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Synthesis and Anti-inflammatory Activity of Novel Pyridazine and Pyridazinone Derivatives as Non-ulcerogenic Agents

Makarem M. Saeed, Nadia A. Khalil, Eman M. Ahmed, and Kholoud I. Eissa
Department of Organic Chemistry, Faculty of Pharmacy, Cairo University, Cairo, Egypt

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Herein, we report the synthesis and pharmacological properties of several series of pyridazine and pyridazinone derivatives. All the synthesized compounds were tested, *in vivo*, for their anti-inflammatory and ulcerogenic properties against indomethacin, as a reference compound. Compounds **4a** and **9d** have shown a potent anti-inflammatory activity more than indomethacin with rapid onset of action and safe gastric profile. The latter compounds were then selected for further investigation. In the MTT assay *in vitro*, both compounds were identified as potent and selective COX-2 inhibitors.

Key words: Pyridazine, Pyridazinone, Anti-inflammatory activity, Ulcerogenicity

INTRODUCTION

Currently available non-steroidal anti-inflammatory drugs (NSAIDs), like ibuprofen and indomethacin, remain widely prescribed medications worldwide for the treatment of pain, fever, and swelling, which is associated with arthritis (Palomer et al., 2002). However, long-term use of these drugs result in gastrointestinal (GI) side effects, which are inseparable from their pharmacological activities, such as ulceration, bleeding and renal toxicity (Sostres et al., 2010). The GI damage from NSAIDs is generally attributed to the suppression of prostaglandin biosynthesis from arachidonic acid via nonselective inhibition of the two isoforms of the cyclooxygenase (COX) enzyme (COX-1 and COX-2) (Warner et al., 1999; Habeeb et al., 2001). COX-1 is expressed in many organs and is responsible for homeostatic processes, such as platelet aggregation, gastric protection and renal function, whereas the COX-2 enzyme is inducible and expressed during inflammation, pain, and oncogenesis. Due to this reason, the non-selective inhibition of COX-1, by traditional NSAIDs, lead to GI complications (Almansa et al., 2003). On the other hand, selective COX-2 inhibitors that achieve the same anti-inflammatory efficacy as traditional NSAIDs, but with improved gastrointestinal safety profile would be of therapeutic value. Diaryl heterocycles have become the major class of selective COX-2 inhibitors, such as celecoxib, rofecoxib, etoricoxib, and valdecoxib, which display lower GI toxicity potential than compared to that of the traditional NSAIDs (Talley et al., 2000; Stichtenoth and Frölich, 2003). However, concerns have been raised about the cardiovascular safety of selective COX-2 inhibitors, as some of them, like rofecoxib and valdecoxib, have been withdrawn from the market (Hsiao et al., 2009). Therefore, synthetic approaches based upon chemical modification of NSAIDs have been taken with the aim of improving their safety profile.

Pyridazines and pyridazinones are an important class of heterocycles, which have been the subject of extensive research due to their broad-activities, such as anti-inflammatory (Gökçe et al., 2009; Asif, 2010), analgesic (Gökçe et al., 2009; Malinka et al., 2011), antihypertensive (Siddiqui et al., 2010, 2011), anticancer (Al-Tel, 2010), anti-diabetic (Rathish et al., 2009), anticonvulsant (Guan et al., 2010), anti-microbial (Kandile et al., 2009) and anti-fungal (Ting et al., 2011) activities.

It has been reported that pyridazine and pyridazinone moieties are an excellent templates for many selective COX-2 inhibitors, such as RS-57067 **I** (Beswick et al., 2004), Syntex compound **II** (Cesari et al., 2006) and

Correspondence to: Eman M. Ahmed, Department of Organic Chemistry, Faculty of Pharmacy, Cairo University, Cairo, Egypt
Tel: 2-100-527-0276
E-mail: dr_eman2001@hotmail.com

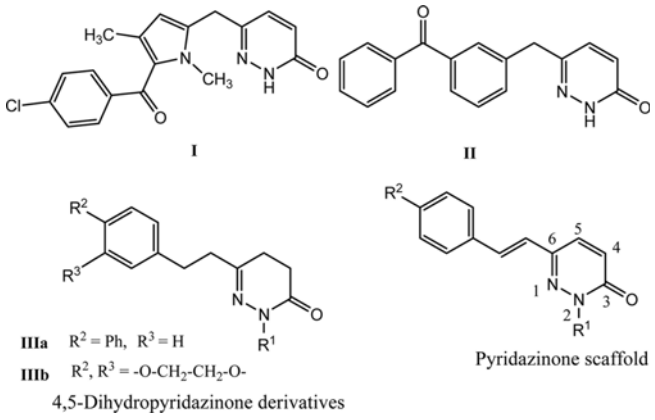
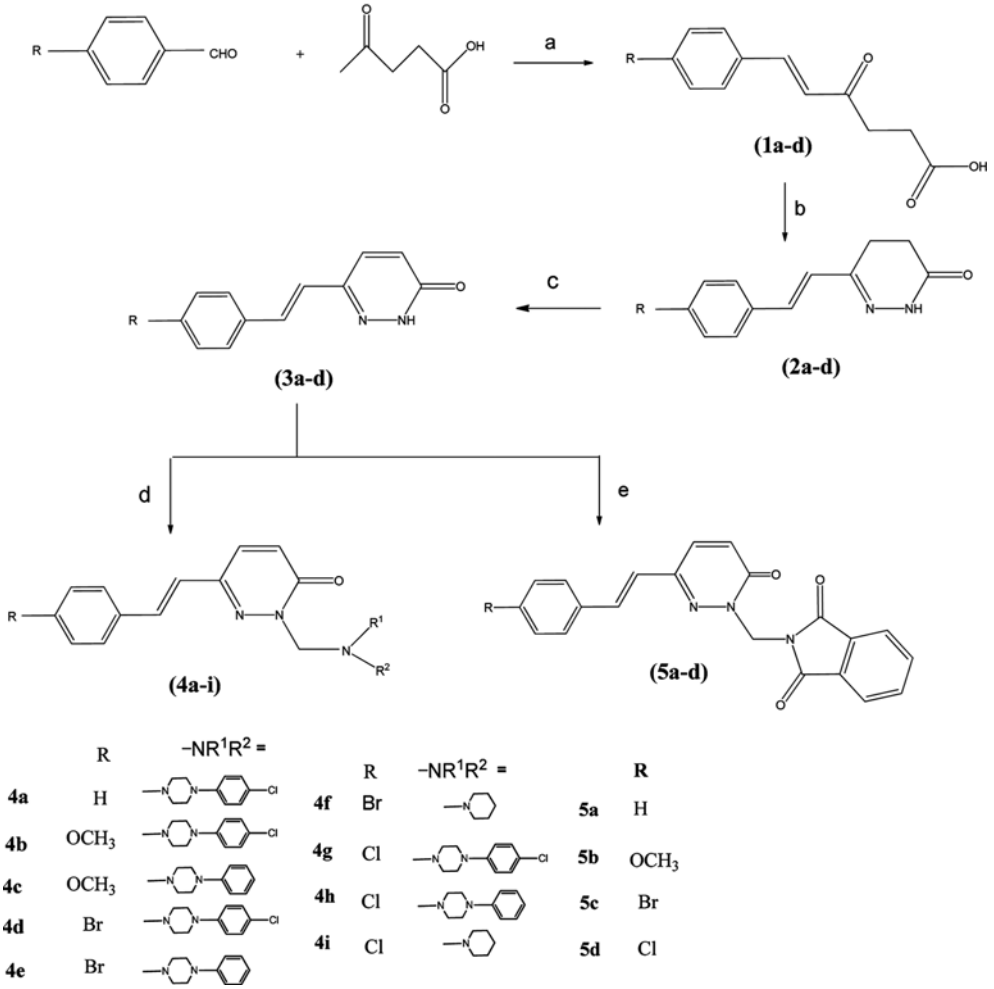


Fig. 1. Representatives of pyridazinone lead compounds and our pyridazinone scaffold.

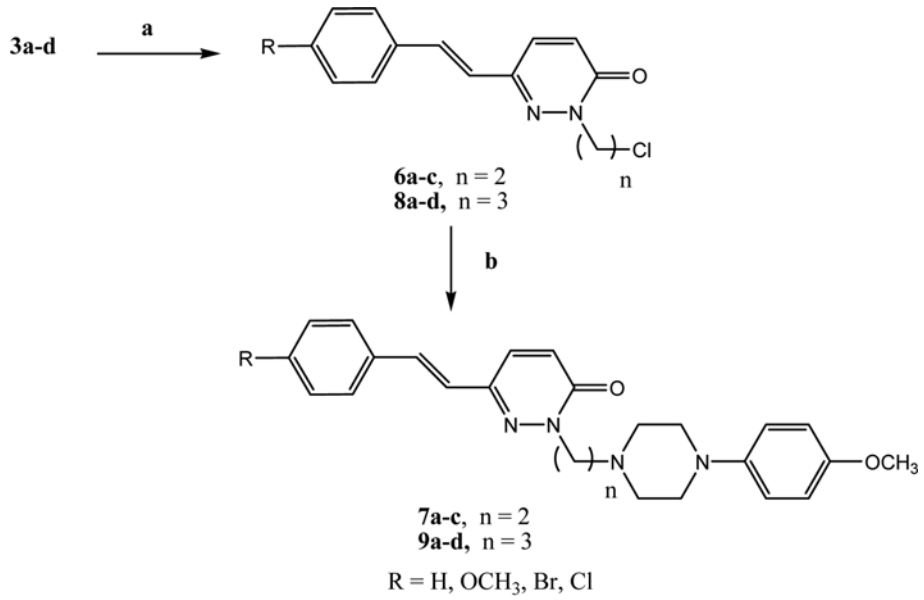
the 4,5-dihydropyridazinone derivatives, **IIIa**,**b** (Abouzid and Bekhit, 2008) (Fig. 1).

Structure activity relationship studies (SAR) employing pyridazinone have shown that *N*-substitution is a requirement for COX-2 selectivity. So, many *N*-substituted pyridazinone derivatives have been reported to possess anti-inflammatory activity (Dogruer et al., 2003; Asif, 2010; Rao and Knaus, 2008).

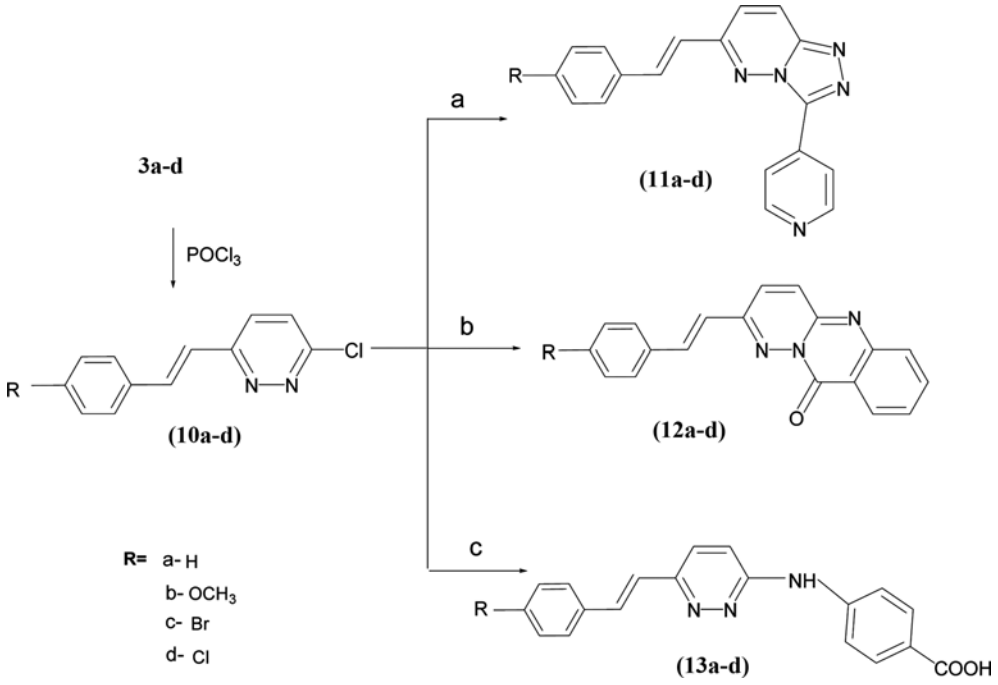
In this study, along with the continuous effort to find distinctive structural template to selective COX-2 inhibitors, we utilized the pyridazinone moiety with a suitable substitution at *N* (2) and connecting the pyridazinone ring at C (6) with an aryl group via an ethenyl spacer, instead of methylene or ethyl spacer, compounds **4a**-**i**, **5a**-**d**, **7a**-**c** and **9a**-**d.** It is assumed that these modifications may provide additional sites of interaction, thus, giving the desired selectivity, low ulcerogenicity and increased potency. Furthermore, the anti-inflammatory activity of certain fused pyridazines had been reported (Abdel-Hakim, 2004). On this basis, we became interested in synthesizing certain triazolopyridazines **11a**-**d** and pyridazinoquinazolines **12a**-**d** for the evaluation of their anti-inflammatory and ulcerogenic properties. Moreover, 6-arylethenyl-



Scheme 1. Reagents and conditions: **a**) morpholine, HOAc, dry benzene, 6 h, **b**) $N_2H_4H_2O$ 99%, 3 h, **c**) anhyd.$CuCl_2$/CH_3CN/ 60°C 3 h, **d**) HCHO/appropriate secondary amine, 20 h, **e**) N-bromomethylphthalimide/anhydrous K_2CO_3/DMF, 24 h.



Scheme 2. Reagents and conditions: **a**) 1-bromo-2-bromoethane or 1-bromo-3-chloropropane/K2CO3/DMF, 24 h, **b**) N-(4-methoxyphenyl)piperazine/KI/K_2CO_3/DMF, 20 h.



Scheme 3. Reagents and conditions: **a**) INH, dioxan/absolute ethanol (1:1), 20 h, **b**) Anthranilic acid, dioxan/absolute ethanol (1:1), 4 h, **c**) PABA, absolute ethanol/3 h.

pyridazine derivatives **13a-d** were also synthesized and tested for anti-inflammatory activity and gastric toxicity.

MATERIALS AND METHODS

Chemistry

All chemicals and reagents were obtained from Aldrich (Sigma-Aldrich), and were used without further purification. Reactions were monitored by TLC, performed on silica gel glass plates that contained 60 GF-254, and visualization on TLC was achieved, by UV light or iodine indicator. IR spectra were determined on Shimadzu IR 435 spectrophotometer (KBr, cm^{-1}). ^{1}H-NMR spectra were carried out using a Mercury 300-BB 300 MHz, using TMS as internal standard. Chemical shifts (δ)

are recorded in ppm on δ scale, Micro analytical Center, Cairo University, Egypt. ^{13}C-NMR spectra were carried out using a Mercury 300-BB 300 MHz that used TMS as an internal standard. Chemical shifts (δ) are recorded in ppm on δ scale, Micro analytical Center, Cairo University, Egypt. Mass spectra were recorded on Shimadzu Qp-2010 plus, spectrometer, Micro analytical Center, Cairo University, Egypt. Elemental analyses were carried out at the Micro analytical Center, Cairo University, Egypt. Melting points were determined with Stuart apparatus and are uncorrected.

6-(4-Aryl)-4-oxo-hex-5-enoic acids (**1a-d**) (Abouzid and Bekhit, 2008), 6-[2-(4-aryl)ethenyl]-4,5-dihydropyridazin-3(2H)-ones (**2a-d**) (Abouzid et al., 2007), 6-[2-(4-aryl) ethenyl]pyridazin-3(2H)-ones (**3a**, **b**, **d**) (Abouzid et al., 2008) and 3-chloro-6-[2-(4-aryl)ethenyl]pyridazines (**10a**, **b**, **d**) (Abouzid et al., 2008) were synthesized according to the reported procedures.

6-[2-(4-Bromophenyl)ethenyl]pyridazin-3(2*H*)-one (3c)

This compound was prepared according to the reported procedure (Abouzid et al., 2008). Yield: 65%; mp 249-250°C; IR (cm^{-1}): 3441 (N-H), 3055 (C-H aromatic), 1666 (C=O), 1593 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6): 6.91 (d, 1H, =CH-olefinic, J = 10.2 Hz), 7.07 (d, 1H, =CH-olefinic, J = 10.2 Hz), 7.12 (d, 2H, ArH), 7.37 (d, 2H, ArH), 7.55-7.58 (m, 2H, pyridazinone H-4 and H-5), 13.08 (s, 1H, NH, D_2O exchangeable); EIMS (% rel. abundance): 128 (100), 276 (M^+, 86.16), 278 (M+2, 85.85). Anal. Calcd for $C_{12}H_9BrN_2O$: C, 52.01; H, 3.27; N, 10.11. Found: C, 52.14; H, 3.28; N, 10.04.

6-[2-(4-Substitutedphenyl)ethenyl]-2-[(substituted) aminomethyl]pyridazin-3(2H)-ones (4a-i)

To a solution of an appropriate **3a-d** (0.001 mol), in absolute ethanol (10 mL), was added a mixture of secondary amino compound (0.001 mol) and formalin 37% (1.5 mL, 0.05 mol) in absolute ethanol (15 mL). The reaction mixture was heated under reflux for 20 h, filtered while hot and concentrated to half its volume. After cooling, the separated solid was filtered and crystallized from ethanol.

2-[4-(4-Chlorophenyl)piperazin-1-yl)methyl]-6-[2-(phenylethenyl)]pyridazin-3(2H)-one (4a)

Yield: 45%; mp 175-176°C; IR (cm^{-1}): 3023 (C-H aromatic), 2951, 2831 (C-H aliphatic), 1670 (C=O), 1603 (C=N); ^{1}H-NMR (300 MHz, $CDCl_3$): 2.85-2.88 (br m, 4H, piperazine), 3.12-3.15 (br m, 4H, piperazine), 4.78 (s, 2H, CH_2-N), 6.80 (d, 1H, =CH-olefinic), 6.83 (d, 1H, =CH-olefinic), 6.91 (d, 1H, pyridazinone H-4), 7.17-7.48 (m, 9H, ArH), 7.51 (d, 1H, pyridazinone H-5); EIMS (% rel. abundance): 209 (100), 406 (M^+, 0.46), 408 (M+2, 8.73). Anal. Calcd for $C_{23}H_{23}ClN_4O$: C, 67.89; H, 5.70; N, 13.77. Found C, 68.24; H, 5.63; N, 13.71.

2-[4-(4-Chlorophenyl)piperazin-1-yl)methyl]-6-[2-(4-methoxyphenyl)ethenyl]-pyridazin-3(2H)-one (4b)

Yield: 75%; mp 155-156°C; IR (cm^{-1}): 3021 (C-H aromatic), 2920, 2850 (C-H aliphatic), 1658 (C=O), 1600 (C=N); ^{1}H-NMR (300 MHz, $CDCl_3$): 2.81-2.90 (br m, 4H, piperazine), 3.19-3.22 (br m, 4H, piperazine), 3.84 (s, 3H, -OCH_3), 4.80 (s, 2H, CH_2-N), 6.81 (d, 1H, =CH-olefinic), 6.89 (d, 1H, =CH-olefinic), 6.92 (d, 1H, pyridazinone H-4, J = 8.7 Hz), 7.17-7.42 (m, 8H, Ar-H), 7.45 (d, 1H, pyridazinone H-5, J = 8.7 Hz); EIMS (% rel. abundance): 209 (100), 436 (M^+, 0.6), 438 (M+2, 8.99). Anal. Calcd for $C_{24}H_{25}ClN_4O_2$: C, 65.97; H, 5.77; N, 12.82. Found: C, 65.93; H, 5.81; N, 12.76.

6-[2-(4-Methoxyphenyl)ethenyl]-2-[4-(phenyl)piperazin-1-yl)methyl]pyridazin-3(2H)-one (4c)

Yield: 68%; mp 157-158°C; IR (cm^{-1}): 3063 (C-H aromatic), 2920, 2823 (C-H aliphatic), 1658 (C=O), 1604 (C=N); ^{1}H-NMR (300 MHz, $CDCl_3$): 2.87-2.89 (br m, 4H, piperazine), 3.17-3.21 (br m, 4H, piperazine), 3.84 (s, 3H, -OCH_3), 4.79 (s, 2H, CH_2-N), 6.82 (d, 1H, =CH-olefinic), 6.89 (d, 1H, =CH-olefinic), 6.93 (d, 1H, pyridazinone H-4), 7.25-7.42 (m, 9H, ArH), 7.45 (d, 1H, pyridazinone H-5); EIMS (% rel. abundance): 175 (100), 402 (M^+, 0.40). Anal. Calcd for $C_{24}H_{26}N_4O_2$: C, 71.62; H, 6.51; N, 13.92. Found: C, 71.73; H, 6.51; N, 13.95.

6-[2-(4-Bromophenyl)ethenyl]-2-[4-(4-chlorophenyl) piperazin-1-yl)aminomethyl]pyridazin-3(2H)-one (4d)

Yield: 85%; mp 121-122°C; IR (cm^{-1}): 3101 (C-H aromatic), 2927, 2820 (C-H aliphatic), 1658 (C=O), 1604 (C=N); ^{1}H-NMR (300 MHz, $CDCl_3$): 2.82-2.91 (br m, 4H, piperazine), 3.18-3.20 (br m, 4H, piperazine), 4.81 (s, 2H, CH_2-N), 6.79 (d, 1H, =CH-olefinic), 6.81 (d, 1H, =CH-olefinic), 6.88 (d, 1H, pyridazinone H-4), 7.18-7.49 (m, 8H, ArH), 7.52 (d, 1H, pyridazinone H-5); EIMS (% rel. abundance): 209 (100), 486 (M+2, 4.20), 488 (M+4, 5.10). Anal. Calcd for $C_{23}H_{22}BrClN_4O$: C, 56.86; H, 4.56; N, 11.53; Found: C, 56.89; H, 4.62; N, 11.56.

6-[2-(4-Bromophenyl)ethenyl]-2-[4-(phenyl)piperazin-1-yl)aminomethyl]pyridazin-3(2H)-one (4e)

Yield: 78%; mp 126-127°C; IR (cm^{-1}): 3067 (C-H aromatic), 2939, 2823 (C-H aliphatic), 1658 (C=O), 1600 (C=N); ^{1}H-NMR (300 MHz, $CDCl_3$): 2.80-2.87 (br m, 4H, piperazine), 3.17-3.19 (br m, 4H, piperazine), 4.78

(s, 2H, CH_2-N), 6.83 (d, 1H, =CH-olefinic), 6.89 (d, 1H, =CH-olefinic), 6.93 (d, 1H, pyridazinone H-4, J = 8.7 Hz), 7.22-7.48 (m, 9H, ArH), 7.51 (d, 1H, pyridazinone H-5, J = 8.7 Hz); ^{13}C-NMR (DMSO-d_6): δ 48.34 (2C of piperazine), 49.92 (2C of piperazine), 68.61 (-CH_2-N), 118.42-151.12 (aromatic C), 166.54 (C=O); EIMS (% rel. abundance): 175 (100), 450 (M^+, 0.36), 452 (M+2, 25.73). Anal. Calcd for $C_{23}H_{23}BrN_4O$: C, 61.20; H, 5.14; N, 12.41. Found: C, 61.26; H, 5.18; N, 12.52.

6-[2-(4-Bromophenyl)ethenyl]-2-[piperidin-1-yl) methyl]pyridazin-3(2H)-one (4f)
Yield: 58%; mp 128-129°C; IR (cm^{-1}): 3054 (C-H aromatic), 2939, 2830 (C-H aliphatic), 1660 (C=O), 1604 (C=N); ^{1}H-NMR (300 MHz, $CDCl_3$): 1.64-1.68 (m, 2H, CH_2 of piperidine), 2.56-2.61 (m, 4H, $2CH_2$ of piperidine), 2.82 (t, 4H, $2CH_2$ of piperidine), 4.93 (s, 2H, CH_2-N), 6.86 (d, 1H, =CH-olefinic), 6.88 (d, 1H, =CH-olefinic), 6.89 (d, 1H, pyridazinone H-4), 7.27-7.52 (m, 4H, ArH), 7.53 (d, 1H, pyridazinone H-5); ^{13}C-NMR (DMSO-d_6 ppm): δ 48.34 (2C of piperazine), 49.92 (2C of piperazine), 68.61 (-CH_2-N), 118.42-151.12 (15 aromatic C, 2 olefinic C), 166.54 (C=O); EIMS (% rel. abundance): 279 (100), 373 (M^+, 0.06), 375 (M+2, 0.73). Anal. Calcd for $C_{18}H_{20}BrN_3O$: C, 57.76; H, 5.39; N, 11.23. Found: C, 57.70; H, 5.43; N, 11.31.

6-[2-(4-Chlorophenyl)ethenyl]-2-[4-(4-chlorophenyl) piperazin-1-yl)methyl] pyridazin-3(2H)-one (4g)
Yield: 66%; mp 159-160°C; IR (cm^{-1}): 3011 (C-H aromatic), 2947, 2831 (C-H aliphatic), 1674 (C=O), 1600 (C=N); ^{1}H-NMR (300 MHz, $CDCl_3$): 2.85-2.87 (br m, 4H, piperazine), 3.13-3.16 (br m, 4H, piperazine), 4.78 (s, 2H, CH_2-N), 6.79 (d, 1H, =CH-olefinic), 6.85 (d, 1H, =CH-olefinic), 6.87 (d, 1H, pyridazinone H-4), 7.17-7.41 (m, 8H, ArH), 7.44 (d, 1H, pyridazinone H-5); EIMS (% rel. abundance): 70 (100), 440 (M^+, 0.78), 442 (M+2, 6.13), 444 (M+4, 4.02). Anal. Calcd for $C_{23}H_{22}Cl_2N_4O$: C, 62.59; H, 5.02; N, 12.69. Found: C, 62.64; H, 5.13; N, 12.73.

6-[2-(4-Chlorophenyl)ethenyl]-2-[4-(phenyl)piperazin-1-yl)methyl]pyridazin-3(2H)-one (4h)
Yield: 76%; mp 128-129°C; IR (cm^{-1}): 3043 (C-H aromatic), 2951, 2881 (C-H aliphatic), 1662 (C=O), 1597 (C=N); ^{1}H-NMR (300 MHz, $CDCl_3$): 2.83-2.89 (br m, 4H, piperazine), 3.17-3.21 (br m, 4H, piperazine), 4.79 (s, 2H, CH_2-N), 6.85 (d, 1H, =CH-olefinic, J = 8.1 Hz), 6.90 (d, 1H, =CH-olefinic, J = 8.1 Hz), 6.93 (d, 1H, pyridazinone H-4), 7.22-7.41 (m, 9H, ArH), 7.44 (d, 1H, pyridazinone H-5); EIMS (% rel. abundance): 175 (100), 406 (M^+, 0.48), 408 (M+2, 6.65). Anal. Calcd for $C_{23}H_{23}ClN_4O$: C, 67.89; H, 5.70; N, 13.77. Found C, 68.21; H, 5.93; N, 13.84.

6-[2-(4-Chlorophenyl)ethenyl]-2-[piperidin-1-yl) methyl]pyridazin-3(2H)-one (4i)
Yield: 72%; mp 96-97°C; IR (cm^{-1}): 3107 (C-H aromatic), 2927, 2804 (C-H aliphatic), 1662 (C=O), 1600 (C=N); ^{1}H-NMR (300 MHz, $CDCl_3$): 1.57-1.61 (m, 2H, CH_2 of piperidine), 2.55-2.62 (m, 4H, $2CH_2$ of piperidine), 2.79 (t, 4H, $2CH_2$ of piperidine), 4.66 (s, 2H, CH_2-N), 6.83 (d, 1H, =CH-olefinic), 6.87 (d, 1H, =CH-olefinic), 6.88 (d, 1H, pyridazinone H-4), 7.26-7.40 (m, 4H, ArH), 7.43 (d, 1H, pyridazinone H-5); EIMS (% rel. abundance): 98 (100), 331 (M+2, 0.50). Anal. Calcd for $C_{18}H_{20}ClN_3O$: C, 65.55; H, 6.11; N, 12.74. Found C, 65.84; H, 6.02; N, 12.60.

2-[(1,3-Dioxo-1,3-dihydro-2H-isoindol-2yl)methyl]-6-[2-(4-substitutedphenyl)ethenyl]pyridazin-3(2H)-ones (5a-d)

A mixture of the respective **3a-d** (0.001 mol), *N*-bromomethylphthalimide (0.24 g, 0.001 mol) and anhydrous K_2CO_3 (0.41 g, 0.003 mol) in dry DMF (10 mL) was stirred, at room temperature for 24 h. The reaction mixture was poured onto ice-cold water (25 mL) and the resulting precipitate was filtered, washed with water and crystallized from ethanol.

2-[(1,3-Dioxo-1,3-dihydro-2H-isoindol-2yl)methyl]-6-[2-(phenyl)ethenyl]pyridazin-3(2H)-one (5a)
Yield: 50%; mp >300°C; IR (cm^{-1}): 3008 (C-H aromatic), 2950, 2835 (C-H aliphatic), 1728, 1639 (C=O), 1600 (C=N); ^{1}H-NMR (300 MHz, $CDCl_3$): 5.65 (s, 2H, CH_2-N), 6.89 (d, 1H, =CH-olefinic), 6.92 (d, 1H, =CH-olefinic), 7.26-7.89 (m, 11H: 9 ArH, 2H of pyridazinone H-4 and H-5); EIMS (% rel. abundance): 55 (100), 357 (M^+, 0.23). Anal. Calcd for $C_{21}H_{15}N_3O_3$: C, 70.58; H, 4.23; N, 11.76. Found: C, 71.02; H, 4.51; N, 11.64.

2-[(1,3-Dioxo-1,3-dihydro-2H-isoindol-2yl)methyl]-6-[2-(4-methoxyphenyl)ethenyl] pyridazin-3(2H)-one (5b)
Yield: 58%; mp 151-152°C; IR (cm^{-1}): 3035 (C-H aromatic), 2970, 2850 (C-H aliphatic), 1730, 1685 (C=O), 1604 (C=N); ^{1}H-NMR (300 MHz, $CDCl_3$): 3.84 (s, 3H, OCH_3), 5.65 (s, 2H, CH_2-N), 6.80 (d, 1H, =CH-olefinic), 6.85 (d, 1H, =CH-olefinic), 6.92 (d, 1H, pyridazinone H-4, J = 8.7 Hz), 7.45 (d, 1H, pyridazinone H-5, J = 8.7 Hz), 7.72-7.74 (m, 4H, ArH), 7.86-7.89 (m, 4H, ArH); EIMS (% rel. abundance): 229 (100), 387 (M^+, 0.02). Anal. Calcd for $C_{22}H_{17}N_3O_4$: C, 68.21; H, 4.42; N, 10.85. Found: C, 68.24; H, 4.44; N, 10.81.

2-[(1,3-Dioxo-1,3-dihydro-2H-isoindol-2yl)methyl]-6-[2-(4-bromophenyl)ethenyl]pyridazin-3(2H)-one (5c)

Yield: 62%; mp 194-195°C; IR (cm^{-1}): 3055 (C-H aromatic), 2931, 2850 (C-H aliphatic), 1728, 1670 (C=O), 1612 (C=N); ^{1}H-NMR (300 MHz, $CDCl_3$): 5.65 (s, 2H, CH_2-N), 6.84 (d, 1H, =CH-olefinic), 6.85 (d, 1H, =CH-olefinic), 7.32 (d, 1H, pyridazinone H-4, J = 8.4 Hz), 7.51 (d, 1H, pyridazinone H-5, J = 8.4 Hz), 7.71-7.73 (m, 4H, ArH), 7.86-7.88 (m, 4H, ArH); ^{13}C-NMR (DMSO-d_6 ppm): δ 72.24 (-CH_2-N), 131.77-144.35 (15 aromatic C, 2 olefinic C), 163.87, 166.98 (3 C=O); EIMS (% rel. abundance): 104 (100), 437 (M+2, 0.18). Anal. Calcd for $C_{21}H_{14}BrN_3O_3$: C, 57.82; H, 3.23; N, 9.63. Found: C, 57.89; H, 3.28; N, 9.68.

2-[(1,3-Dioxo-1,3-dihydro-2H-isoindol-2yl)methyl]-6-[2-(4-chlorophenyl)ethenyl]pyridazin-3(2H)-one (5d)

Yield: 68%; mp 199-200°C; IR (cm^{-1}): 3055 (C-H aromatic), 2939, 2854 (C-H aliphatic), 1728, 1666 (C=O), 1597 (C=N); ^{1}H-NMR (300 MHz, $CDCl_3$): 5.64 (s, 2H, CH_2-N), 6.85 (d, 1H, =CH-olefinic), 6.94 (d, 1H, =CH-olefinic), 7.26-7.42 (m, 6H, 4Ar-H and 2H of pyridazinone H-4 and H-5), 7.73 (d, 2H, ArH), 7.88 (d, 2H, ArH); EIMS (% rel. abundance): 233 (100), 393 (M+2, 0.19). Anal. Calcd for $C_{21}H_{14}ClN_3O_3$: C, 64.37; H, 3.60; N, 10.72. Found C, 64.48; H, 3.69; N, 10.74.

2-(2-Chloroethyl)-6-[2-(4-substitutedphenyl) ethenyl]-pyridazin-3(2H)-ones (6a-c)

A mixture of an appropriate **3a-d** (0.01 mol), 1-bromo-2-chloroethane (7.17 g, 4.16 mL, 0.05 mol) and anhydrous K_2CO_3 (6.9 g, 0.05 mol) in dry DMF (20 mL) was stirred, at room temperature for 24 h. The reaction mixture was poured slowly with continuous stirring onto ice-cold water (50 mL). The formed solid was filtered and washed with ethanol.

2-(2-Chloroethyl)-6-[2-(4-methoxyphenyl)ethenyl]pyridazin-3(2H)-one (6a)

Yield: 82%; mp 169-170°C; IR (cm^{-1}): 3005 (C-H aromatic), 2970, 2827 (C-H aliphatic), 1681 (C=O), 1604 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6): 2.57 (t, 2H, -CH_2-$\underline{CH_2}$-), 2.82 (t, 2H, -$\underline{CH_2}$-CH_2-), 3.84 (s, 3H, -OCH_3), 6.73 (d, 1H, =CH-olefinic), 6.84 (d, 1H, =CH-olefinic), 7.27-7.44 (m, 6H: 4 ArH, 2H of pyridazinone); EIMS (% rel. abundance): 149 (100), 294 (M+2+H, 16.84). Anal. Calcd for $C_{15}H_{15}ClN_2O_2$: C, 61.97; H, 5.20; N, 9.64. Found: C, 62.21; H, 5.24; N, 9.66.

2-(2-Chloroethyl)-6-[2-(4-bromophenyl)ethenyl] pyridazin-3(2H)-one (6b)

Yield: 74%; mp 119-120°C; IR (cm^{-1}): 3055 (C-H aromatic), 2931, 2820 (C-H aliphatic), 1666 (C=O), 1600 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6): 3.79 (t, 2H, -CH_2-$\underline{CH_2}$-), 3.99 (t, 2H, -$\underline{CH_2}$-CH_2-), 6.94 (d, 1H, =CH-olefinic), 6.99 (d, 1H, =CH-olefinic), 7.01 (d, 1H, H-4 of pyridazinone), 7.10-7.58 (m, 5H: 4 ArH, 1H, H-5 of pyridazinone); EIMS (% rel. abundance): 63 (100), 339 (M+H, 9.24), 342 (M+4, 11.30). Anal. Calcd for $C_{14}H_{12}BrClN_2O$: C, 49.51; H, 3.56; N, 8.25. Found: C, 49.49; H, 3.56; N, 8.25.

2-(2-Chloroethyl)-6-[2-(4-chlorophenyl)ethenyl] pyridazin-3(2H)-one (6c)

Yield: 68%; mp 186-187°C; IR (cm^{-1}): 3059 (C-H aromatic), 2931, 2827 (C-H aliphatic), 1666 (C=O), 1600 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6): 3.80 (t, 2H, CH_2-$\underline{CH_2}$-), 4.02 (t, 2H, -$\underline{CH_2}$-CH_2-), 6.93 (d, 1H, =CH-olefinic), 7.02 (d, 1H, =CH-olefinic), 7.41-7.45 (m, 3H: 2 Ar-H, 1H, H-4 of pyridazinone), 7.60-7.63 (m, 3H: 2 ArH, 1H, H-5 of pyridazinone); EIMS (% rel. abundance): 247 (100), 294 (M^+, 2.93), 296 (M+2, 97.58), 298 (M+4, 66.30). Anal. Calcd for $C_{14}H_{12}Cl_2N_2O$: C, 56.97; H, 4.10; N, 9.49. Found: C, 56.97; H, 4.18; N, 9.52.

2-{2-[4-(4-Methoxyphenyl)piperazin-1yl]ethyl}-6-[2-(4-substitutedphenyl)ethenyl]pyridazin-3 (2H)-ones (7a-c)

A mixture of an appropriate **6a-c** (0.001 mol), anhydrous K_2CO_3 (0.69 g, 0.005 mol) and few specs of KI in dry acetonitrile (20 mL) was heated under reflux for 30 min. 4-Methoxyphenylpiperazine (0.58 g, 0.003 mol) was added to the hot reaction mixture, which was heated under reflux for 20 h. After cooling, the reaction mixture was poured onto ice-cold water (25 mL) with continuous stirring. The resulting solid was filtered, dried then washed with dry ether.

2-{2-[4-(4-Methoxyphenyl)piperazin-1yl]ethyl}-6-[2-(4-methoxyphenyl)ethenyl]pyridazin-3(2H)-one (7a)

Yield: 58%; mp 110-111°C; IR (cm^{-1}): 3028 (C-H aromatic), 2935, 2814 (C-H aliphatic), 1685 (C=O), 1604 (C=N); ^{1}H-NMR (300 MHz, $CDCl_3$): 2.56 (t, 2H, -CH_2-$C\underline{H_2}$-), 2.72-2.80 (br m, 4H, piperazine), 2.82 (t, 2H, -$C\underline{H_2}$-CH_2-), 3.11-3.21 (br m, 4H, piperazine), 3.77 (s, 3H, -OCH_3), 3.84 (s, 3H, -OCH_3), 6.79 (d, 1H, =CH-olefinic), 6.86 (d, 1H, =CH-olefinic), 6.90-7.45 (m, 10H: 8 ArH, 2H of pyridazinone); ^{13}C-NMR (DMSO-d_6 ppm): δ 20.19 (-CH_2-$\underline{C}H_2$-), 25.90 (-$\underline{C}H_2$-CH_2-), 49.60 (2C of piperazine), 51.10 (2C of piperazine), 55.17 (2-OCH_3), 114.25-150.78 (aromatic C), 167.05 (C=O); EIMS (% rel. abundance): 205 (100), 219 (3.31), 227 (11.98), 446 (M^+, 0.00). Anal. Calcd for $C_{26}H_{30}N_4O_3$: C, 69.93; H, 6.77; N, 12.55.

Found C, 70.25; H, 6.39; N, 12.43.

2-{2-[4-(4-Methoxyphenyl)piperazin-1yl]ethyl}-6-[2-(4-bromophenyl)ethenyl]pyridazin-3(2H)-one (7b)

Yield: 43%; mp 126-127°C; IR (cm^{-1}): 3050 (C-H aromatic), 2947, 2823 (C-H aliphatic), 1665 (C=O), 1600 (C=N); ^{1}H-NMR (300 MHz, $CDCl_3$): 2.63 (t, 2H, -CH_2-C$\underline{H_2}$-), 2.85 (t, 2H, -C$\underline{H_2}$-CH_2-), 3.08-3.18 (br m, 4H, piperazine), 3.44-3.46 (br m, 4H, piperazine), 3.78 (s, 3H, -OCH_3), 6.79 (d, 1H, =CH-olefinic), 6.86 (d, 1H, =CH-olefinic), 6.90-7.10 (m, 5H, ArH), 7.26 (d, 1H, H-5 of pyridazinone), 7.33 (d, 2H, ArH), 7.52 (d, 2H, ArH); EIMS (% rel. abundance): 205 (1.94), 219 (2.88), 275 (4.72), 277 (62.45), 289 (0.82), 291 (100), 494 (M^+, 0.00), 496 (M+2, 0.00). Anal. Calcd for $C_{25}H_{27}BrN_4O_2$: C, 60.61; H, 5.49; N, 11.31. Found: C, 60.64; H, 5.53; N, 11.29.

2-{2-[4-(4-Methoxyphenyl)piperazin-1yl]ethyl}-6-[2-(4-chlorophenyl)ethenyl]pyridazin-3(2H)-one (7c)

Yield: 46%; mp 130-131°C; IR (cm^{-1}): 3047 (C-H aromatic), 2943, 2823 (C-H aliphatic), 1670 (C=O), 1585 (C=N); ^{1}H-NMR (300 MHz, $CDCl_3$): 2.57 (t, 2H, -CH_2-C$\underline{H_2}$), 2.63-2.78 (br m, 4H, piperazine), 2.82 (t, 2H, -C$\underline{H_2}$-CH_2), 3.05-3.18 (br m, 4H, piperazine), 3.77 (s, 3H, -OCH_3), 6.84 (d, 1H, =CH-olefinic), 6.98 (d, 1H, =CH-olefinic), 7.27-7.43 (m, 10H: 8 ArH, 2H of pyridazinone); EIMS (% rel. abundance): 205 (100), 219 (2.25), 450 (M^+, 13.29), 452 (M+2, 24.15). Anal. Calcd for $C_{25}H_{27}ClN_4O_2$: C, 66.58; H, 6.03; N, 12.42. Found: C, 66.29; H, 6.21; N, 12.81.

2-(3-Chloropropyl)-6-[2-(4-substitutedphenyl) ethenyl]-pyridazin-3(2H)-ones (8a-d)

The compounds were prepared from **3a-d** and 1-bromo-3-chloropropane (4.72 g, 2.96 mL, 0.03 mol), following the procedure described for the compounds **6a-d**.

2-(3-Chloropropyl)-6-[2-(phenyl)ethenyl]pyridazin-3(2H)-one (8a)

Yield: 77%; mp >300°C; IR (cm^{-1}): 3035 (C-H aromatic), 2924, 2835 (C-H aliphatic), 1662 (C=O), 1600 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.38-2.40 (m, 2H, -CH_2-C$\underline{H_2}$-CH_2-), 2.48 (t, 2H, -C$\underline{H_2}$-CH_2-CH_2-), 2.77 (t, 2H, -CH_2-CH_2-C$\underline{H_2}$-), 6.84 (d, 1H, =CH-olefinic), 7.01 (d, 1H, =CH-olefinic), 7.23-7.59 (m, 4H, 3 ArH, 1H, H-4 of pyridazinone), 7.60-7.80 (m, 3H: 2 ArH, 1H, H-5 of pyridazinone); EIMS (% rel. abundance): 199 (100), 274 (M^+, 10.82), 276 (M+2, 10.26). Anal. Calcd for $C_{15}H_{15}ClN_2O$: C, 65.57; H, 5.50; N, 10.20. Found: C, 65.53; H, 5.49; N, 10.32.

2-(3-Chloropropyl)-6-[2-(4-methoxyphenyl)ethenyl]pyridazin-3(2H)-one (8b)

Yield: 72%; mp 79-80°C; IR (cm^{-1}): 3055 (C-H aromatic), 2924, 2854 (C-H aliphatic), 1666 (C=O), 1597 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.15-2.19 (m, 2H, -CH_2-C$\underline{H_2}$-CH_2-), 2.37 (t, 2H, -C$\underline{H_2}$-CH_2-CH_2-), 2.75 (t, 2H, -CH_2-CH_2-C$\underline{H_2}$-), 3.78 (s, 3H, OCH_3), 6.78 (d, 1H, =CH-olefinic), 6.80 (d, 1H, =CH-olefinic), 6.91-7.95 (m, 6H, 4 ArH, 2H, H-4 and H-5 of pyridazinone). EIMS (% rel. abundance): 304 (M^+, 100), 306 (M+2, 34.39). Anal. Calcd for $C_{16}H_{17}ClN_2O_2$: C, 63.05; H, 5.62; N, 9.19. Found C, 62.84; H, 5.83; N, 9.26.

2-(3-Chloropropyl)-6-[2-(4-bromophenyl)ethenyl] pyridazin-3(2H)-one (8c)

Yield: 81%; mp 143-144°C; IR (cm^{-1}): 3051 (C-H aromatic), 2954, 2858 (C-H aliphatic), 1654 (C=O), 1585 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.17-2.20 (m, 2H, -CH_2-C$\underline{H_2}$-CH_2-), 3.70 (t, 2H, -C$\underline{H_2}$-CH_2-CH_2-), 4.19 (t, 2H, -CH_2-CH_2-C$\underline{H_2}$-), 6.99 (d, 1H, =CH-olefinic), 7.02 (d, 1H, =CH-olefinic), 7.14 (d, 2H, ArH), 7.35 (d, 2H, ArH), 7.58 (d, 1H, H-4 of pyridazinone), 7.92 (d, 1H, H-5 of pyridazinone). EIMS (% rel. abundance): 352 (M^+, 48.73), 354 (M+2, 65.55), 356 (M+4, 17.27). Anal. Calcd for $C_{15}H_{14}BrClN_2O$: C, 50.94; H, 3.99; N, 7.92. Found: C, 51.16; H, 4.13; N, 8.11.

2-(3-Chloropropyl)-6-[2-(4-chlorophenyl)ethenyl] pyridazin-3(2H)-one (8d)

Yield: 75%; mp 193-194°C; IR (cm^{-1}): 3047 (C-H aromatic), 2974, 2831 (C-H aliphatic), 1681 (C=O), 1604 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.39-2.45 (m, 2H, -CH_2-C$\underline{H_2}$-CH_2-), 2.49 (t, 2H, -C$\underline{H_2}$-CH_2-CH_2-), 2.77 (t, 2H, -CH_2-CH_2-C$\underline{H_2}$-), 6.93 (d, 1H, =CH-olefinic), 7.02 (d, 1H, =CH-olefinic), 7.41-7.44 (m, 3H: 2ArH, 1H, H-4 of of pyridazinone), 7.60-7.63 (m, 3H: 2ArH, 1H, H-5 of of pyridazinone). EIMS (% rel. abundance): 128 (100), 310 (M+2, 7.94), 312 (M+4, 4.81). Anal. Calcd for $C_{15}H_{14}Cl_2N_2O$: C, 58.27; H, 4.56; N, 9.06. Found: C, 58.48; H, 4.71; N, 9.12.

6-[2-(4-Aryl)ethenyl]-2-{3-[4-(4-Methoxyphenyl) piperazin-1yl]propyl}-pyridazin-3(2H)-ones (9a-d)

The compounds were prepared from **8a-d**, following the procedure described for the compounds **7a-c**. The reaction mixture was cooled and poured slowly onto ice-cold water (25 mL) with continuous stirring, then extracted with methylene chloride (3 × 5 mL). The organic layer was washed with water and dried (Na_2SO_4). After distillation off the solvent, the residue

was triturated with dry ether to give **9a-d**.

2-{3-[4-(4-Methoxyphenyl)piperazin-1yl]propyl}-6-[2-(phenyl)ethenyl]pyridazin-3(2H)-one (9a)
Yield: 66%; mp 144-145°C; IR (cm^{-1}): 3050 (C-H aromatic), 2947, 2831 (C-H aliphatic), 1670 (C=O), 1604 (C=N); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.59-2.61 (m, 2H, -CH_2-C$\underline{H_2}$-CH_2-), 2.73 (t, 2H, -C$\underline{H_2}$-CH_2-CH_2-), 2.90 (t, 2H, -CH_2-CH_2-C$\underline{H_2}$), 3.10-3.16 (br m, 4H, piperazine), 3.24-3.39 (br m, 4H, piperazine), 3.77 (s, 3H, - OCH_3), 6.87 (d, 1H, =CH-olefinic), 6.90 (d, 1H, =CH-olefinic), 7.09-7.50 (m, 11H: 9 Ar-H, 2H of pyridazinone). EIMS (% rel. abundance): 150 (100), 197 (5.40), 205 (24.97), 211 (1.19), 219 (1.40), 225 (0.53), 233 (0.62), 430 (M^+, 1.02). Anal. Calcd for $C_{26}H_{30}N_4O_2$: C, 72.53; H, 7.02; N, 13.01. Found: C, 72.36; H, 7.18; N, 13.20.

2-{3-[4-(4-Methoxyphenyl)piperazin-1yl]propyl}-6-[2-(4-methoxyphenyl)ethenyl]pyridazin-3(2H)-one (9b)
Yield: 42%; mp 134-135°C; IR (cm^{-1}): 3045 (C-H aromatic), 2920, 2850 (C-H aliphatic), 1685 (C=O), 1604 (C=N); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.34-2.39 (m, 2H, -CH_2-C$\underline{H_2}$-CH_2-), 2.49 (t, 2H, -C$\underline{H_2}$-CH_2-CH_2-), 2.75 (t, 2H, -CH_2-CH_2-C$\underline{H_2}$-), 3.01-3.11 (br m, 4H, piperazine), 3.25-3.45 (br m, 4H, piperazine), 3.68 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 6.71 (d, 1H, =CH-olefinic), 6.77 (d, 1H, =CH-olefinic), 6.85-7.54 (m, 10H: 8 ArH, 2H of pyridazinone). EIMS (% rel. abundance): 227 (2.14), 229 (100), 233 (0.34), 460 (M^+, 0.00). Anal. Calcd for $C_{27}H_{32}N_4O_3$: C, 70.41; H, 7.00; N, 12.16. Found: C, 70.52; H, 7.08; N, 12.19.

2-{3-[4-(4-Methoxyphenyl)piperazin-1yl]propyl}-6-[2-(4-bromophenyl)ethenyl]pyridazin-3(2H)-one (9c)
Yield: 63%; mp 174-175°C; IR (cm^{-1}): 3055 (C-H aromatic), 2939, 2850 (C-H aliphatic), 1666 (C=O), 1604 (C=N); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.94-2.00 (m, 2H, -CH_2-C$\underline{H_2}$-CH_2-), 2.59 (t, 2H, -C$\underline{H_2}$-CH_2-CH_2-), 2.82 (t, 2H, -CH_2-CH_2-C$\underline{H_2}$-), 3.00-3.08 (br m, 4H, piperazine), 3.20-3.30 (br m, 4H, piperazine), 3.68 (s, 3H, OCH_3), 6.83 (d, 1H, =CH-olefinic), 6.90 (d, 1H, =CH-olefinic), 7.26-7.51 (m, 10H: 8 ArH, 2H of pyridazinone); EIMS (% rel. abundance): 219 (0.71), 234 (2.88), 277 (91.85), 279 (100), 508 (M^+,0.00), 510 (M+2, 0.00). Anal. Calcd for $C_{26}H_{29}BrN_4O_2$: C, 61.30; H, 5.74; N, 11.00. Found: C, 61.44; H, 5.82; N, 11.13.

2-{3-[4-(4-Methoxyphenyl)piperazin-1yl]propyl}-6-[2-(4-chlorophenyl)ethenyl]pyridazin-3(2H)-one (9d)
Yield: 55%; mp 79-80°C; IR (cm^{-1}): 3055 (C-H aromatic), 2931, 2831 (C-H aliphatic), 1666 (C=O), 1597 (C=N); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.39-2.42 (m, 2H, -CH_2-C$\underline{H_2}$-CH_2-), 2.51 (t, 2H, -C$\underline{H_2}$-CH_2-CH_2-), 2.75 (t, 2H, -CH_2-CH_2-C$\underline{H_2}$-), 2.82-3.05 (br m, 4H, piperazine), 3.25-3.59 (br m, 4H, piperazine), 3.67 (s, 3H, OCH_3), 6.78 (d, 1H, =CH-olefinic), 6.85 (d, 1H, =CH-olefinic), 7.01-7.63 (m, 6H: 4 Ar-H, 2H of pyridazinone), 7.41 (d, 2H, Ar-H, J = 8.4 Hz), 7.63 (d, 2H, Ar-H, J = 8.4 Hz). EIMS (% rel. abundance): 205 (8.77), 231 (7.97), 233 (100), 261 (4.69), 464 (M^+, 0.00). Anal. Calcd for $C_{26}H_{29}ClN_4O_2$: C, 67.16; H, 6.29; N, 12.05. Found: C, 67.31; H, 6.31; N, 12.11.

3-Chloro-6-[2-(4-bromophenyl)ethenyl]pyridazine (10c)
This compound was prepared according to reported procedure (Abouzid et al., 2008). Yield: 67%; mp 184-185°C; IR (cm^{-1}): 3032 (C-H aromatic), 1595 (C=N). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 7.47 (d, 1H, =CH-olefinic), 7.52 (d, 1H, =CH-olefinic), 7.61-7.82 (m, 2H pyridazine H-4 and H-5), 7.94 (d, 2H, Ar-H), 8.10 (d, 2H, Ar-H); EIMS (% rel. abundance): 294 (M^+, 21.60), 295 (M+H, 100), 296 (M+2, 26.21), 298 (M+4, 6.56). Anal. Calcd for $C_{12}H_8BrClN_2$: C, 48.76; H, 2.73; N, 9.48. Found: C, 49.12; H, 2.66; N, 9.53.

3-(Pyridin-4-yl)-6-[2-(4-substitutedphenyl)ethenyl]-1,2,4-triazolo[4,3-b]pyridazines (11a-d)
An equimolar mixture of a respective **10a-d** and isonicotinic acid hydrazide (0.002 mol each) in absolute ethanol/dry dioxan mixture (40 mL, 1:1) was heated under reflux for 20 h. The reaction mixture was filtered while hot, the filtrate was concentrated under reduced pressure to half its volume, then cooled. The separated solid was filtered, washed with $NaHCO_3$ solution, dried and crystallized from the benzene/petroleum ether (60-80°C) mixture.

6-[2-(Phenyl)ethenyl]-3-(pyridin-4-yl)-1,2,4-triazolo[4,3-b]pyridazines (11a)
Yield: 44%; mp >300°C; IR (cm^{-1}): 3032 (C-H aromatic), 1616 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 7.04 (d, 1H, =CH-olefinic), 7.21 (d, 1H, =CH-olefinic), 7.28-8.2 (m, 11H, 9Ar-H and 2H pyridazine H-4 and H-5); EIMS (% rel. abundance): 105 (100), 299 (M^+, 1.31). Anal. Calcd for $C_{18}H_{13}N_5$: C, 72.23; H, 4.38; N, 23.40. Found: C, 72.20; H, 4.36; N, 23.49.

3-(Pyridin-4-yl)-6-[2-(4-methoxyphenyl)ethenyl]-1,2,4-triazolo[4,3-b]-pyridazines (11b)
Yield: 62%; mp 174-175°C; IR (cm^{-1}): 3059 (C-H aromatic), 2920, 2850 (C-H aliphatic), 1604 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.80 (s, 3H, OCH_3), 6.96

(d, 1H, =CH-olefinic), 6.99 (d, 1H, =CH-olefinic), 7.25 (d, 1H, pyridazinone H-4, *J* = 6.9 Hz*)*, 7.27-8.06 (m, 8H, Ar-H), 7.88 (d, 1H, pyridazinone H-5, *J* = 6.9 Hz); EIMS (% rel. abundance): 57 (100), 329 (M^+, 4.01). Anal. Calcd for $C_{19}H_{15}N_5O$: C, 69.29; H, 4.59; N, 21.26. Found: C, 69.34; H, 4.55; N, 21.19.

3-(Pyridin-4-yl)-6-[2-(4-bromophenyl)ethenyl]-1,2,4-triazolo[4,3-b]pyridazines (11c)

Yield: 65%; mp 184-185°C; IR (cm^{-1}): 3035 (C-H aromatic), 1600 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 6.85 (d, 1H, =CH-olefinic), 6.90 (d, 1H, =CH-olefinic), 7.47-7.93 (m, 6H, ArH), 8.06 (d, 2H, Ar-H, *J* = 9 Hz), 8.09 (d, 2H, Ar-H, *J* = 9 Hz); EIMS (% rel. abundance): 128 (100), 377 (M^+, 66.33), 379 (M+2, 56.05). Anal. Calcd for $C_{18}H_{12}BrN_5$: C, 57.16; H, 3.20; N, 18.52. Found: C, 56.94; H, 3.31; N, 18.64.

6-[2-(4-Chlorophenyl)ethenyl]-3-(pyridin-4-yl)-1,2,4-triazolo[4,3-b]pyridazines (11d)

Yield: 71%; mp 193-194°C; IR (cm^{-1}): 3047 (C-H aromatic), 1600 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 6.87 (d, 1H, =CH-olefinic, *J* = 18 Hz), 6.92 (d, 1H, =CH-olefinic, *J* = 18 Hz), 7.36-7.93 (m, 6H, 4ArH and 2H pyridazine H-4 and H-5), 8.06 (d, 2H, Ar-H, *J* = 9 Hz), 8.09 (d, 2H, Ar-H, *J* = 9 Hz); EIMS (% rel. abundance): 233 (100), 334 (M+H, 0.20). Anal. Calcd for $C_{18}H_{12}ClN_5$: C, 64.77; H, 3.62; N, 20.98. Found: C, 64.73; H, 3.62; N, 20.93.

6-[2-(4-Substitutedphenyl)ethenyl]-10H-pyridazino[6,1-b]quinazolin-10-ones (12a-d)

An equimolar mixture of an appropriate **10a-d** and anthranilic acid (0.001 mol each) in absolute ethanol/dry dioxan mixture (40 mL, 1:1) was heated under reflux for 4 h. After cooling, the precipitated solid was filtered and crystallized from absolute ethanol.

6-[2-(Phenyl)ethenyl]-10H-pyridazino[6,1-b]quinazolin-10-ones (12a)

Yield: 45%; mp >300°C; IR (cm^{-1}): 3055 (C-H aromatic), 1635 (C=O), 1605 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 6.80 (d, 1H, =CH-olefinic), 6.95 (d, 1H, =CH-olefinic), 7.35-8.35 (m, 11H, 9Ar-H and 2H pyridazine H-4 and H-5); ^{13}C-NMR (DMSO-d_6 ppm): δ 133.41-145.17 (16 aromatic C, 2 olefinic C), 167.17 (C=O); EIMS (% rel. abundance): 119 (100), 300 (M+H, 1.32). Anal. Calcd for $C_{19}H_{13}N_3O$: C, 76.24; H, 4.38; N, 14.04. Found: C, 76.29; H, 4.35; N, 14.12.

6-[2-(4-Methoxyphenyl)ethenyl]-10H-pyridazino[6,1-b]quinazolin-10-ones (12b)

Yield: 52%; mp 162-163°C; IR (cm^{-1}): 3035 (C-H aromatic), 2920, 2850 (C-H aliphatic), 1670 (C=O), 1604 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.72 (s, 3H, -OCH_3), 6.89 (d, 1H, =CH-olefinic), 6.95 (d, 1H, =CH-olefinic), 7.20-7.66 (m, 10H, 8Ar-H and 2H pyridazine H-4 and H-5); EIMS (% rel. abundance): 45 (100), 329 (M^+, 6.76). Anal. Calcd for $C_{20}H_{15}N_3O_2$: C, 72.94; H, 4.59; N, 12.76. Found: C, 72.96; H, 4.63; N, 12.70.

6-[2-(4-Bromophenyl)ethenyl]-10H-pyridazino[6,1-b]quinazolin-10-ones (12c)

Yield: 43%; mp 194-195°C; IR (cm^{-1}): 3032 (C-H aromatic), 1631 (C=O), 1604 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 6.88 (d, 1H, =CH-olefinic), 6.96 (d, 1H, =CH-olefinic), 7.46-7.81 (m, 6H, 4ArH and 2H pyridazine H-4 and H-5), 7.89 (d, 2H, Ar-H, *J* = 8.7 Hz), 8.05 (d, 2H, Ar-H, *J* = 8.7 Hz); EIMS (% rel. abundance): 295 (100), 377 (M^+, 4.12), 379 (M+2, 4.10). Anal. Calcd for $C_{19}H_{12}BrN_3O$: C, 60.34; H, 3.20; N, 11.11. Found: C, 60.34; H, 3.29; N, 11.08.

6-[2-(4-Chlorophenyl)ethenyl]-10H-pyridazino[6,1-b]quinazolin-10-ones (12d)

Yield: 68%; mp 187-188°C; IR (cm^{-1}): 3035 (C-H aromatic), 1670 (C=O), 1604 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 6.92 (d, 1H, =CH-olefinic), 7.01 (d, 1H, =CH-olefinic), 7.41-7.84 (m, 6H, 4Ar-H and 2H pyridazine H-4 and H-5), 7.90 (d, 2H, Ar-H, *J* = 9 Hz), 8.06 (d, 2H, ArHs, *J* = 9 Hz); EIMS (% rel. abundance): 111 (100), 333 (M^+, 56.45). Anal. Calcd for $C_{19}H_{12}ClN_3O$: C, 68.37; H, 3.62; N, 12.59. Found: C, 68.28; H, 3.57; N, 12.63.

4-{[6-(2-(4-Substitutedphenyl)ethenyl)pyridazin-3-yl]amino}benzoic acids (13a-d)

An equimolar mixture of an appropriate **10a-d** and 4-aminobenzoic acid (0.001 mol each) was heated under reflux for 3 h. The reaction mixture was filtered while hot and the filtrate was concentrated under reduced pressure to half its volume, then cooled. The separated solid was filtered and crystallized from isopropanol.

4-{[6-(2-(Phenyl)ethenyl)pyridazin-3-yl]amino}benzoic acid (13a)

Yield: 35%; mp >300°C; IR (cm^{-1}): 3140 (N-H), 3248 (O-H), 3057 (C-H aromatic), 1688 (C=O), 1608 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 6.74 (d, 1H, =CH-olefinic), 6.80 (d, 1H, =CH-olefinic), 7.38-8.20 (m, 11H, 9Ar-H and 2H of pyridazine H-4 and H-5), 11.90 (s, 1H, D_2O exchangeable). EIMS (% rel. abundance): 120 (100), 317 (M^+, 8.63). Anal. Calcd for $C_{19}H_{15}N_3O_2$: C, 71.91; H, 4.76; N, 13.24. Found: C, 71.99; H, 4.78; N, 13.28.

4-{[6-(2-(4-Methoxyphenyl)ethenyl)pyridazin-3-yl]amino}benzoic acid (13b)

Yield: 32%; mp 129-130ºC; IR (cm^{-1}): 3213 (N-H), 3444 (O-H), 3012 (C-H aromatic), 2966, 2839 (C-H aliphatic), 1681 (C=O), 1604 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.80 (s, 3H, -OCH_3), 6.53 (d, 1H, =CH-olefinic), 6.77 (d, 1H, =CH-olefinic), 7.01 (d, 1H, pyridazine H-4), 7.32-7.84 (m, 4H, Ar-H), 7.87 (d, 1H, pyridazine H-5), 8.02 (d, 2H, Ar-H), 8.05 (d, 2H, Ar-H), 10.77 (s, 1H, D_2O exchangeable); EIMS (% rel. abundance): 231 (100), 349 (M+2H, 8.17). Anal. Calcd for $C_{20}H_{17}N_3O_3$: C, 69.15; H, 4.93; N, 12.10. Found: C, 69.21; H, 4.95; N, 12.19.

4-{[6-(2-(4-Bromophenyl)ethenyl)pyridazin-3-yl]amino}benzoic acid (13c)

Yield: 56%; mp 169-170ºC; IR (cm^{-1}): 3217 (N-H), 3429 (O-H), 3032 (C-H aromatic), 1681 (C=O), 1608 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 6.52 (d, 1H, =CH-olefinic), 6.70 (d, 1H, =CH-olefinic), 7.44 (d, 1H, pyridazine H-4), 7.49-7.89 (m, 8H, Ar-H), 8.04 (d, 1H, pyridazine H-5), 10.90 (s, 1H, D_2O exchangeable); EIMS (% rel. abundance): 295 (100), 394 (M-H, 0.04). Anal. Calcd for $C_{19}H_{14}BrN_3O_2$: C, 57.59; H, 3.56; N, 10.60. Found: C, 57.58; H, 3.56; N, 10.71.

4-{[6-(2-(4-Chlorophenyl)ethenyl)pyridazin-3-yl]amino}benzoic acids (13d)

Yield: 68%; mp 179-180ºC; IR (cm^{-1}): 3305 (N-H), 3217 (O-H), 3032 (C-H aromatic), 1685 (C=O), 1606 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 6.49 (d, 1H, =CH-olefinic), 6.75 (d, 1H, =CH-olefinic), 7.48-8.07 (m, 10H, 8ArHs and 2H pyridazine H-4 and H-5), 10.91 (s, 1H, D_2O exchangeable); EIMS (% rel. abundance): 249 (100), 351 (M^+, 0.13). Anal. Calcd for $C_{19}H_{14}ClN_3O_2$: C, 64.87; H, 4.01; N, 11.94. Found: C, 64.89; H, 4.12; N, 11.82.

Pharmacology

Chemicals and drugs

Carrageenan and indomethacin were purchased from Sigma-Aldrich.

Animals

Male Sprague Dawley rats (120-130 g weight) were purchased from the animal house facility of the National Research Center. Animals were acclimatized in the animal house unit of the Pharmacology Dept., National Research Center of Egypt for at least one week prior to the experiments. Animals were kept at 22 ± 3ºC and 55 ± 5% relative humidity, during the whole experiment. Standard food pellets and water were supplied *ad labium*. All test compounds were dispensed in 10% Tween-80 solution in the distilled water. Animals' treatment protocol was approved by the National Research Center Animal Rights Committee.

Assessment of anti-inflammatory activity

Anti-inflammatory activity of the compounds was assessed using carrageenan-induced paw edema. Rats were divided into groups of six rats each. One hour after oral administration of test compounds (10 mg/kg), 0.1 mL of 1% carragenan solution was injected, sub-planter to the left hind paw of each animal. Paw volumes were measured using standard fluid displacement procedures (7140-plesthysmometer Ugo Basile) by dipping the hind left paw in 0.45% saline solution, at 1, 2, 3, and 4 h, post carrageenan injection. The percent change in paw volume relative to that of the base line measurement was taken as the criteria of comparison. Indomethacin (10 mg/kg) was used as an internal standard anti-inflammatory agent. Significant differences between the control and the treated groups were obtained, using Student's *t*-test and *p* values. The differences in the results were considered significant at $p < 0.05$ (Table I).

Gastric ulcerative effect

Ulcerative effect (gastric hemorrhagic gross lesions) of test compounds, which showed anti-inflammatory action, was assessed after intragastric administration of 10 mg/kg as previously described. Six hours after drug administration, rats were euthanized by cervical dislocation and the stomachs were removed, inflated and opened along the greater curvature. The hemorrhagic lesions were stretched out and scored from 0 to 5, according to the method of Clementi et al. Gastric tissue samples were fixed in neutral buffered formalin for 24 h. indomethacin was used as positive control drug, (Table II, Fig. 2).

Statistical analysis

Data are presented as mean ± S.E.M. Analysis of variance (ANOVA) with Dunnet's post hoc test was used for testing the significance of data, using SPSS® for windows, version 17.0. $p < 0.05$ was taken as a cut off value for significance.

In vitro studies

Cell culture

Raw murine macrophage (RAW 264.7) was purchased from the American Type Culture collections. Cells were routinely cultured in RPMI-1640. Media were supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, containing 100 U/mL penicillin G sodium, 100 U/mL streptomycin sulphate, and 250 mg/mL

Table I. Percent change in rat paw edema after carrageenan sub-planter injection

Compound No.	% Change in paw volume (mean ± S.E.M)[a]			
	1 h	2 h	3 h	4 h
Control	89.7 ± 19.6	79.4 ± 9.9	118.2 ± 26.2	104.8 ± 13
Indomethacin	62.7 ± 16.4	50.8 ± 17.3	48.8* ± 16.4	40.3* ± 14.8
4 a	47.7* ± 10.6	42.0* ± 12.9	33.5* ± 8.8	42.6* ± 6.0
4 b	49.6* ± 10.3	50.4* ± 3.8	48.5* ± 11.8	43.8* ± 6.2
4 c	68.9 ± 12.2	70.7 ± 8.5	88.2 ± 18.5	73.2 ± 22.1
4 d	39.3* ± 13.0	89.2 ± 18.0	104.8 ± 25.9	70.8* ± 13.9
4 e	43.0* ± 8.4	82.1 ± 7.0	100.7 ± 19.0	87.4 ± 4.0
4 f	65.0 ± 8.8	62.4 ± 19.8	52.6* ± 6.8	51.7* ± 12.0
4 g	51.0* ± 11.6	136.5 ± 24.8	94.3 ± 10.2	78.0 ± 17.2
4 h	41.6* ± 5.5	69.1 ± 6.7	112.6 ± 24.1	72.6 ± 12.1
4 i	53.8* ± 6.0	51.8* ± 4.1	48.5* ± 2.1	55.6* ± 7.0
5 a	54.1 ± 8.2	67.0 ± 21.0	56.5* ± 13.0	32.9* ± 7.2
5 b	54.6* ± 6.2	77.4 ± 20.6	47.1* ± 11.0	40.1* ± 5.0
5 c	53.3* ± 6.8	81.9 ± 10.8	73.0 ± 12.1	65.0 ± 21.6
5 d	47.9* ± 4.6	66.9 ± 10.1	35.3* ± 6.5	37.2* ± 7.6
7 a	90.7 ± 4.4	67.7 ± 12.2	56.0* ± 13.7	65.2* ± 16.2
7 b	66.8 ± 16.2	74.7 ± 8.5	89.2 ± 19.5	77.4 ± 21.1
7 c	76.0 ± 13.6	84.6 ± 6.0	59.9* ± 11.8	44.9* ± 7.6
9 a	60.5 ± 7.5	86.6 ± 18.4	95.5 ± 15.1	76.0* ± 8.7
9 b	47.0* ± 8.9	72.1 ± 12.6	43.1* ± 1.2	73.2 ± 17.3
9 c	74.9 ± 12.1	90.8 ± 12.9	99.7 ± 17.8	84.3 ± 7.6
9 d	23.8* ± 5.6	33.1* ± 10.0	31.9* ± 13.1	31.0* ± 14.7
11 a	37.2* ± 4.5	48.2* ± 5.3	63.2* ± 12.9	59.3 ± 17.7
11 b	42.7* ± 4.0	48.7* ± 5.1	69.0 ± 10.6	51.9* ± 10.3
11 c	68.7 ± 7.7	90.8 ± 22.0	65.1* ± 15.9	36.3* ± 4.2
11 d	42.2* ± 10.2	75.0 ± 16.5	57.2* ± 12.7	68.3 ± 21.2
12 a	66.9 ± 8.5	75.8 ± 11.7	47.9* ± 15.3	62.7* ± 4.4
12 b	42.5* ± 12.7	90.9 ± 18.9	76.7 ± 8.2	85.3 ± 10.9
12 c	54.6 ± 14.3	104.6 ± 13.4	115.8 ± 17.0	79.8 ± 19.6
12 d	65.8 ± 17.9	64.2 ± 20.1	85.8 ± 12.9	78.4 ± 17.5
13 a	38.9* ± 4.9	74.2 ± 10.0	78.6 ± 14.1	82.0 ± 21.1
13 b	66.8 ± 16.0	93.1 ± 25.2	102.9 ± 10.5	59.0* ± 14.1
13 c	61.3 ± 10.4	70.9 ± 14.1	85.1 ± 12.8	64.3* ± 12.1
13 d	51.1 ± 17.9	120.1 ± 11.5	86.0 ± 14.7	98.9 ± 19.5

Significantly different from corresponding control value at $^{*}p < 0.05$; [a]The data are the result of six rats/experiment.

amphotericin B. Cells were maintained in humidified air, which contains 5% CO_2 at 37°C. RAW 264.7 cells were collected by scraping. All experiments were repeated four times, unless mentioned, and the data was represented as (mean ± S.D.). All cell culture material was obtained from Cambrex, BioScience.

Cells 0.5×10^5 cells/well in serum-free media were plated in a flat bottom 96-well microplate, and treated with 20 μL of different concentrations of each tested compound for 24 h at 37°C, in a humidified 5% CO_2 atmosphere. After incubation, media were removed and 40 μL MTT solution/well were added and incubated for an additional 4 h. MTT crystals were solubilized, via the addition of 180 μL acidified isopropanol/well and plate was shacked at room temperature, followed by photometric determination of the absorbance at 570 nm using 96 wells microplate ELISA reader. Triplicate repeats were performed for each concentration and the average was calculated. Data was expressed as the percentage of relative viability compared with that of the untreated control cells, as the relative fold of increase in the cell viability (Fig. 3).

The fold of increase in the relative viability was calculated, using the following equation: Absorbance of treated cells/Absorbance of control untreated cells.

Table II. Gastric ulcerative effect of test compounds compared to indomethacin

Compound No.	Ulcer number[a]	Ulcer severity (score)[a]
Control	0.0 ± 0.0	0.0 ± 0.0
Indomethacin	2.6 ± 0.4	6.2 ± 0.7
4 a	0.0 ± 0.0	0.0 ± 0.0
4 b	2.6 ± 0.5	3.0 ± 0.8
4 d	3.0 ± 1.0	3.0 ± 1.0
4 e	2.3 ± 1.2	2.6 ± 1.5
4 f	0.0 ± 0.0	0.0 ± 0.0
4 g	1.3 ± 0.7	1.3 ± 0.7
4 h	3.6 ± 0.5	6.0 ± 2.1
4 i	5.0 ± 0.5	5.3 ± 0.3
5 a	1.2 ± 0.6	1.6 ± 0.8
5 b	4.6 ± 1.0	9.3 ± 0.5
5 c	4.0 ± 1.3	5.0 ± 1.7
5 d	3.6 ± 0.5	6.0 ± 2.1
7 a	1.6 ± 1.1	1.6 ± 1.1
7 c	4.0 ± 1.3	5.0 ± 1.2
9 a	1.6 ± 1.0	2 ± 1.2
9 b	0.6 ± 0.5	0.6 ± 0.5
9 c	1.2 ± 0.6	2.2 ± 1.3
9 d	2.6 ± 1.2	4.3 ± 2.4
11 a	0.0 ± 0.0	0.0 ± 0.0
11 b	7.0 ± 1.8	9.3 ± 3.2
11 c	2.6 ± 1.0	4.3 ± 2.4
11d	2.6 ± 0.5	6.3 ± 0.5
12 a	1.3 ± 0.6	1.6 ± 0.8
12 b	5.3 ± 2.2	6.3 ± 1.6
13 a	0.0 ± 0.0	0.0 ± 0.0
13 b	4.5 ± 2.2	6.3 ± 3.1
13 c	0.0 ± 0.0	0.0 ± 0.0

[a]Data is expressed as mean ± S.E.M.; The data are the result of six rats/experiment.

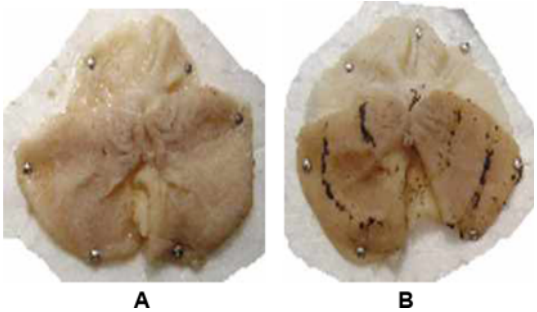


Fig. 2. (**A**) Non-ulcerative stomach (Negative control); (**B**) Ulcerative stomach (indomethacin).

Estimation of COX-2 in the macrophages lysate

The level of COX-2 was measured in the cells by ELISA. The assay uses the quantitative indirect immunoassay technique that uses polyclonal antibody and biotin-linked polyclonal antibody, both of which are specific against murine COX-2. Commercially available matched paired antibodies were used (R&D Systems Inc.).

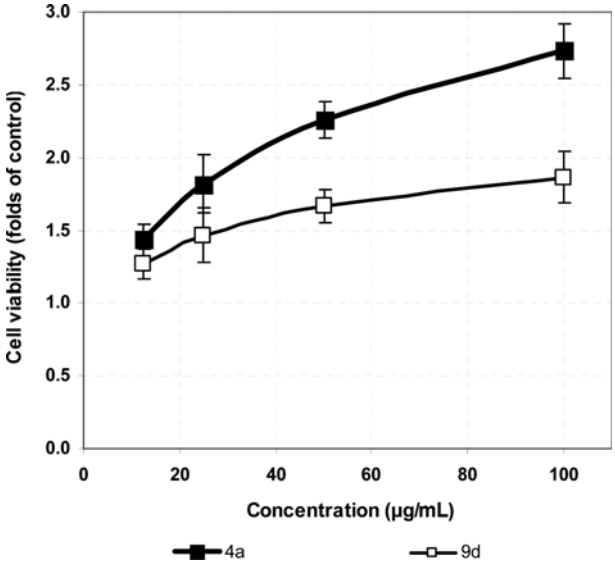


Fig. 3. Cell viability of RAW 264.7 cells after the treatment with different concentrations of the test compounds for 48 h as measured by MTT assay. The data are presented as fold of increase compared to control untreated cells.

Cells were treated for 24 h, with or without bacterial lipopolysaccharide (25 µg/mL), to stimulate COX-2 production in the cells, and then cells were treated with samples (100 µg/mL) for another 24 h. The cell lysate was coated onto 96-well flat bottom microtiter plate (Griener Labortechnik) in diluent, 50 µL/well and incubated 1 h at 37°C, which was then placed in the humidified chamber overnight, at 4°C. Plates were washed three times with washing buffer and blocked with 200 µL/well blocking buffer, which was then incubated, at 37°C for 1.5 h. The plates were washed three times with washing buffer and incubated with the diluted primary antibody. At the end of the incubation period, the plates were washed three times with washing buffer and diluted second biotin labeled antibody was added for 1 h incubation, at 37°C. After washing away any unbound substances, the peroxidase-conjugated streptavidin (Jackson Immunsearch Lab) diluted 1:1000 was added to as 50 µL/well, then the plates were incubated for 1 h, at 37°C. After an intensive washing, the enzyme reaction was carried out by adding a 50 µL/well of substrate solution. Color development was stopped by addition of 50 µL/well of stopping buffer (1M HCl) (Surechern Products, Needham Marker). The intensity of the developed color was measured by reading optical absorbance at 450 nm, using a microplate reader FLUOstar OPTIMA, BMG LABTECH GmbH) (Hansen et al., 1989) (Fig. 4).

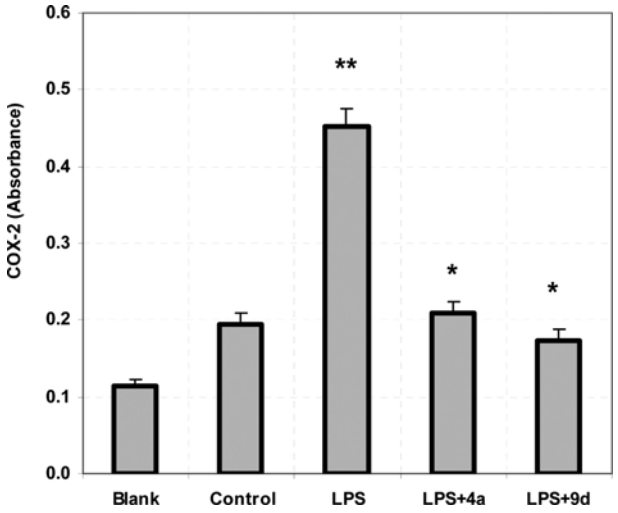


Fig. 4. The level of COX-2 protein in RAW 264.7 cells lysate after the treatment with the samples (100 μg/mL) for 48 h compared LPS treated cells, as measured by ELISA assay. The data are presented as absorbance (mean ± S.E).

RESULTS AND DISCUSSION

The synthetic pathways leading to the target compounds **4a-i** and **5a-d** are presented in Scheme 1. The intermediate 6-(4-substitutedphenyl)-4-oxo-hex-5-enoic acids (**1a-d**) (Abouzid et al., 2007), 6-[2-(4-substitutedphenyl)ethenyl]-4,5-dihydropyridazin-3(2H)-ones (**2a-d**) (Abouzid and Bekhit, 2008) and 6-[2-(4-substitutedphenyl)ethenyl] pyridazin-3(2H)-ones (**3a**, **3b**, **3d**) (Abouzid et al., 2008), were prepared following the previously reported procedures. The Mannich bases **4a-i** were synthesized by condensation of **3a-d** with formaline and a secondary amino function of amino compounds, which follows the standard reaction conditions. On the other hand, the preparation of the target compounds **5a-d** was achieved by alkylation of **3a-d** with N-bromomethylphthalimide.

The final targets **4a-i** and **5a-d** were obtained in 45-85% and 50-68% yields respectively, and were structurally elucidated on the basis of microanalyses and spectral data. The IR spectra revealed the disappearance of a band corresponding to *NH*. ^{1}H-NMR of **4a-i** and **5a-d** proved the presence of a singlet peak, which corresponds to methylene group flanked by the two nitrogen atoms in the range of δ 4.72-4.93 and 5.64-5.65 ppm respectively. In addition, ^{1}H-NMR spectrum of compounds **4a-e**, **g**, **h** showed the appearance of two characteristic broad multiplets of piperazine ring, in the range δ 2.80-2.91 ppm and 3.13-3.21 ppm. All the other aromatic and aliphatic protons were observed in the expected regions.

In the Scheme 2, the intermediates **6a-c** and **8a-d** were prepared by alkylation of **3a-d** with, either 1-bromo-2-chloroethane or 1-bromo-3-chloropropane, respectively. Nucleophilic displacement of chlorine in **6a-c** and **8a-d** with 4-(4-methoxyphenyl) piperazine afforded the desired target compounds **7a-c** and **9a-d**, respectively in 42-66% yields. The structure of the target compounds **7a-c** and **9a-d** was substantiated by elemental analyses, as well as spectroscopic data.

^{1}H-NMR spectra of compounds **7a-c** showed the appearance of two broad signals of piperazine ring, in the range of δ 2.63-3.18 and 3.05-3.46 ppm. Moreover, these compounds displayed a singlet signal in the range of δ 3.76-3.78 ppm assignable to 3H of OCH_3. ^{13}C-NMR spectrum of compound **7a** showed the appearance of 4C of piperazine ring at δ 49.60 ppm and 51.10 ppm. All the other signals in ^{1}H-NMR and ^{13}C-NMR were observed in the expected regions. The mass spectra of **7a-c** showed two fragments at m/z = 219 and m/z = 205, corresponding to the characteristic fragmentation pattern of these compounds. ^{1}H-NMR spectrum of compounds **9a-d** showed the appearance of two characteristic broad signals of piperazine ring, in the range of δ 2.92-3.16 and 3.25-3.50 ppm. Moreover, the spectra showed the appearance of a singlet signal in the range of δ 3.67-3.78 ppm assignable to 3H of OCH_3 group. The mass spectra of compounds **9a-d** displayed two fragments at m/z = 233 and m/z = 219, which explained the fragmentation pattern of these compounds.

Scheme 3 involves the chlorination of **3a-d** with phosphorous oxychloride to give the corresponding chloro derivatives **10a-d**. The physical and spectral data of **10a**,**b**,**d** were in accordance with the literature (Abouzid et al., 2008). Compound **10c** was obtained in 67% yield and was fully characterized by microanalysis and spectral data. The IR spectra revealed the disappearance of a band corresponding to *NH*. Cyclization of **10a-d** was carried out with either, isonicotinic acid hydrazide or anthranilic acid in ethanol/dioxan mixture (1:1) to give the target compounds **11a-d** and **12a-d**, respectively in 43-71% yieds. IR spectra of **12a-d** showed the appearance of a strong band in the range 1631-1670 cm^{-1}, which indicates the presence of C=O stretching band of amide. On the other hand, reaction of **10a-d** with *p*-aminobenzoic acid afforded the target compounds **13a-d** in 32-68% yieds. The IR spectra of **13a-d** showed the appearance of a broad band in the range of 3240-3444, indicating the presence of *NH* and COOH. Moreover, the ^{1}H-NMR spectra displayed D_2O exchangeable signals in the range δ 10.77-11.90 ppm assignable to *NH* protons.

Pharmacology

The synthesized new final compounds were subjected

to the evaluation of anti-inflammatory activity and acute ulcerogenicity studies. Indomethacin was used as reference standard.

Anti-inflammatory activity

Anti-inflammatory activity was determined using carrageenan-induced rat paw edema model (Winter et al., 1962). Carrageenan (1% w/v) was used to produce paw edema. Edema was presented as percentage increase in left hind paw, in comparison to the uninjected right hind paw. Percentage change in paw volume was calculated and expressed as the amount of inflammation. For anti-inflammatory activity, the test compounds were orally administered, at molar equivalent doses of indomethacin (10 mg/kg, p.o.). Sub-planter injection of 0.1 mL of 1% carrageenan in rat paw increased the paw volume (edema) in all the animals of various groups. The onset of action was evident during 1 h in various test groups. The significant ($p < 0.01$) reduction of rat paw edema was observed for most of the test compounds, at 4 h, when compared to that of the control group and indomethacin (Table I).

Among all of the tested compounds, **4a** and **9d** exhibited the most prominent and consistent anti-inflammatory activity, which was higher than indomethacin with rapid onset of action and sustained duration until the fourth hour after the administration of the drug. Also, compounds **4b** and **4i** gave a high rapid and sustained anti-inflammatory effect for 4 h comparable to indomethacin. On the other hand, compounds **4c**, **7b**, **9c**, **12c**, **12d** and **13d** did not show any significant change in paw volume, when compared to that of the untreated group. It is also obvious that the triazolopyridazine derivatives **11a-d** seems to exhibit rapid onset and more potent anti-inflammatory activity than the pyridazinoquinazoline derivatives **12a-d**.

Moreover, it has been shown that compounds **4e**, **4g**, **4h**, **5c**, **12b** and **13a** displayed a rapid short anti-inflammatory effect at the first hour, only after drug administration, whereas the compounds **9a**, **13b** and **13c** revealed a mild anti-inflammatory effect of delayed onset at the fourth hour, only compared to indomethacin.

Gastric ulcerative effect

Compounds with significant anti-inflammatory profile were tested for GI-ulcerogenicity potential. The ulcerative effect of test compounds has been inspected visually, relative to the known ulcerogenic drug, indomethacin, (Fig. 2). After gross visual inspection, it has been obvious that compounds **4a**, **4f**, **9a**, **13a** and **13c** showed no ulcer formation, whereas compounds **4h**, **4i**, **5b**, **5d**, **11b**, **11d**, **12b**, **13b** and indomethacin showed significant ulcerogenic effect. Other test compounds haven't displayed any significant ulcerogenicity (Table II).

In vitro assays

The compounds that showed the most potent anti-inflammatory activity **4a** and **9d** were selected to further investigation for COX-2 selectivity.

Cell viability assay

The effect of test compounds **4a** and **9d**, on the cell viability of RAW 264.7 cells, was measured using the MTT cell viability assay.

3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay is based on the ability of active mitochondrial dehydrogenase enzyme of living cells to cleave the tetrazolium rings of the yellow MTT, which forms a dark blue insoluble formazan crystals, which is largely impermeable to cell membranes, resulting in its accumulation within healthy cell. Solubilization of the cells results in the liberation of crystals, which are then solubilized. The number of viable cells is directly proportional to the level of soluble formazan dark blue color. The extent of the reduction of MTT was quantified by measuring the absorbance at 570 nm (Hansen et al., 1989) (Fig. 3).

Estimation of COX-2 in the macrophages lysate

The level of COX-2 was measured in the cells by ELISA. The assay uses the quantitative indirect immunoassay technique that uses polyclonal antibody and biotin-linked polyclonal antibody, both of which are specific against murine COX-2. Commercially available matched paired antibodies were used (R&D Systems Inc.).

The results indicated that LPS induced COX-2 production up to 2.34 fold of the control and that the compounds **4a** and **9d** (100 μg/mL) possessed a high inhibitory activity of COX-2 to the extent that is nearly to the control level. Treatment with different concentrations of the test compounds revealed that IC_{50} of **4a** and **9d** is 48.4 μg/mL and 56.4 μg/mL, respectively (Fig. 4).

In summary, most of the synthesized compounds exhibited anti-inflammatory activity and superior gastrointestinal safety profile in the animal study. The results showed that among the Mannich bases, **4a-i**, the compounds **4a** and **4b** containing 4-chlorophenylpiperazine in their structure, displayed potent anti-inflammatory effect, with a safe gastric profile. Also, in a series of compounds **9a-d**, in which the 6-arylethenylpyridazinone pharmacophore is linked to the 4-methoxyphenylpiperazine via three carbon spacer, the compound **9d**, in which there is a chlorine atom in the arylethenyl moiety that showed a superior action higher

than indomethacin with very low ulcerogenicity. Also, among the benzoic acid derivatives **13a**-**d**, the unsubstituted and 4-bromo substituted derivatives, **13a** and **13c** have shown high anti-inflammatory activity, without ulcer formation. The results of *in vitro* studies revealed that compounds **4a** and **9d** exhibited a high COX-2 inhibition effect.

Therefore, it can be concluded that the rational, on which these compounds were designed, has been proven to show promising results, which follow the SAR studies previously discussed that revealed the importance of *N*-substitution on pyridazine ring as a requirement for COX-2 selectivity.

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