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Design and synthesis of new pyrido[2,3-*d*]pyrimidine-1,4-dione derivatives as anti-inflammatory agents

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ABSTRACT

*Virtual screening against the active site of cyclooxygenase-2 (COX-2) enzyme identified 3-ethyl-5-methyl-7-phenylpyrido[2,3-d]pyrimidine-2,4(1H,3H)-dione (**1**) as a potential inhibitor. Biological evaluation revealed **1** possesses a moderate level of anti-inflammatory activity in the paw induced edema rat model (60% edema inhibition at a dose of 10 mg/kg). Docking studies highlighted the possibility of hydrogen bonding and hydrophobic interactions between **1** and the COX-2 acrive site. Using an approach based on molecular modeling-guided lead optimisation, new pyridopyrimidine analogues were designed. The designed pyrido[2,3-d]pyrimidine-1,4-dione derivatives were synthesized from the corresponding 6-aminouracil derivatives in a one-step simple reaction with very good to excellent yields. Anti-inflammatory results showed that some drivatives are more potent than indomethacin (relative potency of 105%, 106% and 109%). The ulecerogenic effect of selected derivatives was examined in vivo reavealing greater gastric safety than indomethacin and highlighting possible COX-2 selectivity for these compounds.*

Keywords: pyrido[2,3-d]pyrimidine-1,4-diones, COX-2 inhibitors, molecular modeling, anti-inflammatory.

INTRODUCTION

Since the first use of aspirin in the treatment of inflammation in 1897, many non-steroidal anti-inflammatory drugs (NSAIDs) have entered the clinic [1]. The main indications for this drug class are to treat inflammation and painful symptoms associated with rheumatoid arthritis, tissue lesions, fever and infectious diseases [2]. More recent applications for NSAIDs include cancer chemoprevention [3, 4] and protection against Alzheimer's disease [3, 5, 6]. Despite the many NSAIDs in the current clinical use, inflammation remains a common and poorly controlled problem which can be life threatening in extreme forms of allergy, autoimmune disease and rejection of transplanted organs. NSAIDs can exert their action through the inhibition of cyclooxygenase (COX) enzymes, which mediate the production of prostaglandins, prostacyclins and thromboxanes from arachidonic acid. Two COX isoforms are known, COX-1 which plays a physiological role in the kidneys and the stomach, and COX-2 which is involved in the production of prostaglandins mediating pain and supports the inflammation.

Unfortunately, the use of NSAIDs has been connected with serious side effects, most commonly gastrointestinal ulceration and bleeding even with the use of low prophylactic doses [7-9]. These side effects were attributed to the inhibition of COX-1 isoform, a problem that was thought to be solved with the development of selective COX-2 inhibitors. However, many of the approved COX-2 selective inhibitors were withdrawn from the market due to

associated cardiovascular side effects [10]. For these reasons, the development of new anti-inflammatory agents remains an active area for medicinal chemists to explore.

Interest in pyrido[2,3-*d*]pyrimidine derivatives has increased dramatically in recent years, based upon their diverse range of biological properties. These molecules were reported to show highly species-specific responses as antitumour [11, 12], antibacterial [13-16], anti-inflammatory [17, 18] and antimicrobial agents [19-21]. Additionally, some pyrido[2,3-*d*]pyrimidines compounds have been reported as anti-fungal [22], phosphodiesterase inhibitors [23] and antiviral agents [24]. Therefore, the search for new routes for the synthesis of pyrido[2,3-*d*]pyrimidines derivatives has attracted considerable attention aiming for a rapid entry to these heterocycles. Examples for the reported approaches towards the pyridopyrimidine scaffold include the reaction of benzylidene derivatives of malonitrile and 6-amin-3,4-dihydropyrimidine [24, 25]; the reaction of 6-amino-1-thio uracil with ethyl-3-phenyl-2-cyano-acrylate [26, 27], the three component reaction of aldehydes, alkyl nitriles and aminopyrimidines in water and in the presence of KF-Al_2O_3 or TE-BAC as catalysts [28, 29]. Some of the reported methods have their drawbacks such as the need for a multi-step synthesis procedure with low yielding reactions and the use of expensive and/or potentially toxic reagents. Thus the development of efficient method for synthesis of rapid access to pyrido[2,3-d]pyrimidines would be highly valuable.

Herein, the structure-based design of pyrido[2,3-d]pyrimidine-1,4-diones of potential anti-inflammatory activity and their synthesis from 6-aminouracil derivatives is reported.

MATERIALS AND METHODS

Molecular Modeling

LibDock function implemented in Accelrys Discovery Studio 3.0 package (Accelrys, Inc., San Diego, USA) and the crystal structure 3LN1 (after omission of the co-crystalized ligand and other accessories) were used to virtually screen an *in-house* library of 2000 ligands. A relaxed Lipinski filter (MW<600, HBA<12, and HBD<6) was used to filter non-lead like compounds. Each ligand was assigned a score reflecting the quality of the ligand-receptor complex. The top ranked 10 compounds were visually inspected using Molecular Operating Environment (MOE 2013.08) software (Chemical Computing Group Inc., QC, Canada) to insure proper interactions and were selected for biological evaluation and lead-optimization. Data for only one of the defined hits is presented in this manuscript.
Molecular docking into COX-2 enzyme was carried out using the LigandFit software [30]. In all docking experiments the binding pocket was identified as the volume of the co-crystallized ligand. The input ligands were subjected to systematic conformational search using the "Generate conformations" options. Each conformer was docked into the assigned binding site using Dreiding/Gasteiger as the "Energy Grid Forcefield". All other parametrs were set to their default values by the software. The highest ranked docking pose for each ligand was extracted and saved separately as a PDB file. Each complex was visualized and energy relaxed using OPLS-AA forcefield within MOE software and then inspected for potential interactions.

Chemistry

All reactions were performed using standard laboratory equipment and glassware. Solvents and reagents were purchased from Sigma-Aldrich or MERCK and used as received. Melting points (mp) were determined on an electrothermal Stuart Scientific SMP1 melting point apparatus and were uncorrected. Thin-layer chromatography (TLC, R_f values) was carried out using TLC aluminum sheets kieselgel 60 F254 (MERCK) and dichloromethane-methanol (9:1 or 8.5:1.5) as a mobile phase. Visualization was effected with ultraviolet lamp Spectroline ENF-240C/F at short wavelength (λ = 254 nm). Not all chemical yields were optimized and they generally represented the findings of a single experiment. IR spectra were recorded on a Shimadzu spectrophotometer (IR-470) as potassium bromide discs at the Faculty of Pharmacy, Assiut University. Some ^{1}HNMR spectra were recorded on a Varian EM-360 60 MHz spectrometer at the Faculty of Pharmacy, Assiut University or Bruker DPX 500 MHz at faculty of Pharmacy, King Saud University, Saudia Arabia. DMSO-d_6 was used as a solvent, unless otherwise specified, the chemical shifts were given in δ (ppm), and coupling constants (J) were in Hertz (Hz). Chemical shifts are expressed either relative to tetramethylsilane (TMS) as an internal standard or to the chemical shifts of the remaining protons of DMSO-d_6. Protons of NH and OH groups were confirmed by D_2O exchange experiment. Electrospray mass spectra (ESI) were recorded using or JOEL JMS600 mass spectrometer at the Unit of Microanalysis, Assiut University. Microanalyses for elements C, H, and N were performed on Perkin-Elmer 240 elemental analyzer at the Unit of Microanalysis, Faculty of Science, Cairo University, Egypt.

General procedure for the synthesis of intermediates 2-4 [31, 32]
The di-alkyl urea derivative (0.1 mol) and cyanoacetic acid (0.12 mol) were dissolved in acetic anhydride (40 ml), and the mixture was heated at 80 °C for 2 hours. The resulting mixture was evaporated under reduced pressure and the obtained residue was diluted with water (20 ml). The mixture was cooled to 0-5 °C and aqueous NaOH (70%, 25 ml) was added and the mixture was stirred at room temperature for 1 hour. The obtained precipitate was filtered off, washed with water, dried under vacuum and recrystallized from hot ethanol.

*6-Amino-1,3-dimethylpyrimidine-2,4(1H,2H)-dione (**2**)*
White solid, yield = 77%; mp 305-307 °C (ethanol). ^{1}H-NMR (400 MHz): 6.60 (br s, 2H, NH_2), 6.40 (s, 1H, C5-H), 3.35 (s, 3H, N3-CH_3), 3.15 (s, 3H, N3-CH_3); ^{13}C-NMR (100 MHz): 161.55 C4, 154.32 C6, 151.30 C2, 78.30 C5, 31.50 N3-CH_3, 28.25 N1-CH_3.

*6-Amino-1,3-diethylpyrimidine-2,4(1H,2H)-dione (**3**)*
White solid, yield = 70%; mp 148-150°C (ethanol). ^{1}H-NMR (400 MHz, DMSO-d_6): 6.64 (br s, 2H, NH_2), 6.33 (s, 1H, C5-H), 3.70 (q, *J* = 7.20 Hz, 2H, 1"-CH_2), 3.55 (q, *J* = 7.20 Hz, 2H, 1'-CH_2), 1.35 (t, *J* = 7.20 Hz, 3H, 2"-CH_3), 0.95 (t, *J* = 7.20 Hz, 3H, 2'-CH_3); ^{13}C-NMR (100 MHz): 161.10 C4, 154.34 C6, 151.10 C2, 76.30 C5, 39.32 C1", 37.80 C1', 13.70 C2", 11.95 C2'.

*6-Amino-1,3-dipropylpyrimidine-2,4(1H,2H)-dione (**4**)*
It was prepared as previously reported procedure as white solid, yield = 73%; mp 138-140 °C (ethanol). ^{1}H-NMR (400 MHz, DMSO-d_6): 6.74 (br s, 2H, NH_2), 6.46 (s, 1H, C5-H), 3.72 (t, *J* = 7.20 Hz, 2H, 1"-CH_2), 3.65 (t, *J* = 7.20 Hz, 2H, 1'-CH_2), 1.42-1.55 (m, 4H, 2'-CH_2 & 2"-CH_2), 0.84 (t, *J* = 7.20 Hz, 3H, 3"-CH_3), 0.79 (t, *J* = 7.20 Hz, 3H, 3'-CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6): 161.38 C4, 154.50 C6, 151.45 C2, 75.30 C5, 41.38 C1", 40.20 C1', 20.80 C2", 20.50 C2', 11.30 C3", 10.75 C3'.

*6-Amino-3-ethylpyrimidine-2,4(1H,2H)-dione (**5**)*
It was prepared as previously reported procedure [33] and obtained as a white solid, yield = 72%; mp 260-262°C (ethaol). ^{1}H-NMR (400 MHz, DMSO-d_6): 10.23 (s, 1H, N1-H), 6.16 (br s, 2H, NH_2), 4.55 (s, 1H, C5-H), 3.45 (q, *J* = 7.20 Hz, 2H, 1'-CH_2), 0.98 (t, *J* = 7.20 Hz, 3H, 2'-CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6): 163.10 C4, 154.10 C6, 151.30 C2, 74.30 C5, 38.70 C1', 11.85 C2'.

*6-Amino-3-propylpyrimidine-2,4(1H,2H)-dione (**6**)*
It was prepared as previously reported procedure [33] as white solid, yield = 81%; mp 275-277 °C (ethanol). ^{1}H-NMR (400 MHz, DMSO-d_6): 10.50 (s, 1H, N1-H), 6.10 (br s, 2H, NH_2), 6.56 (s, 1H, C5-H), 3.82 (t, *J* = 7.20 Hz, 2H, 1'-CH_2), 1.32-1.45 (m, 2H, 2'-CH_2), 0.94 (t, *J* = 7.20 Hz, 3H, 3'-CH_3), 0.79 (t, *J* = 7.20 Hz, 3H, 3'-CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6): 164.18 C4, 153.90 C6, 151.70 C2, 74.15 C5, 40.30 C1', 21.50 C2', 11.10 C3'. The data was in accordance with reported data.

General procedure for preparation of pyrido[2,3-d]pyrimidine-2,4(*1H,3H*)-dione derivatives 1 and 7-23.
Substituted 6-aminouracil derivative **2-6** or non-substituted 6-aminouracil (0.1 mol) and the appropriate acetylacetone, benzoylacetone or 4-methoxybenzoylacetone (0.1 mol) were dissolved in glacial acetic acid (10-12 mL) and the resulted solution was refluxed for 6-8 h. The reaction progress was monitored using TLC (9:1 DCM:MeOH as TLC solvent). Upon completion of the reaction, the reaction mixture was poured into ice-cooled water and made basic to litmus paper using concentrated ammonia solution (27%). The formed precipitate was filtered off and recrystallized from the appropriate solvent or purified using column chromatography to afford the final products **1** and **7-23**.

*3-Ethyl-5-methyl-7-phenylpyrido[2,3-d]pyrimidine-2,4(1H,3H)-dione (**1**)*
The compound was prepared by above general procedure and it was obtained as pale yellow solid which was purified via column chromatography (DCM:MeOH; 9:1) , yield = 85%; mp 184-186 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): 11.76 (br s, 1H, N1H/N3H), 8.12-8.15 (m, 2H, 2",6"-H_{arom}), 7.49-7.56 (m, 3H, 3",4",5"-H_{arom}), 6.89 (s, 1H, C6-H), 3.91 (q, *J* = 7.20 Hz, 2H, 1'-CH_2), 2.76 (s, 3H, 5-CH_3), 1.16 (t, *J* = 7.20 Hz, 3H, 2'-CH_3). ^{13}C-NMR (125 MHz, DMSO-d_6): 162.88 C4=O, 161.65 C2=O, 152.83 C8a, 151.57 C7, 149.83 C5, 138.99 C1", 136.78 C3" & C5", 128.79 C2", 128.17 C6", 127.44 C4", 122.11 C6, 106.54 C4a, 35.01 C1', 23.95 5-CH3, 12.73 C2'. IR (KBr) ν, cm^{-1}: 3300 (NH), 3090, 2955 (CH), 1692 (C=O), 1642 (C=O), 1597 (C=N), 1551 (C=C). MS: (m/z) $[M]^+$ 281.10. Anl. Calcd. for $C_{16}H_{15}N_3O_2$: C, 68.31; H, 5.37; N, 14.94. Found: C, 68.55; H, 5.55; N, 14.88.

*5-Methyl-7-phenyl-3-propylpyrido[2,3-d]pyrimidine-2,4(1H,3H)-dione (**7**)*
The compound was prepared by above general procedure and was obtained as yellow solid, yield = 81%; mp 195-197 °C (acetic acid/water). ^{1}H-NMR (60 MHz, DMSO-d_6): 11.20 (br s, 1H, N1H), 8.00-8.25 (m, 2H, 2",6"-H_{arom}), 7.35-7.70 (m, 4H, 3",4",5"-H_{arom} and C6-H), 4.00 (q, *J* = 7.20 Hz, 2H, 1'-CH_2), 2.90 (s, 3H, 5-CH_3), 1.55-1.95 (m, 2H, 2'-CH_2), 1.20 (t, *J* = 7.20 Hz, 3H, 3'-CH_3).; ^{13}C-NMR (125 MHz, DMSO-d_6): 162.67 C4=O, 161.70 C2=O, 153.11 C8a, 150.54 C7, 141.93 C5, 138.89 C1", 136.80 C3" & C5", 128.69 C2", 128.37 C6", 127.44 C4", 122.14 C6, 99.82 C4a, 40.10 C1', 26.50 5-CH_3, 22.80 C2', 11.60 C3'. IR (KBr) ν, cm^{-1}: 3390 (NH), 3105, 2955 (CH), 1700 (C=O), 1643 (C=O), 1604 (C=N), 1560 (C=C). MS: (m/z) $[M+1]^+$ 296.14. Anl. Calcd. for $C_{17}H_{17}N_3O_2$: C, 69.14; H, 5.80; N, 14.23. Found: C, 69.09; H, 5.54; N, 14.24.

*5-Methyl-7-phenylpyrido[2,3-d]pyrimidine-2,4(1H,3H)-dione (**8**)*
The compound was prepared by above general procedure and it was obtained as white solid which was recrystallized from ethanol, yield = 82%; mp 143-145 °C. ^{1}H-NMR (500 MHz, DMSO-d_6): 10.90 (br s, 2H, N1H and N3H), 7.89-7.98 (m, 2H, 2',6'-H_{arom}), 7.51-7.57 (m, 3H, 3',4',5'-H_{arom}), 6.66 (s, 1H, C6-H), 2.51 (s, 3H, 5-CH_3). ^{13}C-NMR (125 MHz, DMSO-d_6): 162.57 C4=O, 161.74 C2=O, 152.78 C8a, 150.14 C7, 140.83 C5, 138.99 C1', 136.78 C3' & C5', 128.79 C2', 128.16 C6', 127.21 C4', 121.44 C6, 96.86 C4a, 25.60 5-CH_3. IR (KBr) ν, cm^{-1}: 3370 (NH), 3145 (NH), 3020, 2905 (CH), 1590 (C=N), 1554 (C=C), 1709(C=O), 1665 (C=O). MS (ESI+) (m/z): $[M]^+$ 253.09. Anl. Calcd. for $C_{14}H_{11}N_3O_2$: C, 66.40; H, 4.38; N, 16.59. Found: C, 66.69; H, 4.78; N, 16.22.

*7-(4"-methoxyphenyl)-5-methyl-3-propylpyrido[2,3-d]pyrimidine-2,4(1H,3H)-dione (**9**)*
The compound was prepared by above general procedure and it was obtained as dark yellow solid, yield = 78%; mp 214-216 °C (ethanol). ^{1}H-NMR (60 MHz, DMSO-d_6): 10.90 (br s, 1H, N1H), 8.20 (d, *J* = 8.50 Hz, 2H, 2",6"-H_{arom}), 7.40 (s, 1H, C6-H), 7.15 (d, *J* = 8.50 Hz, 2H, 3",5"-H_{arom}), 4.10 (q, *J* = 7.20 Hz, 2H, 1'-CH_2), 2.90 (s, 3H, 5-CH_3), 1.70-2.00 (m, 2H, 2'-CH_2), 1.20 (t, *J* = 7.20 Hz, 3H, 3'-CH_3). ^{13}C-NMR (125 MHz, DMSO-d_6): 162.10 C4=O, 161.54 C2=O, 153.38 C8a, 151.83 C7, 140.87 C5, 139.40 C1", 136.42 C3", 136.48 C5", 129.74 C2", 129.40 C6", 128.10 C4", 123.14 C6, 100.86 C4a, 59.86 4"-OCH_3, 40.12 C1', 29.50 5-CH_3, 25.70 C2', 11.40 C3'. IR (KBr) ν, cm^{-1}: 3400 (NH), 3065, 2930 (CH), 1702 (C=O), 1645 (C=O), 1598 (C=N), 1548 (C=C). MS (ESI+): (m/z) $[M]^+$ 325.15. Anl. Calcd. for $C_{18}H_{19}N_3O_3$: C, 66.45; H, 5.89; N, 12.91. Found: C, 66.63; H, 5.68; N, 12.92.

*3-Ethyl-7-(4"-methoxyphenyl)-5-methylpyrido[2,3-d]pyrimidine-2,4(1H,3H)-dione (**10**)*
The compound was prepared by above general procedure and it was obtained as yellow solid, yield = 79%; mp 215-217 °C (ethanol). ^{1}H-NMR (60 MHz, DMSO-d_6): 10.70 (br s, 1H, N1H), 8.10 (d, *J* = 8.50 Hz, 2H, 2",6"-H_{arom}), 7.40 (s, 1H, C6-H), 7.10 (d, *J* = 8.50 Hz, 2H, 3",5"-H_{arom}), 4.20 (q, *J* = 7.40 Hz, 2H, 1'-CH_2), 3.70 (s, 3H, 4"-O-CH_3), 2.90 (s, 3H, 5-CH_3), 1.30 (t, *J* = 7.20 Hz, 3H, 2'-CH_3).; ^{13}C-NMR (125 MHz, DMSO-d_6): 162.17 C4=O, 161.24 C2=O, 152.38 C8a, 150.73 C7, 140.57 C5, 139.30 C1", 136.40 C3", 136.45 C5", 129.64 C2", 129.40 C6", 128.30 C4", 122.74 C6, 99.86 C4a, 58.86 4"-OCH_3, 35.81 C1', 24.95 5-CH3, 12.22 C2'. IR (KBr) ν, cm^{-1}: 3275 (NH), 3065, 2950 (CH), 1707 (C=O), 1645 (C=O), 1591 (C=N), 1565 (C=C). MS (ESI) (m/z): $[M]^+$ 311.12. Anl. Calcd. for $C_{17}H_{17}N_3O_3$: C, 65.58; H, 5.50; N, 13.50. Found: C, 65.38; H, 5.35; N, 13.62.

*7-(4'-Methoxyphenyl)-5-methylpyrido[2,3-d]pyrimidine-2,4(1H,3H)-dione (**11**)*
The compound was prepared by above general procedure and it was obtained as yellow solid which was recrystallized from acetic acid/water mixture, yield = 82%; mp 155-158 °C. ^{1}H-NMR (60 MHz, DMSO-d_6):: 10.60 (br s, 2H, N1H and N3H), 7.94 (d, *J* = 8.50 Hz, 2H, 2',6'-H_{arom}), 7.06 (d, *J* = 8.50 Hz, 2H, 3',5'-H_{arom}), 6.74 (s, 1H, C6-H), 3.85 (s, 3H, 4'-O-CH_3), 2.16 (s, 3H, 5-CH_3). ^{13}C-NMR (125 MHz, DMSO-d_6): 161.87 C4=O, 160.94 C2=O, 152.58 C8a, 150.33 C7, 140.87 C5, 139.12 C1', 136.58 C3' & C5', 129.74 C2', 128.40 C6', 127.30 C4', 122.14 C6, 98.86 C4a, 57.66 4'-OCH_3, 25.60 5-CH_3. IR (KBr) ν, cm^{-1}: 3400 (NH), 3180 (NH), 3045, 2935 (CH), 1710 (C=O), 1660 (C=O), 1593 (C=N), 1524 (C=C). MS: (m/z) $[M+H]^+$ 284.10. Anl. Calcd. for $C_{15}H_{13}N_3O_3$: C, 63.60; H, 4.63; N, 14.83. Found: C, 63.38; H, 5.00; N, 15.27.

*5,7-Dimethyl-3-propylpyrido[2,3-d]pyrimidine-2,4(1H,3H)-dione (**12**)*
The compound was prepared by above general procedure and it was obtained as yellow solid, yield 80%; mp 158-160 °C (ethanol). ^{1}H-NMR (60 MHz, DMSO-d_6): 10.30 (br s, 1H, N1H), 7.00 (s, 1H, C6-H), 4.10 (q, *J* = 7.20 Hz, 2H, 1'-CH_2), 2.90 (s, 3H, 5-CH_3), 2.70 (s, 3H, 7-CH_3), 1.40-1.90 (m, 2H, 2'-CH_2), 1.25 (t, *J* = 7.20 Hz, 3H, 3'-CH_3). ^{13}C-NMR (125 MHz, DMSO-d_6): 162.47 C4=O, 161.64 C2=O, 152.68 C8a, 150.34 C7, 140.93 C5, 121.24 C6, 97.86 C4a, 40.30 C1', 28.70 7-CH_3, 24.50 5-CH_3, 21.80 C2', 11.80 C3'.; IR (KBr) ν, cm^{-1}: 3370 (NH), 3105, 2940

(CH), 1699 (C=O), 1643 (C=O), 1600 (C=N), 1553 (C=C). MS: (m/z) $[M+H]^+$ 234.14. Anl. Calcd. for $C_{12}H_{15}N_3O_2$: C, 61.79; H, 6.48; N, 18.01. Found: C, 61.83; H, 6.21; N, 18.29.

*3-Ethyl-5,7-dimethylpyrido[2,3-d]pyrimidine-2,4(1H,3H)-dione (**13**)*
The compound was prepared by above general procedure and it was obtained as white solid, yield = 87%; mp 179-181°C (ethanol). ^{1}H-NMR (500 MHz, DMSO-d_6): 11.62 (br s, 1H, N1H), 6.91 (s, 1H, C6-H), 3.89 (q, *J* = 7.20 Hz, 2H, 1'-CH_2), 2.63 (s, 3H, 7-CH_3), 2.43 (s, 3H, 5-CH_3), 1.14 (t, *J* = 7.20 Hz, 3H, 2'-CH_3). ^{13}C-NMR (125 MHz, DMSO-d_6): 162.57 C4=O, 161.74 C2=O, 151.72 C8a, 151.33 C7, 149.72 C5, 121.37 C6, 105.31 C4a, 34.93 C1', 29.11 7-CH_3, 26.83 5-CH_3, 11.83 C2'. IR (KBr) ν, cm^{-1}: 3335 (NH), 3095, 2955 (CH), 1699 (C=O), 1643 (C=O), 1602 (C=N), 1551 (C=C). MS (ESI+): (m/z) $[M+H]^+$ 220.12. Anl. Calcd. for $C_{11}H_{13}N_3O_2$: C, 60.26; H, 5.98; N, 19.17. Found: C, 60.01; H, 6.00; N, 18.92.

*5,7-Dimethylpyrido[2,3-d]pyrimidine-2,4(1H,3H)-dione (**14**)*
The compound was prepared by above general procedure and it was obtained as white solid which was purified by column chromatography (DCM:MeOH; 9:1), yield = 83%; mp 290-292 °C. ^{1}H-NMR (60 MHz, DMSO-d_6): 11.10 (br s, 2H, N1H and N3H), 6.70 (s, 1H, C6-H), 2.70 (s, 3H, 7-CH_3), 2.50 (s, 3H, 5-CH_3). ^{13}C-NMR (125 MHz, DMSO-d_6): 162.47 C4=O, 161.64 C2=O, 152.68 C8a, 150.34 C7, 140.93 C5, 121.24 C6, 97.86 C4a, 28.70 7-CH_3, 26.50 5-CH_3. IR (KBr) ν, cm^{-1}: 3380 (NH), 3155 (NH), 3040, 2895 (CH), 1700(C=O), 1645 (C=O), 1596 (C=N), 1553 (C=C). MS (ESI+) (m/z): $[M]^+$ 191.08. Anl. Calcd. for $C_9H_9N_3O_2$: C, 56.54; H, 4.74; N, 21.98. Found: C, 56.88; H, 5.12; N, 21.67.

*1,3,5,7-tetramethylpyrido[2,3-d]pyrimidine-2,4(1H,3H)-dione (**15**)*
The compound was prepared by above general procedure and it was obtained as white solid, yield = 87%; mp 193-195 °C (ethanol). ^{1}H-NMR (500 MHz, DMSO-d_6): 6.95 (s, 1H, C6-H), 3.84 (s, 3H, 7-CH_3), 3.40 (s, 3H, 5-CH_3), 2.79 (s, 3H, 3-CH_3), 2.60 (s, 3H, 1-CH_3). ^{13}C-NMR (125 MHz, DMSO-d_6): 160.82 C4=O, 160.29 C2=O, 153.11 C8a, 150.80 C7, 139.18 C5, 117.07 C6, 106.71 C4a, 32.28 N3-CH_3, 30.68 N1-CH_3, 28.10 7-CH_3, 25.65 5-CH_3. IR (KBr) ν, cm^{-1}: 3065, 2935 (CH), 1689 (C=O), 1649 (C=O), 1588 (C=N), 1549 (C=C). MS (ESI+) (m/z): $[M]^+$ 219.16. Anl. Calcd. for $C_{11}H_{13}N_3O_2$: C, 60.26; H, 5.98; N, 19.17. Found: C, 60.48; H, 6.26; N, 18.89.

*1,3-Diethyl-5,7-dimethylpyrido[2,3-d]pyrimidine-2,4(1H,3H)-dione (**16**)*
The compound was prepared by above general procedure and it was obtained as white solid, yield = 81%; mp 139-141 °C (ethanol). ^{1}H-NMR (500 MHz, DMSO-d_6): 7.10 (s, 1H, C6-H), 4.26 (q, *J* = 7.40 Hz, 2H, N3-1"-CH_2), 3.94 (q, *J* = 7.40 Hz, 2H, N1-1'-CH_2), 2.7 (s, 3H, 7-CH_3), 2.47 (s, 3H, 5-CH_3), 1.20 (t, *J* = 7.40 Hz, 3H, N3-2"-CH_3), 1.15 (t, *J* = 7.40 Hz, 3H, N3-2'-CH_3). 13C-NMR (125 MHz, DMSO-d_6): 161.93 C4=O, 160.73 C2=O, 152.29 C8a, 150.29 C7, 149.70 C5, 121.45 C6, 106.40 C4a, 37.16 N3-C1", 35.98 N1-C1', 24.33 7-CH_3, 21.76 C5-CH_3, 12.84 N3-C2", 11.12 N1-C2'. IR (KBr) ν, cm^{-1}: 3080, 2955 (CH), 1688 (C=O), 1643 (C=O), 1587 (C=N), 1550 (C=C). MS (ESI+) (m/z): $[M+H]^+$ 248.16. Anl. Calcd. for $C_{13}H_{17}N_3O_2$: C, 63.14; H, 6.93; N, 16.99. Found: C, 62.85; H, 7.05; N, 16.78.

*5,7-Dimethyl-1,3-diperopylpyrido[2,3-d]pyrimidine-2,4(1H,3H)-dione (**17**)*
The compound was prepared by above general procedure and it was obtained as white solid, yield = 78%; mp 145-147 °C (ethanol). ^{1}H-NMR (500 MHz, DMSO-d_6): 6.97 (s, 1H, C6-H), 4.18 (t, *J* = 7.40 Hz, 2H, N3-1"-CH_2), 3.85 (t, *J* = 7.40 Hz, 2H, N1-1'-CH_2), 2.70 (s, 3H, 7-CH_3), 2.47 (s, 3H, 5-CH_3), 1.62-1.68 (m, 2H, N3-2"-CH_2), 1.57-1.59 (m, 2H, N1-2'-CH_2), 0.87-0.98 (m, 6H, N3-3"& N1-3'-2XCH_3). ^{13}C-NMR (125 MHz, DMSO-d_6): 161.85 C4=O, 160.90 C2=O, 152.29 C8a, 150.64 C7, 150.18 C5, 121.43 C6, 106.28 C4a, 43.41 N3-C1", 42.26 N1-C1', 29.62 7-CH_3, 27.78 5-CH_3, 21.37 N3-C2", 20.56 N1-C2', 11.12 N3-C3", 11.00 N1-C3'. IR (KBr) ν, cm^{-1}: 3120, 2920 (CH), 1692 (C=O), 1645 (C=O), 1592 (C=N), 1554 (C=C). MS: (m/z) 275.17 $[M]^+$. Anl. Calcd. for $C_{15}H_{21}N_3O_2$: C, 65.43; H, 7.69; N, 15.26. Found: C, 65.30; H, 8.00; N, 15.06.

*1,3,5-Trimethyl-7-phenylpyrido[2,3-d]pyrimidine-2,4(1H,3H)-dione (**18**)*
The compound was prepared by above general procedure and it was obtained as yellow solid which was purified by column chromatography (DCM:MeOH; 9:1), yield = 77%; mp 152-154 °C. ^{1}H-NMR (500 MHz, DMSO-d_6): 7.86-8.20 (m, 2',6'-2H, H_{arom}), 7.20-7.70 (m, 3",4",5"-3H, H_{arom}), 6.97 (s, 1H, C6-H), 3.80 (s, 3H, 5-CH_3), 3.53 (s, 3H, 3-CH_3), 2.80 (s, 3H, 1-CH_3). ^{13}C-NMR (125 MHz, DMSO-d_6): 161.22 C4=O, 160.82 C2=O, 153.20 C8a, 150.19 C7, 139.61 C5, 138.75 C1', 135.40 C3', 135.50 C5', 127.97 C2', 126.82 C6', 126.51 C4', 117.17 C6, 106.21 C4a, 32.38 N3-CH_3, 30.18 N1-CH_3, 25.55 5-CH_3. IR (KBr) ν, cm^{-1}: 3125, 2985 (CH), 1690 (C=O), 1636 (C=O) 1576 (C=N),

154936 (C=C). MS (ESI) (m/z): $[M]^+$ 280.72. Anl. Calcd. for $C_{16}H_{15}N_3O_2$: C, 68.31; H, 5.37; N, 14.94. Found: C, 68.13; H, 5.60; N, 14.96.

*1,3-Diethyl-5-methyl-7-phenylpyrido[2,3-d]pyrimidine-2,4(1H,3H)-dione (**19**)*
The compound was prepared by above general procedure and it was obtained as yellowish white solid, yield = 79%; mp 138-140 °C (CH_2Cl_2/CH_3OH, 9:1, column). ^{1}H-NMR (500 MHz, DMSO-d_6): 7.54-7.60 (m, 2H, 2'", 6'"-ArH), 7.32-7.40 (m, 3H, 3'",4'",5'"-H_{arom}), 6.97 (s, 1H, C6-H), 4.38 (q, *J* = 7.40 Hz, 2H, N3-1"-CH_2), 3.97 (q, *J* = 7.40 Hz, 2H, N1-1'-CH_2), 2.80 (s, 3H, 5-CH_3), 1.19 (t, *J* = 7.40 Hz, 3H, N3-2"-CH_3), 1.16 (t, *J* = 7.40 Hz, 3H, N1-2'-CH_3). ^{13}C-NMR (125 MHz, DMSO-d_6): 162.23 C4=O, 159.28 C2=O, 153.45 C8a, 150.62 C7, 149.78 C1'", 136.83 C3'" & C5'", 129.45 C2'", 129.86 C6'", 127.33 C4'", 121.43 C6, 107.63 C4a, 37.33 N3-C1", 35.95 N1-C1', 22.43 5-CH_3, 12.10 N3-C2", 12.86 N1-C2'. IR (KBr) ν, cm^{-1}: 3030, 2995 (CH), 1687 (C=O), 1644 (C=O), 1579 (C=N), 1545 (C=C).; MS (ESI) (m/z): $[M]^+$ 309.15. Anl. Calcd. for $C_{18}H_{19}N_3O_2$: C, 69.18; H, 6.19; N, 13.58. Found: C, 69.95; H, 6.04; N, 13.38.

*5-Methyl-7-phenyl-1,3-dipropylpyrido[2,3-d]pyrimidine-2,4(1H,3H)-dione (**20**)*
The compound was prepared by above general procedure and it was obtained as yellow solid, yield = 83%; mp 142-144 °C (ethanol). ^{1}H-NMR (500 MHz, DMSO-d_6): 8.10-8.16 (m, 2H, 2'", 6'"-H_{arom}), 7.53-7.66 (m, 3H, 3'", 4'", 5'"-H_{arom}), 7.10 (s, 1H, C6-H), 4.10 (t, *J* = 7.30 Hz, 2H, N3-1"-CH_2), 3.84 (t, *J* = 7.30 Hz, 2H, N1-1'-CH_2), 2.78 (s, 3H, 5-CH_3), 1.70-1.72 (m, 2H, N3-2"-CH_2), 1.62-1.66 (m, 2H, N3-2'-CH_2), 1.28 (t, *J* = 7.30 Hz, 2H, N3-1"-CH_3), 1.17 (t, *J* = 7.30 Hz, 2H, N3-1'-CH_3).; ^{13}C-NMR (125 MHz, DMSO-d_6): 161.29 C4=O, 160.66 C2=O, 152.29 C8a, 150.64 C7, 140.18 C5, 137.98 C1'", 130.41 C3'", 129.23 C5'", 128.71 C2'", 127.10 C6'", 126.55 C4'", 117.10 C6, 106.84 C4, 43.74 N3-C1", 42.34 N1-C1', 27.41 5-CH_3, 20.66 N3-C2", 20.49 N1-C2', 12.76 N3-C3", 11.15 N1-C3'. IR (KBr) ν, cm^{-1}: 3035, 2910 (CH), 1689 (C=O), 1649 (C=O), 1588 (C=N), 1550 (C=C). MS (ESI) (m/z): 338.20 $[M+1]^+$. Anl. Calcd. for $C_{20}H_{23}N_3O_2$: C, 71.45; H, 6.87; N, 12.45. Found: C, 70.96; H, 6.40; N, 12.60.

*7-(4'-Methoxyphenyl)-1,3,5-trimethylpyrido[2,3-d]pyrimidine-2,4(1H,3H)-dione (**21**)*
The compound was prepared by above general procedure and it was obtained as dark yellow solid, yield = 80%; mp 220-22 °C (ethanol). ^{1}H-NMR (60 MHz, DMSO-d_6): 8.10 (d, *J* = 8.60 Hz, 2H, 3',5'-H_{arom}), 7.40 (s, 1H, C6-H), 7.10 (d, *J* = 8.60 Hz, 2H, 2',6'-H_{arom}), 3.90 (s, 3H, 4'-O-CH_3), 3.80 (s, 3H, 5-CH_3), 3.40 (s, 3H, 3-CH_3), 2.80 (s, 3H, 1-CH_3). ^{13}C-NMR (125 MHz, DMSO-d_6): 162.30 C4=O, 161.74 C2=O, 153.48 C8a, 152.13 C7, 140.77 C5, 139.20 C1', 136.32 C3', 136.58 C5', 129.80 C2', 129.32 C6', 128.20 C4', 123.34 C6, 100.10 C4a, 59.74 4'-OCH_3, 32.28 N3-CH_3, 30.28 N1-CH_3, 26.35 5-CH_3. IR (KBr) ν, cm^{-1}: 3120, 2960 (CH), 1691 (C=O), 1640 (C=O), 1598 (C=N), 1542 (C=C).; MS (ESI) (m/z): $[M+H]^+$ 312.12. Anl. Calcd. for $C_{17}H_{17}N_3O_3$: C, 65.58; H, 5.50; N, 13.50. Found: C, 65.57; H, 5.73; N, 13.76.

*1,3-Diethyl-7-(4'"-methoxyphenyl)-5-methylpyrido[2,3-d]pyrimidine-2,4(1H,3H)-dione (**22**)*
The compound was prepared by above general procedure and it was obtained as orange solid, yield = 84%; mp 150-152 °C (ethanol). ^{1}H-NMR (500 MHz, DMSO-d_6): 8.12 (d, *J* = 8.60 Hz, 2'", 6'"-H_{arom}), 7.64 (s, 1H, C6-H), 7.10 (d, *J* = 8.60 Hz, 3'", 5'"-H_{arom}), 4.36 (q, *J* = 7.40 Hz, 2H, N3-1"-CH_2), 3.96 (q, *J* = 7.40 Hz, 2H, N1-1'-CH_2), 3.84 (s, 3H, O-CH_3), 2.76 (s, 3H, 5-CH_3), 1.28 (t, *J* = 7.40 Hz, 3H, N3-2"-CH_3), 1.17 (t, *J* = 7.40 Hz, 3H, N1-2"-CH_3). ^{13}C-NMR (125 MHz, DMSO-d_6): 161.28 C4=O, 160.24 C2=O, 154.55 C8a, 152.45 C7, 141.60 C5, 139.55 C1'", 132.86 C3'", 132.33 C5'", 129.88 C2'", 129.54 C6'", 128.32 C4'", 117.10 C6, 106.81 C4a, 54.60 4'"-OCH_3, 35.89 N3-C1", 33.70 N1-C', 24.95 5-CH_3, 12.88 N3-C2", 1210 N1-C2'. IR (KBr) ν, cm^{-1}: 3045, 2995 (CH), 1686 (C=O), 1643 (C=O), 1589 (C=N), 1544 (C=C). MS (ESI) (m/z): 339.16 $[M]^+$. Anl. Calcd. for $C_{19}H_{21}N_3O_3$: C, 67.24; H, 6.24; N, 12.38. Found: C, 66.97; H, 6.52; N, 12.18.

*7-(4'"-methoxyphenyl)-5-Methyl-1,3-dipropylpyrido[2,3-d]pyrimidine-2,4(1H,3H)-dione (**23**)*
The compound was prepared by above general procedure and it was obtained as orange solid, yield = 84%; mp 147-149 °C (ethanol). ^{1}H-NMR (500 MHz, DMSO-d_6): 8.14 (d, *J* = 8.60 Hz, 2'",6'"-H_{arom}), 7.66 (s, 1H, C6-H), 7.08 (d, *J* = 8.60 Hz, 3'",5'"-H_{arom}), 4.33 (t, *J* = 7.30 Hz, 2H, N3-1"-CH_2), 3.94 (t, *J* = 7.30 Hz, 2H, N1-1'-CH_2), 3.85 (s, 3H, 4'"-O-CH_3), 2.77 (s, 3H, 5-CH_3), 1.73-1.78 (m, 2H, N3-2"-CH_2), 1.58-1.62 (m, 2H, N1-2"-CH_2), 0.98 (t, *J* = 7.30 Hz, 2H, N3-1"-CH_3), 0.92 (t, *J* = 7.30 Hz, 2H, N1-1'-CH_3).; ^{13}C-NMR (125 MHz, DMSO-d_6): 161.29 C4=O, 160.82 C2=O, 153.11 C8a, 150.81 C7, 140.30 C5, 139.55 C1'", 135.80 C3'", 136.10 C5'", 129.19 C2'", 128.65 C6'", 128.15 C4'", 117.07 C6, 106.81 C4a, 55.28 4'"-OCH3, 43.67 N3-C1", 42.28 N1-C1', 27.45 5-CH3, 22.10 N3-C2", 20.66 N1-C2', 11.60 N3-C3", 11.15 N1-C3'. IR (KBr) ν, cm^{-1}: 3070, 2940 (CH), 1690 (C=O), 1644 (C=O), 1604 (C=N),

1560 (C=C). MS (ESI) (m/z): 368.20 $[M+H]^+$. Anl. Calcd. for $C_{21}H_{25}N_3O_3$: C, 68.64; H, 6.86; N, 11.44. Found: C, 68.39; H, 7.02; N, 11.21.

Biological Assays

Anti-inflammatory activity

All biological testing using animals were conducted in accordance with the internationally accepted principles for laboratory animals' use and care as mentioned in the European Community Guidelines and were officially approved. The anti-inflammatory activity of the newly synthesized compounds **1** and **7-23** was determined according to paw induced edema method [34] in comparison to indomethacin as a reference drug. The test is based on the pedal inflammation in rat paws induced by sub plantar injection of carrageenan suspension (0.2 mL of 1% solution in normal saline) in the right hind paw of the rats.

Male albino rats were divided into groups (5/group). The thickness of rat paw was measured by Varnier Caliper (SMIEC, China) before and after 1 h of carrageenan injection to determine the induced inflammation. The tested compounds of a dose (10 mg/kg) were injected i.p. to the animals. The control group received a vehicle (1% NaCMC) while the reference group received indomethacin (10 mg/kg).

Results of anti-inflammatory activity of the tested compounds and the reference drug were listed in (tables 1-2).
The percentage of edema and percentage of edema inhibition were calculated (Valencia et al., 1994) where:

$$\% \text{ Variation (edema)} = \frac{(V_R - V_L) \times 100}{V_R}$$

$$\% \text{ Edema inhibition} = \frac{(V_R - V_L) \text{ control} - (V_R - V_L) \text{ treated} \times 100}{(V_R - V_L) \text{ control}}$$

V_R: Average right paw thickness, V_L: Average left paw thickness.

Gastric ulceration

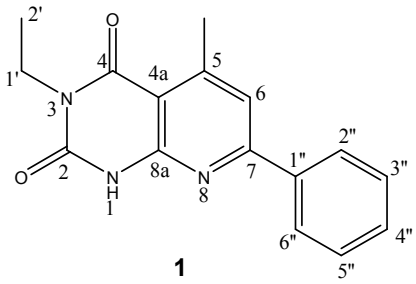
Male albino rats were divided into groups (6/group), the rats were fasted for 24 h. The tested compounds **10**, **12** and **16**-**18** and indomethacin were administered orally as a suspension in 1% NaCMC. After 6 h, the rats were killed, the stomach were removed for macroscopic and microscopic investigation. "Ulcer" was defined as at least one lesion that was 0.5 mm or more in length. All lesions of more than 0.1 mm in length were summed to obtain ulcer index. The results are shown in table 3.

RESULTS AND DISCUSSION

Structure-Based Design

Through our continuing efforts to identify new anti-inflammatory and anti-asthmatic agents [31, 33, 35, 36], pyridopyrimidine-2,4-dione was identified as an attractive scaffold. A structure-based strategy was used to virtually screen an *in-house* compounds library against the active site of COX-2 enzyme (PDB: 3LN1) [37] using LibDock software [38] identifying the pyridopyrimidine-2,4-dione derivative **1** (Figure 1) as one of the potential starting structures. *In vivo* testing of **1** against carrageenan-induced paw edema in rats revealed a moderate activity (60% edema inhibition at a dose of 10 mg/kg) which was encouraging for further optimization aiming for enhancing the activity.

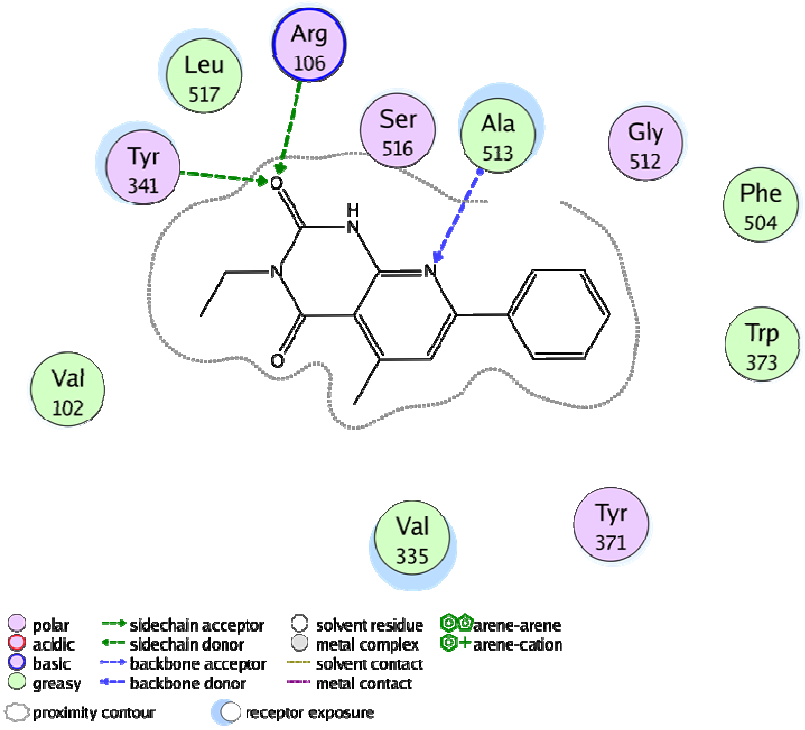
Fig. 1. Structure of the pyridopyrimidin-2,4-dione derivative (1) identified as a potential anti-inflammatory agent through COX-2 inhibition



1

The potential hit **1** was docked into COX-2 active site using a more rigorous method based on LigandFit software [37]. The results revealed the involvement of the pyridopyrimidine-dione core in two hydrogen bond contacts with residues in the enzyme binding pocket (Figure 2). The first involves hydrogen bonding between one of the carbonyl groups and the side chain of Arg106 and/or Tyr341 at the entrance of the binding pocket. The second shows a hydrogen bond interaction between the pyridine nitrogen atom and the backbone of Ala513.

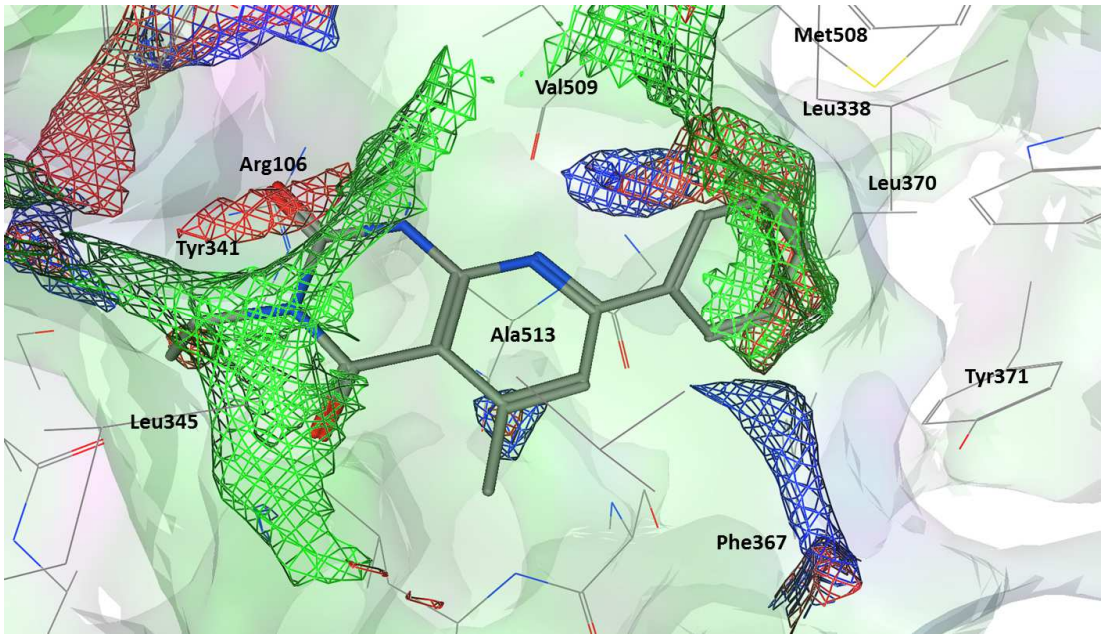
Fig. 2. 2D representation of 1 docked at the binding pocket of COX-2 enzyme. Hydrogen bonding between 1 and binding site residues are shown as dashed-line arrows



An “interaction potential maps” for the COX-2 binding pocket was calculated in order to guide subsequent synthetic efforts (Figure 3). The calculated map shows the alignment of the ethyl substituent at C3 within a hydrophobic potential contour (green mesh) formed by Tyr341 and Leu345. This hydrophobic potential is extended to cover the N1 region (refer to Figure 1 for compound’s numbering), which highlights the possibility for a second alkyl substituent at this position. The phenyl moiety at C7 is aligned well into a hydrophobic potential at the other side of the binding site entrance Val509 and two leucine residues, Leu338 and Leu370. A hydrogen bond acceptor contour (red mesh) was observed within this hydrophobic region, highlighting the possibility for incorporating a hydrogen

bond acceptor to the phenyl moiety. Based on these modeling outcomes, a library of pyridopyrimidine-2,4-diones was designed as potential COX-2 inhibitory activity.

Fig. 3. Predicted orientation of 1 (stick representation) into COX-2 binding pocket (selected amino acids are shown and labeled, line presentation). Interaction potential maps are calculated as hydrophobic contacts (green mesh), hydrogen bond acceptor (red mesh) and hydrogen bond donor (blue mesh)

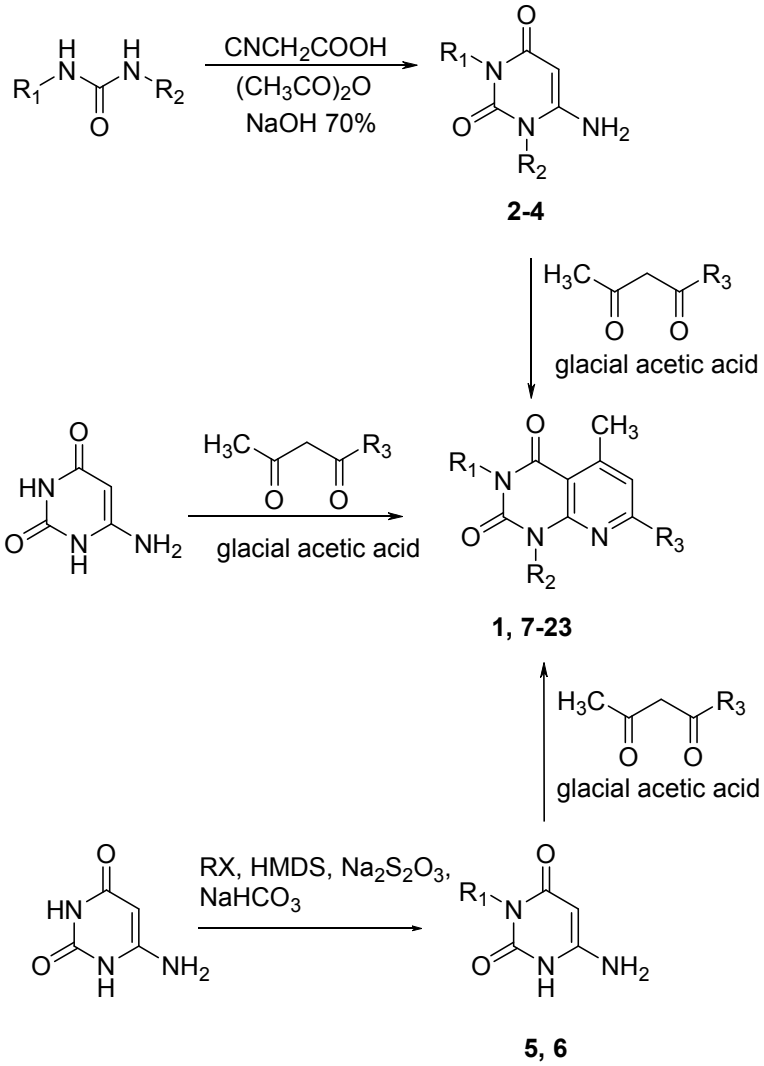


Synthesis

The designed pyridopyrimidine-2,4-dione derivatives were synthesized according to Scheme 1. The key intermediates **2-4** were synthesized from di-substituted urea derivatives by reaction with cyanoacetic acid in acetic anhydride followed by cyclization with sodium hydroxide. Intermediates **5** and **6** were prepared by the regio-selective alkylation of 6-aminouracil in hexamethyldisilazane (HMDS).

The second step was achieved through the condensation between acetylacetone, benzoylacetone or *p*-methoxybenzoylacetone with the substituted 6-aminouracil derivatives **2-6** or 6-aminouracil as illustrated in Scheme 1. The regioselectivity of this condensation reaction was previously reported [39]. It was proposed that the most reactive carbonyl moiety (the methyl carbonyl in this case) reacts first with the 5-position of pyrimidine ring followed by the cyclization mediated by the second carbonyl moiety and the primary amine [39]. The newly synthesized target compounds were characterized and their structures confirmed by IR, ^{1}H-NMR and ^{13}C-NMR spectroscopy and their purities were assessed by elemental analyses. The formation of target pyrido[2,3-d]pyrimidine-1,4-dione derivatives **1** and **7-23** was further confirmed by analyzing their ^{1}H-NMR spectra, which show the disappearance of C5-H at δ 4.55-4.6 ppm and 6-NH_2 singlet (2 protons) at δ 6.16-6.40 ppm of 6-aminouracil ring, in addition to, appearance of a singlet (1H) at δ 6.5-7.4 ppm corresponding to C6-H and signals corresponding to the introduced moieties of pyrido[2,3-d]pyrimidine-1,4-dione derivatives. ^{13}C-NMR spectra further confirmed these new derivatives which revealed the disappearance of C5 of 6-aminouracil derivatives δ 74.3-76.10 ppm and appearance of C4a instead around δ 97-106 ppm which is considered a further evidence for confirmation of pyrido[2,3-d]pyrimidine-1,4-dione derivatives **1** and **7-23**. The previous spectral analyses confirm the formation of the target compounds through the cyclization between the acetylacetone derivatives and 6-aminouracil occurs through 6-NN_2 and C5H rather than 6-NH_2 and N1H.

Scheme 1. Synthesis of the designed pyridopyrimidin-2,4-dione derivatives 1 and 7-23



Anti-inflammatory activity

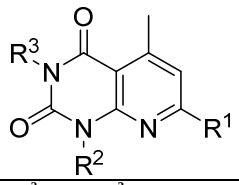
Compounds **1** and **7-23** were tested *in vivo* for their anti-inflammatory effect using carrageenan-induced paw edema in rats and indomethacin as a reference drug [34]. The activity of the tested compounds was recorded as the percentage of edema inhibition observed at time intervals of 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 h from exposure (Supporting information Table S1). The maximum inhibition exerted by each derivative throughout the 5 h monitoring time and its relative potency to indomethacin are presented in Table 1.

Results shown in Table 1 reveal a variation in activities based on the type of the substitution at the pyridopyrimidine core. For the first set of inhibitors (**7-14**), it was found that the replacement of the ethyl substituent at N1 with *n*-propyl group is showing moderate decrease in activity (**1** vs **7**, **9** vs **10** and **12** vs **13**). Howecer, the omission of this alkyl substituent resulted in a dramatic loss of activity (**7** vs **8**, **10** vs **11** and **13** vs **14**). This observation agrees with our initial modelling study about the engagement of this substituent with hydrophobic region in the enzyme active site. The introduction of a methoxy substituent to the phenyl ring had only little effect on the apparent activity (derivatives **9-11**).

For the second set of compounds (**15-23**), it was found that the introduction of a second alkyl group into the pyrimidine ring (N3) leads to an enhanced biological activity (compounds **16** and **17**) which is consistent with the initial model suggestion. Surprisingly, the replacement of the phenyl moiety with a methyl group in derivatives **12-**

17 shows a significant enhancement in the apparent activity. The reason of this activity enhancement may be attributed to the improvement of the pharmacokinetic properties of the methyl containing substituent relative to the corresponding phenyl containing analogues. This was supported by the calculated lipophilicity parameter (ClogP) for the most active derivative **16** (ClogP = 2.6) and its corresponding phenyl analogue **19** (ClogP = 4.2).

Table 1. Maximum % inhibition and relative potencies observed during the first 5 hours after drug administration for compound 1 and 7-23



Compound	R^1	R^2	R^3	Edema inhibition (%)[a]	Potency (%)[b]
IND	-	-	-	79±2.1	100
1	C_6H_5	H	C_2H_5	60±2.5	75.9
7	C_6H_5	H	*n*-C_3H_7	50±1.8	63.3
8	C_6H_5	H	H	27±±2.2	34.2
9	4-OCH_3-C_6H_5	H	*n*-C_3H_7	51±3.4	64.5
10	4-OCH_3-C_6H_5	H	C_2H_5	64±1.4	81.0
11	4-OCH_3-C_6H_5	H	H	33±1.7	41.8
12	CH_3	H	*n*-C_3H_7	61±3.3	77.2
13	CH_3	H	C_2H_5	83±1.9	105.1
14	CH_3	H	H	25±2.6	31.6
15	CH_3	CH_3	CH_3	28±2.7	35.4
16	CH_3	C_2H_5	C_2H_5	86±3.4	108.9
17	CH_3	*n*-C_3H_7	*n*-C_3H_7	84±1.1	106.3
18	C_6H_5	CH_3	CH_3	57±2.9	72.2
19	C_6H_5	C_2H_5	C_2H_5	42±1.4	53.2
20	C_6H_5	*n*-C_3H_7	*n*-C_3H_7	49±3.2	62.0
21	4-OCH_3-C_6H_5	CH_3	CH_3	74±2.4	93.7
22	4-OCH_3-C_6H_5	C_2H_5	C_2H_5	42±2.4	53.2
23	4-OCH_3-C_6H_5	*n*-C_3H_7	*n*-C_3H_7	46±1.3	58.2

[a]*Maximum % inhibition (mean±SEM; n=5) observed during the first 5 hours after drug administration (10 mg/kg i.p).*
[b]*Relative potencies calculated relative to indomethacin activity.*

From the above analysis it can be concluded that for better anti-inflammatory activity both nitrogen atoms of the pyrimidine-dione moiety should be substituted with 2-3 aliphatic groups while position C7 is substituted by a small aliphatic group.

Table 2. Ulcerogenic effects in rats for selected pyridopyrimidine derivatives

Compound	Dose mg /kg	Ulcer score[a]	Ulcer Index ± SEM
IND	10	3/6	1.84 ± 0.47
	30	5/6	4.15 ± 0.64
	50	Not tested	
10	30	0/6	0.00
	50	1/6	1.12 ± 0.22
	75	2/6	2.18 ± 0.29
12	30	0/6	0.00
	50	1/6	1.30 ± 0.12
	75	1/6	1.21 ± 0.35
16	30	0/6	0.00
	50	1/6	1.33 ± 0.82
	75	1/6	1.20 ± 0.28
17	30	0/6	0.00
	50	1/6	0.00
	75	1/6	1.60 ± 0.25
18	30	0/6	0.00
	50	0/6	0.00
	75	2/6	2.10 ± 0.18

[a]*Number of rats with lesions that were more than 0.5 mm in length per total numbers.*

Ulcerogenic effect

Six derivatives (**10**, **12**, **13**, and **16-18**) were selected to be tested for their ulcerogenic effect in rats [40] in order to access their gastric upset, as a common side effect for anti-inflammatory agents. As presented in Table 2, all tested derivatives show no ulcerogenic effect at a dose of 30 mg/kg compared to indomethacin. Even at higher doses up to 75 mg/kg, all compounds show better gastric safety than indomethacin, a result that suggests a selective inhibition of COX-2 by the tested compounds.

CONCLUSION

On the basis of virtual screening against the active site of cyclooxygenase-2 (COX-2) enzyme, 3-ethyl-5-methyl-7-phenylpyrido*[2,3-d]*pyrimidine-2,4(*1H,3H*)-dione (**1**) was identified as a potential inhibitor. Using an approach based on molecular modeling-guided lead optimisation, new pyridopyrimidine analogues were designed. Novel pyrido*[2,3-d]*pyrimidine-1,4-dione derivatives were synthesized from the corresponding 6-aminouracil derivatives in a one-step simple reaction with very good to excellent yields**.** Anti-inflammatory results showed that some drivatives are more potent than indomethacin (relative potency of 105%, 106% and 109%). The ulecerogenic effect of selected derivatives was examined *in vivo* reavealing greater gastric safety than indomethacin and highlighting possible COX-2 selectivity for these compounds.

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