Synthesis and Biological Evaluation of New Heteroaryl Carboxylic Acid Derivatives as Anti-inflammatory-Analgesic Agents

Khaled Abouzid Mohamed Abouzid,[a] Nadia Abdalla Khalil,[b] Eman Mohamed Ahmed,*[,b] and Sawsan Abo-Bakr Zaitone[c]

[a] *Pharmaceutical Chemistry Department, Faculty of Pharmacy, Ain Shams University; Cairo 11566, Egypt:*
[b] *Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy, Cairo University; Cairo 11562, Egypt: and*
[c] *Department of Pharmacology & Toxicology, Faculty of Pharmacy, Suez Canal University; Ismailia 41522, Egypt.*
Received October 28, 2012; accepted November 20, 2012

A series of nicotinic acid derivatives structurally related to niflumic acid and certain pyridazine-containing compounds have been synthesized and characterized by analytical and spectral data. All compounds were screened for their potential analgesic and anti-inflammatory activities. The compounds which displayed analgesic and anti-inflammatory activities were tested for ulcerogenicity and screened for *in vivo* inhibition of certain inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and cyclooxygenase-2 (COX-2). Compounds 1c, 2a, 2b, and 5a have shown potent analgesic and anti-inflammatory activities.

Key words nicotinic acid; niflumic acid; pyridazine; analgesic; anti-inflammatory

Rheumatoid arthritis (RA) is an immune-based chronic inflammatory synovitis presenting with pain, stiffness and swelling of the affected joints. RA results in secondary bone and cartilage destruction causing joint deformity. Current therapies include conventional non-steroidal anti-inflammatory agents (NSAIDs), which inhibit prostaglandin (PG) formation through inhibition of both cyclooxygenase-1 (COX-1) and COX-2 enzymes.[1,2] PGs are important lipid mediators that are produced at elevated levels in inflamed tissues including rheumatoid synovium.[3,4] However, long term NSAID treatment is often limited due to gastrointestinal (GI) ulcerogenicity that may result from the suppression of physiological PG production in these tissues.[5] Certain studies have revealed key roles for inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin-1b (IL-1b) and IL-6, in pathogenesis of rheumatoid arthritis (RA).[6,7] IL-1, TNFα, and PGE2 overproduction play potential pathogenic roles in the establishment of rheumatoid synovitis, in the formation of pannus tissue and in the process of joint destruction.[8] Thus, therapies suppressing these inflammatory cytokines have been focused on as effective treatment for RA.

Selective COX-2 inhibitors demonstrated the ability to block PG production[9] and acute tissue inflammation[10] *in vivo* at dosages that do not affect stomach PG production, suggesting that COX-2 inhibitors may provide a safer therapeutic alternative to NSAIDs. Nevertheless, the voluntary withdrawal of rofecoxib worldwide in 2004 due to an increased risk of cardiovascular events,[11] raised serious concerns about safety of selective COX-2 inhibitors.[12] Therefore, there exists an unmet medical need for 'Safe NSAIDs' that do not cause adverse effects.

Synthetic approaches based upon chemical modification of NSAIDs have been taken with the aim of improving safety profile and in turn, therapeutic window of these NSAIDs. It has been reported in the literature that certain compounds bearing pyridine, pyridazine and pyridazinone heterocycles, possess significant analgesic and anti-inflammatory activities.[13–16] Niflumic acid and flunixin are two traditional NSAIDs belonging to the class of fenamates enclosing pyridine nucleus in their structure (Fig. 1). They bind at the prostaglandin receptor site thus, potentially antagonizing the physiopathological effects of the already produced prostaglandins. However, fenamates are still exhibited most of the GI adverse effects induced by NSAIDs.[16]

Furthermore, a lot of 3(2*H*)-pyridazinone derivatives have been reported as analgesic and anti-inflammatory agents without gastrointestinal side effect.[14,17,18] In the same direction, we planned to design new pyridazinone scaffolds as potential anti-inflammatory agents (Fig. 2). This is agreement with in our experience in the pyridazinone field.[19]

Considering the above results and as a part of our ongoing program to design analgesic and anti-inflammatory agents, herein, we describe the synthesis, and biological evaluation of certain 2-arylamino, heteroarylamino or aryloxynicotinic acid derivatives structurally related to niflumic acid. Keeping the 2-aminonicotinic acid framework, and either modifying the aryl substituents on the benzene ring or replacing the aryl ring by a heterocycle. We also aimed at developing certain pyridazine and pyridazinone derivatives (Fig. 2). The analgesic and anti-inflammatory activities were investigated for the title compounds utilizing the writhing test and the carrageenan-induced paw edema test (CPE test), respectively. The compounds with analgesic and anti-inflammatory potentials were also tested for the irritative and ulcerogenic action on gastric mucosa. The serum cytokine level has been analysed as well, for the compounds that revealed significant anti-inflammatory

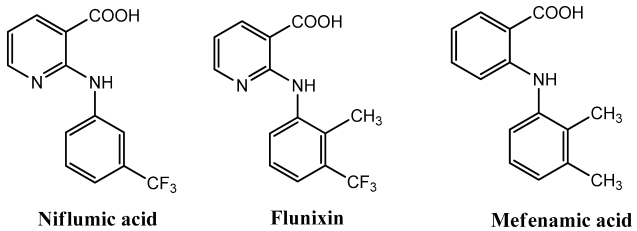


Fig. 1. Selected Fenamate Derivatives

The authors declare no conflict of interest.

* To whom correspondence should be addressed. e-mail: dr_eman2001@hotmail.com

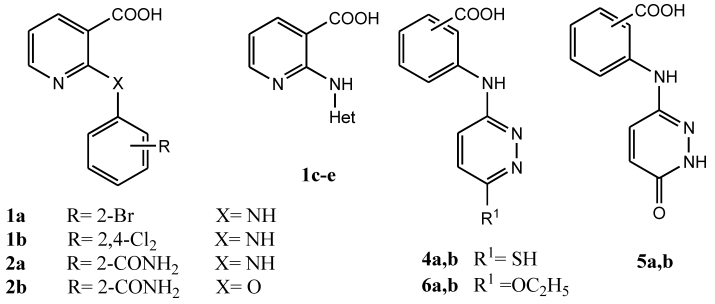


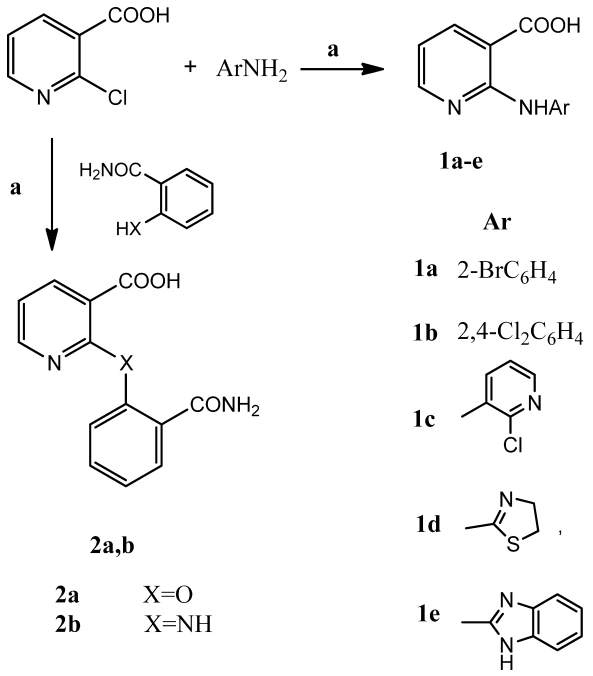
Fig. 2. General Structures of the Synthesized Compounds

activity in the rat paw edema model.

Results and Discussion

Chemistry In Chart 1, new 2-substituted-nicotinic derivatives **1a**–**e** were synthesized through nucleophilic displacement reaction of the commercially available 2-chloronicotinic acid with various aromatic and heterocyclic primary amino compounds following procedure reported for analogous compounds.[15] On the other hand, reaction of 2-chloronicotinic acid with either salicylamide or anthranilamide resulted in the formation of 2-(2-carbamoylphenoxy)- and 2-(2-carbamoylphenylamino)nicotinic acids **2a**, **b**, respectively.

New benzoic acid derivatives **3a**, **b**, **4a**, **b**, **5a**, **b**, and **6a**, **b** were synthesized according to Chart 2. Initially, nucleophilic displacement reaction of commercial 3,6-dichloropyridazine with the appropriate aminobenzoic acid in isopropanol afforded the appropriate 3-(6-chloropyridazine amino)benzoic acids **3a**, **b** according to previously reported procedure.[19] The latter were reacted with thiourea to give the isothiouronium intermediates, which upon alkaline hydrolysis yielded the corresponding thiones **4a**, **b** following procedure reported for analogous compounds.[20] Hydrolysis of the chloropyridazine derivatives **3a**, **b** was carried out upon heating in acetic acid to afford the corresponding 3(2*H*)-pyridazinone derivatives **5a**, **b**. Furthermore, 3-(6-ethoxypyridazinamino)benzoic acids **6a**, **b** were synthesized by heating **3a**, **b** in absolute ethanol in the presence of sodium ethoxide. Analytical and spectral data (IR, ^{1}H-NMR, mass spectra) of the synthesized compounds were in full agreement with the proposed structures.

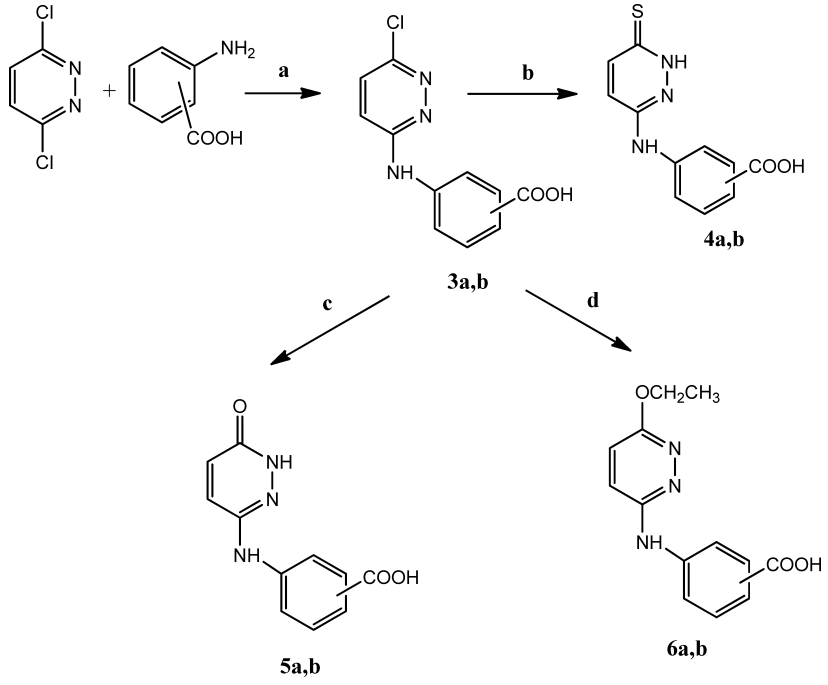


Reagents and conditions: **a**) isopropanol/K_2CO_3/reflux 4 h.

Chart 1

Analgesic Activity In current study, the new compounds were tested for analgesic activity using the writhing test in mice[21] and compared to mefenamic acid, as a standard drug at the same dose level (25 mg/kg, *per os* (*p.o.*)). Writhing was induced by intraperitoneal (i.p.) injection of freshly prepared acetic acid solution (1%, 10 mL/kg, i.p.) in mice. The number of writhes (constriction of abdomen, turning of trunk, extension of hind limbs) due to acetic acid was expressed as a nociceptive response. Vehicle treated control mice were given 1% acetic acid and writhing response was noted for 15 min. The results showed that the tested compounds exhibited analgesic activity ranging from 10.2±1.2% to 78.2±6.8%. Compounds **1c**, **2a**, **2b**, **5a**, and **6a** showed significant reduction in the writhing response (72.1±6.5 to 78.2±6.8) superior to that exhibited by mefenamic acid (70.3±2.5%). Among the compounds **1a**–**e**, the 2-chloropyridin-3-yl derivative **1c**, exhibited the highest analgesic activity. Replacement of the 2-chloropyridine moiety with phenyl ring with halogen substituents in 2 and/or 4 position resulted in reduction of analgesic and anti-inflammatory activities. Furthermore, the thiazoline and benzimidazole conjugates (**1d**, **e**, respectively) showed no analgesic and anti-inflammatory potentials. It was also obvious that replacement oxygen atom in by nitrogen in the nicotinic acid derivatives **2a**, **b** has only slight effect on the analgesic activity. On the other hand, the pyridazine derivatives **3a**, **4a**, and **4b** were almost devoid of activity. However, the pyridazinone analogues **5a**, **b** showed significant protection. The compound **5a** displayed the most potent analgesic activity with % protection 78.2±6.8 (Table 1). Compounds **6a**, **b** with 6-ethoxy substituent in the pyridazine ring showed lower analgesic activity compared to their hydroxyl analogues, with no significant change in anti-inflammatory activity.

Anti-inflammatory Activity Anti-inflammatory activity was determined by using carrageenan induced rat paw edema model.[22] Carrageenan (1% w/v) was used to produce paw edema. Edema is presented as percentage increase in right hind paw, in comparison to the uninjected left hind paw. Percentage change in paw volume was calculated and expressed as the amount of inflammation. Compounds **1c**, **2a**, **b**, **3b**, **5a**, and **6a** significantly induced strong anti-inflammatory activity that was slightly superior to that exhibited by mefenamic acid.



Reagents and conditions: **a**) isopropanol, K_2CO_3, reflux 4 h; **b**) H_2NCSNH_2, abs. C_2H_5OH, reflux; **c**) acetic acid, reflux, 5 h; **d**) abs. C_2H_5OH, CH_3ONa reflux, 0.5 h.

Chart 2

Table 1. Analgesic, Anti-inflammatory and Ulcerogenic Potential of the Tested Compounds

Compound	Analgesic activity (% protection)	Anti-inflammatory activity (% inhibition)	Ulcerogenic potential (Severity index)
Vehicle	0	0	0
Mefenamic acid	70.3±4.5*	54.5±3.45*	1.1±0.03*
1a	46.2±3.3*,#	18.3±1.5*,#	0.4±0.02*,#
1b	53.2±4.3*,#	24.2±1.8*,#	0.5±0.03*,#
1c	74.2±5.3*	64.5±4.9*	0.9±0.04*
1d	0	—	—
1e	0	—	—
2a	72.1±6.5*	61.3±5.6*	0.9±0.03*
2b	73.4±7.2*	56.2±5.7*,#	1.0±0.08*
3a	0	—	—
3b	59.4±5.2*	55.4±4.3*	0.7±0.02*,#
4a	0	—	—
4b	10.2±1.2*,#	0	0.1±0.01#
5a	78.2±6.8*	56.4±4.3*	0.7±0.02*,#
5b	47.2±4.2*,#	23.3±2.1*,#	0.2±0.01*,#
6a	72.4±4.8*	57.4±4.7*,#	1.3±0.09*
6b	56.4±5.3*,#	43.2±4.1*	0.8±0.06*

The vehicle group was pretreated with 1% carboxymethylcellulose solution. All compounds were tested in a dose equals 25 mg/kg (*p.o.*) 30 min before testing. Data are presented as mean±S.E.M. and analyzed using one-way ANOVA followed by Tukey's test for multiple comparisons. *$p<0.05$ compared to the vehicle group and #$p<0.05$ compared to mefenamic acid group.

Table 2. Serum Level of TNF-α, IL-6 and COX-2 in Mice Pretreated with the Mefenamic Acid or the Tested Compounds in Carrageenan-Induced Paw Edema Test

Compound	TNF-α (ng/mL)	IL-6 (ng/mL)	COX-2 (ng/mL)
Vehicle	5.61±0.42	22.1±1.8	43.2±3.5
Mefenamic acid	3.23±0.27*	12.6±0.91	39.2±2.8
1a	4.23±0.39*	18.3±0.15#	45.2±3.5
1b	4.12±0.37*	19.3±1.4#	42.6±3.9
1c	3.25±0.21*	9.2±0.61*	38.5±3.1
1d	—	—	—
1e	—	—	—
2a	2.45±0.18*	14.3±0.56*	35.3±2.9*
2b	2.67±0.15*	10.1±0.82*	41.1±4.3
3a	—	—	—
3b	3.56±0.27*	16.4±0.43*	41.3±3.7
4a	—	—	—
4b	—	—	—
5a	2.69±0.16*	15.4±0.43*	35.1±2.8*
5b	4.62±0.42	20.3±0.21#	42.3±2.6
6a	2.94±0.18*	9.3±0.61*	38.3±2.8
6b	3.54±0.23*	17.2±0.22#	42.5±3.2

All compounds were tested in a dose equals 25 mg/kg (*p.o.*) 30 min before testing. Data are represented as mean±S.E.M. and analyzed using one-way ANOVA followed by Tukey's test for multiple comparisons. *$p<0.05$ compared to the vehicle group and #$p<0.05$ compared to mefenamic acid group.

These results were in agreement with the results shown in the analgesic activity test (Table 1).

Additionally, mefenamic acid as well as the tested compounds **1c**, **2a**, **b**, **3b**, **5a**, and **6a** have shown suppression of serum IL-6 as well as TNF-α levels compared to the control group. However, only compounds **2a** and **5a** significantly reduced serum COX-2 level compared to the vehicle group. In spite of the potent anti-inflammatory activity observed by compounds **1c**, **2b** and **5b**, they did not suppress serum COX-2 level. This may be attributed to inhibition of enzyme activity with no influence on enzyme expression (Table 2).

Acute Ulcerogenicity Studies In current study, testing acute ulcerogenic potential revealed that mefenamic acid showed an ulcer severity index of 1.1±0.03. All tested compounds except **5c** showed lower mean severity index than that observed by mefenamic acid, indicating lower ulcerogenic effect (Table 1).

Histopathological examination revealed that mefenamic

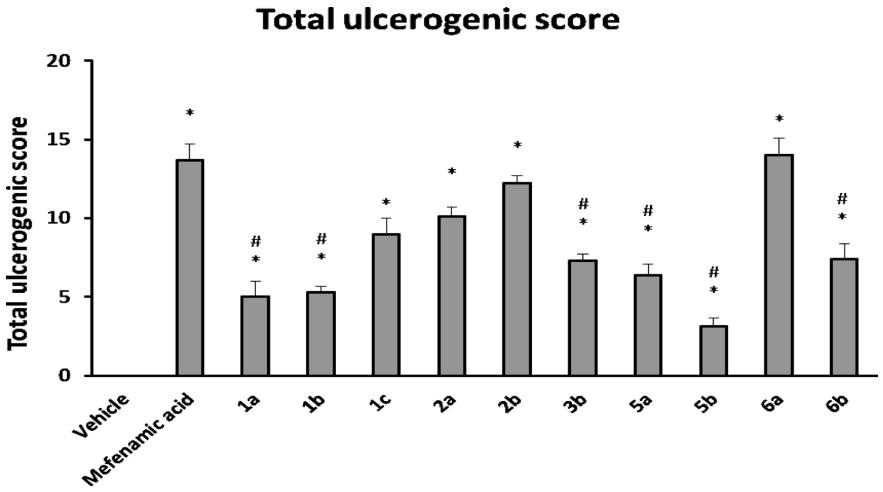


Fig. 3. Total Ulcerogenic Score of the Tested Compounds

*$p<0.05$ compared to the vehicle group. #$p<0.05$ compared to mefenamic acid group.

acid-treated mice showed a marked change in the architecture of the mucosal cells, and erosion of the surface epithelia extending, in some cases to the muscularis mucosa. Compound **6a** showed non-significant increase in the mean total ulcerogenic score compared to mefenamic acid. In addition, the ulcerogenic effect of the compounds **1c**, **6b**, **2a**, and **2b** was comparable to that of the reference drug. On the other hand, the tested compounds **1a**, **b**, **3b**, and **5a** produced a lesser degree of erosion and inflammatory cells infiltration, whereas compound **5b** showed fairly normal mucosal structure (Figs. 3, 4).

Conclusion

Various pyridazine derivatives and nicotinic acid derivatives structurally related to niflumic acid were synthesized and screened for their potential analgesic, anti-inflammatory and ulcerogenic potentials. The results of the analgesic and anti-inflammatory activities of the synthesized compounds showed moderate enhancement in activity with the compounds **1a**, **b**, **5b**, and **6b**. In acetic-acid-induced abdominal constriction test, compounds **1c**, **2a**, **b**, **5a**, and **6a** displayed the most potent analgesic activity. Moreover, these compounds in addition to compound **3b** exhibited the most prominent and consistent anti-inflammatory activity which was superior to mefenamic acid. In contrast, compounds **1d**, **e**, **3a**, **4a**, and **b** were almost devoid of activity. It has been also observed that compounds **1c**, **2a**, **b**, **3b**, **5a**, and **6a** significantly reduced the level of TNF-α and IL-6 in the serum. However, only the compounds, **2a** and **5a** markedly reduced the level of COX-2 as well.

The compounds that displayed significant analgesic and anti-inflammatory activities were also screened for ulcerogenic adverse effect at 50 mg/kg dose level. After microscopic elimination, it was obvious that all compounds which showed higher potency than mefenamic acid (except **6a**) in both analgesic and anti-inflammatory screens, showed lower ulcerogenic potential than the reference drug. No significant ulceration risk was observed in the compound **5b**.

In light of these results, one can say that the most promising results as the anti-inflammatory and analgesic agents was observed with the nicotinic acid derivatives **1c**, **2a**, and **b** as well as the pyridazinone derivative **5a**.

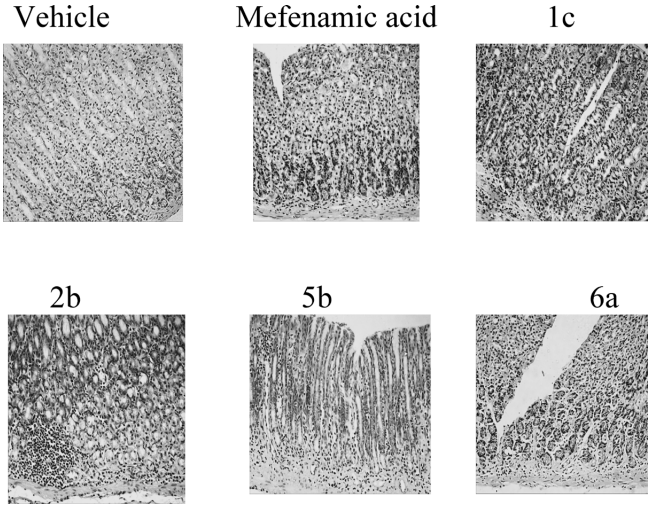


Fig. 4. Histopathological Examination of the Gastric Mucosa of Some of the Tested Compounds

Experimental

Chemistry All chemicals and reagents were obtained from Aldrich (Sigma-Aldrich, St. Louis, MO, U.S.A.), and were used without further purification. Reactions were monitored by TLC, performed on silica gel glass plates containing 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 tetramethylsilane (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 75 MHz using TMS as 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. Progress of the reactions was

monitored using TLC sheets precoated with UV fluorescent silica gel Merck 60F 254 using acetone–benzene (1 : 9) and were visualized using UV lamp.

The starting material 2-chloronicotinic acid and other chemicals were obtained from Aldrich, Fluka, or Merck Chemicals.

General Procedure for 1a–e, 2a, b A mixture of 2-chloronicotinic acid (0.157 g, 0.01 mol), anhydrous K_2CO_3 (2.76 g, 0.02 mol) and an appropriate substituted amino compound (0.01 mol) in isopropanol (30 mL) was heated under reflux for 4 h. The reaction mixture was concentrated under reduced pressure to half its volume then cooled. The separated solid was filtered, dried and crystallized from isopropanol.

2-[(2-Bromophenyl)amino]nicotinic Acid (**1a**): Yield 75%; mp 187–188°C; IR (KBr) cm^{-1}: 3200–2200 (O–H and NH), 3097 (C–H aromatic), 1720 (C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 5.54 (s, 1H, NH, D_2O exchangeable), 7.51–7.55 (m, 5H, 4H arom. and H_5, pyr.), 8.23 (dd, 1H, J=6.0, 1.8 Hz, H_4, pyr.), 8.55 (dd, 1H, J= 4.8, 1.8 Hz, H_6, pyr.), 13.75 (br, 1H, OH, D_2O exchangeable); MS (electron ionization (EI)) m/z (% rel. int.): 294 (M+2, 0.20), 293 (M+H), 171 (9.30), 157 (100), 122 (5.78), 78 (23.63). *Anal.* Calcd for $C_{12}H_9BrN_2O_2$ (293.12): C, 49.17; H, 3.09; N, 9.56; Found: C, 49.34 H, 3.25; N, 9.23.

2-[(2,4-Dichlorophenyl)amino]nicotinic Acid (**1b**): Yield 70%; mp 51–52°C; IR (KBr) cm^{-1}: 3417–2580 (O–H and NH), 3190 (C–H aromatic), 1700 (C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 5.52 (s, 1H, NH, D_2O exchangeable), 6.78 (d, 1H, Ar-H), 7.08 (d, 1H, Ar-H), 7.26 (s, 1H, Ar-H), 7.51–7.55 (m, 1H, H_5, pyr.), 8.22 (d, 1H, H_4, pyr.), 8.55 (d, 1H, H_6, pyr.); MS (EI) m/z (% rel. int.): 286 (M+4, 58.74), 282 ($M^{+\bullet}$, 3.72). *Anal.* Calcd for $C_{12}H_8Cl_2N_2O_2$ (283.11): C, 50.91; H, 2.85; N, 9.89; Found: C, 50.86; H, 2.98; N, 10.08.

2-[(2-Chloropyridin-3-yl)amino]nicotinic Acid (**1c**): Yield 65%; mp 174–175°C; IR (KBr) cm^{-1}: 3452–2360 (O–H and NH), 1720 (C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 5.55 (s, 1H, NH, D_2O exchangeable), 7.10 (d, 1H, H_4, pyr.), 7.51–7.57 (m, 2H, Ar-H), 8.22 (d, 1H, H_4, pyr.), 8.53–8.56 (m, 2H, H_6, pyr.), 13.79 (br, 1H, OH, D_2O exchangeable); MS (EI) m/z (% rel. int.): 249 (M, 0.19), 251 (M+2, 0.18). *Anal.* Calcd for $C_{11}H_8ClN_3O_2$ (249.65): C, 52.92; H, 3.23; N, 16.83; Found: C, 52.68; H, 3.42; N, 16.98.

2-[(4,5-Dihydrothiazol-2-yl)amino]nicotinic Acid (**1d**): Yield 50%; mp 110–111°C; IR (KBr) cm^{-1}: 3400–2353 (O–H and NH), 1643 (C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 3.45 (t, 2H, J=7.5 Hz, CH_2), 3.86 (t, 2H, J=7.5 Hz, CH_2), 5.55 (s, 1H, NH, D_2O exchangeable), 7.33–7.37 (m, 1H, H_5, pyr.), 7.89 (d, 1H, H_4, pyr.), 8.31 (d, 1H, H_6, pyr.); MS (EI) m/z (% rel. int.): 223 ($M^{+\bullet}$, 3.86), 224 (M+H, 7.26). *Anal.* Calcd for $C_9H_9N_3O_2S$ (223.25): C, 48.42; H, 4.06; N, 18.82; Found: C, 48.16; H, 4.25; N, 18.79.

2-[(1*H*-Benzo[*d*]imidazol-2-yl)amino]nicotinic Acid (**1e**): Yield 65%; mp 189–190°C; IR (KBr) cm^{-1}: 3290–2588 (O–H and NH), 1681 (C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 7.07–7.10 (m, 2H, Ar-H), 7.25–7.28 (m, 2H, Ar-H), 7.39–7.43 (m, 1H, H_5, pyr), 8.24 (s, 2H, 2NH, D_2O exchangeable), 8.01 (dd, 1H, J=6.0, 1.8 Hz, H_4, pyr.), 8.37 (dd, 1H, J= 4.8, 1.8 Hz, H_6, pyr.); MS (EI) m/z (% rel. int.): 254 ($M^{+\bullet}$, 0.92). *Anal.* Calcd for $C_{13}H_{10}N_4O_2$ (254.24): C, 61.41; H, 3.96; N, 22.04; Found: C, 61.25; H, 3.77; N, 21.96.

2-(2-Carbamoylphenoxy)nicotinic Acid (**2a**): Yield 85%; mp 164–165°C; IR (KBr) cm^{-1}: 3398–2360 (O–H and NH_2), 1716, 1681 (2C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.82–6.88 (m, 1H, Ar-H), 7.36–7.50 (m, 1H, Ar-H), 7.52–7.55 (m, 1H, Ar-H), 7.82–7.90 (m, 1H, H_5, pyr.), 8.22 (dd, 1H, J= 5.4, 1.8 Hz, H_4, pyr.), 8.40 (s, 2H, NH_2, D_2O exchangeable), 8.54 (dd, 1H, J=4.5, 1.8 Hz, H_6, pyr.), 13.79 (br, 1H, O–H, D_2O exchangeable); MS (EI) m/z (% rel. int.): 258 ($M^{+\bullet}$, 0.17). *Anal.* Calcd for $C_{13}H_{10}N_2O_4$ (258.23): C, 60.47; H, 3.90; N, 10.85; Found: C, 60.33; H, 4.05; N, 10.78.

2-(2-Carbamoylphenylamino)nicotinic Acid (**2b**): Yield 80%; mp 150–151°C; IR (KBr) cm^{-1}: 3429–2400 (O–H and NH_2), 1716, 1680 (2C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 7.51–7.55 (m, 3H, 2Ar-H, H_5, pyr.), 8.13–8.28 (m, 2H, Ar-H and H_4, pyr.), 8.53–8.55 (m, 2H, Ar-H, H_6, pyr.), 13.70 (br, 1H, O–H, D_2O exchangeable); MS (EI) m/z (% rel. int.): 256 (M−H, 67.86), 213 (15.48). *Anal.* Calcd for $C_{13}H_{11}N_3O_3$ (257.24): C, 60.70; H, 4.31; N, 16.33; Found: C, 60.53; H, 4.25; N, 16.57.

General Procedure for 3a, b A mixture of 3,6-dichloropyridazine (1.49 g, 0.01 mol), anhydrous K_2CO_3 (2.76 g, 0.02 mol) and the appropriate substituted amino benzoic acid (1.37g, 0.01 mol) in isopropanol (30 mL) was heated under reflux for 4 h. The reaction mixture was concentrated under reduced pressure to half its volume then cooled. The separated solid was filtered, dried and crystallized from isopropanol.

3-[(6-Chloropyridazin-3-yl)amino]benzoic Acid (**3a**): Yield 80%; mp 189–190°C; IR (KBr) cm^{-1}: 3298–2553 (O–H and NH), 1685 (C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.43 (d, 1H, Ar-H), 6.77–6.81 (m, 2H, Ar-H), 7.06 (d, 1H, Ar-H), 7.96 (d, 1H, pyridazine H-4), 8.37 (d, 1H, pyridazine H-5), 9.80 (s, 1H, NH, D_2O exchangeable); MS (EI) m/z (% rel. int.): 251 (M+2, 10.51), 249 ($M^{+\bullet}$, 32.00), 248 (M−H, 100). *Anal.* Calcd for $C_{11}H_8ClN_3O_2$ (249.65): C, 52.92; H, 3.23; N, 16.83; Found: C, 52.75; H, 3.45; N, 16.66.

4-[(6-Chloropyridazin-3-yl)amino]benzoic Acid (**3b**): Yield 70%; mp 244–245°C; IR (KBr) cm^{-1}: 3294–2549 (O–H and NH), 1666 (C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 7.27 (d, 2H, Ar-H), 7.64 (d, 2H, Ar-H), 7.81 (d, 1H, pyridazine H-4), 7.92 (d, 1H, pyridazine H-5), 9.91 (s, 1H, NH, D_2O exchangeable); MS (EI) m/z (% rel. int.): 251 (M+2, 1.11), 249 ($M^{+\bullet}$, 0.92), 248 (M–H, 0.92). *Anal.* Calcd for $C_{11}H_8ClN_3O_2$ (249.65): C, 52.92; H, 3.23; N, 16.83; Found: C, 52.65; H, 3.18; N, 16.78.

General Procedure for 4a, b A mixture of an appropriate **3a**, **b** (0.5 g, 0.002 mol) and thiourea (0.23 g, 0.003 mol) in absolute ethanol (20 mL) was heated under reflux for 3 h. The reaction mixture was allowed to cool to room temperature and the excess solvent was removed under diminished pressure. The crude isothiouronium salt was combined with sodium hydroxide solution (10%, 20 mL) and the mixture was heated under reflux for 2h. After cooling, the mixture was acidified with glacial acetic acid, the precipitated orange solid product was filtered, washed with water and crystallized from ethanol.

3-[(6-Sulphanylpyridazin-3-yl)amino]benzoic Acid (**4a**): Yield 50%; mp 149–150°C; IR (KBr) cm^{-1}: 3444–2650 (O–H and NH), 1681 (C=O), ; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 7.05–7.91 (m, 4H, Ar-H), 7.99 (d, 1H, pyridazine H-4), 8.10 (d, 1H, pyridazine H-5), 8.98 (s, 1H, NH or SH, D_2O exchangeable), 9.55 (s, 1H, NH or SH, D_2O exchangeable); MS (EI) m/z (% rel. int.): 247 ($M^{+\bullet}$, 20.97), 246 (M−H, 20.14). *Anal.* Calcd for $C_{11}H_9N_3O_2S$ (247.27): C, 53.43; H, 3.67; N,

16.99; Found: C, 53.15; H, 3.50; N, 17,35.

4-[(6-Sulphanylpyridazin-3-yl)amino]benzoic Acid (**4b**): Yield 55%; mp 269–270°C; IR (KBr) cm^{-1}: 3500–2538 (O–H and NH), 1701 (C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 7.07 (d, 2H, J=9.3 Hz, Ar-H), 7.44 (d, 2H, J=9.3 Hz, Ar-H), 7.60 (d, 1H, J=9 Hz, pyridazine H-4), 7.87 (d, 1H, J=9 Hz, pyridazine H-5), 9.45 (s, 1H, NH or SH, D_2O exchangeable), 9.78 (s, 1H, NH or SH, D_2O exchangeable); MS (EI) *m/z* (% rel. int.): 247 ($M^{+\bullet}$, 77.89), 246 (M−H, 100). *Anal.* Calcd for $C_{11}H_9N_3O_2S$ (247.27): C, 53.43; H, 3.67; N, 16.99; Found: C, 53.58; H, 3.43; N, 17.05.

General Procedure for 5a, b An appropriate **3a**, **b** (0.25 g, 0.001 mol) in glacial acetic acid (25 mL) was heated under reflux for 5 h. The reaction mixture was concentrated to half its volume under reduced pressure then cooled. The precipitated crystalline solid was filtered, washed with water, and recrystallized from ethanol to give **5a**, **b**.

3-[(6-Oxo-1,6-dihydropyridazin-3-yl)amino]benzoic Acid (**5a**): Yield 60%; mp 273–274°C; IR (KBr) cm^{-1}: 3336–2507 (O–H and NH), 1693, 1674 (2C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.86 (d, 1H, J=9.3 Hz, pyridazine-H4), 7.20 (d, 1H, Ar-H), 7.34–7.40 (m, 1H, Ar-H), 7.45 (d, 1H, Ar-H), 7.74 (d, 1H, J=9.3 Hz, pyridazine-H5), 8.15 (s, 1H, Ar-H), 9.13 (s, 1H, NH, D_2O exchangeable), 12.10 (s, 1H, OH, D_2O exchangeable); MS (EI) *m/z* (% rel. int.): 231 ($M^{+\bullet}$, 89.85); 230 (100). *Anal.* Calcd for $C_{11}H_9N_3O_3$ (231.21): C, 57.14; H, 3.92; N, 18.17; Found: C, 57.23; H, 3.88; N, 18.10.

4-[(6-Oxo-1,6-dihydropyridazin-3-yl)amino]benzoic Acid (**5b**): Yield 55%; mp 329–330°C; IR (KBr) cm^{-1}: 3414–2500 (O–H and NH), 1685, 1670 (2C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.88 (d, 1H, pyridazine-H4), 7.25 (d, 1H, pyridazine-H5), 7.77 (d, 2H, Ar-H), 7.85 (d, 2H, Ar-H), 9.33 (s, 1H, NH, D_2O exchangeable), 12.14 (brs, 1H, OH, D_2O exchangeable); MS (EI) *m/z* (% rel. int.): 231 ($M^{+\bullet}$, 11.74). *Anal.* Calcd for $C_{11}H_9N_3O_3$ (231.21): C, 57.14; H, 3.92; N, 18.17; Found: C, 57.05; H, 4.15; N, 18.35.

General Procedure for 6a, b An appropriate **3a**, **b** (0.25 g, 0.001 mol) in absolute ethanol (25 mL) containing metallic sodium (0.46 g, 0.02 mol) was heated under reflux for 0.5 h. The reaction mixture was concentrated under reduced pressure, poured into ice cold water then acidified by dilute hydrochloric acid. The precipitated crystalline solid was filtered, washed with water, and recrystallized from ethanol.

3-[(6-Ethoxypyridazin-3-yl)amino]benzoic Acid (**6a**): Yield 50%; mp 214–215°C; IR (KBr) cm^{-1}: 3417–2546 (O–H and NH), 1666 (C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 1.05 (t, 3H, J= 6.9 Hz, CH_3), 3.42 (q, 2H, J=6.9 Hz, CH_2), 7.23 (t, 1H, Ar-H), 7.35 (d, 1H, pyridazine H-4), 7.52–7.56 (m, 2H, Ar-H), 7.85 (d, 1H, pyridazine H-5), 8.17 (s, 1H, Ar-H), 9.90 (s, 1H, NH, D_2O exchangeable); MS (EI) *m/z* (% rel. int.): 259 ($M^{+\bullet}$, 0.35). *Anal.* Calcd for $C_{13}H_{13}N_3O_3$ (259.26): C, 60.22; H, 5.05; N, 16.21; Found: C, 60.45; H, 5.32; N, 16.05.

4-[(6-Ethoxypyridazin-3-yl)amino]benzoic Acid (**6b**): Yield 45%; mp 255–256°C; IR (KBr) cm^{-1}: 3417–2546 (O–H and NH), 1666 (C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 1.15 (t, 3H, CH_3), 3.55 (q, 2H, CH_2), 7.29 (d, 1H, pyridazine H-4), 7.81 (d, 1H, pyridazine H-5), 7.84 (d, 2H, Ar-H), 7.92 (d, 2H, Ar-H), 9.93 (s, 1H, NH, D_2O exchangeable); MS (EI) *m/z* (% rel. int.): 259 ($M^{+\bullet}$, 74.39), 258 (100). *Anal.* Calcd for $C_{13}H_{13}N_3O_3$ (259.26): C, 60.22; H, 5.05; N, 16.21; Found: C, 60.38; H, 5.11; N, 16.48.

Pharmacological Activity. Animals The investigations were carried out using healthy male Swiss albino mice with a weight of 20–25 g. Mice were supplied by the Modern Veterinary Office for Laboratory Animals (Cairo, Egypt). All animals were naive to drug treatment and experimentation at the beginning of the study. Mice were allowed to acclimatize for 1–2 weeks prior to use with standard pellet diet and water *ad libitum*. The animals were randomly assigned to experimental groups, 6 mice each. All experiments were conducted between 10:00 a.m. and 16:00 p.m. to eliminate circadian influence on animal behaviour. All experimental protocols were approved by the Institutional Animal Care and Use Committee at the Faculty of Pharmacy, Suez Canal University.

Preparation of the Tested Compounds and the Standard Drug Test compounds were given orally to test animals after suspending in a 1% sodium carboxymethylcellulose (CMC) aqueous solution. The control group animals received the same experimental handling as those of the test groups except that the drug treatment was replaced with appropriate volumes of the dosing vehicle. Mefenamic acid (25 mg/kg) was kindly provided by Al-Qahira Pharmaceutical Company (Cairo, Egypt). It was prepared in 1% CMC and used as a reference drug.

Analgesic Activity Analgesic activity was carried out by acetic acid induced writhing method.[21] A 1% aqueous acetic acid solution (i.p. injection; 0.1 mL) was used to induce writhing. Mice were kept individually in the test cage, before acetic acid injection and habituated for 30 min. Screening of analgesic activity was performed after *p.o.* administration of test drugs at the dose of 25 mg/kg. All compounds were dissolved in 1% CMC solution. One group was kept for the control experiment and received *p.o.* administration of 1% CMC. Mefenamic acid was used as a reference drug. After 30 min of drug administration, 0.1 mL of 1% acetic acid solution was given to each mouse intraperitoneally.

Stretching movements consisting of arching of the back, elongation of body and extension of hind limbs were counted for 5–15 min of acetic acid injection. The analgesic activity was expressed as follows: % analgesic activity=$\{(n-n')/n\}\times100$ where n=mean number of writhes of control group and n'=mean number of writhes of test group.

Anti-inflammatory Activity Anti-inflammatory activity test was performed following the method of Winter *et al.*[22] Carrageenan (Sigma, St. Louis, MO, U.S.A.) was freshly prepared as suspension (0.05 mL, 1% w/v solution in 0.9% saline). Carrageenan solution was injected into the subplantar tissue of the right hind paw of each mouse. Control animals were injected with saline into that of the left hind paw.

Animals were divided in groups of six in each group. One group was kept as control and the animals of other groups were pre-treated with the test drugs suspended in 1% CMC given orally 30 min before carrageenan injection. The paw volume was measured before and after 4 h of carrageenan injection. The difference in thickness between the right and left hind paw was measured with a pair of dial thickness gauge callipers (Ozaki Co., Tokyo, Japan). Mean values of treated groups were compared with those of control group and analysed using statistical methods. The percentage inhibition of inflammation was calculated by applying the following formula:

$$\text{anti-inflammatory activity (\% inhibition)} = (V_c - V_t) / V_c \times 100$$

where V_c=oedema volume in control group, V_t=oedema volume in groups treated with the test compounds.

Furthermore, the mice were anesthetized with a mixture of ketamine (50 mg/kg, i.p.)–xylazine (10 mg/kg, i.p.) and then sacrificed by decapitation. Thereafter, a midline incision was made, and blood samples were withdrawn from the heart. Thirty min after collection, blood samples were processed by centrifugation at 2000×***g*** for 15 min. Then, serum samples were separated, collected in clean tubes and stored at −80°C until used for ELISA assays. Enzyme linked immunosorbent assay (ELISA) kits for interlukin-6 (IL-6) and cyclooxygenase 2 (COX-2) (Glory Science Co., Ltd., Del Rio, TX, U.S.A.) were used for determination of tissue levels of these markers. The assays were carried out following the instructions of the manufacturer using an automated ELISA reader (Europe S.A., Belgium).

Acute Ulcerogenesis Acute ulcerogenesis test was determined according to Cioli *et al.*[23] Ulcerogenic activity was evaluated after *p.o.* administration of the test compound or mefenamic acid at the dose of 50 mg/kg. Control mice were treated orally with the vehicle (suspension of 1% CMC). Food but not water was removed 24 h before administration of the test compounds. After the drug treatment, mice were fed normal diet for 17 h. Mice were anesthetized with a mixture of ketamine (50 mg/kg, i.p.)–xylazine (10 mg/kg, i.p.) and then sacrificed by decapitation. The stomach was removed and opened along the greater curvature, washed with distilled water and cleaned gently by dipping in saline. The gastric mucosa of the mice was examined by means of a magnifying glass. For each stomach, the severity of mucosal damage was assessed by measuring severity index *i.e.* severity of drug to cause mucosal damage. It is measured according to the following scoring system: 0.5, redness; 1.0, spot ulcers; 1.5, haemorrhagic streaks; 2.0, ulcers >3, but ≤5; 3.0, ulcers >5. The mean score of each treated group minus the mean score of the control group was considered as severity index of gastric mucosal damage.

Statistical Analysis Data was collected, tabulated and expressed as mean±S.E.M. Statistical differences between the treatments and the control were evaluated using one-way ANOVA followed by Tukey's test for multiple comparisons. All statistical analyses were carried out using The Statistical Package for Social Sciences, version 17 (SPSS Inc., Chicago, IL, U.S.A.). A $p<0.05$ was considered to be statistically significant.

Acknowledgements Authors wish to acknowledge Al-Qahira Pharmaceutical Company for the generous gift of mefenamic acid.

References

1) Meade E. A., Smith W. L., DeWitt D. L., *J. Biol. Chem.*, **268**, 6610–6614 (1993).
2) Abbott J. D., Moreland L. W., *Expert Opin. Investig. Drugs*, **13**, 1007–1018 (2004).
3) Bombardieri S., Cattani P., Ciabattoni G., Di Munno O., Pasero G., Patrono C., Pinca E., Pugliese F., *Br. J. Pharmacol.*, **73**, 893–901 (1981).
4) Davies P., Bailey P. J., Goldenberg M. M., Ford-Hutchinson A. W., *Annu. Rev. Immunol.*, **2**, 335–357 (1984).
5) Lanas A., García-Rodríguez L. A., Arroyo M. T., Gomollón F., Feu F., González-Pérez A., Zapata E., Bástida G., Rodrigo L., Santolaria S., Güell M., de Argila C. M., Quintero E., Borda F., Piqué J. M., Asociación Española de Gastroenterología, *Gut*, **55**, 1731–1738 (2006).
6) Feldmann M., Brennan F. M., Maini R. N., *Annu. Rev. Immunol.*, **14**, 397–440 (1996).
7) Maini R. N., Taylor P. C., *Annu. Rev. Med.*, **51**, 207–229 (2000).
8) Koch A. E., *Arthritis Rheum.*, **41**, 951–962 (1998).
9) Masferrer J. L., Zweifel B. S., Manning P. T., Hauser S. D., Leahy K. M., Smith W. G., Isakson P. C., Seibert K., *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 3228–3232 (1994).
10) Seibert K., Zhang Y., Leahy K., Hauser S., Masferrer J., Perkins W., Lee L., Isakson P. C., *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 12013–12017 (1994).
11) Dogne J. M., Supuran C. T., Pratico D., *J. Med. Chem.*, **48**, 2251–2257 (2005).
12) Hsiao F.-Y., Tsai Y.-W., Huang W.-F., *Clin. Ther.*, **31**, 2618–2627 (2009).
13) Gökçe M., Utku S., Küpeli E., *Eur. J. Med. Chem.*, **44**, 3760–3764 (2009).
14) Abouzid K., Bekhit S. A., *Bioorg. Med. Chem.*, **16**, 5547–5556 (2008).
15) Moradi A., Navidpour L., Amini M., Sadeghian H., Shadnia H., Firouzi O., Miri R., Ebrahimi S. E. S., Abdollahi M., Zahmatkesh M. H., Shafiee A., *Arch. Pharm. Chem. Life Sci.*, **343**, 509–518 (2010).
16) Cocco M. T., Congiu C., Onnis V., Morelli M., Felipo V., Cauli O., *Bioorg. Med. Chem.*, **12**, 4169–4177 (2004).
17) Chintakunta V. K., Akella V., Vedula M. S., Mamnoor P. K., Mishra P., Casturi S. R., Vangoori A., Rajagopalan R., *Eur. J. Med. Chem.*, **37**, 339–347 (2002).
18) Süküroglu M., Caliskan Ergün B., Unlü S., Sahin M. F., Küpeli E., Yesilada E., Banoglu E., *Arch. Pharm. Res.*, **28**, 509–517 (2005).
19) Abouzid K. A. M., Khalil N. A., Ahmed E. M., Esmat A. A., Al-Abd A. M., *Med. Chem. Res.*, **21**, 3581–3590 (2012).
20) Badran M. M., Abouzid K. A., Hussein M. H., *Arch. Pharm. Res.*, **26**, 107–113 (2003).
21) Seigmund E., Cadmus R., Lu G., *Proc. Soc. Exp. Biol. Med.*, **95**, 729–731 (1957).
22) Winter C. A., Risley E. A., Nuss G. W., *Proc. Soc. Exp. Biol. Med.*, **111**, 544–547 (1962).
23) Cioli V., Putzolu S., Rossi V., Scorza Barcellona P., Corradino C., *Toxicol. Appl. Pharmacol.*, **50**, 283–289 (1979).