Synthesis and Anti-Inflammatory Activities of Novel Salicylic Acid and Diflunisal Amide Derivatives

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ABSTRACT

Several compounds were derived from the conversion of the carboxyl group in salicylic acid and diflunisal into amides of various heterocyclic rings such as 2-amino-5-methyl-2- thiazole, 3-amino-5-methylisooxazole, 2-amino-5-methylthio-1,3,4-thiadiazole and 2- aminothiazole. The synthetic steps involve esterification of the phenolic group in diflunisal, followed by activation of the carboxyl group in aspirin and the esterified difluninal. Coupling of the corresponding anhydride with the above mentioned heterocyclic rings yielded the intermediates 8-11 and 18-21. Removal of the acetate generated the designed compounds 12-15 and 22-25. The anti-inflammatory activities of these compounds were tested using the % inhibition of granuloma. The results were 64%, 50% and 67% for compounds 25, Roficoxib and Indomethacin respectively. The ulcerogenic potential of tested compounds indicate that compound 25 in this novel series showed better anti-inflammatory activity and least ulcerogenic side effect relative to Roficoxib and Indomethacin.

Keywords: Anti-inflammatory, Salicylic acid and Diflunisal derivatives.

INTRODUCTION

Cyclooxygenase (COX) enzymes which catalyze the formation of prostaglandins (PGs) from arachidonic acid play an important role in various types of inflammation and ulceration. COX exists at least in two mammalian isoform, COX1 and COX2. Constitutive COX1 has house keeping function including gastro protective PGs, whereas COX2 is induced in inflammation. Conventional nonsteroidal drugs (NSAIDs), mainly inhibit COX1 and are associated with reduction in gastro protection, while COX2 inhibitors (Valdecoxib, Rofecoxib, Celecoxib) exert their anti–inflammatory, and analgesic effect with less GI toxicity than traditional NSAIDs but suffer from cardiovascular, renal and even GI irritation or ulceration with long term use or at higher doses[(1-14)].

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These clinical observations associated with COX1 or COX2 inhibitors necessitated the need for new selective, potent COX2 inhibitors with no or reduce risk of side effects.

A novel hybrid structure generated from a combination of COX1 inhibitors as represented by salicylic acid and Diflunisal and the side chain analogous found in meloxicam as represented by (2-aminothiazole, 2-amino-5-methyl thiazole, 3-amino–5- methylisooxazole and, 2–amino–5-methylthio-thiadiazole), resulted in the formation of the desired compounds as shown in chart (1).

MATERIALS AND METHODS

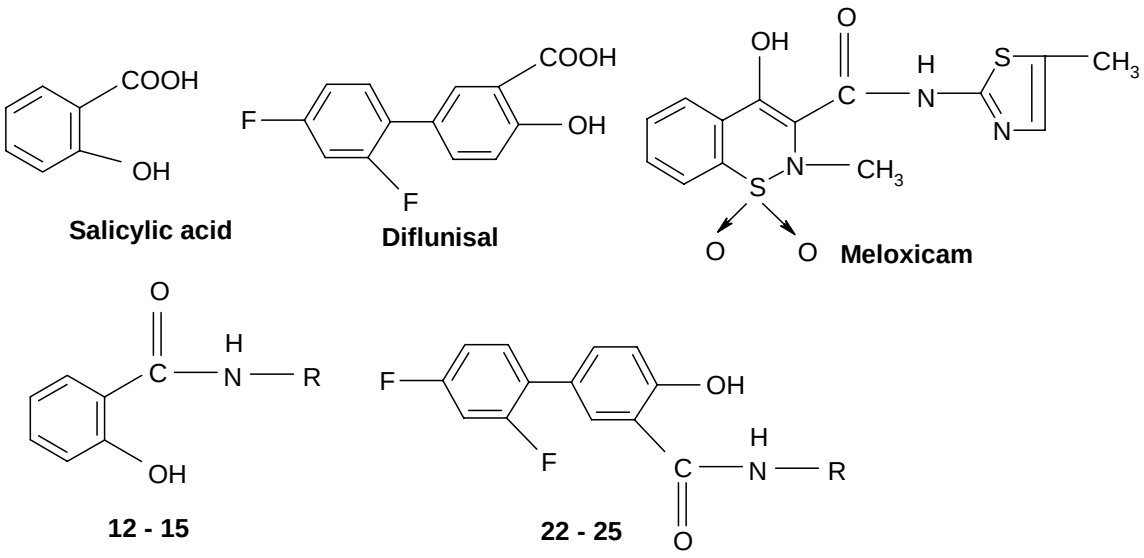
Experimental:

Chemical methods

Melting points were determined by using a calibrated Thomas–Hoover melting apparatus. IR spectra were recorded using Shimadza FT- (8101 IR) spectrophotometers (Japan) and were performed in the laboratories of Faculty of Pharmacy, Petra University.

(^{1}H–NMR) spectra were carried out on, mercury 300 MHz spectrometer (Aldenmark), using tetramethylsilane as the internal reference and were performed in the laboratories of Faculty of Science, Baghdad University. Microanalyses were performed in the laboratories of Micro – Analytical Center, Faculty of Science, Cairo University and were carried out with Yanagimoto C H N Corder MT-5.

Chart 1



Where R = thiazole, 5 - methylthiazole, 5 - methyl isooxazole, 5 - methylthiothiazole.

Chemistry:

Aspirin Anhydride (3)

Aspirin, (10 g, 55.5 mmol) was dissolved in 150 ml methylene chloride; dicyclohexylcarbodimide (5.72 g, 27.7 mmol) was added. The reaction mixture was continuously stirred at room temperature for 3 hrs. A white precipitate of dicyclohexylurea was formed and removed by filtration. The solvent was evaporated under vacuum, and an oily product was formed to yield the desired anhydride (70% yields) as described in the literature [15].

N – (2 – Thiazolyl) – acetylsalicylamide (8)

Aspirin anhydride 3, (5 g, 14.60 mmol), 2-aminothiazole (1.60 mmol), zinc dust (0.013 g), glacial acetic acid (1.4 ml, 24.481 mmol), and dioxane (40 ml) were placed in 100 ml round bottom flask, equipped with reflux condenser, and boiling stones were added. The reaction mixture was refluxed for about 1 hr with continuous stirring. The reaction was checked with TLC to make sure of the completion of the reaction. The solvent was evaporated under vacuum; the residue was dissolved in ethyl acetate, washed with $NaHCO_3$ (10%, 3X), HCL (IN, 3X) and 3 times with distilled water, and filtered over anhydrous sodium sulphate. The filtrate was evaporated and the residue was redissolved in ethyl acetate and filtered. The recrystallization was carried out by adding petroleum ether (60 – 80 °C) on the filtrate until turbidity occurred and kept in cold place over-night. Then the mixture was filtered while it was cold and the precipitate was collected to give compound **8** in 44% yield as faint yellow powder. Mp. 141-143° C, IR (KBr, Cm^{-1}) 3250 (NH, amide), 3050 (CH, ArH), 1775 (C= O, ester) 1675 (C=O, amide), 1600, 1550, 1500, (Ar). ^{1}H–NMR (C_2D_6SO): δ, 2.2 (s, 3H, $COCH_3$), 3.5 (br,1H, CONH), 7.05 (d, 1H, J=3Hz, N- CH, thiazolyl), 7.15 (d,1H, J=3Hz, S-CH,thiazolyl), 7.60, 745, 720 (m, 4H,

ArH), Anal. Calcd: ($C_{12}H_{10}N_2SO_3$): C, 54.96; H, 3.81; N, 10.68. Found: C, 54.92; H, 3.85; N, 10.64.

N–(5–Methyl–2–thiazolyl)–acetyl salicylamide (9)

Aspirin anhydride **3**, (5 g, 14.60 mmol), 2-amino–5-methylthiazole (1.66 g, 14.60 mmol), zinc dust (0.013 g), glacial acetic acid (1.4 ml , 24.481 mmol), dioxane (40 ml) were prepared as previously described in **8** to liberate compound **9** in 45% yield as faint yellow powder. Mp.126–127 °C. IR (KBr, Cm^{-1}) 3250 (NH, amide), 3050 (CH, ArH), 1760 (C=O ester), 1650 (C=O, amide), 610, 1550, 1450 (Ar). ^1H – NMR (C_2D_6SO): δ, 2.2 (s, 3H, $COCH_3$), 2.55 (s, 3H, 5-CH_3, thiazolyl), 3.5 (br. 1H, CONH), 7.15 (s, 1H, thiazolyl), 7.30, 7.40, 7.55 (m, 4H, ArH). Anal. Calcd: ($C_{13}H_{12}N_2SO_3$): C, 56.52; H, 4.34; N, 10.14. Found: C, 56.33; H, 4.3; N, 10.08.

N–(5–Methyl–3–isooxazolyl)–acetyl salicylamide (10)

Aspirin anhydride **3**, (5 g , 14.60 mmol), 3-amino-5-methylisooxazole (1.432 g , 14.60 mmol), zinc dust (0.013 g), glacial acetic acid (1.4 ml , 24.481 mmol), dioxane (40 ml), were prepared as previously described in **8** to give compound **10**, in 38 % yield as faint yellow crystals. Mp. 65 °C decomposes. IR (KBr, Cm^{-1}): 3200 (NH, amide), 3030 (CH, ArH), 1760 (C=O ester), 1630 (CO, amide), 1620, 1530, 1520 (Ar). ^1H–NMR (C_2D_6SO): δ, 2.2 (s, 3H, $COCH_3$), 2.56 (s,3H, 5-CH_3, isooxalyl), 3.5 (br. 1H, CONH), 7.15 (s, 1H, isooxazole), 7.30, 7.45, 7.55 (m, 4H, ArH). Anal. Calcd: ($C_{13}H_{12}N_2O_4$): C, 60.00; H, 4.61; N, 10.76. Found: C, 60.03; H, 4.59; N, 10.70

N–(5–Methylthio–2–(1,3,4–thiadiazolyl)–acetylsalicylamide (11)

Aspirin anhydride **3**, (5 g , 14.60 mmol), 2-amino–5-(methylthio-1,3,4) thiadiazole (2.149 g , 14.6 mmol), zinc dust (0.013 g), glacial acetic acid (1.4 ml , 24.481 mmol), dioxane (50 ml) were prepared as described in **8** to liberate compound **11** in 48 % yield as a faint yellow powder. Mp. 137 °C decomposes. IR (KBr, Cm^{-1}) 3250 (NH, amide), 3050 (ArH), 1740 (C=O ester), 1630 (C=O, amide), 1610, 1560, 1450 (Ar), 1020 (S-CH_3). ^1H–NMR (C_2D_6SO): δ, 2.2 (s, 3H,$COCH_3$), 2.55 (s, 3H, S-CH_3), 3.5 (br, 1H, CONH), 7.35, 7.42, 7.50 (m, 4H, ArH). Anal. Calcd; ($C_{12}H_{11}N_3S_2O_3$): C, 46,80; H, 3.58; N, 13.65. Found: C, 46.56; H, 3.60; N, 13.61.

5–(2,4–Difluorophenyl) acetylsalicylic acid (16)

A dry Diflunisal **2**, (10 g, 40 mmol) was placed in 200 ml round conical flask. Acetic anhydride (25 ml, 262 mmol) was added, and 5 drops of concentrated sulfuric acid was added dropwise, mixing the contents by rotating the conical flask for 5 minutes, warm in water bath to about 50-60 °C, with stirring for 20 minutes. The reaction mixture was allowed to cool with occasional stirring, and then cold distilled water was added until precipitate was formed, and filtered by using suction pump, washed with cold distilled water several times, and the crude product was collected. Recrystallization was carried out by using ethanol 95%, the precipitate was collected and dried to give compound **16** in 89 % yield as a white crystals. Mp. 175–176 °C, (KBr, Cm^{-1}): 3450 – 2000 (COOH, ArH), 1760 (C=O, ester), 1650 (C=O, COOH), 1600, 1550, 1450 (Ar). ^1H – NMR (C_2D_6SO): δ, 2.2(s, 3H, $COCH_3$), 3.5(br,1H, CONH), 7.15(1H, d, J=3.0Hz, S-CH, thiazolyl), 7.1(d, 1H, J=3.0Hz, N-CH, thiazolyl), 7.25, 7.35, 7.50 (m, 3H, ArH), 7.75, (m, 2H, $ArHF_2$), 8.08 (s, 1H, $ArHF_2$). Anal. Calcd: ($C_{15}H_{10}F_2O_4$): C, 61.60; H, 3.45. Found C, 61.68; H, 3.43.

5–(2,4–Difluorophenyl)–acetyl salicylic acid anhydride (17)

Compound **16**, (10 g, 34.22 mmol) was dissolved in (160 ml) methylene chloride;

dicyclohexylcarbodimide (3.53 g, 17.11 mmol) was added. The reaction mixture was continuously stirred at room temperature for about 3 hrs. A white precipitate of dicyclohexylurea was formed and removed by filtration. The solvent was evaporated under vacuum; a solid product was obtained to yield the desired anhydride **17** [18].

5–(2,4–Difluorophenyl)–N–(2–thiazolyl) acetyl salicylamide (18)

Compound **17**, (5 g , 8.8 mmol), 2- aminothiazole (0.883 g , 8.82 mmol), zinc dust (0.008), glacial acetic acid (0.85 ml, 14.864 mmol), dioxane (50 ml) were prepared as previously described in **8**, afforded compound **18** in 42% yield as faint reddish powder. Mp. 85 °C, decompose: IR, (KBr, Cm^{-1}) 3220 (NH, amide), 3050 (ArH), 1760 (C=O, ester), 1670 (C=O, amide)

1620, 1560, 1520 (Ar).^{1}H – NMR (C_2D_6SO): δ, 2.2 (s, 3H, $COCH_3$), 3.5 (br, 1H, CONH), 7.10 (d, 1H, J=3.0Hz, N–CH_3, thiazolyl), 7.15 (d, 1H, J=3.0Hz, S–CH, thiazolyl), Anal. Calcd: ($C_{18}H_{12}N_2F_2O_3S$): C, 57.75; H, 3.20; N, 7.48. Found: C, 57.68; H, 3.20; N, 7.50.

5–(2,4–Difluorophenyl)–N–(5–methyl–2– thiazolyl)–acetyl salicylamide (19)

Compound **17**, (5 g , 8.82 mmol), 2– amino–5–methylthiazole (1.00 g , 8.82 mmol), zinc dust (0.008 g), glacial acetic acid (0.85 ml , 14.864 mmol), dioxane (50 ml), were prepared as described before in **8**, to generate compound **19** in 46 % yield as a faint yellow powder. Mp. 96 °C decomposes. The IR (KBr, Cm^{-1}): 3450 (NH, amide),3040 (ArH), 1750 (C=O, ester), 1650 (C=O, amide), 1610, 1550, 1450 (Ar), 1110 (5- CH_3, thiazolyl). ^{1}H–NMR (C_2D_6SO): δ, 2.2 (s, 3H, $COCH_3$), 2.45 (s, 3H, 5-CH_3, thiazolyl), 3.5 (br, 1H, CONH), 7.15 (s, 1H, thiazolyl), 7.40, 7.35, 7.20 (m, 3H, ArH), 7.70, 7.45 (m, 2H, ArH_2F_2), 8.20 (s, 1H, $ArHF_2$). Anal. Calcd: ($C_{19}H_{14}N_2F_2O_3S$): C, 58.76; H, 3.60; N, 7.21. Found: C, 58.55; H, 3.6; N, 7.11.

5–(2,4–Difluorophenyl)–N–(5–methyl–3–isooxazolyl)–acetyl salicylamide (20)

Compound **17**, (5 g , 8.82 mmol), 3- amino-5-methylisooxazole (0.865 g , 8.82 mmol), zinc dust (0.008 g), glacial acetic acid (0.85 ml , 14.864 mmol), dioxane (40 ml), were prepared as described before in **8** to give compound **20** in 39% yield as a faint yellow powder. Mp. 150–152 °C. The IR (KBr, Cm^{-1}): 3255 (NH, amide), 3050 (ArH), 1745 (C=O, ester), 1630 (C=O, amide), 1605, 1560, 1450 (Ar), 1120 (5-CH_3, isooxazolyl). ^{1}H–NMR (C_2D_6SO): δ, 2.2 (s, 3H, $COCH_3$), 2.5 (s, 3H, 5-CH_3, isooxazole), 3.5 (br, 1H, CONH), 7.15 (s, 1H, isooxazole), 7.35, 7.20 (m, 3H, ArH), 7.65, 7.45 (m, 2H, ArH_2F_2), 8.15 (s, 1H, $ArHF_2$). Anal. Calcd: ($C_{18}H_{14}N_2F_2O_4$): C, 61.29; H, 3.76; N, 7.52. Found: C, 61.10; H, 3.8; N, 7.54.

5–(2,4–Difluorophenyl)–N–(5–(methylthio)–2–(1,3,4–thiadiazolyl)1– acetylsalicylamide (21)

Compound **17**, (5 g, 8.82 mmol), 2–amino-5-(methylthio)–1,3,4–thiadiazole (1.298 g , 8.82 mmol), zinc dust (0.008 g), glacial acetic acid (0.85 ml , 14.864 mmol), dioxane (60 ml), were prepared as described before in **8** to give Compound **21** in 47% as a faint yellow powder. Mp.164-167 °C. The IR (KBr, Cm^{-1}) 3450 (NH, amide), 3030 (ArH), 1750 (C=O, ester), 1650 (C=O, amide), 1610, 1550, 1450, (Ar). ^{1}H – NMR (C_2D_6SO): δ, 2.2 (s, 3H, $COCH_3$), 2.5 (s, 3H, S-CH_3), 3.5 (br, 1H, CONH), 7.35, 7.25, 7.20 (m, 3H, ArH), 7.60, 7.45 (m, 2H, ArH_2F), 8.20 (s, 1H, $ArHF_2$). Anal. Calcd: ($C_{18}H_{13}N_3F_2O_3S_2$): C, 51.35; H, 3.08; N, 9.97. Found: C, 51.22; H, 3.1; N, 9.95

N–(2–Thiazolyl) salicylamide (12)

Compound **8**, (2.62 g, 10 mmol) was dissolved in minimum volume of ethanol (95%). The solution was cooled to 18 °C, and then sodium hydroxide (6 ml, 12 mmol, 2 N) was added dropwise, with continuous stirring at 18 °C, during which the reaction mixture was checked by TLC, until the disappearance of methyl ester group in compound **8**, indicating a complete alkaline hydrolysis. Then the reaction mixture was acidified with HCL (6 ml, 12 mmol, 2 N), excess of cold water was added and the crude phenolic compound was precipitated. TLC showed a single spot. The recrystallization was carried out by using ethanol and water to yield compound **12** in 75% yield as white crystals. Mp. 280 °C decomposes. IR (KBr, Cm-1): 3600 – 3350 (br, OH), 3250 (NH, amide), 3050(CH, ArH), 1650 (C=O), 1610, 1560, 1450 (Ar). ^{1}H–NMR (C_2D_6SO): δ, 3.5 (br, 2H, OH, CONH), 7.10 (d, 1H, J=3.0Hz, N–CH, thiazolyl), 7.20 (d, 1H, J=3.0Hz, S–CH, thiazolyl), 7.60, 7.45, 7.25 (m, 4H, ArH), Anal. Calcd: (C_{10} $H_8N_2O_2S$): C, 54.32; H, 3.62; N, 12.367. Found: C, 54.12; H, 3.68; N, 12.71.

N–(5-Methyl–2–thiazolyl) salicylamide (13)

Compound **9**, was treated as described in **12** afforded compound **13** in 53 % yields as a white crystals, Mp. 243 - 245 °C. IR (KBr, Cm^{-1}): 3600 – 3350 (OH), 3250 (NH, amide), 2950 (CH, ArH), 1650 (C=O, amide), 1610, 1560, 1450 (Ar). ^{1}H–NMR (C_2D_6SO): δ, 2.5 (s, 3H, J=3.0Hz, 5-CH_3, thiazolyl), 3.5 (br, 2H, OH, CONH), 7.20 (s, 1H, thiazolyl), 7.50, 7.45, 7.30, (m, 4H, ArH), Anal. Calcd: ($C_{11}H_{10}N_2O_2S$): C, 56.33; H, 4.26; N, 11.94. Found: C, 56.40; H, 4.47; N, 11.85.

N–(5–Methyl–3–isooxazolyl) Salicylamide (14)

Compound **10**, was treated as described in **12** to give compound **14** in 67% yield as faint reddish crystals. Mp.176-177 °C. IR (KBr, Cm^{-1}): 3650 – 3350 (OH), 3250 (NH, amide), 2950 (CH, ArH), 1670 (C=O), 1600, 1550, 1450 (Ar). ^1H – NMR (C_2D_6SO): δ, 2.45 (s, 3H, 5-CH_3, isooxalyl), 3.5 (br, 2H, OH, CONH), 7.15 (s, 1H, isooxazole), 7.60, 7.40, 7.25, (m, 4H, ArH). Anal. Calcd: ($C_{11}H_{10}N_2O_3$): C, 60.48; H, 4.58; N, 12.70. Found: C, 60.43; H, 4.51; N, 12.83.

N–(5–Methylthio–2–(1,3,4–thiadiazolyl) Salicylamide (15)

Compound **11** was treated as described in the preparation of compound **12** to yield compound **15** in 63.5% as white crystalline powder. Mp. 251–253 °C. IR (KBr, Cm^{-1}): 3600 – 3250 (OH), 3250 (NH, amide), 1675 (C = O), 1600, 1450, 1400 (Ar). ^1H–NMR (C_2D_6SO): δ, 2.5 (s, 3H, 5-CH_3, thiadiazolyl), 3.5 (br, 2H, OH, CONH), 7.60, 7.45, 7.25 (m, 4H, ArH). Anal. Calcd: ($C_{10}H_9N_3O_2S_2$): C, 44.88; H, 3.36; N, 15.70. Found: C, 44.60; H, 3.74; N, 15.76.

5–(2,4–Difluorophenyl)–N–(2–thiazolyl) salicylamide (22)

Compound **18**, (5.67 g, 10 mmol) was treated as described in the preparation of **12** to liberate compound **22** in 63 .4 % yields as white crystalline powder. Mp. 251-253 °C. IR (KBr, Cm^{-1}), 3600 – 3350 (OH, phenolic), 3250 (NH, amide), 3035 (CH, ArH), 1675 (C=O, amide), 1600, 1550, 1500, 1450 (Ar), 1325, 1200 (C-O). ^1H–NMR (C_2D_6SO): δ, 3.5 (br, 2 H, OH, CONH), 7.10 (d, 1H, J=3.0Hz, N–CH, thiazolyl), 7.20 (d, 1H, J=3.0Hz, S – C, thiazolyl), 7.40, 7.2 (m, 3H, ArH), 7.45 (m, 2H, ArH_2F_2), 8.20 (s,1H, $ArHF_2$). Anal. Calcd: ($C_{16}H_{10}N_2F_2O_2S$): C, 57.77; H, 3.00; N, 8.42. Found: C, 57.78; H, 3.08; N, 8.4.

5–(2,4–Difluorophenyl)–N–(5–methyl–2–thiazolyl–salicylamide (23)

Compound **19** was treated through the same condition for the synthesis of compound **12** afforded compound **23** in 40% yields as white crystals. Mp. 297–299 °C. IR (KBr, Cm^{-1}) 3350 (OH), 3150 (NH, amide), 1675 (C=O, amide), 1600, 1550, 1450 (Ar). ^1H–NMR (C_2D_6SO): δ, 2.55 (s, 3H, 5-CH_3, thiazolyl), 3.5 (br, 2H, OH, CONH), 7.15 (s. 1H, thiazolyl), 7.45, 7.40, 7.2 (m, 3H, ArH) 7.65, 7.60, (m, 2H, ArH_2F_2), 8.15 (s, 1H, $ArHF_2$). Anal. Calcd: ($C_{17}H_{12}N_2F_2O_2S$): C, 58, 89; H, 3.46; N, 8.08. Found: C, 58.87; H, 3.45; N, 8.00.

5–(2,4–Difluorophenyl)N–(5–methyl–3–isooxazolyl)–salicylamide (24)

Compound **20** was treated as described for **12** to afford compound **24** in 45% yields as white crystals. Mp. 230-232 °C. IR (KBr, Cm^{-1}) 3350 (OH), 3200 (NH, amide), 1680 (C=O), 1610, 1550, 1450, (Ar). ^1H–NMR (C_2D_6SO): δ, 2.50 (s, 3H, 5-CH_3, isooxazolyl), 3.60 (br, 2 H, OH, CONH), 7.0 (s, 1H, isooxazolyl), 7.40, 7.30, 7.20 (m, 3H, ArH), 7.65 (m, 2H, ArH_2F_2), 8.2 (s, 1H, $ArHF_2$). Anal. Calcd: ($C_{17}H_{12}N_2F_2O_3$): C, 61.75; H, 3.63; N, 8.47. Found: C, 61.79; H, 3.65; N, 8.35.

5–(2,4–Difluorophenyl)–N–(5–methylthio–2–(1,3,4–thiadiazolyl)-salicylamide (25)

Compound **21** was treated as described for compound 1**2** to generate compound **25** in 45% yields as a white crystalline powder. Mp. 281–282 °C. IR (KBr, Cm^{-1}) 3500 (OH), 3150 (NH, amide), 3050(CH, ArH), 1650 (C=O), 1610, 1540, 1450 (Ar). ^1H–NMR (C_2D_6SO): δ, 2.5 (s, 3H, 5-CH_3, thiadiazolyl), 3.5 (br, 2H, OH, CONH), 7.45, 7.33 (m, 3H, ArH), 7.69, 7.50 (m, 2H, ArH_2F_2),8.1, (s, 1H, $ArHF_2$). Anal. Calcd: ($C1_6H_{11}N_3F_2O_2S_2$): C, 50.60; H, 2.89; N, 11.06. Found: C, 50.49; H, 2.81; N, 11.00

Pharmacology

Materials and methods:

Animals:

Adult male guinea pigs weighing 400±50 gm were used throughout the study. These were fed fresh plants, and provided with water ad libitum.

The animals were divided into 7 different groups as follows:

Group 1: 6 animals served as control received propylene glycol 50% vlv only.

Group 2: 6 animals received 2.55 mg/400 gm body weight of Indomethacin.

Group 3: 6 animals received Rofecoxib (in a dose based on the equimolecular dose to that of Indomethacin).

Group 4: 6 animals received test Compound #**15**.

Group 5: 6 animals received test Compound #**22**.

Group 6: 6 animals received test Compound #**23**.

Group 7: 6 animals received test Compound # **25**.

Note: All other prepared compounds were pharmacologically inactive.

Route of schedule of treatment:

The compounds and Rofecoxib were given (I.P.) as single daily doses for 7 days. Indomethacin was given twice daily (every 12 hours) for 7 days.

Control animals were given propylene glycol 50% v/v for 7 days.

The 7 – day treatment started after the induction of inflammation.

Experimental design:

A model of chronic inflammation was induced in all animals by S.C. implantation of cotton pellets of fixed weight (35±1 mg) according to the method described in[20, 21].

After 7 days administration of drug molecules, all animals were sacrificed by decapitation cotton pellets were removed, dried and weighed. The increase in the weight of cotton pellet is considered as an indication of inflammatory response by implanted cotton pellets. The anti-inflammatory effect of test compounds is inversely proportional to the increasing in the weight of cotton pellets. The presence of compound-induced gastric ulceration was examined both macroscopically and microscopically. The confirmed ulceration indicates the inhibition of COX-1 enzyme, and the non-selectivity of the test compounds.

Statistical processing of the results was done by using the test of analysis of variance (ANOVA).

RESULTS:

Determination of the anti-inflammatory activities of test drugs:

Table 1 shows the biological evaluation of the test compounds as indicated by changing the weight of granuloma induced by S.C. implantation of cotton pellets. According to the method, the most potent anti-inflammatory compound was compound **25**, followed by compounds **22** and **23**, and Indomethacin, then compound **15** and Rofecoxib. The respective values for granuloma reduction (% inhibition) were 64%, 59%, 57%, 52% and 51% for compounds **25**, **22**, **23**, indomethacin, **15** and Rofecoxib, respectively.

Table (1): The effect of compounds 15, 22, 23, 25, Indomethacin and Rofecoxib on changing the weight of Granuloma, induced by S.C. implantation of cotton pellets.

Group	Dose (mg/Kg)	Mean weight of dry gran-uloma(mg)*	S.D.	% inhibition	P*	P**
1 (vehicle)	7.5 ml/Kg	31.25	1.2145		<0.01	
2 (Indomethacin)	6.375	13.333	1.4376	57.227	<0.01	<0.01
3 (Rofecoxib)	5.575	15.416	1.1143	50.536	<0.01	<0.01
Compound 15	4.75	15.0833	1.4289	51.6044	<0.01	<0.01
Compound 22	5.9	12.75	1.8371	59.09	<0.01	<0.01
Compound 23	6.075	13.25	1.2942	57.486	<0.01	<0.01
Compound 25	6.75	11.00	0.7071	64. 7059	<0.01	<0.01

* The mean of granuloma of 6 animals (right and left body sides)

Determination of ulcerogenic effect of test drugs:

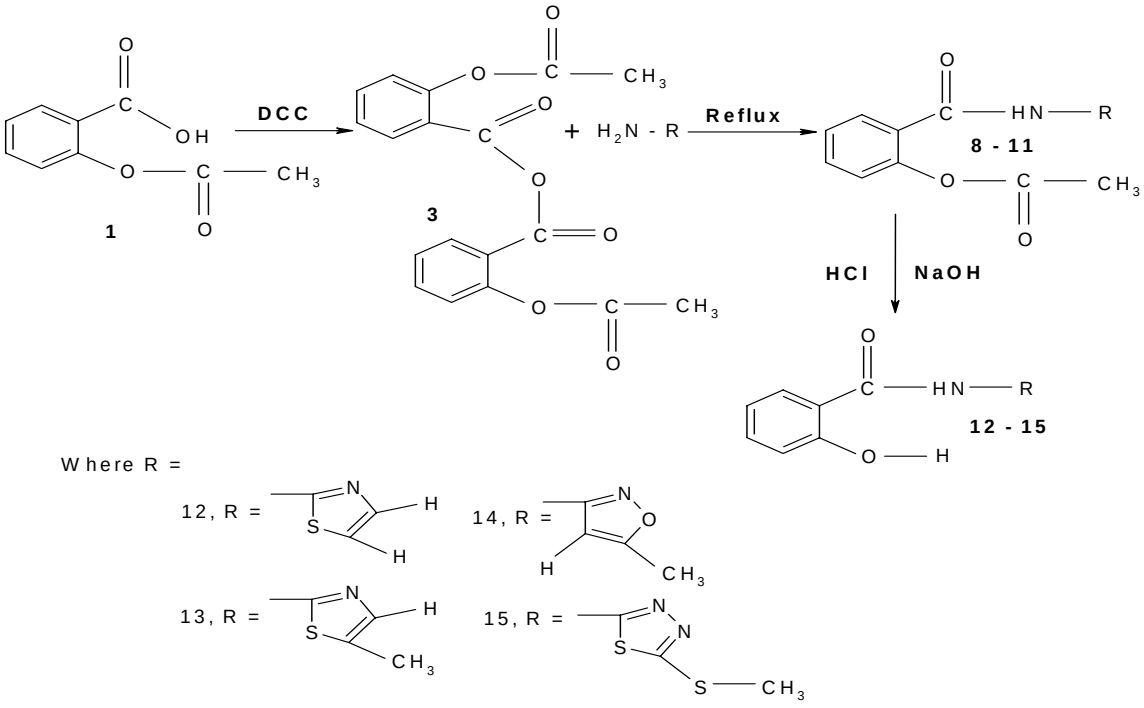
Macroscopic examination of the possible ulcerogenic effects of the equimolar dose of test compounds, indomethacin and rofecoxib using method described by Daidene *et al* (1994) showed that compound **22** had the most marked diffused hyperemia but less than indomethacin and rofecoxib. The microscopic examination showed results parallel to those of macroscopic evaluation.

Higher doses of test drugs and indomethacin (ulcerogenic dose) showed there were marked diffuse, thinning and diffuse hyperemia with numerous and deep ulceration, some of them perforated (e.g. indomethacin), others are not perforated (e.g. compound **15** and **22**). Compound **23** caused hyperemia without ulceration, while compound **25** was not toxic to gastric mucosa at all.

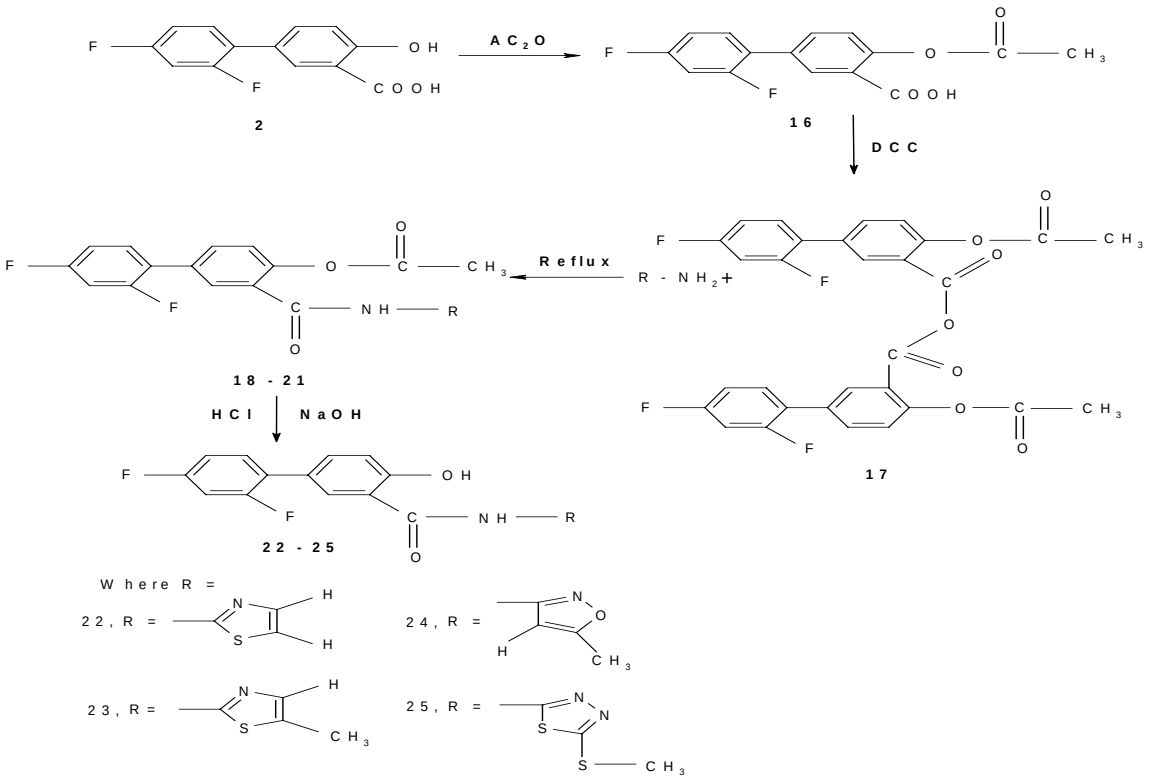
DISCUSSION:

Chemistry:

The synthetic routes for preparation of the target compounds are outlined in schemes (**I** and **II**). Aspirin (**1**) was converted to the corresponding aspirin anhydride (**3**) through reaction with dicyclohexylcarbodimide (DCC) as coupling reagent in methylene dichloride[15-17]. Conversion of the (**3**) to amides (**8-11**) upon treatment with various aminosubstituted heterocyclic rings in the presence of Zn dust as catalyst to accelerate the formation of compounds (**8-11**). Hydrolysis of the acetate group in compounds (**8-11**) generated the designed salicylamide derivatives (**12-15**). IR, H-NMR spectra and Elemental analysis were consistent with assigned structures as shown in the experimental part.



Scheme I



Scheme II

Diflunisal derivatives (**22- 25**) were prepared according to the method shown in scheme (II). Acylation of the phenolic group in diflunisal with acetic anhydride as protecting group to prevent the interference of the phenolic group in subsequent reactions. Compound (**16**) was converted to its corresponding anhydride (**17**) upon treatment with (DCC) in acidified methylene chloride. The acylated diflunisal anhydride (**17**) upon treatment with various selected aminosubstituted heterocyclic rings, yielded the amide derivatives of acylated diflunisal (**18 - 21**). Removal of the acetate group in (**18-21**) resulted in the desired diflunisal derivatives (**22-25**)[18, 19]. IR, H–NMR and Elemental analysis were consistent with the assigned structures as described in the experimental part.

Pharmacology

The anti-inflammatory activity of the proposed compounds indicate that 5–(2,4–difluorophenyl)–N–[5–methylthio–2–(1,3–4–thiadiazolyl)] salicylamide (**25**) is the most active one among the active synthesized salicylamide derivatives (**15**, **22**, **23**, **25)** and to indomethacin and rofecoxib as reference drugs. The least activity was showed by compound **15** in our series compared to compounds **25**, **22** and **23** may be attributed to the absence of 2,4–difluorophenyl group at the p–position of salicylamide, which is required for effective overlap with COX enzyme, and this is in agreement with what is seen with many anti – inflammatory drugs such as mefenamic acid, ketoprofen, nabumetone and many others[7,24,25]. Comparison of the activity of **25**, **23** and **22** can recognize the importance of the substituent on the heterocyclic ring; methylthio contributes more than methyl and least in unsubstituted heterocyclic ring (see compounds **23** and **22**). However, this can not rule out the contribution of the heterocyclic ring as seen in compound **25**.

Early reports indicated that indomethacin showed effective anti–inflammatory activity with profound

ulcerogenic side effects while rofecoxib showed less potent anti–inflammatory effect with mild toxicity on gastric mucosa. This property of rofecoxib was attributed to COX2 selectivity[23]. Accordingly, the microscopic and macroscopic examinations of ulcerogenic effect of the newly synthesized compounds **15**, **22**, **23**, **25**, indomethacin and rofecoxib indicate that indomethacin showed greater ulcerogenic effect as characterized by marked diffuse, thinning and diffuse hyperemia with numerous and deep ulceration, some of which with perforation. Compounds **15** and **22** showed similar effect to indomethacin but no sign of perforation; compound **23** caused hyperemia without ulceration, while compound **25** was not toxic to gastric mucosa at all.

CONCLUSIONS

The result presented in the pharmacology section suggest that compound **25** in this novel series possess a profound anti–inflammatory activity with no or negligible ulcerogenic activity. The above observations indicate selectivity to inhibit COX2.

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التخليق والتقييم الفارماكولوجي لمضادات الإلتهاب لمشتقات الامايد لحامض السلسليك والداي فلونيسال الجديدة

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ملخـص

تم تخليق مجموعة من المركبات الجديدة من خلال تحويل مجموعة الكاربوكسيل في حامض السلسليك ومركب الداي فلونيسال إلى مشتقات الأمايد لمجاميع مختلفة من الحلقات غير المتجانسة متمثلة في 2-امينو-5-مثيل-ثايوزول و 3-امينو-5-مثيل-آزوكسوزول، 2-امينو-5-مثيل-1,3,4- ثايودايوزول و2-أمينو- ثايوزول. فاعلية هذه المركبات في تثبيط الاحتقان تم قياسها من خلال النسبة المئوية لتثبيط تكون الاحتقان الناتج من وضع حبيبات قطنية تحت الجلد وأما قابليتها على إحداث القرحة المعوية فقد قيست بدراسة التغيرات النسيجية التي طرأت على غشاء معدة الحيوانات بعد إعطاء جرع مختلفة من المركبات المخلفة مقارنة مع جرع علاجية لأدوية معروفة كعقار الاندوميثاسين والروفيكوكسب. أظهرت النتائج أن مركبنا المرقم 25 أظهر فعالية أفضل كمضاد للاحتقان وبدون التسبب بالقرحة من عقاري الاندوميثاسين والروفيكوكسب.

الكلمـات الدالـة: مثبط الاحتقان، مشتقات السلسليك، مشتقات الداي فلونيسال.

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