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Synthesis and antinociceptive evaluation of bioisosteres and hybrids of naproxen, ibuprofen and paracetamol

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ABSTRACT

The aim of this work was to design, synthesize and characterize the potential anti-nociceptive and anti-inflammatory activities of a new series of bioisosteres and hybrids from known non-steroidal anti-inflammatory drugs (NSAIDs). The compounds 4-(acetylamino)phenyl (2*S*)-2-(6-methoxy-2-naphthyl)propanoate (**GUF-1**) and 4-(acetylamino)phenyl 2-(*R,S*)-(4-isobutylphenyl)propanoate (**GUF-2**) were synthesized as hybrids (also known as heterodimers); whereas those named 2-(*R,S*)-(4-isobutylphenyl)-*N*-1*H*-tetrazol-5-ylpropanamide (**GUF-3**), (2*S*)-2-(6-methoxy-2-naphthyl)-*N*-1*H*-tetrazol-5-ylpropanamide (**GUF-4**), [2-(*R,S*)-*N*-hydroxy-2-[4-(2-methylpropyl)phenyl]propanamide] (**GUF-5**), and (2*S*)-*N*-hydroxy-2-(6-methoxy-2-naphthyl)propanamide (**GUF-6**) were synthesized as bioisosteres of the NSAIDs paracetamol, ibuprofen, and naproxen, respectively. All these compounds were characterized by spectroscopic and spectrometric analysis. Antinociceptive activity of **GUF-1** to **GUF-6** was evaluated using the formalin test in rats. Pharmacological responses of **GUF-1, GUF-2** (hybrids), and **GUF-5** (bioisostere) demonstrated significant antinociceptive effects; thus these compounds were assayed in an inflammation test like carrageenan-induced paw oedema in rats. Complete molecular docking of cyclooxygenase and the **GUF-1** and **GUF-2** hybrids showed high docking scores, compared to the reference drugs. Our data demonstrate that compounds **GUF-1**, **GUF-2,** and **GUF-5** possesses antinociceptive and antiinflammatory activities resembling and improving those known for the traditional NSAIDs, paracetamol, naproxen and ibuprofen.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are a chemically heterogeneous group of compounds sharing certain therapeutic actions related to nociception, inflammation and pyretic process [1–5]. The principal therapeutic effect of NSAIDs is the inhibition of cyclooxygenase (COX) family proteins that catalyze the rate-limiting steps in prostaglandin synthesis (PG) [1,2]. The acidic moiety is essential for COX inhibitory activity and it is linked to a planar aromatic group. The latter is also connected to a lipophilic part through a polar group. Thus, NSAIDs are classified as non-selective and COX-2-selective inhibitors (COXIBs) based on their selectivity for COX inhibition. As a result, the inhibition of COX-2 is thought to be mediated by the antipyretic, analgesic, and anti-inflammatory actions of NSAIDs, while the simultaneous inhibition of COX-1 largely but not exclusively accounts for unwanted adverse effects that complicate chronic NSAID therapies. The major side effects of NSAIDs include gastrointestinal complications, renal disturbances and cardiovascular events [6–9].

The market introduction of novel drugs with greater selective inhibition on the COX-2 activity in the last decade has offered therapeutic alternatives with similar efficacy to non-selective inhibitors but better gastrointestinal tolerability, mainly for patients with chronic use of traditional NSAIDs. However, concerns regarding the cardiovascular safety of selective COX-2 inhibitors has led to the withdrawal of two drugs (rofecoxib and valdecoxib) and focused attention on the non-specific COX inhibitors. It is now understood that some degree of gastrointestinal and cardiovascular risk is associated with the mechanism of action of all NSAIDs [10–13].

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Drug discovery for pain medications aims to develop new NSAIDs with a good analgesic effect to side effect ratio. In order to look for therapeutic options for pain, inflammation and fever reducing adverse drug reactions, such as damage to the digestive, kidney and cardiovascular systems; the search for new bioactive molecules with non-steroidal anti-inflammatory effects that produce minimal adverse effects continues. Therefore, our present study assesses the anti-nociceptive and/or anti-inflammatory activities of designed and synthesized molecules as bioisosteres and hybrids of paracetamol, ibuprofen and naproxen in experimental pain.

2. Material and methods

2.1. Biological activity spectra prediction

The *in silico* biological activity spectra of the NSAIDs as bioisosteres and hybrids of paracetamol, ibuprofen and naproxen were obtained using the *Prediction of Activity Spectra for Substances* (PASS) on line software (http://www.way2drug.com). Estimation of general biological potential for these compounds was performed based on their structural formula. It is known that PASS predicts more than 7000 types of biological activity with an average accuracy > 95% [14]. This program was based on a robust analysis of structure-activity relationship from a heterogeneous training set of about a million compounds. A biological spectrum for a substance is the list of pharmacological activity types for which the probability to be exposed (*Pa*) and not be exposed (*Pi*) values are independent, and their values ranges from 0 to 1 [14].

2.2. Chemistry

All the starting materials and reagents were obtained commercially from Sigma-Aldrich (St. Louis, MO, USA). Melting points were determined on an SRS EZ Melt MPA120 automated apparatus from Stanford Research Systems and are uncorrected. Thin layer chromatography on 2 × 5 cm pre-coated silica gel 60 F254 plates (E. Merck KGaA, Darmstadt, Germany) visualized at 254–365 nm UV light was used to monitor reactions.

The chemical structures of the synthesized compounds were confirmed based on of their spectral data (^{1}H NMR, ^{13}C NMR and M(*S*)-FAB$^+$). NMR studies were performed on INOVA-400 MHz instrument. Chemical shifts (δH, δC) and coupling constant values (*J*) are given in ppm and Hz, respectively. A standard reference of TMS ($\delta_H = 0$, $\delta_C = 0$) in $CDCl_3$ and DMSO-d_6 as solvents was used. Mass spectra were recorded on a JEOL JM(*S*)-700 instrument (JEOL USA Inc., Peabody, MA, USA).

2.2.1. General preparation of **GUF-1** and **GUF-2**

Synthesis of the **GUF-1** and **GUF-2** hybrids started from the carboxylic acids of (*S*)-naproxen and (*R,S*)-ibuprofen, which were reacted with paracetamol (**GUF-1** and **GUF-2**) through Steglich esterification. This reaction consisted of placing the carboxylic acid NSAIDs and phenol (paracetamol) in the presence of a catalytic amount of 4-dimethylaminopyridine (4-DMAP) (< 10%) with stirring at 0 °C for 30 min in anhydrous chloroform ($CHCl_3$). Then, the mixtures were reacted with 1.5 equivalents of dicyclohexylcarbodiimide (DCC) as a coupling agent and chloroform as a solvent at room temperature for 30 h. The reactions were monitored by TLC analysis until they were complete.

*2.2.1.1. 4-(Acetylamino)phenyl (2S)-2-(6-methoxy-2-naphthyl)propanoate (**GUF-1**).*. It was obtained as white crystals with a needle shape in an 89% yield after recrystallization from ethanol. Mp: 181.5–182.9 °C. ^{1}H NMR (400 MHz, DMSO-d6) δ: 1.58 (*d*, 3H, $\underline{CH_3}CH$), 2.03 (s, 3H, $\underline{CH_3}CO$), 3.87 (*s*, 3H, -$O\underline{CH_3}$), 4.18 (*q*, 1H, $\underline{CH}$), 6.95 (*d*, 2H, H-2″, H-6″, J_o= 8.8 Hz), 7.17 (*dd*, 1H, H-6, J_m= 2.0 Hz, J_o= 9.0 Hz), 7.31 (*d*, 1H, H-4, J_m= 2.4 Hz), 7.50 (*dd*, 1H, H-2, J_m= 1.6 Hz), 7.57 (*d*, 2H, H-3″, H-5″, J_o= 8.8 Hz), 7.84 (*m*, 3H, H-3, H-7, H-8) and 9.99 (s, 1H, NH) ppm. ^{13}C NMR (100 MHz, DMSO-d6) δ: 18.5 ($\underline{CH_3}CH$), 24.0 ($\underline{CH_3}CO$), 44.6 ($CH_3\underline{CH}$), 55.3 (-$O\underline{CH_3}$), 105.9 (C-4), 118.9 (C-6), 119.9 (C.2″, C-6″), 121.7 (C-3″, C-5″), 125.9 (C-3), 126.3 (C-2), 127.3 (C-8), 128.6 (C-7a), 129.3 (C-7), 133.6 (C-3a), 135.4 (C-1), 137.1 (C-4″), 145.8 (C-1″), 157.4 (C-5), 168.3 (C═O) and 173.1 (O−C═O) ppm. MS (FAB$^+$): *m/z* 364 (M + H)$^+$.

*2.2.1.2. 4-(Acetylamino)phenyl 2-(R,S)-(4-isobutylphenyl)propanoate (**GUF-2**).*. This compound was characterized as light brown powder with an 87% yield after purification by acid-base extraction from a 1:1 ethyl acetate: water mixture. Mp: 83.5–85.5 °C. ^{1}H NMR (400 MHz, DMSO-d6) δ: 0.86 (*d*, 6H, $(\underline{CH_3})_2CHCH_2$), 1.48 (*d*, 3H, $\underline{CH_3}CH$), 1.82 (*m*, 1H, $(CH_3)_2\underline{CH}CH_2$), 2.03 (s, 3H, $\underline{CH_3}CO$), 2.43 (*d*, 2H, $(CH_3)_2CH\underline{CH_2}$), 4.00 (*q*, 1H, $\underline{CH}$), 6.93 (*d*, 2H, H-2”, H-6”, J_o = 8.8 Hz), 7.16 (*d*, 2H, H-3, H-5, J_o = 8.4 Hz), 7.29 (*d*, 2H, H-2, H-6, J_o= 8 Hz), 7.57 (*d*, 2H, H-3″, H-5″, J_o = 8.8 Hz) and 9.99 (*s*, 1H, NH) ppm. ^{13}C NMR (100 MHz, DMSO-d6) δ: 18.5 ($\underline{CH_3}CH$), 22.2 ($(\underline{CH_3})_2CHCH_2$), 23.9 ($\underline{CH_3}CO$), 29.6 ($(CH_3)_2\underline{CH}CH_2$), 44.2 ($(CH_3)_2CH\underline{CH_2}$), 44.3 ($CH_3\underline{CH}$), 119.9 (C-2″, C-6″), 121.6 (C-3″, C-5″), 127.2 (C-3, C-5), 129.3 (C-2, C-6), 137.0 (C-4″), 140.0 (C-4), 145.7 (C-1″), 168.3 (C═O) and 173.0 (O−C═O) ppm. MS (FAB$^+$): *m/z* 340 (M + H)$^+$.

2.2.2. General preparation of**GUF-3** and **GUF-4**

(*R,S*)-ibuprofen and (*S*)-naproxen (2.2 mmol) were reacted with 3 equivalents of $SOCl_2$ (6.5 mmol) under toluene reflux (110 °C) for 5 h to obtain the corresponding acid chloride. Then, the last compound was reacted with 5-aminotetrazole (2.8 mmol) via Schötten-Baumann reaction using triethylamine (TEA, 1.3 equivalents) as a base and dichloromethane as the solvent. The reaction was maintained under nitrogen atmosphere [15].

*2.2.2.1. 2-(R,S)-(4-Isobutylphenyl)-N-1H-tetrazol-5-ylpropanamide (**GUF-3**).* It is represented by a white powder with a 48% yield purification by extraction from a 1:1 ethyl acetate:water mixture. Mp: 195.5–197.5 °C. ^{1}H NMR (400 MHz, DMSO-d6) δ: 0.82 (*d*, 6H, $(\underline{CH_3})_2CHCH_2$), 1.43 (*d*, 3H, $\underline{CH_3}CH$), 1.78 (*m*, 1H, $(CH_3)_2\underline{CH}CH_2$), 2.39 (*d*, 2H, $(CH_3)_2CH\underline{CH_2}$), 3.94 (*q*, 1H, $\underline{CH}$), 7.11 (*d*, 2H, H-3, H-5, J_o = 8 Hz), 7.27 (*d*, 2H, H-2, H-6, J_o = 8 Hz) and 12.17 (*s*, NH) ppm. ^{13}C NMR (100 MHz, DMSO-d6) δ: 18.2 ($\underline{CH_3}CH$), 22.1 ($(\underline{CH_3})_2CHCH_2$), 29.5 ($(CH_3)_2\underline{CH}CH_2$), 44.2 ($(CH_3)_2CH\underline{CH_2}$), 44.6 ($CH_3\underline{CH}$), 127.1 (C-3, C-5), 129.1 (C-2, C-6), 137.8 (C-1), 140.0 (C-4), 149.8 (C-2″) and 173.1 (C═O) ppm. MS (FAB$^+$): *m/z* 274 (M + H)$^+$.

*2.2.2.1. (2S)-2-(6-Methoxy-2-naphthyl)-N-1H-tetrazol-5-ylpropanamide (**GUF-4**).* It was obtained as a light brown powder with a 31% yield after recrystallization from methanol. Mp: 253.9–255.6 °C. ^{1}H NMR-(400 MHz, DMSO-d6) δ: 1.54 (*d*, 3H, $\underline{CH_3}CH$), 3.85 (*s*, 3H, -$O\underline{CH_3}$), 4.15 (*q*, 1H, $\underline{CH}$), 7.16 (*dd*, 1H, H-6), 7.28 (*d*, 1H, H-4), 7.50 (*dd*, 3H, H-2, H-4, H-6) and 7.80 (*m*, 3H, H-3, H-7, H-8) ppm. ^{13}C NMR (100 MHz, DMSO-d6) δ: 18.1 ($\underline{CH_3}CH$), 44.8 ($CH_3\underline{CH}$), 55.2 (-$O\underline{CH_3}$), 105.8 (C-4), 118.8 (C-6), 125.8 (C-3), 126.1 (C-2), 127.0 (C-8), 128.3 (C-7a), 129.2 (C-7), 133.4 (C-3a), 135.5 (C-1), 149.8 (C-2″), 157.2 (C-5) and 173.1 (O−C═O) ppm. MS (FAB$^+$): *m/z* 298 (M + H)$^+$.

2.2.3. General preparation of**GUF-5** and **GUF-6**

Both compounds were synthesized by converting the (*R,S*)-ibuprofen and (*S*)-naproxen carboxylic group into hydroxamic acid. The compounds were prepared using (*R,S*)-ibuprofen (4.8 mmol) and (*S*)-naproxen (4.3 mmol) dissolved in acetone at room temperature and reacted with sodium bicarbonate (5.8 mmol) for 1 h to generate the carboxylate salts. The naproxen and ibuprofen carboxylates were reacted with dimethyl sulfate (1.2 equivalents) for 24 h to produce the corresponding methyl esters. The methyl esters were purified and dissolved in methanol. Subsequent, solid KOH (in 1:3 ratio with respect to the initial mole of NSAIDs) and hydroxylamine hydrochloride (at a 1:2 ratio) were added. The reaction was maintained under a nitrogen atmosphere at room temperature until its completion.