 h, stirring occasionally to break up the solid lumps of tetraacetate of glucose **4** which were separated, filtered off, washed well with cold water, and recrystallized from methanol.

Synthesis of Schiff base of naproxen

For this purpose different amino acids namely glycine, valine, alanine, cysteine, leucine, threonine, phenylalanine, tyrosine, histidine, tryptophan, aspartic acid, glutamic acid, ornithine, and 3,4-dihydroxyphenylalanine (DOPA) were employed. Appropriate quantity of amino acid (0.005 mol) was added to the compound **4** after dissolving it in a solution of water and ethanol and the solution was refluxed for 2 h. The product was allowed to evaporate till title compounds **5a**–**n** were obtained.

N-{6-[1″-methyl-1″-(6-methoxynaphth-1-yl)]ethoxy}-2′,3′,4′,5′-tetraacetylhex-1′-yl-iminoethanoic acid **5a**. Yield 64%; m.p. 83–86°C. IR (KBr, cm^{-1}): 1749.2 (Carboxylic C=O *str*), 1631.5 (C=N *str*), 1165 (C–O), 1038 (C–O–C *str*). ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.51–1.82 (3H, d, CH–CH_3), 2.08–2.18 (12H, s, $OCOCH_3$), 3.65 (3H, s, OCH_3), 3.84–4.10 [5H, m, (1H, CH–CH_3). (2H, $COOCH_2$) and (2H, N–CH_2)], 4.24 (1H, s, CH–$OCOCH_3$ {C_4}), 5.09–5.20 (3H, m, CH–$OCOCH_3$ {C_1–C_3}, 6.91–7.46 [7H, m, (6H, ArH) and (1 H, N=CH)]. MS, m/z: 617.

2-N-{6-[1″-methyl-1″-(6-methoxynaphth-1-yl)]ethoxy}-2′,3′,4′,5′-tetraacetylhex-1′-yl-2-iminopropanoic acid **5b**. Yield 59%; m.p. 76–79°C. IR (KBr, cm^{-1}): 1749.6 (Carboxylic C=O *str*), 1632.8 (C=N *str*), 1159.9 (C–O), 1039.3 (C–O–C *str*). ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.55–1.58 (6H,

m, CH–CH_3), 2.01–2.21 (12H, s, $OCOCH_3$), 2.62 (1H, q, =N–CH), 3.70 (3H, s, OCH_3), 3.81–3.91 (1H, q, CH–CH_3), 4.12–4.26 [3H, m, (2H, $COOCH_2$) and (1H, CH–$OCOCH_3$ {C_4})], 5.13–5.25 [3H, m, (1H, CH–$OCOCH_3$ {C_3}) and (2H, CH–$OCOCH_3$ {C_1–C_2}], 7.12–7.70 [7H, m, (6H, ArH) and (1H, N=CH)]. MS, m/z: 631.

3-methyl-2-N-{6-[1″-methyl-1″-(6-methoxynaphth-1-yl)] ethoxy}-2′,3′,4′,5′-tetraacetylhex-1′-yl-iminobutanoic acid **5c**.Yield 66%; m.p. 77–78°C. IR (KBr, cm^{-1}): 1755.9 (Carboxylic C=O *str*), 1632.7 (C=N *str*), 1163.1 (C–O), 1065.4 (C–O–C *str*). ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.25–1.58, 9H, m, [(6H, –CH(CH_3)) and (3H, CH–CH_3)], 2.01–2.03 (1H, q, –CH(CH_3)), 2.07–2.20 (12H, s, $OCOCH_3$), 3.66 (1H, d, =N–CH), 3.79 (3H, s, OCH_3), 3.82–4.22 [3H, m, (1H, CH–CH_3) and (2H, $COOCH_2$)], 4.26–4.29 (1H, s, CH–$OCOCH_3$ {C_4}), 5.07–5.17 [3H, m, (1H, CH–$OCOCH_3$ {C_3}) and (2H, CH–$OCOCH_3$ {C_1–C_2})], 7.01–7.72 [(7H, m, (6H, ArH) and (1H, N=CH)]. MS, m/z: 659.

3-sulphanyl-2-N-{6-[1″-methyl-1″-(6-methoxynaphth-1-yl)]ethoxy}-2′,3′,4′,5′-tetraacetylhex-1′-yl-2-iminopropanoic acid **5d**. Yield 61%; m.p. 70–71°C. IR (KBr, cm^{-1}): 1749.2 (Carboxylic C=O *str*), 1632.7 (C=N *str*), 1152.5 (C–O), 1038 (C–O–C *str*). ^{1}H-NMR: δ (ppm) ($CDCl_3$): 0.88–0.91 (1H, s, SH), 1.51–1.82 (3H, d, CH–CH_3), 2.02–2.18 (12H, s, $OCOCH_3$), 2.49–2.63 (2H, d, CH–CH_2), 3.84 (3H, s, OCH_3), 4.11–4.14 (1H, q, CH–CH_3), 3.92 (1H, t, =N–CH), 4.14–4.30 [3H, m, (2H, $COOCH_2$) and (1H, CH–$OCOCH_3$ {C_4})], 5.09–5.17 [3H, m, (1H, CH–$OCOCH_3$ {C_3}) and (2H, CH–$OCOCH_3$ {C_1–C_2})], 6.8–7.6 [7H, m, (6H, ArH) and (1H, N=CH). MS, m/z: 663.

4-methyl-2-N-{6-[1″-methyl-1″-(6-methoxynaphth-1-yl)] ethoxy}-2′,3′,4′,5′-tetraacetylhex-1′-yl-iminopentanoic acid **5e**. Yield 64%; m.p. 75–76°C. IR (KBr, cm^{-1}): 1749.1 (Carboxylic C=O *str*), 1632 (C=N *str*), 1160.8 (C–O), 1040 (C–O–C *str*). ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.55–1.88 [9H, m, (6H –CH(CH_3)$_2$) and (3H CH–CH_3)], 1.95–2.02 [3H, m, (2H, –CH_2–CH) and (1H, –CH(CH_3))], 2.08–2.22 (12H, s, $OCOCH_3$), 3.70 (3H, s, OCH_3), 3.76–4.14 [4H, m, (1H, =N–CH), (1H, CH–CH_3) and (2H, $COOCH_2$)], 4.24–4.36 (1H, s, CH–$OCOCH_3$ {C_4}), 5.06–5.15 [3H, m, (1H, CH–$OCOCH_3$ {C_3}) and (2H, m, CH–$OCOCH_3$ {C_1–C_2})], 7.12–7.72 [7H, m, (6H, ArH) and (1H, N=CH)]. MS, m/z: 673.

3-hydroxy-2-N-{6′-[1″-methyl-1″-(6-methoxynaphth-1-yl)] ethoxy}-2′,3′,4′,5′-tetraacetylhex-1′-yl-iminobutanoic acid **5f**. Yield 65%; m.p. 71–72°C. IR (KBr, cm^{-1}): 1756.2 (Carboxylic C=O *str*), 1632.2 (C=N *str*), 1150.9 (C–O), 1041.5 (C–O–C *str*). ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.18–1.25 (1H, d, –CH–CH_3), 1.61 (3H, d, CH–CH_3), 2.05–2.19 (12H, s, $OCOCH_3$), 2.30–2.49 (1H, d, =N–CH), 2.72 (1H, m, CH–CH_3), 3.77 (3H, s, OCH_3), 4.07–4.28 [4H, m, (1H, CH–CH_3), (2H, $COOCH_2$) and (1H, CH–$OCOCH_3$ {C_4})], 5.09–5.19 [3H, m, (1H, CH–$OCOCH_3$ {C_3}) and (2H, CH–$OCOCH_3$ {C_1–C_2})], 6.80–7.51 [7H, m, (6H, ArH) and (1H, d, N=CH)]. MS, m/z: 661.

3-phenyl-2-N-{6-[1″-methyl-1″-(6-methoxynaphth-1-yl)] ethoxy} -2′,3′,4′,5′-tetraacetylhex-1′-yl-2-iminopropanoic acid **5g**. Yield 61%; m.p. 76–78°C. IR (KBr, cm^{-1}): 1765.2 (Carboxylic C=O *str*), 1632.1 (C=N *str*), 1151.4 (C–O), 1037.5 (C–O–C *str*). ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.51–1.83 (3H, d, CH–CH_3), 2.09–2.31 [13H, m, (12H, –$OCOCH_3$) and (1H, =N–CH)], 2.63 (2H, m, –CH_2–C_6H_5), 3.98 (3H, s, OCH_3), 4.04–4.27 [4H, m, (1H, CH–CH_3), (2H, $COOCH_2$) and (1H, CH–$OCOCH_3$ {C_4})], 5.08–5.18(1H, s, CH–OC-OCH_3 {C_3}), 5.12–5.18 (2H, m, CH–$OCOCH_3$ {C_1–C_2}), 7.21–7.80 [12H, m, (11H, ArH) and (1H, N=CH). MS, m/z: 707.

3-(4′-hydroxyphenyl)-2-N-{6-[1″-methyl-1″-(6-methoxynaphth-1-yl)]ethoxy}-2′,3′,4′,5′-tetraacetylhex-1′-yl-2-iminopropanoic acid **5h**. Yield 68%; m.p. 75–76°C. IR (KBr, cm^{-1}): 1755.3 (Carboxylic C=O *str*), 1632 (C=N *str*), 1152.5 (C–O), 1041.7 (C–O–C *str*). ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.52–1.80 (3H, d, CH–CH_3), 2.11–2.20 (12H, s, $OCOCH_3$), 2.63 (2H, m, –CH_2–C_6H_4), 4.07–4.13 [7H, m, (3H, –OCH_3), (1H, CH–CH_3), (2H, $COOCH_2$) and (1H, =N–CH)], 4.24–4.30 (1H, s, CH–$OCOCH_3$ {C_4}), 5.08–5.16 [3H, m, (1H, CH–$OCOCH_3$ {C_3}) and (2H, CH–$OCOCH_3$ {C_1–C_2})], 7.04–7.81 [11H, m, (10H, ArH) and (1H, N=CH)]. MS, m/z: 723.

3-(1H-imidazol-4′-yl)-2-N-{6-[1″-methyl-1″-(6-methoxynaphth-1-yl)]ethoxy}-2′,3′,4′,5′-tetraacetylhex-1′-yl-2-iminopropanoic acid **5i**. Yield 67%; m.p. 81–83°C. IR (KBr, cm^{-1}): 1749 (Carboxylic C=O *str*), 1632.1 (C=N *str*), 1160.6 (C–O), 1038.1 (C–O–C *str*). ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.44–1.54 (3H, d, CH–CH_3), 2.0–2.19 (12H, s, $OCOCH_3$), 2.58–2.62 (2H, m, –CH_2), 3.65 (1H, t, =N–CH), 3.77 (3H, s, OCH_3), 3.81–4.28 [4H, m, (1H, CH–CH_3), (2H, $COOCH_2$) and (1H, CH–$OCOCH_3$ {C_4})], 5.05–5.13 [3H, m (1H, CH–$OCOCH_3$ {C_3}) and (2H, CH–$OCOCH_3$ {C_1–C_2})], 7.14–7.75 [7H, m, (6H, ArH) and (1H, N=CH)]. MS, m/z: 697.

3-(1H-indol-3-yl)-2-N-{6-[1″-methyl-1″-(6-methoxynaphth-1-yl)]ethoxy}-2′,3′,4′,5′-tetraacetylhex-1′-yl-2-iminopropanoic acid **5j**. Yield 61%; m.p. 79–80°C. IR (KBr, cm^{-1}): 1749.1 (Carboxylic C=O *str*), 1631.8 (C=N *str*), 1160.8 (C–O), 1037.9 (C–O–C *str*). ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.58–1.60 (3H, d, CH–CH_3), 2.01–2.10 (12H, s, $OCOCH_3$), 2.63 (2H, d, –CH_2), 3.68 (1H, t, =N–CH), 3.76 (3H, s, OCH_3), 4.03–4.26 [4H, m, (1H, CH–CH_3), (2H, $COOCH_2$) and (1H, CH–$OCOCH_3$ {C_4})], 5.11–5.14 [3H, m, (1H, CH–$OCOCH_3$ {C_3}) and (2H, CH–$OCOCH_3$ {C_1–C_2})], 6.92–7.71 [12H, m, (11H, ArH) and 7.26 (1H, N=CH)]. MS, m/z: 746.

2-N-{6-[1″-methyl-1″-(6-methoxynaphth-1-yl)]ethoxy}-2′,3′,4′,5′-tetraacetylhex-1′-yl-2-iminobutanedioic acid **5k**. Yield 65%; m.p. 75–76°C. IR (KBr, cm^{-1}): 1755.6

(Carboxylic C=O *str*), 1632.4 (C=N *str*), 1151.7 (C–O), 1040.6 (C–O–C *str*). ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.51–1.80 (3H, d, CH–CH_3), 2.05–2.20 (12H, s, $OCOCH_3$), 2.34–2.71 [3H, m, (2H, –CH_2–C_6H_6) and (1H, =N–CH)], 3.86 (3H, s, OCH_3), 3.96–4.13 [3H, m, (1H, CH–CH_3) and (2H, $COOCH_2$)], 4.19–4.28 (1H, s, CH–$OCOCH_3$ {C_4}), 5.08–5.17 [3H, m, (1H, CH–$OCOCH_3$ {C_3}) and (2H, CH–$OCOCH_3$ {C_1–C_2})], 7.05–7.76 [7H, m, (6H, ArH) and (1H, N=CH)]. MS, m/z: 675.

5-amino-5-oxo-2-N-{6-[1″-methyl-1″-(6-methoxynaphth-1-yl)]ethoxy}-2′,3′,4′,5′-tetraacetylhex-1′-yl-2-iminopentanoic acid **5l**. Yield 60%; m.p. 76–77°C. IR (KBr, cm^{-1}): 1752.9 (Carboxylic C=O *str*), 1632.4 (C=N *str*), 1151.5 (C–O), 1041 (C–O–C *str*). ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.03–1.18 (2H, m, –CH_2), 1.54–1.72 (3H, d, CH–CH_3), 2.03–2.17 [14, m, (2H, –CH_2) and (12H, $OCOCH_3$)], 2.31 (1H, t, =N–CH), 3.70 (3H, s, OCH_3), 3.81 (1H, q, CH–CH_3), 4.11 (2H, d, $COOCH_2$), 4.25–4.28 (1H, s, CH–OC-OCH_3 {C_4}), 5.09–5.19 [3H, m, (1H, CH–$OCOCH_3$ {C_3}) and (2H, CH–$OCOCH_3$ {C_1–C_2})], 6.34 (2H, s, –$CONH_2$), 6.80–7.57 [7H, m, (6H, ArH) and (1H, N=CH)]. MS, m/z: 688.

5-amino-2-N-{6-[1″-methyl-1″-(6-methoxynaphth-1-yl)]ethoxy}-2′,3′,4′,5′-tetraacetylhex-1′-yl-2-iminopentanoic acid **5m**. Yield 67%; m.p. 75–78°C. IR (KBr, cm^{-1}): 1741.3 (Carboxylic C=O *str*), 1632.4 (C=N *str*), 1151.9 (C–O), 1042.1 (C–O–C *str*). ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.58–1.61 (3H, d, CH–CH_3), 2.02 (2H, t, –NH_2), 2.09–2.18 (12H, s, $OCOCH_3$), 2.34–2.92 (1H, t, =N–CH), 3.86 (3H, s, OCH_3), 3.90–4.30 [4H, m (1H, CH–CH_3), (2H, $COOCH_2$) and (1H, CH–$OCOCH_3$ {C_4})], 5.06–5.17 [3H, m, (1H, CH–$OCOCH_3$ {C_3}) and (2H, CH–$OCOCH_3$ {C_1–C_2})], 7.15–7.84 [7H, m. (6H, ArH) and (1H, N=CH)]. MS, m/z: 646.

3-(3,4-dihydroxyphenyl)-2-N-{6-[1″-methyl-1″-(6-methoxynaphth-1-yl)]ethoxy}-2′,3′,4′,5′-tetraacetylhex-1′-yl-2-iminopropanoic acid **5n**. Yield 68%; m.p. 75–76°C. IR (KBr, cm^{-1}): 1756.4 (Carboxylic C=O *str*), 1632.4 (C=N *str*), 1151.7 (C–O), 1042.9 (C–O–C *str*). ^{1}H-NMR: δ (ppm) ($CDCl_3$): 1.58–1.88 (3H, d, CH–CH_3), 2.02–2.18 (12H, s, $OCOCH_3$), 2.37–2.41 [3H, m (1H, =N–CH) and (2H, –CH_2–C_6H_3)], 4.08–4.12 [6H, m, (3H, OCH_3), (1H, CH–CH_3) and (2H, $COOCH_2$)] 4.24–4.30 (1H, s, CH–$OCOCH_3$ {C_4}), 5.08–5.21 [3H, m, (1H, CH–$OCOCH_3$ {C_3}) and (2H, CH–$OCOCH_3$ {C_1–C_2})] 7.14–7.78 [7H, m, (6H, ArH) and (1H, N=CH)]. MS, m/z: 739.

Hydrolysis studies

The rates of chemical hydrolysis of selected prodrugs were determined in phosphate buffer of pH 7.4 and acetate buffers of pH 3.0, 4.0, 5.0 at 37°C. Samples (1.0 ml) were withdrawn at appropriate time intervals and diluted with 4.0 ml of methanol and absorbance was measured at 231 nm (λmax) against a blank obtained by similar treatment (Rautio *et al*., 1998).

Biological evaluation

Naproxen and its prodrugs were administered orally as a suspension in Tween 80 (0.5% w/v) solutions. Protocol of the animal experimentation was approved by the IAEC (Institutional Animal Ethics Committee) as registered under CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Government of India.

Analgesic activity

Screening of the synthesized compounds for analgesic activity was carried out by acetic acid induced writhing method (Seigmund *et al*., 1957). The animals were divided in groups (control, standard and test) consisting of six mice each having weight 18–22 g. Control group received 0.5% w/v Tween 80 (10 ml/kg), standard group received naproxen (50 mg/kg) and test groups received dose equivalent to molecular weight of the prodrugs **5a**–**n** by oral route. After 1 h of drug administration, the writhing response was elicited by the intraperitonieal injection of (10 ml/kg body weight of 0.6% v/v in normal saline) acetic acid. Number of writhes for each mice was counted for a period of 10 min, and the average number of writhes was determined for each group and the degree of analgesia was expressed as percentage inhibition calculated according to the formula:

$$\text{Percentage inhibition of writhing} = (1 - T/S) \times 100$$

where S and T are the number of writhes in the control and drug treated group, respectively.

Anti-inflammatory activity

Carrageenan induced rat paw edema method was used to determine anti-inflammatory activity of the compounds (Winter *et al*., 1962). The experiment was carried out on healthy Wistar rats of either sex weighing between 150 and 200 g. The rats were divided by randomization into different groups consisting of 6 animals in each. The animals were fasted for 24 h with water ad libitum. Each animal was injected with carrageenan suspension (0.1 ml, 1% w/v; s.c.) into the subplantar region of the left hind paw after 30 min of drug administration. The paw volume was measured after a time interval of 15, 30, 60, 90, and 120 min using a plethysmometer (Ugo Basile, Italy). Naproxen **1** was given at a dose of 50 mg/kg body weight and its prodrugs at dose equivalent to 50 mg/kg of

naproxen. Results have been expressed as percentage inhibition of edema formation, calculated via the formula:

$$\text{Percentage inhibition of paw edema} = \left(1 - \text{ED}_{\text{drug}}/\text{ED}_{\text{control}}\right) \times 100$$

where ED_{drug} and $\text{ED}_{\text{control}}$ are the edema volume in drug treated and control groups, respectively.

Ulcerogenicity

The ulcerogenic potential of naproxen and synthesized prodrugs was evaluated by the method of Kunchandy et al. 1984. Healthy Wistar rats of either sex weighing between 150 and 200 g were used and divided by randomization into different groups with six animals in each. All the test compounds and standard drug in vehicle were administered orally to rats over a period of seven successive days. All the rats were fasted for 24 h on 8th day. The animals were killed with excessive anesthesia. The stomach was removed, opened along the greater curvature and washed gently in running tap water. The gastric mucosa of rats was examined by means of magnifying lens. The ulcer score was measured. The score of ulcers were as followed: 0.0 = normal colored stomach, 0.5 = pink to red coloration of stomach, 1.0 = spot ulcer, 1.5 = haemorrhagic streak, 2.0 = number of ulcers <5, 3.0 = number of ulcers >5, 4.0 = ulcers with bleeding.

Results and discussion

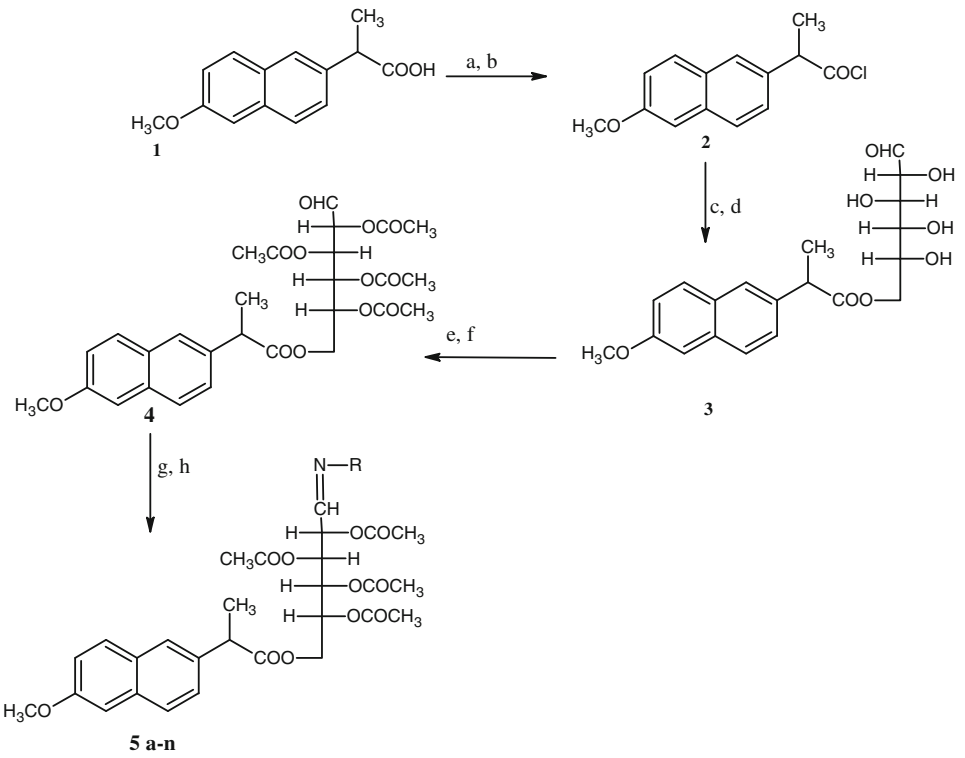
Synthesis

In the present study, a series of 14 novel compounds has been synthesized. Scheme 1 illustrates the synthetic route for the preparation of target compounds. Reaction of naproxen with glucose to form ester was followed by acetylation in presence of $ZnCl_2$ as a catalyst. The tetra-acetate thus formed was reacted with different amino acids to yield the title compounds. All the synthesized compounds were characterized by suitable spectral methods such as IR, NMR, and MS. The IR spectrum of compounds **5a**–**n** showed absorption peaks at 1,631–1,632 cm^{-1} and 1,749–1,765 cm^{-1} due to C=N and C=O stretching vibrations. The structure was further supported by ^{1}H-NMR spectrum, which showed doublet at δ 3.7–4.24 and δ 7.26–7.45 for $COOCH_2$, –CH=N–, respectively. The IR spectrum of compound **5a** showed characteristic absorption peak at 3020.7 (Ar CH), 2974 (Aliphatic CH), 1749.2 (Carboxylic C=O), 1631.5 (C=N), 1165 (C–O), 1038 (C–O–C). The structure was further confirmed by its ^{1}H-NMR spectrum, which showed a doublet at δ 1.5–1.8 CH–CH_3 proton. The signal of two protons ($COOCH_2$) was observed as a doublet at δ 3.9 whereas signal for two protons (=N–CH_2) was obtained as a singlet at δ 3.9–4.0. Further, the signal of one proton (N=CH) was found as a doublet at δ 7.3. The IR spectrum of compound **5b** showed characteristic absorption peak at 3020.7 (Ar CH), 2973.6–2843.3 (Aliphatic CH), 1749.6 (Carboxylic C=O), 1632.5 (C=N), 1159.9 (C–O), 1039.3 (C–O–C). Its ^{1}H-NMR spectrum, showed doublet at δ 1.58 for CH–CH_3 proton. The two protons ($CO-OCH_2$) were observed as doublet at δ 4.12. The signal for one proton (N=CH) was seen as doublet at δ 7.43–7.45. The IR spectrum of compound **5c** depicted characteristic absorption peak at 3020.7 (Ar CH), 1755.9 (Carboxylic C=O), 1632.7 (C=N), 1163.1 (C–O), 1065.4 (C–O–C). The structure was further confirmed by its ^{1}H-NMR spectrum, which showed doublet at δ 1.25–1.56 CH–CH_3 proton. The two protons ($COOCH_2$) were obtained as doublet at δ 4.11–4.24. The signal for one proton (N=CH) was obtained as doublet at δ 7.3. Mass spectral data of synthesized compounds was also in agreement with the proposed structures and hence from spectral analysis, the assigned structures of synthesized compounds were confirmed.

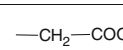
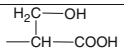
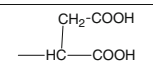
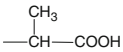
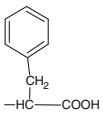
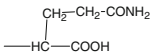
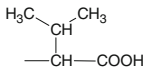
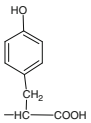
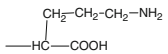
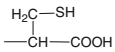
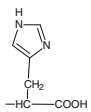
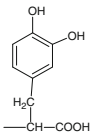
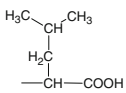
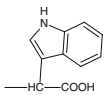
In vitro hydrolysis studies

These studies were carried out on six compounds **5a** to **5d**, **5h**, **5j** in aqueous buffers to investigate the extent of hydrolysis of novel prodrugs. In vitro hydrolysis kinetics of the synthesized prodrugs were evaluated in different aqueous buffer solution of pH 3.0, 4.0, 5.0, and 7.4. At constant pH and temperature, the reaction displayed strict first order kinetics with fair rate constant (K_{obs}) and a straight plot was obtained on plotting log concentration of residual prodrug versus time. The K_{obs} and corresponding half-lives ($t_{1/2}$) for the linear regression equation correlating the log concentration of residual prodrug versus time was calculated.

To examine the degradation of prodrugs of naproxen in stomach, pH 3, 4, 5, and 7.4 were selected. The corresponding half-lives in buffer solution are mentioned in Table 1. In vitro hydrolytic studies revealed that the compounds depict a remarkable stability under acidic conditions and this indicates that compounds fulfill the requirements for oral delivery system of NSAIDs, whereby the masking group should be acid stable to prevent the direct contact effects on gastric mucosa. This would prevent the intracellular entrapment of these compounds and hence leading to local inhibition of the cytoprotective PG synthesis. Results indicate the possibility of these esters to survive in GI conditions and enter the circulation as intact compounds, thereby successfully overcoming the local GI irritation.



Scheme 1 Synthetic route of Naproxen Prodrugs, reagents and reaction conditions: (**a**) $SOCl_2$; (**b**) Benzene; (**c**) Glucose; (**d**) Pyridine; (**e**) $(CH_3CO)_2O$; (**f**) $ZnCl_2$; (**g**) Amino acid; (**h**) Ethanol

Comp	=N-R Amino acid	R	Comp	=N-R Amino acid	R	Comp	=N-R Amino acid	R
5a	Glycine		**5f**	Threonine		**5k**	Aspartic acid	
5b	Valine		**5g**	Phenylalanine		**5l**	Glutamic acid	
5c	Alanine		**5h**	Tyrosine		**5m**	Ornithine	
5d	Cysteine		**5i**	Histidine		**5n**	DOPA	
5e	Leucine		**5j**	Tryptophan				

Hydrolytic pattern of the tested compounds indicated that at pH 3.0, compound **5b** demonstrated a swift hydrolysis with $t_{1/2}$ of 15 h followed by compound **5h** ($t_{1/2}$ 22 h). Compound **5a** depicted least tendency of hydrolysis with a long $t_{1/2}$ of 115 h. At pH 4.0 compound **5b** and **5d** were most readily hydrolyzed ($t_{1/2}$ 15 h) and at the same pH compound **5c** and **5j** depicted slowest rate of hydrolysis with $t_{1/2}$ 115 h. The $t_{1/2}$ of 50 and 88 h were observed for all the tested compounds at pH 5.0. Under alkaline conditions (pH 7.4) all the compounds depicted facile hydrolytic conversion with $t_{1/2}$ ranging from 18 to 30 h. In general, time required for hydrolysis of compounds under acidic condition was longer as compared to alkaline condition indicating that the synthesized prodrugs are capable of tolerating acidic

Table 1 Chemical hydrolysis of prodrugs

Compound	$t_{1/2}$(h) pH 3.0	$t_{1/2}$(h) pH 4.0	$t_{1/2}$(h) pH 5.0	$t_{1/2}$(h) pH 7.4
5a	115	44	88	25
5b	15	15	77	22
5c	44	115	88	19
5d	44	15	77	30
5h	22	67	50	18
5j	88	115	57	18

environment of stomach. However, swift conversion with alkaline pH indicates their suitability to act as prodrugs.

Biological evaluation

All the newly synthesized novel compounds **5a–n** were evaluated for analgesic and anti-inflammatory activity. In acetic acid induced writhing method for evaluation of peripheral analgesic activity, compound **5b** was found to be most active with percentage protection of 98.15 followed by **5n** (92.26%), **5g** (91.24%), **5h** (82.03%), and **5m** (82.03%), respectively. Compound **5c** and **5f** showed comparable activity with percentage protection of 63.59 and 60.38% as compared to naproxen (61.36%) whereas **5d** (30.41%) was least active compound of the series. The observations are presented in Table 2.

Table 3 Ulcerogenic potential of naproxen and its prodrugs (**5a–e**)

Treatment	Dose (mg/kg)	Average number of ulcer score ± SEM
Control	–	0.00 ± 0.00
Naproxen	50	2.7 ± .0.26*
5a	62.8	0.4 ± 0.19$^{\Delta}$
5b	63.2	0.5 ± 0.16$^{\Delta}$
5c	66.0	0.2 ± 0.12$^{\Delta}$
5d	66.4	0.3 ± 0.20$^{\Delta}$
5e	69.4	0.4 ± 0.19$^{\Delta}$

Ulcerogenic potential is expressed as average number of ulcer score ± SEM

* $P < 0.01$ as compared to control; $^{\Delta}$ $P < 0.01$ as compared to naproxen

All the compounds were evaluated for their anti-inflammatory activity by carrageenan induced paw edema method in rats. The derivatives showed highly potent anti-inflammatory activity. At 120 min, all compounds (except **5f**, **5i**, and **5k**) were more potent than parent drug. Compound **5a** was most active compound of the series with percentage inhibition of 76% as compared to naproxen (58% inhibition). It was followed by **5n** (73%) and **5j** (70%). Compound **5k** (45%) was observed to be least active whereas compound **5f** and **5i** showed comparable percentage inhibition of edema of 58% (Table 2). Research findings also reveal that naproxen and its prodrugs showed their maximum inhibitory effects at 120 min. In addition, the synthesized prodrugs protected edema formation in a

Table 2 Analgesic and anti-inflammatory activities of synthesized prodrugs (**5a–n**)

Compound	Dose	No. of writhing (% inhibition)	Edema volume (% inhibition)			
			30 min	60 min	90 min	120 min
Control	–	36.17 ± 0.6	0.50 ± 0.05	0.62 ± 0.06	0.73 ± 0.05	0.84 ± 0.06
Naproxen	50.0	13.67 ± 1.97**(61.36)	0.45 ± 0.04**(10)	0.42 ± 0.05*(32)	0.42 ± 0.04**(42)	0.35 ± 0.05**(58)
5a	62.8	13.17 ± 3.5**(63.59)	0.21 ± 0.02*(58)	0.39 ± 0.03**(37)	0.30 ± 0.04**(58)	0.20 ± 0.04**(76)
5b	63.2	0.67 ± 0.7**(98.15)	0.19 ± 0.04$^{\Delta}$(62)	0.44 ± 0.06$^{\Delta}$(29)	0.37 ± 0.07**(49)	0.27 ± 0.06**(67)
5c	66.0	13.17 ± 2.4**(63.59)	0.35 ± 0.02**(30)	0.50 ± 0.02$^{\Delta}$(19)	0.41 ± 0.03**(43)	0.30 ± 0.03**(64)
5d	66.4	25.2 ± 2.6**(30.41)	0.29 ± 0.05**(42)	0.43 ± 0.03$^{\Delta}$(30)	0.31 ± 0.03**(57)	0.27 ± 0.02**(67)
5e	67.4	10.67 ± 3.6**(70.5)	0.27 ± 0.02$^{\Delta}$(46)	0.43 ± 0.02*(30)	0.36 ± 0.03**(32)	0.30 ± 0.03**(64)
5f	66.2	14.33 ± 5.2**(60.38)	0.41 ± 0.03$^{\Delta}$(18)	0.47 ± 0.03$^{\Delta}$(24)	0.44 ± 0.02**(39)	0.35 ± 0.03**(58)
5g	67.8	3.2 ± 1.7**(91.24)	0.39 ± 0.03**(22)	0.49 ± 0.03$^{\Delta}$(13)	0.38 ± 0.04**(47)	0.26 ± 0.03**(69)
5h	69.4	6.5 ± 2.8**(82.03)	0.20 ± 0.05$^{\Delta}$(60)	0.46 ± 0.08$^{\Delta}$(25)	0.38 ± 0.08**(47)	0.27 ± 0.08**(67)
5i	67.0	12.33 ± 1.2**(65.91)	0.37 ± 0.05$^{\Delta}$(26)	0.49 ± 0.05$^{\Delta}$(13)	0.44 ± 0.05**(39)	0.35 ± 0.05**(58)
5j	71.9	9.5 ± 4.8**(73.74)	0.41 ± 0.04$^{\Delta}$(18)	0.52 ± 0.04$^{\Delta}$(16)	0.46 ± 0.04**(36)	0.25 ± 0.06**(70)
5k	67.6	9.0 ± 5.2**(75.12)	0.40 ± 0.05$^{\Delta}$(20)	0.61 ± 0.05$^{\Delta}$(1)	0.52 ± 0.04*(28)	0.46 ± 0.05**(45)
5l	68.9	13.0 ± 1.5**(64.06)	0.34 ± 0.04$^{\Delta}$(32)	0.42 ± 0.03*(32)	0.34 ± 0.03**(53)	0.28 ± 0.04**(66)
5m	64.7	6.5 ± 2.9**(82.03)	0.38 ± 0.03$^{\Delta}$(24)	0.42 ± 0.03*(32)	0.36 ± 0.04**(50)	0.27 ± 0.04**(67)
5n	69.6	2.8 ± 1.2**(92.26)	0.28 ± 0.04**(44)	0.36 ± 0.03**(41)	0.29 ± 0.04**(50)	0.22 ± 0.04**(73)

** $P < 0.01$, * $P < 0.05$, $^{\Delta}$ $P > 0.05$ as compared to control

Results are expressed as mean ± SEM

dose dependent manner. All tested doses exhibited highly significant activities as compared to control. Remarkable reduction was observed in the ulcerogenic potential of compounds **5a–e** when compared to their parent drug (Table 3). Results indicated that GI toxicity due to direct contact of the carboxylic group has been protected.

In conclusion, the present study undertakes the synthesis of 14 novel prodrugs of naproxen. All the newly synthesized compounds have been screened for their analgesic and anti-inflammatory activities. Selected compound were also tested for their ulcerogenic potential and in vitro hydrolysis pattern. From the observations of present investigation, it can be inferred that these novel prodrugs represent potentially useful naproxen derivatives for oral administration as they (1) are stable in aqueous solutions, (2) retain the analgesic and anti-inflammatory activity of parent acid, and (3) notably inhibit GI irritation induced by naproxen.

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