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Synthesis and Anti-Inflammatory Activities of Some New Pyridopyridine, Pyridopyrimidine and Pyridopyrimidotriazine Derivatives

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Abstract: In continuation to our search for new heterocyclic systems based anti-inflammatories, the suggestion and synthesis of some phenyl-1, 8-naphthyridines and phenylpyrido [2, 3-d] pyrimidines and their derivatives, were herein realized. The pharmacological screening showed that many of these compounds have good anti-inflammatory activities comparable to Valdicoxib® used as reference drug. The structure assignments of the new compounds based on chemical and spectroscopic evidence. The detailed synthesis, spectroscopic data and pharmacological properties are reported.

Key words: 2-Amino-6-methyl-4-phenylnicotinonitrile · Pyridopyrimidine · Pyridopyrimidotriazine · Antiinflammatory activity

INTRODUCTION

In previous work we have found that certain substituted pyridine derivatives show antimicrobial and anti-inflammatory [1-6] and antitumor activities [7-9]. Many of these derivatives were found to possess a variety of pronounced activities such as antimalarial [10], antimicrobial [11], anti-inflammatory [12], anti-cancer [13]. On the other hand, substituted pyrimidine derivatives have promising biological and anticancer activities [14-17]. Recently, some new substituted pyrimidine derivatives have been synthesized, which exhibit analgesic, anti-inflammatory, anticancer, anticonvulsant and antiparkinsonian activities [18-21]. In view of these observations and in continuation of our previous work in heterocyclic chemistry [1], we synthesized some new heterocyclic compounds containing the pyridine or pyrimidine moieties and tested their anti-inflammatory activities.

EXPERIMENTAL

Melting points were determined on open glass capillaries using an Electrothermal IA 9000 digital melting point apparatus. Elemental analyses were performed on Elementar, Vario EL, Microanalytical Unit, National Research Center, Cairo Egypt and were found within ±0.4% of the theoretical values. Infrared spectra were recorded on Carlzeise Spectrophotometer model "UR 10" spectrophotometer using the KBr disc technique. ^{1}H-NMR spectra were recorded on Varian Gemini 270 MHz spectrometer (*DMSO*-d_6) and the chemical shifts are given in δ (ppm) downfield from tetramethylsilane (*TMS*) as an internal standard. The mass spectra were measured using a Finnigan SSQ 7000 mass spectrometer. Follow up of the reactions and checking the purity of the compounds was made by TLC on silica gel-precoated aluminum sheets (Type 60 F_{254}, Merck, Darmstadt, Germany).

Synthesis of Pyrimidone and Pyrimidinthione Derivatives 2a *and* 2b: A mixture of compound 1 (2.09 g, 0.01 mol) and urea or thiourea (0.01 mol) was fused together at 180°C on sand bath for 4 h. The residue was dissolved in 50 ml absolute ethanol; the obtained precipitate was collected by filtration, dried and crystallized to give 2a and 2b, respectively.

4-Aamino-7-methyl-5-phenylpyrido [2, 3-d] Pyrimidin-2(1H)-one (2a): Yield 70%, mp 290-292°C (EtOH); IR (KBr, cm^{-1}): 3346-3298 (NH_2 and NH), 1685 (C=O), 1658 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 2.48 (s, 3H, CH_3), 3.45 (s, 2H, NH_2 exchangeable with D_2O), 7.05-7.46 (m, 6H, Ph-H + CH-pyridine), 8.18 (s, 1H, NH exchangeable with D_2O); MS (EI, 70 eV): m/z = 252 (M^+, 12) corresponding to the molecular formula $C_{14}H_{12}N_4O$ and at 174 (100, base peak).

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4-Amino-7-methyl-5-phenylpyrido [2, 3-d] pyrimidine-2(1H)-thione (2b): Yield 72%, mp 248-250 °C (EtOH); IR (KBr, cm^{-1}): 3356-3300 (NH_2 and NH), 1660 (C=N), 1245 (C=S); ^{1}H-NMR (DMSO-d_6) δ: 2.50 (s, 3H, CH_3), 3.38 (s, 2H, NH_2 exchangeable with D_2O), 7.15-7.48 (m, 6H, Ph-H + CH-pyridine), 8.05 (s, 1H, NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6) δ: 23.10 (CH_3), 125.42, 127.30, 127.45, 136.05 (Ph-C), 103.15, 106.20, 145.60, 146.52, 157.12 (Pyr-C), 154.62 (C-NH_2) and 181.25 (C=S); MS (EI, 70 eV): m/z = 269 (M^++1, 22) corresponding to the molecular formula $C_{14}H_{12}N_4S$ and at 91 (100, base peak).

***Synthesis of 2, 4-diamino-7-methyl-5-phenyl-1, 8-naphthyridine-3-carbonitrile* (3):** A mixture of 1 (2.09 g, 0.01 mol) and malononitrile (0.66 g, 0.01 mol) in glacial acetic acid (50 ml) was stirred under reflux for 8h. After cooling, the reaction mixture was poured onto water; the obtained solid was filtered off, washed with water, dried and crystallized to give 3. Yield 60%, mp 189-190°C (EtOH); IR (KBr, cm^{-1}): 3380-3325 (2NH_2), 2224 (CN), 1656 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 2.50 (s, 3H, CH_3), 4.12, 4.20 (2s, 4H, 2NH_2 exchangeable with D_2O), 7.18-7.48 (m, 5H, Ph-H), 7.56 (s, 1H, CH-pyridine); MS (EI, 70 eV): m/z = 275 (M^+, 8) corresponding to the molecular formula $C_{16}H_{13}N_5$ and at 144 (100, base peak).

***Synthesis of 4-amino-2-hydroxy-7-methyl-5-phenyl-1, 8-naphthyridine-3-carbonitrile* (4):** A mixture of 1 (2.09 g, 0.01 mol) and ethylcyanoacetate (1.13 g, 0.01 mol) in dry dioxane (50 ml) containing a few drops of triethylamine was refluxed for 8h. The reaction mixture was cooled, poured onto water and neutralized with acetic acid. The formed precipitate was filtered off, washed with water, dried and crystallized to give 4. Yield 65%, mp 228-230 (dioxane); IR (KBr, cm^{-1}): 3465-3350 (OH and NH_2), 2218 (CN), 1662 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 2.48 (s, 3H, CH_3), 3.15 (s, 2H, NH_2 exchangeable with D_2O), 5.10 (s, 1H, OH exchangeable with D_2O), 7.15-7.46 (m, 5H, Ph-H), 7.58 (s, 1H, CH-pyridine); ^{13}C-NMR (DMSO-d_6) δ: 23.35 (CH_3), 41.08 (CH_2), 125.45, 127.18, 127.38, 136.15 (Ph-C), 91.70, 103.46, 153.90, 154.10, 158.40 (Pyr-C), 117.85 (C≡N) and 163.45 (C=O); MS (EI, 70 eV): m/z = 276 (M^+, 22) corresponding to the molecular formula $C_{16}H_{12}N_4O$ and at 182 (100, base peak).

***Synthesis of 7-methyl-5-phenylpyrido [2, 3-d] pyrimidin-4-amine* (5):** A solution of 1 (1.05 g, 0.005 mol) in formamide (20 ml) was refluxed for 1h., after cooling; the reaction mixture was poured onto cold water. The obtained solid was filtered off, washed with water, dried and crystallized to give 5. Yield 65%, mp 200-202 °C (dioxane); IR (KBr, cm^{-1}): 3435 (NH_2), 1658 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 2.47 (s, 3H, CH_3), 3.78 (s, 2H, NH_2 exchangeable with D_2O), 7.12-7.49 (m, 6H, Ph-H + CH-pyridine), 8.12 (s, 1H, CH-pyrimidine); MS (EI, 70 eV): m/z = 236 (M^+, 5) corresponding to the molecular formula $C_{14}H_{12}N_4$ and at 210 (100, base peak).

***Synthesis of 2-chloro-N-(3-cyano-6-methyl-4-phenylpyridin-2-yl) acetamide* (6):** To a solution of 1 (2.09 g, 0.01 mol) in dioxane (30 ml), chloroacetylechloride (1.11g, 0.01 mol) was added with stirring at room temperature and then heated under reflux for 30 min. The reaction mixture was stirred at room temperature for 12 h and poured onto cold water; the obtained solid was collected by filtration, dried and crystallized to give 6. Yield 60%, mp 140-142 °C (MeOH); IR (KBr, cm^{-1}): 3318 (NH), 2222 (CN), 1685 (C=O), 1660 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 2.48 (s, 3H, CH_3), 4.32 (s, 2H, CH_2), 7.06-7.46 (m, 5H, Ph-H), 7.65 (s, 1H, CH-pyridine), 8.15 (s, 1H, NH exchangeable with D_2O); MS (EI, 70 eV): m/z = 284 (M^+-1, 6) corresponding to the molecular formula $C_{15}H_{12}ClN_3O$ and at 230 (100, base peak).

***Synthesis of 5-amino-2-methyl-9-oxo-4-phenyl-8,9-dihydropyrrolo[1,2-a][1,8]naphthpyrimidine-7-carbonitrile* (7):** To a solution of 6 (2.85 g, 0.01 mol) in dioxane (30 ml) containing triethylamine (0.5 ml), malononitrile (0.66 g, 0.01 mol) was added and heated under reflux for 4h. After cooling, the reaction mixture was poured onto water containing hydrochloric acid (pH ~7). The formed precipitate was collected by filtration, dried and crystallized to give 7. Yield 60%, mp 190-192°C (dioxane); IR (KBr, cm^{-1}): 3398 (NH_2), 2224 (CN), 1688 (C=O), 1659 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 2.52 (s, 3H, CH_3), 2.80 (s, 2H, CH_2), 3.39 (s, 2H, NH_2 exchangeable with D_2O), 6.62 (s, 1H, Ar-H), 7.15-7.48 (m, 6H, Ph-H + pyridine); MS (EI, 70 eV): m/z = 312 (M^+-2, 15) corresponding to the molecular formula $C_{19}H_{14}N_4O$ and at 77 (100, base peak).

***Synthesis of 4-chloro-6-methyl-8-phenylpyrido [3, 2-d] [1, 2, 3] triazine* (8):** A solution of sodium nitrite (0.01 mol) in water (10 ml) was added to a cold solution (0°C) of 1 (1.05 g, 0.005 mol) in acetic acid (30 ml) and concentrated hydrochloric acid (15 ml) with stirring. The reaction mixture was stirred at room temperature for 2 h; the obtained crude solid was filtered off, dried and crystallized to give 8. Yield 65%, mp 198-200°C (EtOH); IR (KBr, cm^{-1}): 1622 (CN); ^{1}H-NMR (DMSO-d_6)δ: 2.52 (s, 3H, CH_3), 7.18-

7.52 (m, 6H, Ph-H + pyridine); MS (EI, 70 eV): m/z = 258 (M^++2, 32) corresponding to the molecular formula $C_{13}H_9ClN_4$ and at 51 (100, base peak).

***Synthesis of 1-(6-methyl-8-phenylpyrido [3, 2-d] [1, 2, 3] triazin-4-yl) hydrazine* (9):** A mixture of 8 (0.01 mol) and hydrazine hydrate (3 ml) in absolute ethanol (20 ml) was refluxed for 1h after cooling, the formed precipitate was filtered off, dried and crystallized to give 9. Yield 65%, mp 258-260°C (dioxane); IR (KBr, cm^{-1}): 3415-3318 ($NHNH_2$), 1620 (CN); ^{1}H-NMR (DMSO-d_6) δ: 2.47 (s, 3H, CH_3), 2.78 (s, 2H, NH_2 exchangeable with D_2O), 7.12-7.50 (m, 6H, Ph-H + pyridine-H), 8.65 (s, 1H, NH exchangeable with D_2O); MS (EI, 70 eV): m/z = 254 (M^++2, 8) corresponding to the molecular formula $C_{13}H_{12}N_6$ and at 131 (100, base peak).

***Synthesis of N-(3-cyano-6-methyl-4-phenylpyridin-2-yl) acetamide* (10):** A solution of 1 (2.09 g, 0.01 mol) in 20 cm^3 acetic anhydride was heated under reflux for 5h. After cooling, the reaction mixture was poured into water, filtered off, dried and crystallized to give 10. Yield 60%, mp 118-120 °C (AcOH); IR (KBr, cm^{-1}): 3346 (NH), 2222 (CN), 1682 (C=O), 1658 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 2.35 (s, 3H, $COCH_3$), 2.48 (s, 3H, CH_3), 7.12-7.49 (m, 5H, Ph-H), 7.68 (s, 1H, CH-pyridine), 8.15 (s, 1H, NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6) δ: 23.18, 22.08 ($2CH_3$), 125.39, 127.28, 127.24, 136.25 (Ph-C), 91.62, 103.56, 153.75, 154.65, 159.05 (Pyr-C), 118.05 (C≡N) and 166.85 (C=O); MS (EI, 70 eV): m/z = 251 (M^+, 24) corresponding to the molecular formula $C_{15}H_{13}N_3O$ and at 221 (100, base peak).

***Synthesis of 2, 7-dimethyl-5-phenylpyrido [2, 3-d] pyrimidin-4(3H)-one* (11):** A solution of 1 (2.09 g, 0.01 mol) in a mixture of hydrochloric acid and glacial acetic acid (40 ml, 3:1) was heated under reflux for 3h. The reaction mixture was poured onto water and the formed solid was filtered off, dried and crystallized to give 11. Yield 70%, mp 135-137 °C (AcOH); IR (KBr, cm^{-1}): 3355 (NH), 1678 (C=O), 1660 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 0.95 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 7.09-7.42 (m, 5H, Ph-H), 7.70 (s, 1H, CH-pyridine), 8.05 (s, 1H, NH exchangeable with D_2O); MS (EI, 70 eV): m/z = 252 (M^++1, 16) corresponding to the molecular formula $C_{15}H_{13}N_3O$ and at 159 (100, base peak).

***Synthesis of 7-methyl-5-phenylpyrido [2, 3-d] pyrimidin-4(3H)-one* (12):** A solution of 1 (2.09 g, 0.01 mol) in formic acid (85%) (10 ml) was refluxed for 10 h. The solvent was evaporated under reduced pressure and the obtained residue was triturated with ethanol, the solid formed was filtered off, dried and crystallized to give 12. Yield 60%, mp 152-154°C (EtOH); IR (KBr, cm^{-1}): 3298 (NH), 1682 (C=O), 1658 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 2.48 (s, 3H, CH_3), 7.10-7.50 (m, 6H, Ph-H + CH-pyrimidine), 7.68 (s, 1H, CH-pyridine), 8.12 (s, 1H, NH exchangeable with D_2O); MS (EI, 70 eV): m/z = 237 (M^+, 12) corresponding to the molecular formula $C_{14}H_{11}N_3O$ and at 208 (100, base peak).

***Synthesis of 4-chloro-7-methyl-5-phenylpyrido [2, 3-d] pyrimidine* (13):** A mixture of 12 (2.37 g, 0.01 mol), phosphorous pentachloride (2.05 g, 0.03 mol) and phosphorus oxychloride (20 ml) was heated under reflux for 2h. The reaction mixture was cooled and poured into ice; the resulting precipitate was filtered off, dried and crystallized to give 13. Yield 60%, mp 274-276°C ($CHCl_3$); IR (KBr, cm^{-1}): 1662 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 2.50 (s, 3H, CH_3), 7.20-7.52 (m, 6H, Ph-H+CH-pyridine), 8.40 (s, 1H, CH-pyrimidine); MS (EI, 70 eV): m/z = 257 (M^++2, 28) corresponding to the molecular formula $C_{14}H_{10}ClN_3$ and at 77 (100, base peak).

***Synthesis of 1-(7-ethyl-5-phenylpyrido [2, 3-d] pyrimidin-4-yl) hydrazine* (14):** A mixture of 13 (0.01 mol) and hydrazine hydrate (3 ml) in absolute ethanol (20 ml) was refluxed for 1h. After cooling, the formed precipitate was filtered off, dried and crystallized to give 14. Yield 68%, mp 235-237 °C (dioxane); IR (KBr, cm^{-1}): 3398-3265 ($NHNH_2$), 1662 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 2.52 (s, 3H, CH_3), 2.65 (s, 2H, NH_2 exchangeable with D_2O), 7.08-7.48 (m, 6H, Ph-H + CH-pyridine), 8.30 (s, 1H, CH-pyrimidine), 8.72 (s, 1H, NH exchangeable with D_2O);; MS (EI, 70 eV): m/z = 253 (M^++2, 6) corresponding to the molecular formula $C_{14}H_{13}N_5$ and at 205 (100, base peak).

***Synthesis of 7-methyl-9-phenyl-2H-1, 2, 3a, 5, 6-pentaaza-cyclopenta[a]naphthalene-3-thione* (15):** A mixture of 14 (1.26 g, 0.005 mol) and carbon disulphide (4 ml) in pyridine (10 ml) was heated on water bath for 4 h. The solid formed was filtered off, dried and crystallized to yield 15. Yield 70%, mp 168-170°C (dioxane); IR (KBr, cm^{-1}): 3365 (NH), 1659 (C=N), 1242 (C=S); ^{1}H-NMR (DMSO-d_6) δ: 2.52 (s, 3H, CH_3), 7.16-7.65 (m, 7H, Ph-H + CH-pyridine + CH-pyrimidine), 8.05 (s, 1H, NH exchangeable with D_2O); ^{13}C NMR (DMSO-d_6) δ: 22.98 (CH_3), 125.41, 126.99, 127.16, 136.15 (Ph-C), 105.60, 114.15, 145.32, 146.10, 159.12 (Pyr-C), 153.05, 161.04 (Pyrimidine-C), 162.36 (C=S); MS (EI, 70 eV): m/z = 293 (M^+, 24) corresponding to the molecular formula $C_{15}H_{11}N_5S$ and at 168 (100, base peak).

***Synthesis of 3, 7-dimethyl-9-phenyl-1, 2, 3a, 5, 6-pentaaza-cyclopenta[a]naphthalene* (16):** A mixture of 14 (1.26 g, 0.005 mol) and acetic anhydride (10 ml) in absolute ethanol (15 ml) was refluxed for 3h. The formed precipitate was filtered off, washed with ethanol, dried and crystallized to give 16. Yield 55%, mp 170-172°C (dioxane); IR (KBr, cm^{-1}): 1658 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 2.38, 2.49 (2s, 6H, $2CH_3$), 7.12-7.50 (m, 6H, Ph-H + CH-pyridine), 8.45 (s, 1H, CH-pyrimidine); MS (EI, 70 eV): m/z = 275 (M^+, 14) corresponding to the molecular formula $C_{16}H_{13}N_5$ and at 171 (100, base peak).

***Synthesis of 2-methyl-4-phenyl-6H-1, 5, 6, 8a, 10-pentaaza-phenanthrene-7, 8-dione* (17):** A mixture of 14 (2.51 g, 0.01 mol) and diethyleoxalate (1.46 g, 0.01 mol) in absolute ethanol (30 ml) was refluxed for 3h. The reaction mixture was concentrated under reduced pressure; the obtained solid was filtered off, dried and crystallized to give 17. Yield 75%, mp 246-248°C (EtOH); IR (KBr, cm^{-1}): 3330 (NH), 1685, 1680 (2 C=O), 1660 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 2.50 (s, 3H, CH_3), 7.18-7.70 (m, 7H, Ph-H + CH-pyridine + CH-pyrimidine), 7.98 (s, 1H, NH exchangeable with D_2O); MS (EI, 70 eV): m/z = 305 (M^+, 14) corresponding to the molecular formula $C_{16}H_{11}N_5O_2$ and at 77 (100, base peak).

***Synthesis of 7-methyl-3-(4-nitrophenyl)-9-phenyl-1,2,3a,5,6-pentaaza-cyclopenta[a]naphthalene* (18):** A mixture of 14 (2.51 g, 0.01 mol) and 4-nitrobenzaldehyde (1.50 g, 0.01 mol) in glacial acetic acid (50 ml) was heated under reflux for 6 h. After cooling, the obtained solid was collected by filtration, dried and crystallized to give 18. Yield 70%, mp 188-190 °C (EtOH); IR (KBr, cm^{-1}): 1658 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 2.49 (s, 3H, CH_3), 7.14-7.50 (m, 6H, Ph-H + CH-pyridine), 7.70 (d, 2H, Ar-H), 8.15 (d, 2H, Ar-H), 8.25 (s, 1H, CH-pyrimidne); MS (EI, 70 eV): m/z = 381 (M^+-1, 4) corresponding to the molecular formula $C_{21}H_{14}N_6O_2$ and at 130 (100, base peak).

***Synthesis of 7-methyl-4-(3, 5-dimethyl-1H-pyrazol-1-yl)-5-phenylpyrido [2, 3-d] pyrimidine* (19):** A mixture of 14 (2.51 g, 0.01 mol) and acetyl acetone (1.0 g, 0.01 mol) in methanol (50 ml) was refluxed for 5 h. After cooling, the separated solid was filtered off, dried and crystallized to give 19. Yield 80%, mp 170-172°C (benzene/pet. Ether); IR (KBr, cm^{-1}): 1665 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 2.48, 2.80, 2.84 (3s, 9H, $3CH_3$), 6.25 (s, 1H, CH-pyrazole), 7.08-7.52 (m, 6H, Ph-H + CH-pyridine), 8.30 (s, 1H, CH-pyrimidine); ^{13}C-NMR (DMSO-d_6) δ: 11.15, 16.45, 23.24 ($3CH_3$), 125.32, 127.18, 127.12, 136.16 (Ph-C), 107.06, 109.65, 146.95, 153.56, 156.38 (Pyr-C), 155.68, 165.48 (Pyrimidine-C), 103.52, 142.18, 145.85 (Pyrazolo-C); MS (EI, 70 eV): m/z = 314 (M^+-1, 10) corresponding to the molecular formula $C_{19}H_{17}N_5$ and at 238 (100, base peak).

***Synthesis of 4, 5, 6,7-tetrachloro-2-(7-methyl-5-phenyl-pyrido[2,3-d]pyrimidin-4-ylamino)-isoindole-1,3-dione* (20):** A mixture of 14 (0.251 g, 0.001 mol) and 3,4,5,6-tetrachlorophthalic anhydride (2.85 g, 0.01 mol) in glacial acetic acid (50 ml) was refluxed for 6 h. The solvent was evaporated under reduced pressure and the obtained residue solidified with dry ether, the solid product was collected by filtration, dried and crystallized from AcOH to yield 20. Yield 82%, mp 252-254°C (DMF/H_2O); IR (KBr, cm^{-1}): 3362 (NH), 1695 (C=O), 1659 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 2.50 (s, 3H, CH_3), 7.12-7.52 (m, 6H, Ph-H + CH-pyridine), 8.32 (s, 1H, CH-pyrimidine), 8.72 (s, 1H, NH exchangeable with D_2O); MS (EI, 70 eV): m/z = 519 (M^+, 8) corresponding to the molecular formula $C_{22}H_{11}Cl_4N_5O_2$ and at 77 (100, base peak).

Pharmacological Screening

Determination of Acute Toxicity (LD_{50}): The LD_{50} for compounds were determined by injected different gradual increased doses of the tested compounds to adult mail albino rats and then calculate the dose cause 50% animal death, according to Austen *et al.* [25].

Anti-Inflammatory Activity: Carrageenan® induced rat's paw

Procedure: Groups of adult male albino rats (150-180 g), each of 8 animals were orally dosed with tested compounds at a dose level of 25-50 mg/kg one hour before Carrageenan® challenge. Foot paw edema was induced by subplenter injection of 0.05 ml of 1% suspension of Carrageenan® in saline into the planter tissue of one hind paw. An equal volume of saline was injected to the other hind paw and served as control. Four hours after drug administration the animals were decapitated, blood was collected and the paws were rapidly excised. The average weight of edema was examined for the treated as well as the control group and the percentage inhibition of weight of edema was also evaluated. Valdicoxib® (5 mg/kg) was employed as standard reference against which the tested compounds were compared.

Calculation and Evaluation: Thirty minutes after the rats are challenged by subcutaneous injection of 0.05 ml of 1% solution of carrageenan into the planter side of the lift hind paw. The paw is marked with ink at the level of the lateral malleolus; the paw volume was measured by a sensitive method developed by Webb and Griswold [26] that calculated by interfacing anti-i Delta Range top-loading balance with a micro computer.

$$\% \text{ Protection} = (A - B) \times 100 / A$$

A = The paw volume of non-treated group
B = The paw volume of treated group

Estimation of Plasma Prostaglandin E2 (PGE2)
Procedure
Heparinized blood samples were collected from rats obtained from the previous anti-inflammatory examined groups (n = 8), plasma was separated by centrifugation at 12000 g for 2 min at 40°C and immediately stored frozed -2°C until use.

The design correlate EIA prostaglandin E2 (PGE2) kit (Merck, Darmstadt, Germany) is a competitive anti-assay for the quantitative determination of PGE2 in biological fluids. The kit uses a monoclonal antibody to PGE2 to bind, in a competitive manner, the PGE2 in the sample after a simultaneous incubation at room temperature. The excess reagents were washed away and the substrate was added, after a short incubation time the enzyme reaction was stopped and the yellow colour generated was read on a micro plate reader (DYNATCh, MR 5000) at 405 nm. The intensity of the bound yellow colour is inversely proportional to the concentration of PGE2 in either standard or samples.

Calculation and Evaluation: The PGE2 was calculated for the treated and control groups, then the PGE2 percentage inhibition is determined by the following equation:

$$\% \text{ inhibition} = (A - B) \times 100 / A$$

A = PGE2 in the control group
B = PGE2 in the treated group

RESULTS AND DISCUSSION

2-Amino-6-methyl-4-phenylnicotinonitrile (1) [1] was sprepared according to the literature procedure [4] and using as starting material to synthesize a series of phenyl-1,8-naphthyridine and phenyl-pyrido-[2,3-d]pyrimidine derivatives. Compound 1 was fused with urea or thiourea at 180°C to give the derivatives 2a, b. While, 1 was refluxed with malononitrile or ethylcyanoacetate or formamide to afford substituted pyridopyridine and pyridopyrimidine derivatives 3-5, respectively. Compound 1 was treated with chloroacetylchloride or sodium nitrite to afford compounds 6 and 8, which were reacted with malononitrile and/or hydrazine hydrate to afford compounds 7 [22] and 9, respectively (Scheme 1).

In addition, treatment of 1 with refluxing acetic anhydride, acetic acid/HCl and/or formic acid gave the corresponding N-acetylpyridine 10 and pyridopyrimidine 11 and 12 derivatives, respectively. Treatment of 12 with phosphorus oxychloride afforded chloropyridopyrimidine 13, which was reacted with hydrazine hydrate to give the hydrazinopyridopyrimidine 14. The latter compound was treated with carbon disulfide or acetic anhydride to afford the tricyclic derivatives 15 and 16, respectively (Scheme 2).

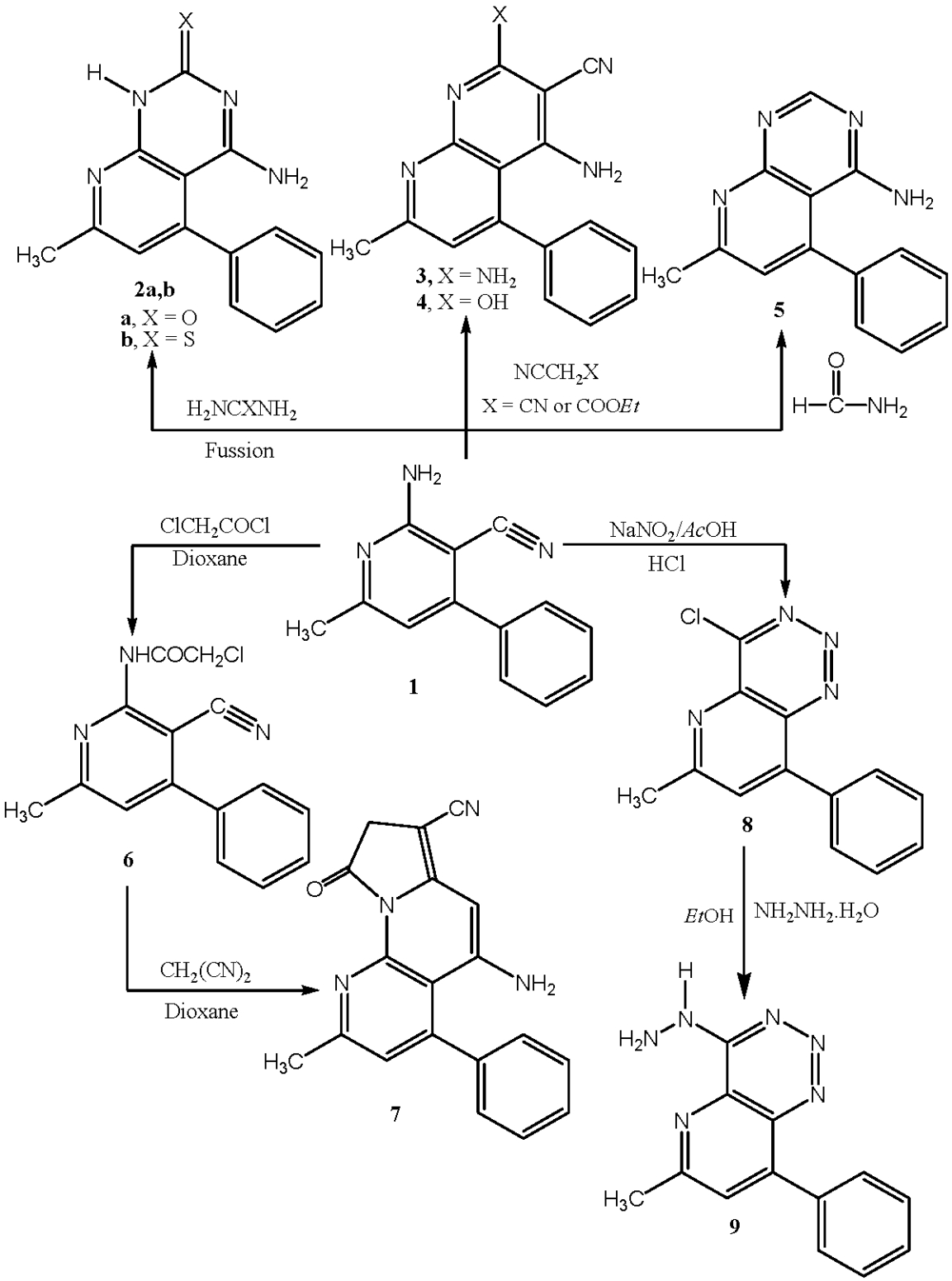
On the other hand, compounds 17 and 18 were prepared by condensation of 14 with diethyl oxalate and/or 4-nitribenzaldehyde and also compounds 19 and 20 were obtained by reaction of 14 with acetyl acetone and/or 3, 4, 5, 6-tetrachlorophthalic anhydride, respectively.

Pharmacological Screening: All animals were obtained from the Animal House Colony, Research Institute of Ophthalmology, Giza, Egypt. Initially, the acute toxicity of the compounds was assayed determining their LD_{50}.

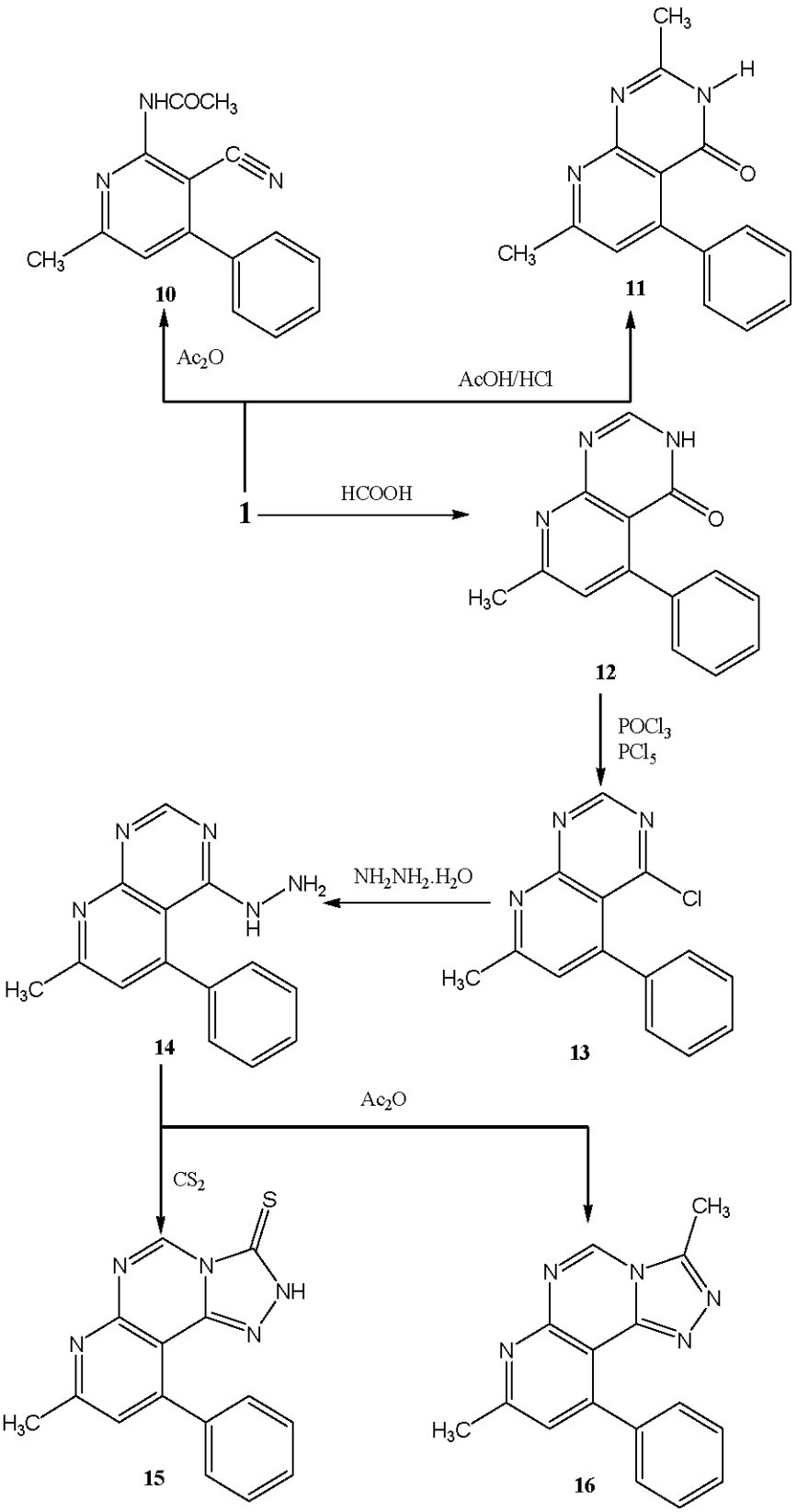
Anti-Inflammatory Potency: Initially the acute toxicity of the compounds was assayed via the determination of their LD_{50}. All the compounds except 2b were interestingly less toxic than Valdecoxib® as the reference drug (Table 1).

The newly synthesized compounds were then pharmacologically screened on male albino rats for their anti-inflammatory potency (Table 2). The evaluation of the anti-inflammatory activities based on a strong biological rational, this involved two criteria present in the tested molecules.

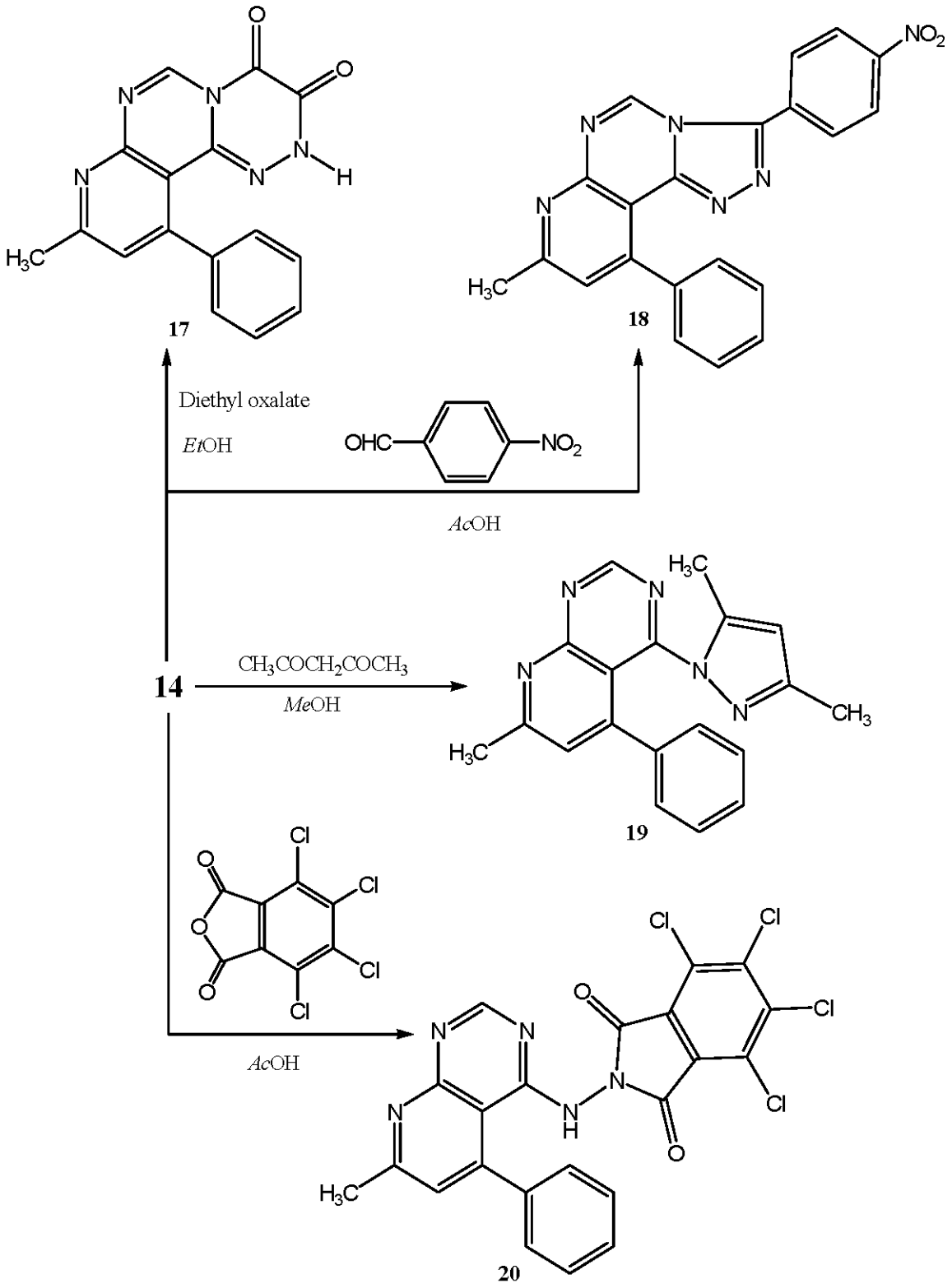
Purpose and Rational: For the determination of the antiphlogistic potency of the synthesized compounds, two standard tests were realized at 25 and 50 mg/kg rat body weight namely, the protection against Carrageenan® induced edema according Winter *et al.* [23] and the inhibition of plasma PGE2. The later is known as a good confirming indicator for the Carrageenan® induced rat paw edema [24].


X
H
N
N
N
NH2
H3C
2a,b
a, X = O
b, X = S
X
CN
N
N
NH2
H3C
3, X = NH2
4, X = OH
N
N
N
NH2
H3C
5
NCCH2X
X = CN or COOEt
H2NCXNH2
Fussion
O
H—C—NH2
NH2
C
N
N
H3C
1
ClCH2COCl
Dioxane
NaNO2/AcOH
HCl
NHCOCH2Cl
C
N
N
H3C
6
Cl
N
N
N
N
H3C
8
CN
O
N
N
NH2
H3C
7
CH2(CN)2
Dioxane
EtOH
NH2NH2.H2O
H
H2N
N
N
N
N
H3C
9

Scheme 1:


NHCOCH3
CN
CH3
10
CH3
H
N
O
11
Ac2O
AcOH/HCl
1
HCOOH
NH
H3C
12
POCl3
PCl5
NH2
NH2NH2.H2O
Cl
14
13
Ac2O
CS2
S
15
16

Scheme 2:


17
18
Diethyl oxalate
EtOH
OHC
NO2
AcOH
14
CH3COCH2COCH3
MeOH
19
AcOH
20

Scheme 3:

Table 1: Acute toxicity (LD_{50}) of the synthesized compounds

Compound N°	LD_{50} [mg/kg]
2a	1.763± 0.010
2b	1.066 ± 0.011
3	1.796 ± 0.010
4	2.115 ± 0.010
6	1.910 ± 0.011
8	2.710 ± 0.011
9	2.315 ± 0.010
10	2.710 ± 0.014
11	3.106 ± 0.011
13	2.070 ± 0.010
14	3.050 ± 0.012
15	2.615 ± 0.010
16	2.420 ± 0.010
17	2.115 ± 0.010
18	2.403 ± 0.012
19	2.100 ± 0.014
20	3.500 ± 0.010
Valdicoxib®	1.635 ± 0.014

Table 2: Anti-inflammatory potencies of the synthesized compounds

Compound N°	Dose [mg/kg]	Protection against carrageenan-induced edema [%]*	Inhibition of plasma PGE2 [%]*
2a	25	90.65 ± 0.082	97.80 ± 0.084
	50	93.90 ± 0.080	91.15 ± 0.089
2b	25	91.56 ± 0.085	88.40 ± 0.086
	50	96.50 ± 0.081	92.70 ± 0.102
3	25	88.75 ± 0.073	91.16 ± 0.087
	50	94.80 ± 0.072	90.62 ± 0.102
4	25	91.25 ± 0.078	77.99 ± 0.095
	50	94.15 ± 0.073	83.05 ± 0.086
6	25	87.46 ± 0.058	80.76 ± 0.107
	50	95.45 ± 0.070	92.98 ± 0.109
8	25	55.74 ± 0.066	51.10 ± 0.100
	50	75.10 ± 0.072	69.96 ± 0.097
9	25	88.85 ± 0.076	77.05 ± 0.077
	50	95.90 ± 0.066	83.88 ± 0.080
10	25	53.99 ± 0.065	47.55 ± 0.064
	50	72.98 ± 0.047	69.95 ± 0.086
11	25	92.88 ± 0.064	77.35 ± 0.088
	50	93.15 ± 0.074	81.55 ± 0.086
13	25	53.33 ± 0.076	46.28 ± 0.090
	50	65.15 ± 0.065	61.32 ± 0.110
14	25	-	-
	50	39.10 ± 0.080	32.99 ± 0.084
15	25	91.88 ± 0.064	82.16 ± 0.075
	50	93.10 ± 0.068	78.96 ± 0.076
16	25	-	-
	50	83.98 ± 0.065	77.50 ± 0.086
17	25	55.24 ± 0.054	43.18 ± 0.088
	50	66.25 ± 0.066	62.13 ± 0.078
18	25	-	-
	50	44.78 ± 0.054	36.18 ± 0.088
19	25	-	-
	50	51.98 ± 0.079	46.61 ± 0.090
20	25	46.88 ± 0.079	41.16 ± 0.077
	50	63.15 ± 0.055	54.17 ± 0.091
Valdicoxib®	25	80.95 ± 0.088	77.00 ± 0.084
	50	92.98 ± 0.075	91.00 ± 0.087

* The doses tested were 25, 50 mg/kg and carryout three determinations for each dose

Regarding the protection against Carrageenan® induced edema, eight compounds namely 2a, 2b, 3, 4, 11, 6, 9 and 15 were found more potent than Valdecoxib®. Their protection percentage against carrageenan induced edema at two-dose levels 25 and 50 mg/kg are 90.65/93.90, 91.56/96.50, 88.75/94.80, 91.25/94.15, 92.88/93.15, 87.46/95.45, 88.85/95.90 and 91.88/93.10, respectively (Valdecoxib® 80.95/92.98). On the other hand,

the inhibition of plasma PGE2 for the compounds 2a, 2b, 3 and 6 were found more potent than Valdecoxib® at two tested doses levels 25 and 50 mg/kg. The inhibition percentages for the latter compounds were found as: 97.80/91.15, 88.40/92.70, 80.16/91.62 and 80.76/92.98, respectively.

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