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Nicotinic acid conjugates of nonsteroidal anti-inflammatory drugs (NSAID's) and their anti-inflammatory properties

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ABSTRACT

A series of nicotinic acid conjugates with non-steroidal anti-inflammatory drugs (NSAID's) have been effectively synthesized using TBTU in high yield and purity. All the synthesized conjugates were evaluated for their *in vitro* anti-inflammatory activity.

1. Introduction

Variety of inflammatory diseases including rheumatoid arthritis, ankylosing spondylitis, osteoarthritis of large joints, etc. are treated by use of the nonsteroidal antiinflammatory drugs NSAID's including diclofenac (Skoutakis et al., 1998; Moser et al., 1990; Sallmann, 1986); antirheumatic drugs (Lee et al., 1976; Mason et al., 1967; Bandarage et al., 2000); aspirins' antiplatelet properties (Cena et al., 2003; Zadrazil, 2006), nonsteroidal anti-inflammatory drugs gastropathy (Laine, 1996), ibuprofen glucopyranoside conjugates (Zhao et al., 2006; Shanbhag et al., 1992) indomethacin, farsenil (Kumakura et al., 1990), prodrugs of flufenamic acid and diclofenac (Ribeiro et al., 2007) derivatives of flurbiprofen (Halen et al., 2006; Kakuta et al. (2008)). NSAID's act by inhibiting cyclooxygenase derived prostaglandin synthesis and through local action exerted by direct contact of the drugs with gastric mucosa due to the acidic nature. However upper GI irritation, ulceration, dyspepsia, bleeding, gastrotoxicity and in some cases death are the major side effects observed in patients undergoing a long-term NSAID's treatment (Albert, 1958). A major drawback with use of NSAID's is development of gastric ulcers during treatment. Nicotinic acid is used for conjugation with NSAID's because it has been known in literature that nicotinic acid helps to reduce gastric irritation associated with use of anti-inflammatory agents. Nicotinic acid is a vitamin constituent and usually prescribed along with the NSAID's for pain, inflammation and arthritis treatment.

We are making mutual prodrugs of NSAID's with Nicotinic acid and principle of mutual prodrug is combining two therapeutic agents in a single molecule using a linker. Linker has to cleave in the biological system under biological pH to release both the agents. We have used the simplest linker i.e. 1,3-propane diol. As both partners of mutual prodrugs i.e. NSAID's and Nicotinic acid contain carboxylic acid functional groups in the molecule, the simplest linkage we can make is ester linkage. As there are enough concentration of esterases present in body, they act on the ester linkage and cleave the components at a time in effective concentration. Also as we are connecting both components in a single molecule they traverse through all the biological membranes together and will be probably targeted to the actual site of action in effective concentration. Thus our principle of mutual prodrug worked as there is no reduction in biological activity. Also, we can expect these ester containing conjugates to release the drugs over a longer period and in a sustained manner, and thus, reducing the doses and the intervals of the medication. To overcome these side effects associated with such drugs, converting carboxylic acid containing drugs into their prodrugs containing ester linkages is the most successful approach (Bundgaard, 1985, 1986, 1989, 1991; Manon and Sharma, 2009; Gund et al., 2011).

Such derivatives are expected to release active parent drug upon administration either chemically or enzymatically which in turn exert the desired biological action (Bundgaard, 1985; Bundgaard, 1986). Several literature reports containing ester containing prodrugs of NSAID's having improved bioavailability, anti-inflammatory activity and reduced gastric toxicity are well documented (Bundgaard, 1991; Bundgaard, 1989). Diclofenac–antioxidant conjugates devoid of ulcerogenic side effects are known in literature

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(Manon and Sharma, 2009). In continuation of our research interest on C-C, C-O and C-N bond formation (Nawaz Khan et al., 2002, Khan et al., 2010; Hathwar, et al., 2007a, 2007b; Tajudeen, 2007; Maiyalagan, 2009; Prabakaran, 2010). The present work was initiated with the aim to develop prodrugs of indomethacin (Gund et al., 2011). Thus with the promising results in hands we have prepared a series of novel ester containing prodrugs (**3a–f**) of other NSAID's as depicted in the scheme below (Scheme 1) in good to excellent yields. Condensation of nicotinic acid with the appropriate hydroxyl derivative of NSAIDs in the presence of TBTU (*O*-(Benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate) reagent gave the desired nicotinic acid conjugates which were evaluated for anti-inflammatory activity. A nicotinic acid is mainly play a role to reduce the gastric irritation associated with use of NSAID's (Nylander, 2011). Further investigation on the actual quantitative reduction in gastric ulcers associated with use of NSAID's is underway and will be communicated soon. The structures of all prodrugs were established by ^{1}H NMR, ^{13}C NMR and mass spectral techniques with HPLC purities more than 95%. All the synthesized conjugates were stable at room temperature and up to 40 °C and at different pH.

2. Materials and methods

2.1. Materials

Penicillin, streptomycin, Roswell Park Memorial Institute medium1640 (RPMI-1640), fetal bovine serum (FBS), phorbol myristate acetate (PMA), lipopolysaccharide (LPS), Ficoll Hypaque-1077 and phosphate buffered saline (PBS) were from Sigma. Duosets for TNF-α, IL-6 enzyme-linked immunosorbent assays (ELISAs) were from BD, Biosciences. CCK-8 reagent was from DoJindo and dimethyl sulfoxide (DMSO) is obtained from Sigma Aldrich, USA.

2.2. Cell lines

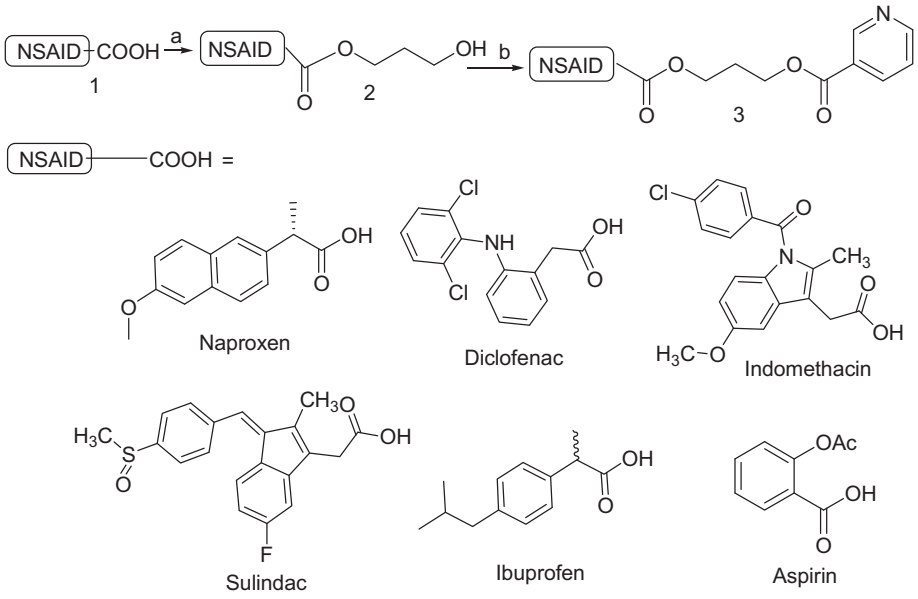
The human monocytic cell line THP-1 (American Type Culture Collection, Manassas, VA.) was maintained in RPMI-1640 supplemented with 10% FBS. Prior to cell-contact experiments, cells were cultured for 24 h in the presence of 10 ng/mL of PMA to enhance their response to activation stimuli.

2.3. Cell based assay for THP-1 cytokine release assay

The human monocytic cell line THP-1 (American Type Culture Collection, Manassas, Va.) was maintained in RPMI supplemented with 2 mM L-glutamine, 100 mg of penicillin per mL, 100 mg of streptomycin per mL with 25 mM HEPES and 10% fetal bovine serum. Induction of cell differentiation was obtained with 100 nM PMA for 24 h. After incubation, non-adherent cells were removed by aspiration and the adherent cells were washed with RPMI three times. For cell stimulation, the cells were further incubated with or without LPS for 24 h, in fresh complete medium with 10% fetal bovine serum. After cell plating, the test compounds or vehicle (0.5% DMSO) were added to each well and the plate was incubated for 30 min at 37 °C. Finally, 20 μL (10 μg/mL) per well of LPS was added for a final concentration of 1 μg/mL.

2.3.1. General synthesis procedure

Synthesis of **2a**: To a stirred solution of naproxen, **1a** (1.0 g, 4.34 mmol) in DMF (8 mL) at 0 °C under nitrogen was added Cs_2CO_3 (1.41 g, 4.34 mmol) and stirred for 20 min. A solution of 3-bromo-1-propanol (0.38 mL, 4.34 mmol) in DMF (2 mL) was added to above mixture at 0 °C. Reaction mixture was stirred at room temperature for ~10 h, poured in ice water (70 mL) and extracted with ethylacetate (2 × 40 mL). Ethyl acetate layer was washed with satu. $NaHCO_3$ (40 mL), brine (40 mL), dried over Na_2SO_4 and concentrated. Crude compound was purified by silica gel column chromatography using 30% EtOAc/Pet.ether to afford the hydroxy derivative, **2a** as a colorless oil. (1.04 g, 84%, R_f = 0.50 in 40% EtOAc/Pet.ether) ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.60 (d, 3H, J = 7.2 Hz), 1.77–1.85 (m, 2H), 3.55 (t, 2H, J = 6.0 Hz), 3.88 (q, 1H, J = 7.2 Hz), 3.93 (s, 3H), 4.25 (t, 2H, J = 5.8 Hz), 7.13–7.18 (m, 2H), 7.40, 7.42 (dd, 1H, J = 1.5 Hz), 7.71 (t, 3H, J = 8.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.95, 31.16, 45.02, 54.84, 58.63, 61.28, 105.13, 118.58, 125.46, 125.61, 126.73, 128.78, 133.23, 135.07,



Where: a)Cs_2CO_3, 3-bromo 1-propanol, DMF; b) Nicotinic acid, TBTU, Diisopropylamine, THF.

Scheme 1. Synthesis of Nicotinic acid-NSAID's conjugates. Where (a) Cs_2CO_3, 3-bromo 1-propanol, DMF; and (b) nicotinic acid, TBTU, diisopropylamine, TMF.

151.19, 174.68. MS *m/z*: 289 $[M+H]^+$, 311 $[M+Na]^+$. HRMS ESI *m/z*: $[M+H]^+$ calculated for $C_{17}H_{21}O_4$: 289.1434, found: 289.1446.

Synthesis of **3a**: To a stirred solution of nicotinic acid (1.1 eq) and NSAID-alcohol **2a** (1.0 eq) in acetonitrile was added TBTU (1.2 eq) followed by diisopropyl ethylamine (1.3 eq) at room temperature under nitrogen atmosphere. Reaction mixture was then stirred vigorously for 30–120 min. The reaction completion was monitored by TLC analysis, the reaction mixture was poured in ice cold water and extracted with ethyl acetate. Ethyl acetate layer was successively with saturated $NaHCO_3$ solution and brine. Organic layer was dried over Na_2SO_4 and concentrated. Crude compound was purified by silica gel column chromatography using 50% EtOAc/Pet.ether to afford the compound **5a** as a colorless oil. (0.60 g, 89%, R_f = 0.39 in 50% EtOAc/Pet.ether) ^1H NMR (300 MHz, $CDCl_3$): δ 1.59 (d, 3H, J = 6.9 Hz), 2.03–2.12 (m, 2H), 3.87 (q, 1H, J = 7.2 Hz), 3.92 (s, 3H), 4.26 (t, 2H, J = 6.3 Hz), 4.31–4.36 (m, 2H), 7.13–7.16 (m, 2H), 7.35–7.42 (m, 2H), 7.69 (t, 3H, J = 6.6 Hz), 8.22, 8.24 (dt, 1H, J = 1.9 Hz), 8.77, 8.79 (dd, 1H, J = 1.8, 1.5 Hz), 9.19 (d, 1H, J = 1.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 18.44, 27.90, 31.66, 45.49, 55.30, 59.05, 61.76, 105.59, 119.05, 123.30, 125.93, 126.10, 127.20, 129.26, 133.70, 135.57, 137.02, 150.82, 153.42, 157.66, 175.12. MS *m/z*: 394.2 $[M+H]^+$, 416.2 $[M+Na]^+$. HRMS ESI *m/z*: $[M+H]^+$ Calculated for $C_{23}H_{24}NO_5$: 394.1649, found: 394.1682.

The spectral analysis data of compounds **2b–2f** and **3b–3f** are given below along with spectral traces as a Supplementary document.

2b: Colorless oil, yield: 69%, R_f = 0.50 in 50% EtOAc/Pet.ether. ^1H NMR (300 MHz, $CDCl_3$): δ 1.87–1.95 (m, 2H), 3.68 (t, 2H, J = 6.0 Hz), 3.48 (s, 2H), 4.34 (t, 2H, J = 6.1 Hz), 6.57 (d, 1H, J = 8.1 Hz), 6.89 (bs, 1H), 6.93–7.03 (m, 2H), 7.15 (dt, 1H, J = 7.6 Hz), 7.24 (d, 1H, J = 6.9 Hz), 7.36 (d, 1H, J = 8.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 30.42, 31.65, 35.04, 36.54, 38.60, 58.96, 60.33, 62.30, 118.23, 122.03, 124.09, 128.04, 128.88, 129.50, 130.82, 137.73, 142.66. MS *m/z*: 354.1 $[M+H]^+$, 376.0 $[M+Na]^+$. HRMS ESI *m/z*: $[M+Na]^+$ Calculated for $C_{17}H_{17}ClNO_3$: 376.0478, found: 376.0459.

3b: Colorless oil, yield: 80%, R_f = 0.39 in 50% EtOAc/Pet.ether. ^1H NMR (300 MHz, $CDCl_3$): δ 2.14–2.23 (m, 2H), 3.84 (s, 2H), 4.32–4.37 (m, 2H), 4.45 (t, 2H, J = 6.1 Hz), 6.56 (d, 1H, J = 8.1 Hz), 6.90 (bs, 1H), 6.94–7.03 (m, 2H), 7.11–7.17 (m, 1H), 7.22–7.25 (m, 1H), 7.34–7.42 (m, 3H), 8.28, 8.30 (dt, 1H, J = 1.9 Hz), 8.79, 8.80 (dd, 1H, J = 1.8, 1.5 Hz), 9.24 (d, 1H, J = 1.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 27.99, 31.69, 38.53, 58.99, 61.90, 62.31, 118.30, 122.07, 123.33, 124.08, 125.94, 128.07, 128.88, 129.47, 130.83, 137.05, 137.74, 142.68, 150.89, 153.52, 165.11, 172.34. MS *m/z*: 459.1 $[M+H]^+$, 481.1 $[M+Na]^+$. HRMS ESI *m/z*: $[M+H]^+$ Calculated for $C_{23}H_{21}ClN_2O_4$: 459.0873, found: 459.0885.

2c: Yellow oil, yield: 59%, R_f = 0.42 in 5% MeOH/CH_2Cl_2. ^1H NMR (300 MHz, $CDCl_3$): δ 1.79–1.91 (m, 2H), 2.40 (s, 3H), 3.53–3.68 (m, 2H), 3.70 (s, 3H), 3.85 (s, 3H), 4.28 (t, 2H, J = 6.0 Hz), 6.68, 6.71 (dd, 1H, J = 2.4 Hz), 6.88 (d, 1H, J = 9.3 Hz), 6.97 (d, 1H, J = 2.4 Hz), 7.49 (d, 2H, J = 8.4 Hz), 7.68 (d, 2H, J = 8.4 Hz). MS *m/z*: 416.1 $[M+H]^+$, 438.1 $[M+Na]^+$. HRMS ESI *m/z*: $[M+Na]^+$ Calculated for $C_{22}H_{22}ClNO_5$: 438.1079, found: 438.1118.

3c: Yellow oil, yield: 83%, R_f = 0.37 in 5% MeOH/CH_2Cl_2. ^1H NMR (300 MHz, $CDCl_3$): δ 2.09–2.18 (m, 2H), 2.40 (s, 3H), 3.69 (s, 2H), 3.83 (s, 3H), 4.29 (t, 2H, J = 6.3 Hz), 4.39 (t, 2H, J = 6.3 Hz), 6.65, 6.68 (dd, 1H, J = 5.4, 2.4 Hz), 6.86 (d, 1H, J = 9.0 Hz), 6.96 (d, 1H, J = 2.4 Hz), 7.38–7.43 (m, 1H), 7.47–7.50 (m, 2H), 7.66–7.70 (m, 2H), 8.26, 8.28 (dt, 1H, J = 1.95 Hz), 8.78, 8.80 (dd, 1H, J = 1.8 Hz), 9.21 (d, 1H, J = 1.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.32, 27.97, 30.36, 31.66, 55.71, 59.16, 62.10, 101.27, 111.62, 114.98, 123.34, 125.90, 129.13, 130.81, 131.19, 133.85, 135.96, 137.04, 139.29, 150.84, 153.52, 156.04, 165.05, 171.24. MS *m/z*: 521.1 $[M+H]^+$, 543.1 $[M+Na]^+$. HRMS ESI *m/z*: $[M+H]^+$ Calculated for $C_{28}H_{26}ClN2O_6$: 521.1474, found: 521.1522.

2d: Yellow solid, yield: 63%, MP: 177–179 °C, R_f = 0.42 in 10% ACN/CH_2Cl_2. ^1H NMR (300 MHz, $CDCl_3$): δ 1.76 (t, 1H, J = 5.5 Hz), 1.84–1.92 (m, 2H), 2.23 (s, 3H), 2.82 (s, 3H), 3.59 (s, 2H), 3.67 (1, 2H, J = 5.7 Hz), 4.29 (t, 2H, J = 6.1 Hz), 6.58 (t, 1H, J = 8.7 Hz), 6.88, 6.91 (dd, 1H, J = 2.4 Hz), 7.14–7.19 (m, 2H), 7.67–7.75 (q, 4H, J = 8.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 10.52, 31.66, 31.78, 43.85, 59.08, 62.18, 105.88, 106.20, 110.65, 110.95, 123.61, 123.73, 123.82, 128.28, 129.48, 129.51, 130.25, 131.71, 131.74, 170.56. MS *m/z*: 415.2 $[M+H]^+$, 437.1 $[M+Na]^+$. HRMS ESI *m/z*: $[M+H]^+$ Calculated for $C_{23}H_{24}FO_4S$: 415.1374, found: 415.1364.

3d: Yellow solid, yield: 74%, MP: >200 °C, R_f = 0.47 in 10% ACN/CH_2Cl_2. ^1H NMR (300 MHz, $CDCl_3$): δ 2.10–2.19 (m, 2H), 2.22 (s, 3H), 2.83 (s, 3H), 3.59 (s, 2H), 4.30 (t, 2H, J = 6.3 Hz), 4.41 (t, 2H, J = 6.3 Hz), 6.56 (t, 1H, J = 9.0 Hz), 6.88, 6.91 (dd, 1H, J = 2.4, 2.1 Hz), 7.13–7.17 (m, 2H), 7.38–7.43 (m, 1H), 7.67–7.75 (q, 4H, J = 8.3 Hz), 8.27, 8.30 (dt, 1H, J = 1.95 Hz), 8.78, 8.80 (dd, 1H, J = 1.5, 1.8 Hz), 9.21 (d, 1H, J = 1.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 10.53, 27.92, 31.73, 43.89, 61.51, 61.74, 105.86, 106.18, 110.66, 110.96, 123.32, 123.62, 123.81, 125.90, 128.34, 129.34, 130.25, 131.59, 137.02, 138.24, 139.62, 141.57, 145.50, 150.86, 153.51, 170.10. MS *m/z*: 520.2 $[M+H]^+$.

2e: Colorless oil, yield: 83%, R_f = 0.48 in 40% EtOAc/Pet. ether. ^1H NMR (300 MHz, $CDCl_3$): δ 0.91 (d, 6H, J = 6.6 Hz), 1.51 (d, 3H, J = 7.2 Hz), 1.63 (bs, 1H), 1.76–1.90 (m, 3H), 2.46 (d, 2H, J = 7.2 Hz), 3.55 (t, 2H, J = 5.4 Hz), 3.71 (q, 1H, J = 7.2 Hz), 4.22–4.27 (m, 2H), 7.11 (d, 2H, J = 8.1 Hz), 7.21 (d, 2H, J = 7.8 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 18.34, 22.36, 30.18, 31.65, 45.01, 45.17, 59.11, 61.63, 127.09, 129.37, 137.63, 140.66, 175.24. MS *m/z*: 265.1 $[M+H]^+$, 287.2 $[M+Na]^+$. HRMS ESI *m/z*: $[M+Na]^+$ Calculated for $C_{16}H_{24}O_3$: 287.1618, found: 287.1643.

3e: Colorless oil, yield: 66%, R_f = 0.47 in 50% EtOAc/Pet.ether. ^1H NMR (300 MHz, $CDCl_3$): δ 0.89 (d, 6H, J = 6.6 Hz), 1.50 (d, 3H, J = 7.2 Hz), 2.04–2.12 (m, 2H), 2.44 (d, 2H, J = 7.2 Hz), 3.71 (q, 1H, J = 7.1 Hz), 4.25 (t, 2H, J = 6.3 Hz), 4.29–4.34 (m, 2H), 7.09 (d, 2H, J = 8.1 Hz), 7.20 (d, 2H, J = 8.1 Hz), 7.38–7.42 (m, 1H), 8.26, 8.29 (dt, 1H, J = 1.8 Hz), 8.78, 8.80 (dd, 1H, J = 1.8 Hz), 9.21 (d, 1H, J = 1.8 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 18.36, 22.35, 27.94, 30.15, 44.99, 45.09, 61.04, 61.87, 123.30, 125.98, 127.07, 129.35, 137.02, 137.58, 140.62, 150.88, 153.47, 165.05, 174.66. MS *m/z*: 370.2 $[M+H]^+$. HRMS ESI *m/z*: $[M+H]^+$ Calculated for $C_{22}H_{28}NO_4$: 370.2013, found: 370.2041.

2f: Colorless oil, 69%, R_f = 0.48 in 30% EtOAc/Pet.ether. ^1H NMR (300 MHz, $CDCl_3$): δ 1.95–2.06 (m, 2H), 2.36 (s, 3H), 3.76 (t, 2H, J = 6.0 Hz), 4.46 (t, 2H, J = 6.1 Hz), 7.11, 7.13 (dd, 1H, J = 0.9 Hz), 7.33 (t, 1H, J = 7.5 Hz), 7.58 (t, 1H, J = 7.8 Hz), 8.01, 8.03 (dd, 1H, J = 1.8 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.03, 31.72, 59.01, 62.03, 123.23, 123.79, 126.05, 131.67, 133.96, 150.63, 164.88, 169.80. ESI–MS *m/z*: 239.1 $[M+H]^+$.

3f: Colorless oil, 81%, R_f = 0.51 in 60% EtOAc/Pet.ether. ^1H NMR (300 MHz, $CDCl_3$): δ 2.24–2.31 (m, 2H), 2.36 (s, 3H), 4.47 (t, 2H, J = 6.3 Hz), 4.53 (t, 2H, J = 6.3 Hz), 7.11 (d, 1H, J = 8.1 Hz), 7.30–7.33 (m, 1H), 7.37, 7.40 (dd, 1H, J = 4.8 Hz), 7.57 (t, 1H, J = 7.8 Hz), 8.00, 8.02 (dd, 1H, J = 1.5 Hz), 8.29 (d, 1H, J = 8.1 Hz), 8.78, 8.80 (dd, 1H, J = 1.5 Hz), 9.24 (d, 1H, J = 2.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.01, 28.08, 61.71, 62.14, 122.97, 123.29, 123.83, 126.00, 131.59, 134.00, 137.05, 150.88, 153.49, 164.20, 165.14, 169.63. MS *m/z*: 344.1 $[M+H]^+$, 366.1 $[M+Na]^+$. HRMS ESI *m/z*: $[M+Na]^+$ Calculated for $C_{18}H_{17}NO_6$: 366.0948, found: 366.0938.

3. Results and discussion

Our investigation begins with synthesis of hydroxy scaffold, **2** through the reaction of NSAIDs with the 1-bromo-3-hydroxypropane (Scheme 1). The NSAIDs were reacted with the later in the presence of caesium carbonate and dimethylformamide to afford

the desired scaffold, **2** which in turn condensed with nicotinic acid in the presence of TBTU to afford the desired prodrugs, **3**.

The coupling reagent TBTU, has been extensively used due to its high efficiency, high stability, long shelf life, solubility in organic solvents, ease of handling and availability. In the present study, TBTU is applied for the esterification of nicotinic acid with the diverse NSAID's containing hydroxyl derivatives in good yields. The advantages of this reaction includes low toxicity of by-products, easy handling, simple work-up. The proposed mechanism of the reaction is depicted below (Scheme 2). The reaction takes place through the tautomeric forms of TBTU, of which the form I reacts with the carboxylate ion to produce the intermediate which then couples with the hydroxyl derivative to form the desired ester product.

The optimization of the reaction was carried out by varying the amount of TBTU reagent, diisopropyl ethylamine and the solvents at room temperature (Tables 1 and 2). The experimental results indicated that with an optimized amount of equimolar TBTU, diisopropyl amine is required for the reaction of **2** (1.0 eq) and nicotinic acid (1.1 eq) and THF as a solvent is necessary for the effective conversion. With this optimization result in hand various hydroxyl derivatives of NSAID's were condensed with nicotinic acid and the results are reported in Table 3. The purified products (HPLC purities more than 95%) were characterized by different spectral techniques including ^{1}H NMR, ^{13}C NMR, IR and MS techniques.

Table 1
Effect of amount of TBTU and diisopropyl ethyl amine loading in the esterification of nicotinic acid with **2a**.[a]

Entry	TBTU eq	DIPEA eq	Yield[b] (%)
1	1.0	1.0	76
2	1.2	1.0	79
3	1.4	1.0	80
4	1.2	1.1	81
5	1.2	1.2	84
6	1.2	1.3	85
7	1.2	Nil	24

[a] Reaction conditions: nicotinic acid (1.1 eq); alcohol, **2** (1.0 eq); diisopropylethyl amine.
[b] Isolated yield.

Table 2
Effect of solvent in the esterification reaction of nicotinic acid and **2**.[a]

Entry	Solvent	Yield[b] (%)
1	Acetonitrile	64
2	Tetrahydrofuran	84
3	2-Methyl THF	71
4	Dichloromethane	54
5	N,N-dimethyl formamide	63

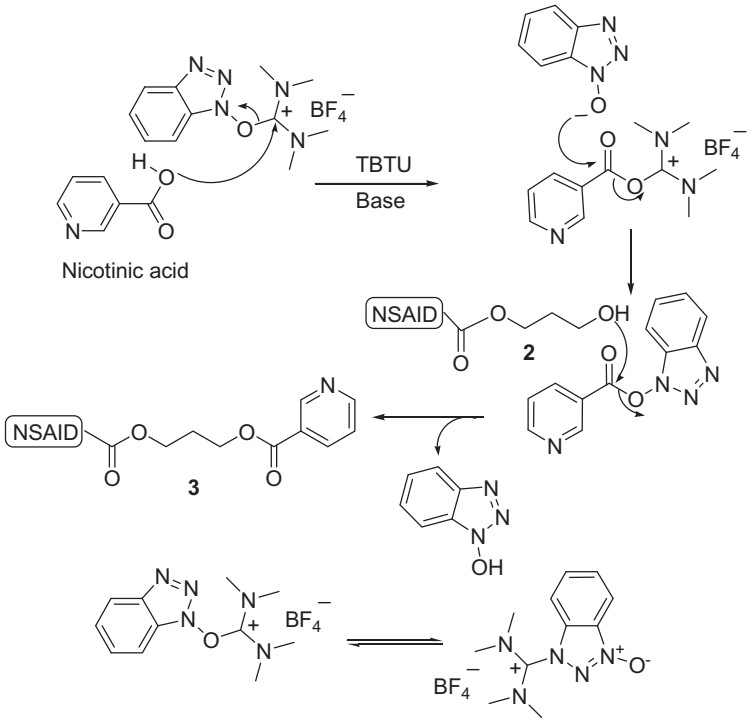
[a] Reaction conditions: nicotinic acid (1.1 eq); alcohol, **2** (1.0 eq); diisopropylethyl amine.
[b] Isolated yield. TBTU; solvent (8 mL).

4.1. Screening for anti-inflammatory activity

All the synthesized conjugates were screened for anti-inflammatory activity at 30 μM concentration and the percentage inhibition data is presented in Table 4. The anti-inflammatory effects of the synthesized conjugates and that of standard NSAID's used for conjugation were very similar. Thus our principle of mutual prodrug worked as there is no reduction in biological activity. Also, we can expect these ester containing conjugates to release the drugs over a longer period and in a sustained manner, and thus, reducing the doses and the intervals of the medication (Uddin et al., 2010). In the traditional NSAID's the aspirin and indomethacin are few examples of selective COX-1 inhibitors has been identified from the literature, similarly conjugates of NSAID's with nicotinic acid have been known in literature (Perrone et al., 2010) and they retain COX-inhibitory activity. The NSAID's possess potent COX-1 inhibitory activity and COX-1 is the main culprit in causing gastric ulceration (Nylander, 2011).

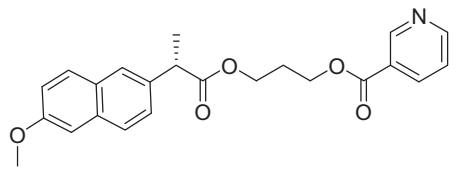
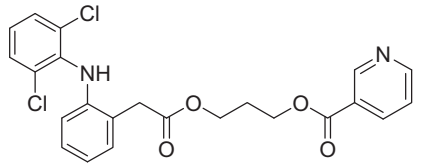
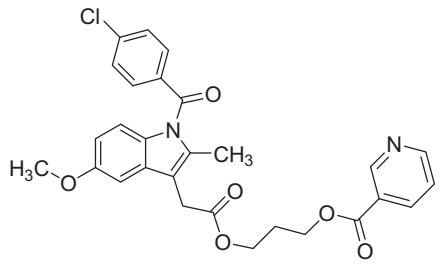
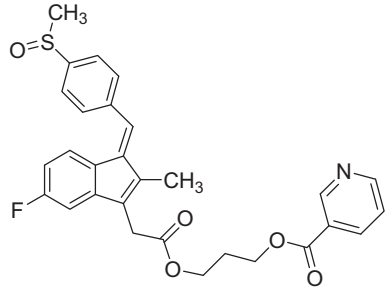
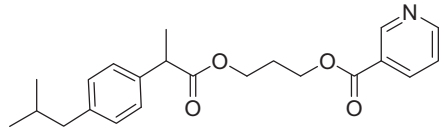
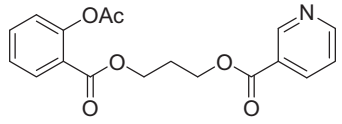
4.1.1. Detection of secreted cytokines

Human TNF-α and IL-6 was quantified by an enzyme linked immunosorbent assay (ELISA) as previously described. Briefly,



Scheme 2. Mechanism of esterification mediated by TBTU reagent.

Table 3
Esterification of nicotinic acid with **2**.

S. no.	Product, **3a–f**	Yield[a] (%)
1		90
2		89
3		92
4		88
5		84
6		87

Reaction conditions: nicotinic acid (1.0 eq); alcohol, **2** (1.1–2.0 eq); diisopropylethyl amine; TBTU (1.1 eq); acetonitrile solvent (8 mL) were stirred at ambient temperature for 20–30 min.
[a] Isolated yield.

96-well ELISA plates (Maxisorp; Nunc, Naperville, Ill.) were coated with an anti-human TNF-α/IL-6 monoclonal antibody (BD, Biosciences) in a coating buffer (carbonate–bicarbonate buffer, pH 9.6), followed by overnight incubation at 4 °C. The wells were blocked for 2 h, at room temperature with 10% FBS prepared in assay buffer. Biotinylated anti-human TNF-α/IL-6 polyclonal antibody (BD, Biosciences) was added, followed by avidin-horse radish peroxidase conjugate, which used tetramethylbenzidine as the substrate. The reaction was stopped by the addition of 2 N sulfuric acid and optical density was read in a Saphire microplate reader (Tecan) at 490–600 nm.

6. Conclusion

In short, an efficient, experimentally simple TBTU mediated coupling methodology is applied for the synthesis of nicotinic acid-NSAID's conjugates with the advantages of low toxicity of by-products, easy handling and simple work-up procedures. All the synthesized conjugates are equally or slight better potent *in vitro* when compared with the respective parent drug.

Table 4
Anti-inflammatory activities of the conjugates.

S. no.	NSAID/ compound	IL-6		TNF-α	
		% Inhibition @ 30 μM	IC_{50}	% Inhibition @ 30 μM	IC_{50}
1	Naproxen	21 ± 2.1	>30	32 ± 4.5	>30
	3a	35 ± 3.2	>30	39 ± 1.9	>30
2	Diclofenac	35 ± 3.2	>30	42 ± 3.2	>30
	3b	44 ± 4.1	>30	48 ± 1.9	>30
3	Indomethacin	21 ± 3.5	>30	32 ± 7.2	>30
	3c	37 ± 3.9	>30	40 ± 2.1	>30
4	Sulindac	36 ± 2.1	>30	38 ± 8.8	>30
	3d	42 ± 3.1	>30	44 ± 7.1	>30
5	Ibuprofen	21 ± 1.1	>30	27 ± 2.2	>30
	3e	26 ± 2.1	>30	29 ± 3.7	>30
6	Aspirin	12 ± 1	>30	12 ± 1.9	>30
	3f	10 ± 2	>30	10 ± 2.1	>30

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejps.2013.02.007.

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