Pharmacological and Acute Toxicity Studies of some Synthesized Macrocyclic Bis-Schiff-Base Candidates

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Abstract: A series of macrocyclic Schiff-bases have been prepared via the cyclo-condensation of pyridine-2,6-dicarbonyl dichloride **(1)** with appropriate dibasic amino acids. The macrocyclic tricyclo-bis-acid hydrazide **3** was chemically synthesized, starting from the acid chloride **1** by coupling with L-ornithine methyl esters to afford the corresponding bis-ester **2**, followed by coupling with hydrazine hydrate. Condensation of bis-hydrazide **3** with diacid anhydrides or aromatic aldehydes in refluxing acetic acid or ethanol gave the corresponding macrocyclic bis-imides **4, 5** and macrocyclic bis-hydrazones **6a-j,** respectively. The pharmacological screening showed that many of these newly synthesized compounds have good anti-inflammatory and analgesic activities comparable to diclofenac potassium and valdecoxib as reference drugs. The structure assignment of the new compounds was based on chemical and spectroscopic evidences.

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1. Introduction:

Among the different areas of macrocyclic chemistry, the synthesis and complexing properties of azacrown compounds have been a subject of intensive exploration [1-7]. Synthesis of chemical modifications of existing antibacterial agents in order to generate novel macro-molecules with better therapeutic properties is necessary because of the emergence of multidrug resistant bacteria [8]. In the other hand, peptides rarely function well as drugs due to their low bioavailability and rapid degradation within cells [9]. In this concept, we reported the synthesis of some macrocyclic candidates from dipicolinic acid with amino acids and their biological activity screening [10-14]. On the other hand, the synthesis of chemosensors as an interesting approaches providing accurate analytical tools in different analytical fields. In particular, 2,6-peptido-pyridines exhibited a general ionophoric potency [15] and were used for inventing novel thiocyanate-selective membrane sensors [16]. Recently, some of new macrocyclic derivatives have been studied as anti-inflammatory [17], anticonvulsant and antiparkinsonian [18], antimicrobial activities [19,20]. In view of these observations and as continuation of our previous work in macrocyclic and heterocyclic chemistry, we have synthesized some new macrocyclic compounds containing amino acid and pyridine moiety, and tested their selected as anti-inflammatory and analgesic agents.

2. Experimental

Chemistry

Melting points were determined in open glass capillaries using in Electrothermal IA 9000 Series digital melting point apparatus (Electrothermal, Essex, U.K.) and are uncorrected. Elemental analyses were performed with all final compounds with an Elementar, Vario EL, Microanalytical Unit, National Research Centre, Cairo Egypt and were in good agreement (±0.2%) with the calculated values. The IR spectra (KBr) were recorded on an FT IR-8201 PC spectrophotometer (Schimadzu, Japan). The ^{1}H- and ^{13}C-NMR spectra were measured with a Jeol 270 MHz spectrometer (FTGNM-EX 270, Japan) in DMSO-d_6 or $CDCl_3$. The chemical shifts were recorded relative to TMS. The Mass spectra (EI) were run at 70 eV with a Finnegan SSQ 7000 spectrometer (Thermoinstrument System Incorporated, USA), *m/z* values are indicated in Dalton. TLC (Silica gel, aluminum sheets 60 F_{254}, Merck, Darmstadt, Germany) was used for tracing the reactions. The starting material **3** was prepared according to reported procedure [13].

Synthesis of Bis-imido-tricyclo-[3,23,1,1^{11,15}] triaconta-1(28),11,13,15,25,27-hexene derivatives 4 and 5a,b

A suspension of the hydrazide derivative **3** (0.554 g, 1 mmol) and 1,8-naphthalindicarboxylic anhydride, phthalic anhydride or 2,3,4,5-tetrachlorophthalic anhydride (2 mmol) in acetic acid (50 mL) was refluxed for 7 h. The solid was collected by filtration, washed with acetic acid and crystallized from dimethylformamide/water to give the corresponding macrocyclic octaamide dipyridyl derivatives **4** and **5a,b**, respectively.

4,20-Di-(oxo-[N-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)amino]-3,9,17,23,29,30-hexaaza-2,10,16, 24-tetraoxo-tricyclo[3,23,1,1^{11,15}]triaconta-1(28), 11,13,15,25,27-hexene (4). IR (KBr, cm^{-1}): ν 3340 (NH, amide), 1640 (C=N), 1660, 1534, 1320 (amide I, II and III). ^{1}H-NMR (DMSO-d$_6$): δ 1.32-1.36 (m, 4H, 2 ×C*H*$_2$), 1.54-1.62 (m, 4H, 2 × C*H*$_2$), 3.18-3.26 (m, 4H, 2 × C*H*$_2$), 4.38-4.54 (m, 2H, 2 ×C*H*-N), 7.75-8.10 (m, 12H, Ar-H), 8.24-8.36 (m, 6H, 2×pyr-*H*), 8.92 (m, 2H, 2 × N*H*, exchangeable with D$_2$O), 9.16 (m, 2H, 2×N*H*, exchangeable with D$_2$O) and 10.08 (m, 2H, 2×N*H*, exchangeable with D$_2$O). ^{13}C-NMR: 27.62, 30.54, 38.54 (*6CH*$_2$), 52.26 (2*CH*NH), 125.10, 125.16, 137.10, 137.14, 148.10, 148.24 (10pyr-*C*), 122.45, 124.98, 127.24, 128.95, 137.43, 137.76 (20Ar-C), 163.46, 169.50 (4*CO*NH), 157.88 (4*CO*-imide), 170.38 (*2CO*-amide). MS, *m/z* (%): 914 [M$^+$, 24], 703 (14), 675 (45), 464 (72), 436 (35), 303 (76), 239 (100).

4,20-Di-[oxo-(2-aminoisoindoline-1,3-dioxo)]-3,9,17,23,29,30-hexaaza-2,10,16,24-tetraoxotri-cyclo-[3,23,1,1^{11,15}]triaconta-1(28),11,13,15, 25,27-hexene (5a). IR (KBr, cm^{-1}): ν 3338 (NH, amide), 1642 (C=N), 1665, 1540, 1322 (amide I, II and III). ^{1}H-NMR (DMSO-d$_6$): δ 1.23-1.32 (m, 4H, 2 ×C*H*$_2$), 1.48-1.58 (m, 4H, 2 × C*H*$_2$), 3.15-3.20 (m, 4H, 2 × C*H*$_2$), 4.42-4.52 (m, 2H, 2 ×C*H*-N), 7.65-7.80 (m, 8H, Ar-H), 8.30-8.38 (m, 6H, 2×pyr-*H*), 8.88 (m, 2H, 2 × N*H*, exchangeable with D$_2$O), 9.12 (m, 2H, 2×N*H*, exchangeable with D$_2$O) and 10.15 (m, 2H, 2×N*H*, exchangeable with D$_2$O). ^{13}C-NMR: 27.65, 30.52, 38.58 (*6CH*$_2$), 52.12 (2*CH*NH), 125.08, 125.12, 137.10, 137.18, 148.16, 148.32 (10pyr-*C*), 123.18, 131.78, 132.45 (12Ar-C), 163.62, 169.58 (4*CO*NH), 164.35 (4*CO*-imide), 170.15 (*2CO*-amide). MS, *m/z* (%): 814 [M$^+$, 33], 653 (22), 522 (62), 492 (42), 464 (55), 436 (24), 189 (100).

4,20-Di-[oxo-(2-amino-4,5,6,7-tetrachloroisoindo-line-1,3-dioxo)]-3,9,17,23,29,30-hexaaza-2,10,16, 24-tetraoxo-tricyclo-[3,23,1,1^{11,15}]triaconta-1(28), 11,13,15,25,27-hexene (5b). IR (KBr, cm^{-1}): ν 3346 (NH, amide), 1645 (C=N), 1662, 1538, 1318 (amide I, II and III). ^{1}H-NMR (DMSO-d$_6$): δ 1.26-1.33 (m, 4H, 2 ×C*H*$_2$), 1.38-1.55 (m, 4H, 2 × C*H*$_2$), 3.18-3.24 (m, 4H, 2 × C*H*$_2$), 4.46-4.54 (m, 2H, 2 ×C*H*-N), 8.26-8.34 (m, 6H, 2×pyr-*H*), 8.92 (m, 2H, 2 × N*H*, exchangeable with D$_2$O), 9.18 (m, 2H, 2×N*H*, exchangeable with D$_2$O) and 10.08 (m, 2H, 2×N*H*, exchangeable with D$_2$O). ^{13}C-NMR: 27.55, 30.58, 38.60 (*6CH*$_2$), 52.22 (2*CH*NH), 124.98, 125.05, 137.12, 137.16, 148.22, 148.30 (10pyr-*C*), 127.12, 132.96, 134.75 (12Ar-C), 163.58, 169.54 (4*CO*NH), 164.48 (4*CO*-imide), 170.26 (*2CO*-amide). MS, *m/z* (%):1086 [M$^+$, 8], 789 (15), 492 (64), 464 (32), 436 (24), 324 (100).

Synthesis of 4,20-di[oxo (substituted) carbo-hydrazonylmethyl)-3,9,17,23,29,30-hexaaza-2,10,16,24-tetraoxotricyclo[3,23,1,1^{11,15}]triaconta-1(28),11,13,15,25,27-hexene (6a-j)

A mixture of the hydrazide derivative **3** (0.554 g, 1 mmol) and the appropriate aldehydes (2 mmol) in absolute ethanol (50 ml) was heated under reflux for 6 h. The solvent was evaporated under reduced pressure and the residue was solidified with ether. The solid was collected by filtration, washed with ether and crystallized from a proper solvent to afford the corresponding macrocyclic hydrazone derivatives **6a-j**, respectively.

4,20-Di-(oxo-phenylcarbohydrazonylmethyl)-3,9,17,23,29,30-hexaaza-2,10,16,24-tetraoxo-tricyclo-[3,23,1,1^{11,15}]triaconta-1(28),11,13,15,25, 27-hexene (6a). IR (KBr, cm^{-1}): ν 3342 (NH, amide), 1646 (C=N), 1668, 1542, 1318 (amide I, II and III). ^{1}H-NMR (DMSO-d$_6$): δ 1.28-1.34 (m, 4H, 2 ×C*H*$_2$), 1.50-1.60 (m, 4H, 2 × C*H*$_2$), 3.10-3.18 (m, 4H, 2 × C*H*$_2$), 4.44-4.56 (m, 2H, 2 ×C*H*-N), 7.45-7.68 (m, 12H, 2Ph-*H* + 2CH=N), 8.25-8.38 (m, 6H, 2×pyr-*H*), 8.86 (m, 2H, 2 × N*H*, exchangeable with D$_2$O), 8.94 (m, 2H, 2×N*H*, exchangeable with D$_2$O) and 10.08 (m, 2H, 2×N*H*, exchangeable with D$_2$O). ^{13}C-NMR: 27.82, 30.40, 38.52 (*6CH*$_2$), 52.05 (2*CH*NH), 147.12 (*2CH*=N), 125.16, 125.24, 137.15, 137.22, 148.20, 148.42 (10pyr-*C*), 123.85, 127.94, 129.38, 132.46 (12Ar-C), 163.66, 169.64 (4*CO*NH), 171.98 (*2CO*-hydrazone). MS, *m/z* (%):730 [M$^+$, 6], 611 (34), 492 (45), 436 (55), 218 (100).

4,20-Di-[oxo-(3-bromophenyl)carbohydrazonyl-methyl]-3,9,17,23,29,30-hexaaza-2,10,16,24-tetra-oxo-tricyclo-[3,23,1,1^{11,15}]triaconta-1(28),11,13,15, 25,27-hexene (6b). IR (KBr, cm^{-1}): ν 3343 (NH, amide), 1640 (C=N), 1660, 1542, 1318 (amide I, II and III). ^{1}H-NMR (DMSO-d$_6$): δ 1.26-1.34 (m, 4H, 2 ×C*H*$_2$), 1.50-1.62 (m, 4H, 2 × C*H*$_2$), 3.18-3.24 (m,

4H, 2 × CH_2), 4.50-4.56 (m, 2H, 2 ×C*H*-N), 7.35-7.66 (m, 6H, Ar-*H*), 7.78 (s, 2H, Ar-H), 7.92 (s, 2H, 2CH=N), 8.24-8.38 (m, 6H, 2×pyr-*H*), 8.96 (m, 2H, 2 × N*H*, exchangeable with D_2O), 9.15 (m, 2H, 2×N*H*, exchangeable with D_2O) and 10.16 (m, 2H, 2×N*H*, exchangeable with D_2O). ^{13}C-NMR: 27.70, 30.38, 38.52 (*6*CH_2), 51.98 (2*CH*NH), 147.08 (*2CH*=N), 125.06, 125.12, 137.10, 137.16, 148.08, 148.16 (10pyr-*C*), 123.82, 127.94, 129.15, 132.18, 133.82, 135.32 (12Ar-C), 163.68, 169.76 (4*CO*NH), 172.14 (*2CO*-hydrazone). MS, *m/z* (%): 888 [M^++2, 23], 886 [M^+, 7], 691 (45), 689 (76), 492 (100), 436 (55), 218 (82).

4,20-Di-[oxo-(p-bromophenyl) carbohydrazonyl-methyl]-3,9,17,23,29,30-hexaaza-2,10,16,24-tetra-oxotricyclo-[3,23,1,1^{11,15}]triaconta-1(28),11,13,15, 25,27-hexene (6c). IR (KBr, cm^{-1}): ν 3338 (NH, amide), 1644 (C=N), 1663, 1545, 1322 (amide I, II and III). ^{1}H-NMR (DMSO-d_6): δ 1.25-1.35 (m, 4H, 2 ×CH_2), 1.52-1.64 (m, 4H, 2 × CH_2), 3.15-3.20 (m, 4H, 2 × CH_2), 4.54-4.58 (m, 2H, 2 ×C*H*-N), 7.55-7.78 (m, 10H, 2Ph-*H* + 2CH=N), 8.18-8.35 (m, 6H, 2×pyr-*H*), 8.88 (m, 2H, 2 × N*H*, exchangeable with D_2O), 8.96 (m, 2H, 2×N*H*, exchangeable with D_2O) and 10.12 (m, 2H, 2×N*H*, exchangeable with D_2O). ^{13}C-NMR: 27.75, 30.42, 38.55 (*6*CH_2), 52.15 (2*CH*NH), 146.98 (*2CH*=N), 125.18, 125.22, 137.05, 137.12, 148.18, 148.25 (10pyr-*C*), 123.80, 127.96, 129.34, 133.48 (12Ar-C), 163.65, 169.72 (4*CO*NH), 172.08 (*2CO*-hydrazone). MS, *m/z* (%):888 [M^++2, 12], 886 [M^+, 12], 691 (34), 689 (32), 492 (100), 436 (35), 218 (94).

4,20-Di-[oxo-(2,6-dichlorophenyl)carbohydra-zonylmethyl]-3,9,17,23,29,30-hexaaza-2,10,16,24-tetraoxo-tricyclo-[3,23,1,1^{11,15}]triaconta-1(28),11, 13,15,25,27-hexene (6d). IR (KBr, cm^{-1}): ν 3344 (NH, amide), 1648 (C=N), 1660, 1541, 1319 (amide I, II and III). ^{1}H-NMR (DMSO-d_6): δ 1.26-1.32 (m, 4H, 2 ×CH_2), 1.50-1.62 (m, 4H, 2 × CH_2), 3.18-3.22 (m, 4H, 2 × CH_2), 4.52-4.60 (m, 2H, 2 ×C*H*-N), 7.40-7.48 (m, 8H, 2Ph-*H* + 2CH=N), 8.23-8.38 (m, 6H, 2×pyr-*H*), 8.78 (m, 2H, 2 × N*H*, exchangeable with D_2O), 8.98 (m, 2H, 2×N*H*, exchangeable with D_2O) and 10.15 (m, 2H, 2×N*H*, exchangeable with D_2O). ^{13}C-NMR: 27.66, 30.39, 38.65 (*6*CH_2), 52.09 (2*CH*NH), 147.068 (*2CH*=N), 125.23, 125.25, 137.08, 137.10, 148.16, 148.22 (10pyr-*C*), 126.56, 127.48, 129.36, 133.52 (12Ar-C), 163.58, 169.68 (4*CO*NH), 172.15 (*2CO*-hydrazone). MS, *m/z* (%): 866 [M^+, 8], 679 (18), 492 (58), 436 (42), 245 (100), 205 (78).

4,20-Di-[oxo-(3,4-dichlorophenyl)carbohydrazonyl methyl]-3,9,17,23,29,30-hexaaza-2,10,16,24-tetra-oxotricyclo-[3,23,1,1^{11,15}]triaconta-1(28),11,13,15, 25,27-hexene (6e). IR (KBr, cm^{-1}): ν 3346 (NH, amide), 1626 (C=N), 1662, 1539, 1322 (amide I, II and III). ^{1}H-NMR (DMSO-d_6): δ 1.28-1.35 (m, 4H, 2 ×CH_2), 1.49-1.60 (m, 4H, 2 × CH_2), 3.24-3.26 (m, 4H, 2 × CH_2), 4.46-4.58 (m, 2H, 2 ×C*H*-N), 7.55-7.65 (m, 6H, 4H-Ar + 2CH=N), 7.86 (s, 2H, Ar-H), 8.18-8.26 (m, 6H, 2×pyr-*H*), 8.84 (m, 2H, 2 × N*H*, exchangeable with D_2O), 9.05 (m, 2H, 2×N*H*, exchangeable with D_2O) and 10.18 (m, 2H, 2×N*H*, exchangeable with D_2O). ^{13}C-NMR: 27.34, 30.42, 37.98 (*6*CH_2), 51.96 (2*CH*NH), 147.08 (*2CH*=N), 124.95, 125.05, 137.10, 137.14, 148.08, 148.12 (10pyr-*C*), 126.94, 129.45, 129.55, 132.65, 132.76, 134.68 (12Ar-C), 163.45, 169.72 (4*CO*NH), 171.88 (*2CO*-hydrazone). MS, *m/z* (%): 866 [M^+, 12], 868 {M^++2, 5], 679 (22), 492 (25), 436 (56), 245 (78), 214 (100).

4,20-Di-[oxo-(2-chloro-6-flourophenyl)carbohydrazonylmethyl]-3,9,17,23,29,30-hexaaza-2,10,16,24-tetraoxo-tricyclo-[3,23,1,1^{11,15}]triaconta-1(28),11,13,15,25,27-hexene (6f). IR (KBr, cm^{-1}): ν 3352 (NH, amide), 1618 (C=N), 1660, 1538, 1324 (amide I, II and III). ^{1}H-NMR (DMSO-d_6): δ 1.34-1.38 (m, 4H, 2 ×CH_2), 1.44-1.58 (m, 4H, 2 × CH_2), 3.30-3.36 (m, 4H, 2 × CH_2), 4.50-4.57 (m, 2H, 2 ×C*H*-N), 7.45-7.85 (m, 8H, Ar-H + 2CH=N), 8.15-8.30 (m, 6H, 2×pyr-*H*), 8.86 (m, 2H, 2 × N*H*, exchangeable with D_2O), 9.10 (m, 2H, 2×N*H*, exchangeable with D_2O) and 10.16 (m, 2H, 2×N*H*, exchangeable with D_2O). ^{13}C-NMR: 27.54, 30.36, 37.84 (*6*CH_2), 52.04 (2*CH*NH), 147.12 (*2CH*=N), 125.12, 125.16, 137.16, 137.24, 147.96, 148.05 (10pyr-*C*), 113.68, 117.88, 124.82, 133.52, 134.56, 161.02 (12Ar-C), 163.62, 169.76 (4*CO*NH), 171.94 (*2CO*-hydrazone). MS, *m/z* (%): 834 [M^+, 17], 8636 [M^++2, 6], 663 (18), 492 (15), 464 (8), 245 (62), 199 (100).

4,20-Di-[oxo-(p-methylphenyl) carbohydrazonyl-methyl]-3,9,17,23,29,30-hexaaza-2,10,16,24-tetra-oxotricyclo-[3,23,1,1^{11,15}]triaconta-1(28),11,13,15, 25,27-hexene (6g). IR (KBr, cm^{-1}): ν 3340 (NH, amide), 1638 (C=N), 1660, 1552, 1324 (amide I, II and III). ^{1}H-NMR (DMSO-d_6): δ 1.32-1.38 (m, 4H, 2 ×CH_2), 1.55-1.65 (m, 4H, 2 × CH_2), 2.25 (s, 6H, 2 x CH_3), 3.18-3.24 (m, 4H, 2 × CH_2), 4.50-4.60 (m, 2H, 2 ×C*H*-N), 7.48-7.85 (m, 10H, 2Ph-*H* + 2CH=N), 8.24-8.32 (m, 6H, 2×pyr-*H*), 8.78 (m, 2H, 2 × N*H*, exchangeable with D_2O), 8.95 (m, 2H, 2×N*H*, exchangeable with D_2O) and 10.18 (m, 2H, 2×N*H*, exchangeable with D_2O). ^{13}C-NMR: 20.32 (CH_3), 27.45, 30.32, 38.64 (*6*CH_2), 52.18 (2*CH*NH), 147.08 (*2CH*=N), 124.96, 125.05, 137.08, 137.10, 148.14,

148.18 (10pyr-*C*), 125.80, 128.05, 129.30, 139.48 (12Ar-C), 163.75, 169.77 (4*CO*NH), 172.15 (*2CO*-hydrazone). MS, *m/z* (%): 625 [M^+, 8], 492 (100), 464 (15), 436 (25), 351 (9), 218 (78).

4,20-Di-[oxo-(2-methoxyphenyl)carbohydrazonyl-methyl]-3,9,17,23,29,30-hexaaza-2,10,16,24-tetra-oxo-tricyclo-[3,23,1,$1^{11,15}$]triaconta-1(28),11,13,15, 25,27-hexene (6h). IR (KBr, cm^{-1}): ν 3338 (NH, amide), 1640 (C=N), 1662, 1552, 1320 (amide I, II and III). ^{1}H-NMR (DMSO-d_6): δ 1.34-1.38 (m, 4H, 2 ×CH_2), 1.62-1.68 (m, 4H, 2 × CH_2), 3.18-3.25 (m, 4H, 2 × CH_2), 3.78 (s, 6H, 2 x OCH_3), 4.45-4.55 (m, 2H, 2 ×C*H*-N), 7.36-7.76 (m, 10H, 2Ph-*H* + 2CH=N), 8.20-8.32 (m, 6H, 2×pyr-*H*), 8.88 (m, 2H, 2 × N*H*, exchangeable with D_2O), 9.10 (m, 2H, 2×N*H*, exchangeable with D_2O) and 10.32 (m, 2H, 2×N*H*, exchangeable with D_2O). ^{13}C-NMR: 27.45, 30.68, 38.82 (*6*CH_2), 52.48 (2*CH*NH), 55.14 (2C, 2OCH_3), 147.30 (*2CH*=N), 124.86, 125.02, 136.95, 137.04, 148.06, 148.18 (10pyr-*C*), 112.75, 115.86, 120.86, 131.14, 132.05, 156.95 (12Ar-C), 163.68, 170.08 (4*CO*NH), 172.55 (*2CO*-hydrazone). MS, *m/z* (%):790 [M^+, 15], 657 (12), 641 (45), 528 (22), 379 (95), 351 (35), 218 (100), 149 (18).

4,20-Di-[oxo-(4-methoxyphenyl)carbohydrazonyl-methyl]-3,9,17,23,29,30-hexaaza-2,10,16,24-tetra-oxo-tricyclo-[3,23,1,$1^{11,15}$]triaconta-1(28),11,13,15, 25,27-hexene (6i). IR (KBr, cm^{-1}): ν 3346 (NH, amide), 1642 (C=N), 1662, 1555, 1319 (amide I, II and III). ^{1}H-NMR (DMSO-d_6): δ 1.28-1.35 (m, 4H, 2 ×CH_2), 1.60-1.67 (m, 4H, 2 × CH_2), 3.20-3.26 (m, 4H, 2 × CH_2), 3.68 (s, 6H, 2 x OCH_3), 4.44-4.58 (m, 2H, 2 ×C*H*-N), 7.58-7.90 (m, 10H, 2Ph-*H* + 2CH=N), 8.22-8.30 (m, 6H, 2×pyr-*H*), 8.84 (m, 2H, 2 × N*H*, exchangeable with D_2O), 9.06 (m, 2H, 2×N*H*, exchangeable with D_2O) and 10.24 (m, 2H, 2×N*H*, exchangeable with D_2O). ^{13}C-NMR: 27.52, 30.62, 38.74 (*6*CH_2), 52.22 (2*CH*NH), 54.66 (2C, 2OCH_3), 147.12 (*2CH*=N), 125.05, 125.10, 137.10, 137.14, 148.16, 148.24 (10pyr-*C*), 113.98, 125.64, 129.68, 162.62 (12Ar-C), 163.82, 169.76 (4*CO*NH), 172.25 (*2CO*-hydrazone). MS, *m/z* (%):790 [M^+, 24], 657 (9), 641 (76), 528 (12), 379 (100), 351 (45), 218 (78), 149 (68).

4,20-Di-[oxo-(3,4,5-trimethoxyphenyl)carbo-hydrazonylmethyl]-3,9,17,23,29,30-hexaaza-2,10,16,24-tetraoxotricyclo-[3,23,1,$1^{11,15}$]triaconta-1(28),11,13,15,25,27-hexene (6j). IR (KBr, cm^{-1}): ν 3336 (NH, amide), 1640 (C=N), 1662, 1556, 1322 (amide I, II and III). ^{1}H-NMR (DMSO-d_6): δ 1.32-1.36 (m, 4H, 2 ×CH_2), 1.58-1.65 (m, 4H, 2 × CH_2), 3.18-3.28 (m, 4H, 2 × CH_2), 3.72 (s, 18H, 6 x OCH_3), 4.34-4.42 (m, 2H, 2 ×C*H*-N), 7.25 (s, 4H, 2Ph-H), 7.88 (s, 2H, 2CH=N), 8.26-8.34 (m, 6H, 2×pyr-*H*), 8.92 (m, 2H, 2 × N*H*, exchangeable with D_2O), 9.14 (m, 2H, 2×N*H*, exchangeable with D_2O) and 10.16 (m, 2H, 2×N*H*, exchangeable with D_2O). ^{13}C-NMR: 27.46, 30.68, 38.66 (*6*CH_2), 52.18 (2*CH*NH), 55.16 (4C, 4OCH_3), 58.78 (2C, 2OCH_3), 147.24 (*2CH*=N), 124.98, 125.02, 137.15, 137.18, 148.10, 148.14 (10pyr-*C*), 105.12, 127.35, 140.64, 152.76 (12Ar-C), 163.86, 169.69 (4*CO*NH), 172.22 (*2CO*-hydrazone). MS, *m/z* (%): 910 [M^+, 15], 777 (8), 701 (12), 673 (25), 692 (76), 436 (58), 303 (22), 237 (100), 218 (78).

Pharmacological screening

Determination of acute toxicity (LD_{50})

The LD_{50} was determined by using rats. They were injected with different increasing doses of the synthesized compounds. The dose that killed 50% of the animals was calculated according to Austen et al. [21] (Table 4).

Table (4): Acute toxicity (LD_{50}) of the synthesized compounds

Compound	LD_{50} [mg/kg]
4	1548.14 ± 0.12
5a	2113.87 ± 0.17
6a	1520.14 ± 0.22
6b	1249.87 ± 0.14
6c	1680.89 ± 0.16
6d	2144.89 ± 0.11
6e	2115.55 ± 0.14
6f	2095.78 ± 0.19
6g	1445.98 ± 0.13
6h	1224.87 ± 0.16
6i	2185.32 ± 0.14
6j	1420.00 ± 0.10
Valdecoxib	1180.01 ± 0.23

All results were significantly different from the normal control value at $P \leq 0.05$.

Anti-inflammatory activity

Carrageenan-induced edema (rats paw test)

Groups of adult male albino rats (150–180 g), each of eight animals were orally dosed with tested compounds at a dose level of 2.5-5 mg/kg one hour before the carrageenan challenge. Foot paw edema was induced by subplantar injection of 0.05 cm^3 of a 1% suspension of carrageenan in saline into the plantar 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 for the control group, and the percentage inhibition of weight of edema was evaluated. Diclofenac potassium (5 mg/kg) was employed as standard reference to which the tested compounds were compared (Table 2).

Estimation of plasma prostaglandin E2 (PGE2)

Heparinized blood samples were collected from rats (n = 8), plasma was separated by centrifugation at 12000g for 2 min at 408C, immediately frozen, and stored at 208C until use. The design correlate EIA prostaglandin E2 (PGE2) kit (Aldrich, Steinheim, Germany) is a competitive immuno 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 color generated was read on a microplate reader DYNATech, MR 5000 at 405 nm (Dynatech Industries Inc., McLean, VA, USA). The intensity of the bound yellow color is inversely proportional to the concentration of PGE2 in either standard or samples.

Analgesic activity

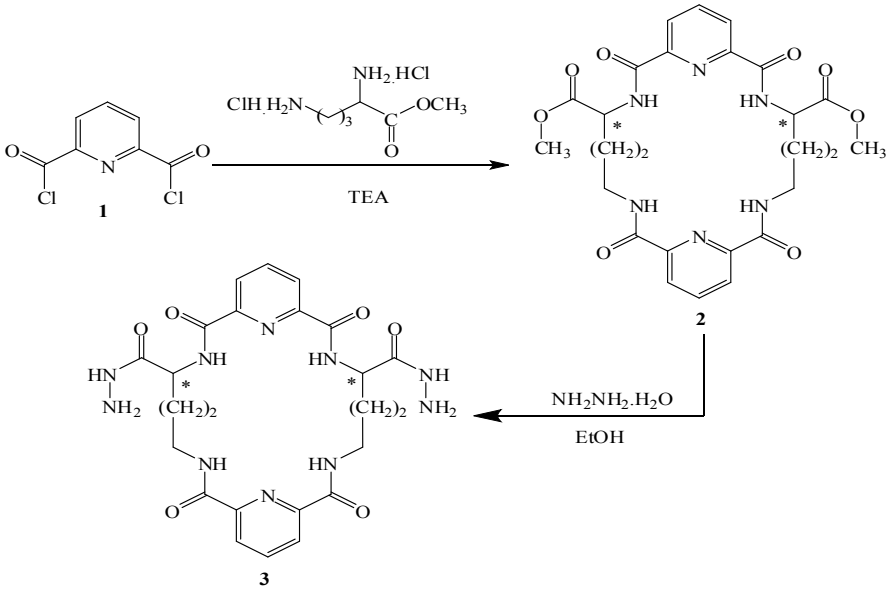
Sixty Webster mice of both sexes weighting 20-25 g were divided into ten groups. One group was kept as control (received saline), the second group received vehicle (gum acacia), and the third one received valdecoxib as a reference drug, whereas the other groups received the test compounds (s.c. administration). Mice were dropped gently in a dry glass beaker of 1 dm3 capacity maintained at 55–55.58C. Normal reaction time in seconds for all animals was determined at time intervals of 10, 20, 30, 45, 60, 90, and 120 minutes. This is the interval extending from the instant the mouse reaches the hot beaker till the animals licks its feet or jump out of the beaker (dose 5 mg/kg) [22]. The relative potencies to valdecoxib were determined (Table 3).

3. Results and discussion

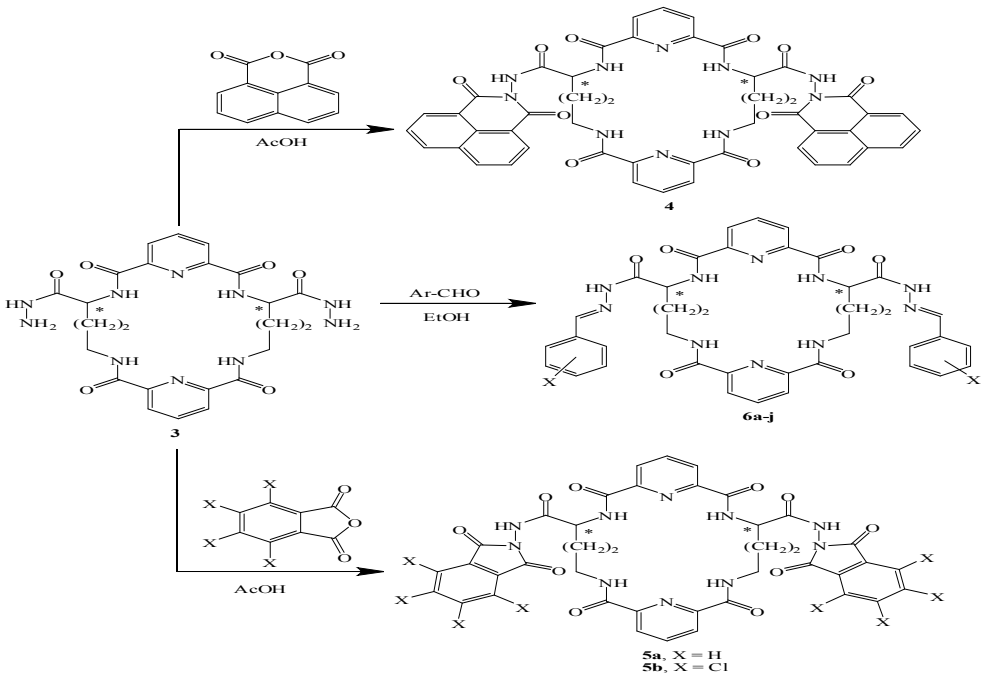
Chemistry

In our previous work we reported the synthesis and a preliminary biological activity screening of several chiral macrocyclic derivatives based on macrocyclic bis-hydrazide **3** [13], which was obtained from the corresponding ester **2** according to the published procedure [23,24] (Scheme 1).

Condensation of the same hydrazide **3** with selected acid anhydrides, namely, 1,8-naphthaline dicarboxylic anhydride, phthalic anhydride or 2,3,4,5-tetrachlorophthalic anhydride, or in refluxing acetic acid afforded the corresponding tricyclo-bis-diimide derivatives **4** and **5a,b**, respectively. Additionally, in light of the aforementioned biological interest of hydrazone derivatives [25-27], the tricycle-bis-hydrazide **3** was condensed with selected aromatic aldehydes in refluxing ethanol to afford the corresponding 4,20-di[oxo(substituted phenyl)carbohydrazonylmethyl)-3,8,16,21,27,28-hexaaza-2,9,15,22-tetraoxotricyclo-[3,21,1,110,14]-octacosa-1(26),10,12,14,23,25-hexene derivatives as tricyclo-bis-hydrazones **6a-j** (Scheme 2). The physical data for the synthesized compounds are summarized in Table 1.



Scheme 1. Synthetic Pathway for Starting Compound 3



Scheme 2. Synthetic Pathway for Compounds 4, 5a,b and 6a-k

Table (1): The physical data of the newly synthesized compounds

Comp. No.	X	Mp (ºC)	Cryst. Solv.	Yield (%)	Molecular Formula (Mol. Wt.)
4	-	276-278	DMF/H_2O	65	$C_{48}H_{38}N_{10}O_{10}$ (914.28)
5a	H	243-245	DMF/H_2O	72	$C_{40}H_{34}N_{10}O_{10}$ (814.25)
5b	Cl	296-298	DMF/H_2O	88	$C_{40}H_{26}C_{l8}N_{10}O_{10}$ (1085.93)
6a	H	178-180	EtOH/Ether	85	$C_{38}H_{38}N_{10}O_6$ (730.30)
6b	3-Br	232-234	MeOH	79	$C_{38}H_{36}Br_2N_{10}O_6$ (886.12)
6c	4-Br	254-256	Dioxane	87	$C_{38}H_{36}Br_2N_{10}O_6$ (886.12)
6d	2,6-Cl_2	198-200	EtOH	68	$C_{38}H_{34}Cl_4N_{10}O_6$ (866.14)
6e	3,4-Cl_2	188-190	EtOH/Ether	78	$C_{38}H_{34}Cl_4N_{10}O_6$ (866.14)
6f	2-Cl-6-F	168-170	AcOH/H_2O	84	$C_{38}H_{34}Cl_2F_2N_{10}O_6$ (834.20)
6g	4-CH_3	155-157	EtOH/H_2O	82	$C_{40}H_{42}N_{10}O_6$ (758.33)
6h	2-OCH_3	210-212	AcOH/H_2O	90	$C_{40}H_{42}N_{10}O_8$ (790.32)
6i	4-OCH_3	216-218	EtOH/H_2O	80	$C_{40}H_{42}N_{10}O_8$ (790.32)
6j	3,4,5-$(OCH_3)_3$	235-257	AcOH/H_2O	75	$C_{44}H_{50}N_{10}O_{12}$ (910.36)

Pharmacological screening

All animals were obtained from the Animal House Colony, Research Institute of Ophthalmology, Giza, Egypt. The newly synthesized compounds were screened pharmacologically for their analgesic and anti-inflammatory activities using male albino rats (Tables 2 and 3). Initially, the acute toxicity of the compounds was assayed determining their LD_{50}. Interestingly, all the synthesized compounds were less toxic than valdecoxib (Table 4).

Anti-inflammatory activity

Purpose and rational

For the determination of the antiphlogistic potency of the synthesized compounds, two standard tests wererealized at a dose level 2.5 and 5 mg/kg body weight of the rats, namely, the protection against carrageenaninduced edema according to Winter *et al.* [28] and the inhibition of plasma PGE2. The latter is known as a good confirming indicator for the carrageenan-induced rat paw edema [29]. Regarding the protection against carrageenan-induced edema, all tested compounds, were found to be more potent than diclofenac potassium. For these compounds, a similar activity profile was realized for the inhibition of plasma PGE2. Concerning the anti-inflammatory activities, the descending order of activity is **6f, 6d, 6e, 6j, 4, 6i, 6c, 6b, 6h, 5a, 6g,** and **6a**. Compounds **6f, 6d, 6e, 6j**, and **4** are the most active products.

Table (2): Anti-inflammatory activities of some synthesized compounds.

Group	Dose [mg/kg]	% Protection against edema	% Inhibition of plasma PGE2
4	2.5 5	86.54 ± 0.072 93.95 ± 0.060	72.70 ± 0.036 78.10 ± 0.058
5a	2.5 5	80.08 ± 0.072 90.25 ± 0.062	58.85 ± 0.030 73.54 ± 0.040
6a	2.5 5	75.88 ± 0.086 78.00 ± 0.060	57.45 ± 0.041 76.05 ± 0.040
6b	2.5 5	78.50 ± 0.075 91.88 ± 0.060	60.10 ± 0.040 77.84 ± 0.032
6c	2.5 5	80.24 ± 0.054 92.29 ± 0.065	57.12 ± 0.040 77.05 ± 0.040
6d	2.5 5	86.22 ± 0.075 96.75 ± 0.060	81.55 ± 0.035 84.05 ± 0.041
6e	2.5 5	88.66 ± 0.037 95.88 ± 0.035	61.86 ± 0.052 82.10 ± 0.050
6f	2.5 5	90.42 ± 0.086 97.55 ± 0.080	64.16 ± 0.041 85.16 ± 0.072
6g	2.5 5	74.32 ± 0.078 89.88 ± 0.095	55.85 ± 0.040 75.21 ± 0.040
6h	2.5 5	85.36 ± 0.060 90.85 ± 0.072	70.45 ± 0.050 75.66 ± 0.048
6i	2.5 5	80.24 ± 0.055 93.06 ± 0.051	57.45 ± 0.041 78.22 ± 0.031
6j	2.5 5	92.35 ± 0.050 94.90 ± 0.042	86.31 ± 0.041 80.52 ± 0.052
Diclofenac potassium	2.5 5	70.14 ± 0.061 75.23 ± 0.083	54.00 ± 0.041 70.00 ± 0.051

All results were significantly different from the standard and normal control value at $P \leq 0.05$.

Analgesic activity

All tested compounds exhibited analgesic activity in a hot-plate assay (Table 3). Interestingly, the analgesic activities of all the compounds **4, 5a** and **6a-j** were more potent than valdecoxib as a reference drug (Table 3) and, compared to valdecoxib after 120 min these analgesic activities were increased. Compounds **6d, 6e, 6f, 6i, 6j, 4, 6b, 6c, 5a, 6h, 6a**, and **6g** are arranged in descending order of analgesic potency. Compound **6d** showed more than three times the activity of valdecoxib, while compounds **6e, 6f, 6i,** and **6j** showed double activity as compared to valdecoxib after two hours.

Table (3): Analgesic activities of some synthesized compounds.

Compound	Comparative analgesic potency to Valdecoxib after time [in min]					
	10 min	**20 min**	**30 min**	**60 min**	**90 min**	**120 min**
4	0.55 ± 0.02	0.55 ± 0.05	0.80 ± 0.07	0.83 ± 0.08	1.12 ± 0.10	1.78 ± 0.10
5a	0.48 ± 0.01	0.47 ± 0.03	0.55 ± 0.05	0.68 ± 0.10	0.85 ± 0.10	1.35 ± 0.12
6a	0.43 ± 0.02	0.43 ± 0.02	0.50 ± 0.05	0.62 ± 0.06	0.96 ± 0.10	1.22 ± 0.13
6b	0.46 ± 0.01	0.46 ± 0.03	0.61 ± 0.06	0.76 ± 0.07	0.98 ± 0.09	1.65 ± 0.08
6c	0.44 ± 0.02	0.45 ± 0.03	0.59 ± 0.05	0.74 ± 0.07	0.98 ± 0.10	1.46 ± 0.05
6d	0.70 ± 0.05	0.80 ± 0.08	0.95 ± 0.09	1.05 ± 0.10	1.56 ± 0.13	3.54 ± 0.12
6e	0.64 ± 0.05	0.69 ± 0.08	0.98 ± 0.09	0.96 ± 0.05	1.18 ± 0.10	2.36 ± 0.14
6f	0.65 ± 0.02	0.77 ± 0.07	0.80 ± 0.07	1.10 ± 0.14	1.22 ± 0.10	2.25 ± 0.12
6g	0.42 ± 0.01	0.42 ± 0.03	0.45 ± 0.04	0.58 ± 0.05	0.89 ± 0.08	1.05 ± 0.06
6h	0.46 ± 0.01	0.44 ± 0.03	0.52 ± 0.04	0.64 ± 0.06	0.80 ± 0.08	1.28 ± 0.08
6i	0.60 ± 0.02	0.64 ± 0.05	0.88 ± 0.03	1.00 ± 0.01	1.10 ± 0.09	2.184 ± 0.10
6j	0.56 ± 0.01	0.58 ± 0.03	0.84 ± 0.05	0.90 ± 0.08	1.05 ± 0.12	2.14 ± 0.15
Valdecoxib	1.0	1.0	1.0	1.0	1.0	1.0

All results were significantly different from the standard and normal control value at $P = 0.05$.

Structure activity relationship (SAR)

The pyridine and amino acid residues are essential for both the anti-inflammatory and analgesic activities. The nucleophilicity of the substituents at hydrazone positions increases the activities. As the aromaticity increases with minimal steric hindrance,

the activities increase. But an increase in steric hindrance alongside with an increase in molecular weight decreases the activities.

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