Journal of Enzyme Inhibition and Medicinal Chemistry, August 2008; 23(4): 454–461

informa
healthcare

Synthesis and antinociceptive-antimicrobial activities of some new amide derivatives of 3,5-di/-and 1,3,5-trimethylpyrazoles

BEDIA KOCYIGIT-KAYMAKCIOGLU[1], HALE Z. TOKLU[2], SERKAN İKIZ[3], A. FUNDA BAGCIGIL[3], SEVIM ROLLAS[1], N. YAKUT OZGUR[3], & SEYYAL AK[3]

[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Marmara University, Haydarpasa, 34668 Istanbul, Turkey,* [2]*Department of Pharmacology, Faculty of Pharmacy, Marmara University, Haydarpasa, 34668 Istanbul, Turkey, and* [3]*Department of Microbiology, Veterinary Faculty, Istanbul University, Avcilar, 34320 Istanbul, Turkey*

(Received 11 June 2007; accepted 7 August 2007)

Abstract

Some N-(3,5-di-/1,3,5-trimethylpyrazole-4-yl)-4-substitutedbenzamide derivatives were prepared as possible antiociceptive-antimicrobial agents. New amide derivatives (**3**–**12**) were synthesized by reacting 4-amino-3,5-di and 1,3,5-trimethyl-pyrazoles with 4-substitutedbenzoyl chlorides. Hotplate and tail-immersion tests were used for the determination of the antinociceptive activity. Morphine, was used as a standard test drug. All compounds were administered at a dose of 100 mg/kg ip and some of them had significant antinociceptive activity in both tests. Compound **10** (*N-(1,3,5-trimethylpyrazole-4-yl)-4-bromobenzamide)*, was the most active one in both tests among the compounds. The antinociceptive activity of the compounds **10**, **11** (*N-(1,3,5-trimethylpyrazole-4-yl)-4-chlorobenzamide)*, and **12** (*N-(1,3,5-trimethylpyrazole-4-yl)-4-fluorobenzamide)*, started at 30 minutes and continued up to 150 minutes in the hotplate test. Also compounds were tested for their in vitro antimicrobial activity, but exhibited weak antibacterial activity.

Keywords: *3,5-di/1,3,5-timethylpyrazoles, amides, antinociceptive activity, antimicrobial activity*

Introduction

Nonsteroidal antiinflammatory and analgesic drugs are important therapeutic agents for treatment of pain and inflammation. However, prolonged use of these drugs causes gastrointestinal ulcers and hence, there is a need to develop new analgesic and antiinflammatory agents with better gastrointestinal safety profile. As the extension of our interest for the search of new compounds as potent analgesic agents and at the same time which are devoid of side effects like ulcerogenic activity, we have synthesized some pyrazole derivatives.

Among the already marketed nonsteroidal analgesic-antiinflammatory drugs that comprise pyrazole nucleus, celecoxib (Celebrex™), occupies a unique position as a potent and safe anti-inflammatory and analgesic agent [1]. Also deracoxib (Deramaxx™) is already used for the treatment of pain and inflammation in dogs [2,3]. Furthermore, there are various reports which have demonstrated the analgesic-antiinflammatory effects of pyrazole derivatives [4–13].

Besides, pyrazole nucleus comprises an important class in the heterocylic chemistry and it has pronounced pharmacological actions as anxiolytic [14] and anticonvulsant [15–18] activities. On the other hand much attention has been focused towards pyrazoles as antimicrobial [19–21], antiviral [22] and anticancer [23] agents of the discovery of the natural pyrazole C-glycoside, pyrazofurin; 4-hydroxy-3-β-D-ribofuranosyl-1H-pyrazole-5-carboxamide. This antibiotic was reported to possess a broad spectrum of antimicrobial and antiviral activities in addition to being active against several tumor cell lines [23]. Furthermore, 1H-pyrazole-4-carboxylates and the corresponding amide derivatives were exhibited good antimicrobial activity [24]. Traditionally small

Correspondence: B. Kocyigit Kaymakcioglu, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Marmara University, Haydarpasa, 34668 Istanbul, Turkey. Tel: 90 216 4142962. Fax: 90 216 3452952. E-mail: bkaymakcioglu@marmara.edu.tr

ISSN 1475-6366 print/ISSN 1475-6374 online
DOI: 10.1080/14756360701631686

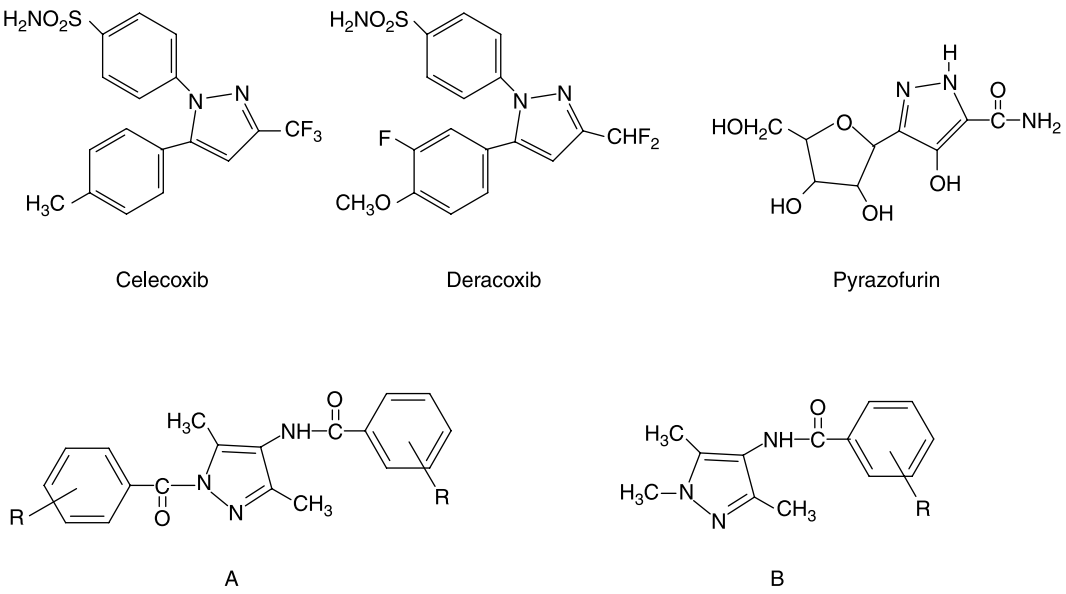


Figure 1. Structures of celecoxib, deracoxib, pyrazofurin and novel pyrazole derivatives A and B.

molecules have been a reliable source for discovering novel biologically active compounds. By considering these active substituted pyrazoles with potential analgesic-antiinflammatory and antimicrobial effects, a new series of similar type of substituted amides (**A** and **B**) obtained from 4-aminopyrazoles were synthesized and their antinociceptive and antimicrobial properties were evaluated (Figure 1).

Experimental

Chemistry

All chemicals were purchased from Aldrich and Merck. Melting points were determined by Buchi 530 melting point apparatus (Flawil, Switzerland). UV spectra were recorded on a Beckman DU 530 spectrophotometer (California, United States). The purity of the compounds was checked by high performance liquid chromatography (HPLC). Chromatographic separation was performed using a Novapak C_{18} (Phenomenex) (150 mm × 4.6 mm i.d., 5 μm particle size) by using a mobile phase consisting of acetonitrile-water (50:50, v/v). The chromatographic system used to develop this technique was a Hewlett Packard 1100 featuring a column oven at 22°C (G1316 A), a quaternary pump (G 1311 A), a manual injector (G 1328 B) and DAD detector (G 13115 B) which was set at 254 nm. Data acquisition was performed using a chromatography software package (Agilent Chemstation version 9.01(1206). Infrared spectra were recorded on Perkin Elmer 1600 FT-IR spectrophotometer (Beaconsfield-England). ^{1}H-NMR spectra (DMSO-d_6) were run on Bruker AVANC -DPX 400 MHz NMR (Rheinstetten, Germany) with TMS internal standard (chemical shift in δ, ppm and coupling constant J Hz). Mass spectra were recorded in Fisons Instruments VG Platform II mass spectrometer (California, United States) (70 eV) with Electron Impact methods. Elemental analysis was performed on a Perkin Elmer 240C (Michigan, United States).

*General procedure for synthesis of amide derivatives of 3,5-di/1,3,5-trimethyl-pyrazole (**3**–**12**).* The 4-aminopyrazoles **1** and **2** were prepared according to previously described method [18,19]. The amide derivatives were obtained by using two different methods.

Method I. The appropriate benzoyl chloride (1.0 mmol) was dissolved in ether (10 mL) and the solution was added to ice cooled mixture of 4-aminopyrazole (0.5 mmol), sodium bicarbonate (0.5 mmol), ether (10 mL) and water (10 mL). The mixture was kept stirring over night at room temperature and then filtered. The precipitate was washed with water, 2N HCl, water and ether respectively. The product was recrystallized from ethanol [25].

Method II. Equimolar amounts (20 mmol) of 4-aminopyrazoles and the substituted benzoyl chlorides in dry chloroform (100 mL) were reacted under reflux for 5 h. One hour later, triethylamine was added in four portions of 1.40, 0.70, 0.35 and 0.25 mL, at 1 h intervals. The solution was evaporated and the residue was washed with water until solid form was obtained. The product was crystallized with the appropriate solvent.

N-(3,5-dimethylpyrazole-4-yl)-4-bromobenzamide (3). HPLC Retention time (Rt): 2.033 min. 58% yield, mp 255°C. UV (EtOH) $\lambda_{max.nm}$. log ε 295 (4.62). IR (KBr) cm^{-1} 3240 (NH pyrazole and amide), 1650

(C═O amide). ^{1}H-NMR (DMSO-d_6) δ(ppm) 2.91 (s, 6H, methyl protons to pyrazole), 7.58 (d, 2H, J: 8.3 Hz, *C_3-H* and *C_5-H*), 7.77 (d, 2H, J: 8.3 Hz, *C_2-H* and *C_6-H*), 9.42 (s, 1H, CONH), 11.99 (s, 1H, NH-pyrazole). Anal. Calcd. for $C_{12}H_{12}BrN_3O$: C, 49.00; H, 4.11; N, 14.29. Found: C, 48.65; H, 4.01; N, 13.95%. EI-MS (m/z) 294 (M^+), 295 ($M^+ + 1$), 184, 182, 110, 98, 90, 42.

N-(1-benzoyl-3,5-dimethylpyrazole-4-yl)-benzamide (4). HPLC Rt: 2.410 min. 48% yield, mp 78–80°C. UV (EtOH) $\lambda_{max.nm}$. log ε 295 (5.00). IR (KBr) cm^{-1} 3260 (NH amide), 1650, 1690 (C═O amide and C═O N^1). ^{1}H-NMR (DMSO-d_6) δ(ppm) 2.10 (s, 6H, methyl protons to pyrazole), 7.44–7.88 (m, 10H, *Ar*), 10.53 (s, 1H, CONH). Anal. Calcd. for $C_{19}H_{17}N_3O_2$: C, 71.47; H, 5.32; N, 13.16. Found: C, 71.52; H, 5.23; N, 14.02%. EI-MS (m/z) 307 (M^+), 241, 242, 165, 160, 110, 111, 105 (rel.%, 100).

N-(1-(4-chlorobenzoyl)-3,5-dimethylpyrazole-4-yl)-4-chlorobenzamide (5). HPLC Rt: 11.81 min. 52% yield, mp 205–206°C. UV (EtOH) $\lambda_{max.nm}$, log ε 297 (4.44). IR (KBr) cm^{-1} 3250 (NH amide), 1660, 1710 (C═O amide and C═O N^1). ^{1}H-NMR (DMSO-d_6) δ(ppm): 2.12 (s, 3H, methyl protons to pyrazole-*C_5*), 2.48 (s, 3H, methyl protons to pyrazole-*C_3*), 7.60–8.02 (m, 8H, *Ar*), 9.80 (s, 1H, CONH). Anal. Calcd. for $C_{19}H_{15}Cl_2N_3O_2$: C, 58.78; H, 3.89; N, 10.82. Found: C, 58.81; H, 4.32; N, 10.70%. EI-MS (m/z) 388 (M^+), 248, 249, 140, 115, 110, 77, 42 (rel. % 100).

N-(1-(4-fluorobenzoyl)-3,5-dimethylpyrazole-4-yl)-4-fluorobenzamide (6). HPLC Rt: 11.10 min. 41% yield, mp 198–200°C. UV (EtOH) $\lambda_{max.nm}$, log ε 297 (4.08). IR (KBr) cm^{-1} 3230 (NH amide), 1660, 1695 (C═O amide and C═O N^1). ^{1}H-NMR (DMSO-d_6) δ(ppm) 2.06 (s, 3H, methyl protons to pyrazole-*C_5*), 2.43 (s, 3H, methyl protons to pyrazole-*C_3*), 7.55 (dd, 4H, J: 2.73 Hz, *Ar*), 7.85 (d, 2H, J: 8.5 Hz, *C_3-H* and *C_5-H*), 7.95 (d, 2H, J: 8.5 Hz, *C_2-H* and *C_6-H*), 9.95 (s, 1H, CONH). Anal. Calcd. for $C_{19}H_{15}F_2N_3O_2$: C, 64.22; H, 4.25; N, 11.83. Found: C, 65.56; H, 4.24; N, 11.64%. EI-MS (m/z) 355 (M^+), 356 ($M^+ + 1$), 111, 110 (rel. %, 100), 104, 77, 42.

N-(1-(4-nitrobenzoyl)-3,5-dimethylpyrazole-4-yl)-4-nitrobenzamide (7). HPLC Rt: 5.791 min. 47% yield, mp 212°C. UV (EtOH) $\lambda_{max.nm}$, logε 291 (5.20). IR (KBr) cm^{-1} 3110 (NH amide), 1695, 1710 (C═O amide and C═O N^1). ^{1}H-NMR (DMSO-d_6) δ(ppm) 2.07 (s, 3H, methyl protons to pyrazole-*C_3*), 2.50 (s, 3H, methyl protons to pyrazole-*C_5*), 8.01–8.29 (m, 8H, *Ar-CH*), 10.72 (s, 1H, CONH). Anal. Calcd. for $C_{19}H_{15}F_2N_3O_2$: C,55.40; H, 3.19; N, 16.92. Found: C, 55.40; H, 3.77; N, 17.63%.

N-(1-(3,5-dinitrobenzoyl)-3,5-dimethylpyrazole-4-yl)-3,5-dinitrobenzamide (8). HPLC Rt: 6.23 min. 51% yield, mp 220–222°C. UV (EtOH) $\lambda_{max.nm}$, log ε 291 (5.20). IR (KBr) cm^{-1} 3100 (NH amide), 1690, 1700 (C═O amide and C═O N^1). ^{1}H-NMR (DMSO-d_6) δ(ppm) 2.15 (s, 3H, methyl protons to pyrazole-*C_3*), 2.16 (s, 3H, methyl protons to pyrazole-*C_5*), 8.83–9.10 (m, 6H, *Ar*), 10.31 (s, 1H, CONH). Anal. Calcd. for $C_{18}H_{13}N_7O_9$: C,45.87; H, 2.78; N, 20.80. Found: C, 45.02; H, 2.96; N, 20.11%.

N-(1-(4-trifluoromethoxybenzoyl)-3,5-dimethylpyrazole-4-yl)-4-trifluoromethoxy-benzamide (9). HPLC Rt: 5.61 min. 50% yield, mp 212–214°C UV (EtOH) $\lambda_{max.nm}$, log ε 296 (5.20). IR (KBr) cm^{-1} 3120 (NH amide), 1690, 1710 (C═O amide and C═O N^1). ^{1}H-NMR (DMSO-d_6) δ(ppm) 1.91 (s, 3H, methyl protons to pyrazole-*C_3*), 2.48 (s, 3H, methyl protons to pyrazole-*C_5*), 7.39 (m, 4H, *Ar-*), 7.92 (m, 4H, Ar), 9.90 (s, 1H, CONH). Anal. Calcd. for $C_{20}H_{15}F_6N_3O_3$: C,52.30; H, 3.29; N, 9.15. Found: C, 51.95; H, 3.16; N, 9.88%.

N-(1,3,5-trimethylpyrazole-4-yl)-4-bromobenzamide (10). HPLC Rt: 2.124 min. 45% yield, mp 201°C UV (EtOH) $\lambda_{max.nm}$, log ε 292 (4.91). IR (KBr) cm^{-1} 3250 (NH amide), 1660 (C═O amide). ^{1}H-NMR (DMSO-d_6) δ(ppm) 1.92 (s, 3H, methyl protons to pyrazole-*C_3*), 2.02 (s, 3H, methyl protons to pyrazole-*C_5*), 3.60 (s, 3H, methyl protons to pyrazole-C_1), 7.64–7.84(m, 4H, *Ar-H*), 9.52 (s, 1H, CONH). Anal. Calcd. for $C_{13}H_{14}BrN_3O$: C,50.67; H, 4.58; N, 13.64. Found: C, 49.79; H, 4.41; N, 14.03%. EI-MS (m/z) 308 (M^+), 309 ($M^+ + 1$), 124, 104, 77, 56 (rel. %, 100).

N-(1,3,5-trimethylpyrazole-4-yl)-4-chlorobenzamide (11). HPLC Rt: 2.074 min. 65% yield, mp 110–112°C. UV (EtOH) $\lambda_{max.nm}$, log ε 295 (4.51). IR (KBr) cm^{-1} 3200 (NH amide), 1700 (C═O amide). ^{1}H-NMR (DMSO-d_6) δ(ppm) 2.00 (s, 3H, methyl protons to pyrazole-*C_3*), 2.15 (s, 3H, methyl protons to pyrazole-*C_5*), 3.75 (s, 3H, methyl protons to pyrazole-*C_1*), 7.60 (d, 2H, J: 6.7 Hz, *C_3-H* and *C_5-H*), 8.05 (d, 2H, J: 6.7 Hz, *C_2-H* and *C_6-H*), 9.60 (s, 1H, CONH). Anal. Calcd. for $C_{13}H_{14}ClN_3O$: C, 59.21; H, 5.35; N, 15.93. Found: C, 59.42; H, 5.20; N, 15.70%. EI-MS (m/z) 263 (M^+, rel.%, 100), 264 ($M^+ + 1$), 124, 104, 77, 56.

N-(1,3,5-trimethylpyrazole-4-yl)-4-fluorobenzamide (12). HPLC Rt: 2.283 min. 50% yield, mp 206–208°C. UV (EtOH) $\lambda_{max.nm}$, log ε 292 (4.69). IR (KBr) (cm^{-1}) 3220 (NH amide), 1690 (C═O amide). ^{1}H-NMR (DMSO-d_6) δ(ppm) 2.01(s, 3H, methyl protons to pyrazole-*C_3*), 2.08 (s, 3H, methyl protons to pyrazole-*C_5*), 5.46 (s, 3H, methyl protons to pyrazole-*C_1*), 7.27(t, 2H, J: 8.76, *C_3-H* and *C_5-H*), 7.98 (m, 2H, *C_2-H* and *C_6-H*), 9.66 (s, 1H, CONH). Anal. Calcd. for $C_{13}H_{14}FN_3O$: C, 63.48; H, 5.66; N,

16.98. Found: C, 63.71; H, 5.25; N, 16.79%. EI-MS (m/z) 247 (M^+), 248 ($M^+ + 1$), 124, 104, 95, 77, 56 (rel. %, 100).

Microbiology

The strains of microorganisms employed were *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Bacillus cereus* ATCC 11778, *Proteus mirabilis* CCM1944, *Escherichia. col*i ATCC 25922, *Klebsiella pneumonia* ATCC 4352, *Pseudomonas aeruginosa* ATCC, *Salmonella enteritidis* KUEN 349. The bacteria were maintained on Muller Hinton agar (MHA, Oxoid) and defibrinated sheep blood agar (Oxoid) plates.

Quantative antibacterial evaluation. Tested compounds were dissolved in DMSO and sterile distilled water, respectively for the preparation of stock solution. In all the experiments, Mueller-Hinton Broth (MHB, Oxoid) enriched Ca^{++} and Mg^{++} cations (CAMHB) were used. The in vitro antimicrobial activities of the tested compounds were carried out by broth macrodilution using the standard method (National Committee for Clinical Laboratory Standards: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. Approved Standard-Fifth Edition M7-A5 January 2000). Briefly, the compounds were serially diluted from 4.096 $mgmL^{-1}$ to 0.008 $mgmL^{-1}$ in doubling dilution in CAMHB. Bacterial suspension of log phase cultures in Mueller-Hinton Broth were adjusted by turbidity to yield an inoculum of 10^5 $cfu.mL^{-1}$, and added (500 μL) to the tubes. End-points for MIC testing were read as the lowest concentration of compounds completely prevented visible turbidity in individual tubes after 24 h of incubation at 37°C. Positive (without tested compound) and negative (without bacterium) control samples were added into the all series tested. The same test was also carried out with DMSO as control.

Pharmacology

Experimental protocol. All experimental protocols were approved by the Marmara University School of Medicine Animal Care and Use Committee. Adult Balb/C male and female mice (25–30 g) were used in the study. They were housed in a quiet, temperature (20 ± 2°C) and humidity (60 ± 3%) controlled room where a 12/12 h light–dark cycle was maintained (07:00–19:00 light). The mice were fed standard lab chow and tap water ad lib during the study. The thermal techniques (tail immersion and hot-plate) [26,27] were used to evaluate both basal nociceptive threshold and the antinociceptive effect of the compounds **1**–**12**. All compounds were suspended in 0.5% methyl cellulose and administered at a dose of 100 mg/kg ip. Briefly in the hot-plate test, the licking of the hindpaw or jumping was measured as hotplate latency at 55°C and in the tail immersion test, the mice tails were immersed in warm water (55°C) which provokes an abrupt movement of the tail and sometimes the recoiling of the whole body. Analgesia is defined as the increase in the baseline latency. Mice were either injected vehicle (0.5% methyl cellulose, control group), morphine hydrochloride (reference analgesic for both tests) 5 mg/kg ip or compounds 100 mg/kg ip. Test duration was 120 min after the injection of the drug. Each animal served as its own control and 30 s (for hot-plate), 10 s (for tail-immersion) were used as a cut-off latency to avoid tissue damage.

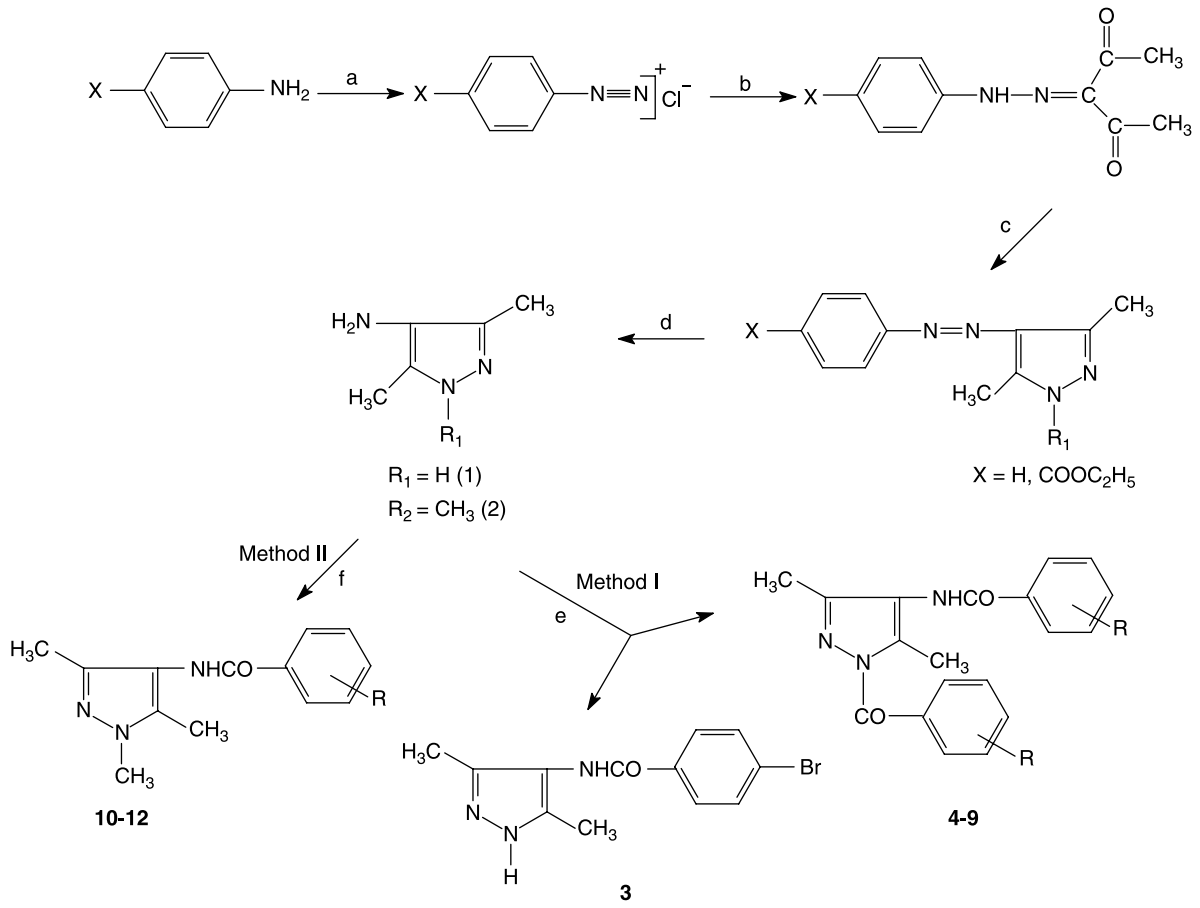
Statistical analysis. Statistical analysis was carried out using GraphPad Prism 3.0 (GraphPad Software, San Diego; CA; USA). All data were expressed as means ± SEM. Groups of data for analgesia tests were compared with an analysis of variance (ANOVA) of repeated measures, followed by Tukey's multiple comparison tests. Values of $p < 0.05$ were regarded as significant.

Results and discussion

Chemisty

The synthetic approach of the pyrazole amides is outlined in Scheme 1. The starting compounds 4-amino-3,5-dimethylpyrazole **1** and 4-amino-1,3,5-trimethylpyrazole **2** were prepared in four steps from aniline or ethyl 4-aminobenzoate following the reported procedure [18,19]. Two different methods were used for the synthesis of the amides **3**–**12**. Method II was prefered to prepare **10**–**12** because of the purification problem and poor yield. The purities of the synthesized compounds were checked by reverse phase-HPLC. The chemical structures of the compounds were supported by the results of the elemental analysis as well as by spectral data.

The IR spectra of the amides showed an absorption band in the range 1650–1695 cm^{-1} corresponding to C═O streching bond. The streching vibration of C═O at pyrazole N^1 (except **3**) was observed at 1690–1710 cm^{-1}. The NH streching bands were found in 3110–3295 cm^{-1}. In the ^{1}H-NMR spectra of the compounds, no peaks belong —NH_2 were observed, indicating the absence of an NH_2 group which is an evidence for the substitution reaction. The methyl protons of pyrazole ring at the third and fifth position appeared as a singlet at 2.91 ppm for compound **3**. However, the same protons that bear substitution at first position for **5**–**12** appeared as two different singlets (except **4**) at 2.00–2.12 ppm and 2.08–2.50 ppm. The reason of this could be the destruction of tautomer form of pyrazole ring that bears substitution at first position



Scheme 1. Synthetic route to **1**–**12**. Reagents. **a**: $NaNO_2$, HCl, 0–5°C, **b**: acetylacetone, CH_3COONa, **c**: hydrazine hydrate, AcOH, reflux, **d**: hydrazine hydrate, EtOH, 50°C, **e**: appropriate benzoyl chloride, $NaHCO_3$, Eter, H_2O, **f**: appropriate benzoyl chloride, $CHCl_3$, triethylamine.

and differing electronic equivalency at third and fifth positions. The amide NH protons were seen as singlet at 9.42–10.53 ppm. The pyrazole NH peak of compounds **4**–**9** was not observed at about 12.00–12.40 ppm. This data show that the pyrazole NH was reacted with benzoyl chlorides. Besides, in the mass spectra, molecular ion peaks were dedected which confirmed the molecular weights of examined compounds.

The pyrazole NH proton was determined the compound **3** at 11.99 ppm. The signals of aromatic ring protons were observed at about 7.27–8.29 ppm.

EI-MS spectra of the **3**–**6**, **10**–**12** showed correct molecular ion peaks [M^+] in different intensity. The main way of cleavage is the broken of NH—CO bond of amide moiety as shown in figure. In addition, in the mass spectrum of compounds **4**–**6** bearing benzoyl moiety at the first position of pyrazole ring, the removal of the benzoyl fragments as a radical is associated with the cleavage of the N—CO bond. Besides, novel compounds exhibited the expected fragmentation pattern of pyrazole structures according to the literature [18].

Table I. Antimicrobial activity of new synthesized compounds (MIC, $mgmL^{-1}$).

Comp.	*K. pneumonia*	*P. aeruginosa*	*B. subtilis*	*S. aureus*	*S. epidermidis*	*B. cereus*	*S. enteritidis*	P. *mirabilis*	*E. coli*
3	1.024	1.024	1.024	0.512	1.024	1.024	1.024	1.024	1.024
4	2.048	1.024	1.024	2.048	1.024	1.024	1.024	1.024	1.024
5	1.024	1.024	1.024	1.024	1.024	1.024	1.024	1.024	1.024
6	2.048	1.024	2.048	2.048	1.024	2.048	2.048	2.048	2.048
7	2.048	2.048	2.048	2.048	2.048	2.048	2.048	2.048	2.048
8	2.048	2.048	2.048	1.024	2.048	2.048	2.048	2.048	2.048
9	1.024	2.048	2.048	1.024	2.048	2.048	1.024	1.024	2.048
10	2.048	2.048	2.048	2.048	2.048	2.048	2.048	2.048	2.048
11	1.024	1.024	2.048	2.048	1.024	1.024	2.048	2.048	2.048
12	2.048	2.048	2.048	2.048	1.024	2.048	2.048	2.048	2.048

Table II. Hotplate latencies of the compounds.

Time (min)	saline	morphine	Comp. 1	Comp. 2	Comp. 3	Comp. 4	Comp. 5	Comp. 6	Comp. 7	Comp. 8	Comp. 9	Comp.10	Com.11	Comp. 12
0	9.49 ± 0.20	9.55 ± 0.20	10.56 ± 0.50	9.65 ± 0.90	10.09 ± 1.00	10.61 ± 0.60	10.25 ± 0.90	10.31 ± 0.50	10.88 ± 0.70	9.89 ± 0.30	10.06 ± 0.70	10.64 ± 0.50	9.76 ± 0.30	10.76 ± 0.60
30	9.79 ± 0.10	19.5 ± 0.70 $^{\star,++}$	15.23 ± 1.10$^{\star,++}$	10.55 ± 1.40	14.47 ± 1.90	10.3 ± 0.70	13.46 ± 1.30	10.66 ± 0.60	10.40 ± 0.40	10.43 ± 0.80	13.87 ± 1.20*	15.70 ± 2.80	13.70 ± 2.80	16.49 ± 1.30*, +
60	9.41 ± 0.4	24.7 ± 1.7 $^{\star,++}$	12.71 ± 1.3	11.86 ± 0.70	12.57 ± 0.60	10.48 ± 0.50	11.70 ± 1.40	10.97 ± 1.00	11.91 ± 0.9	11.60 ± 0.80	13.02 ± 1.30	16.31 ± 2.0*, +	14.17 ± 2.30*	17.69 ± 2.90*, +
90	9.38 ± 0.4	18.2 ± 0.7 ***	10.17 ± 1.00	9.63 ± 1.00	12.13 ± 1.0	11.14 ± 1.00	12.62 ± 1.80	10.57 ± 1.00	11.16 ± 0.80	10.10 ± 0.60	12.46 ± 1.20	14.13 ± 1.80	13.04 ± 1.40	13.72 ± 1.3
120	9.94 ± 0.2	14.1 ± 0.9	9.45 ± 0.80	11.49 ± 1.00	12.70 ± 0.80	12.89 ± 0.90	10.09 ± 1.50	11.09 ± 1.10	10.70 ± 0.5	10.82 ± 0.7	9.79 ± 1.02	16.37 ± 2.80^{+}	12.37 ± 1.00	12.54 ± 1.00

The results were expressed as mean ± SEM. Repeated measures of ANOVA were performed for statistical analysis (n = 8 for each group). 0 minutes refer to the baseline values. *: $p < 0.05$, **:$p < 0.01$, ***: $p < 0.001$ when compared with its own baseline value or control group (+: $p < 0.05$).

Microbiology

All of the synthesized compounds were tested in vitro for their antimicrobial activity against gram-positive and gram-negative bacteria. DMSO which used in dissolving of the compounds, had no effect on microorganism. The antimicrobial activity of the compounds was tested on the gram-positive strains *B. subtilis* ATCC 6633, *S. aureus* ATCC 29213, *S. epidermidis* ATCC, 12228, and *B. cereus* ATCC 11778; gram-negative strains *P. mirabilis* CCM1944, *E. coli* ATCC 25922, *K. pneumonia* ATCC 4352, *P. aeruginosa* ATCC and *S. enteritidis* KUEN 349.

The preliminary results of antimicrobial activities indicated that some of the compounds exhibited weak activity against gram-positive and gram-negative bacteria (Table I). Among the tested compounds, compound **3** which is not substituted at the first position of the pyrazole ring, exhibited highest inhibition ($0.512\,\mathrm{mgmL^{-1}}$) against *S. aureus*. Compound **5** bearing a 4-chloro group on the phenyl ring, showed better inhibition when compared to the other tested compounds. Pyrazole-4-carboxamides have already been reported to be antimicrobial [24]. Here, we expected that the amide derivatives of pyrazole would have similar activity to pyrazole-4-carboxamides or pyrazole-4-carboxylates. Nevertheless, results of the MIC test of the amide derivatives of 4-aminopyrazoles were generally not promising and they exhibited weak activity with a MIC in the range of $0.512-2.048\,\mathrm{mgmL^{-1}}$.

Pharmacology

Two different analgesia tests were used for the determination of the antinociceptive activity. Hotplate test was used for the supraspinal analgesia, while tail-immersion test was used for spinal analgesia. Morphine, an analgesic through both spinal and supraspinal pathways, was used as a standard test drug at a dose of 5 mg/kg. All compounds were administered at a dose of 100 mg/kg ip. Our results demonstrate that most of these compounds exert significant antinociceptive effects in both tests, indicating that these effects were mediated by both spinal and supraspinal pathways (Table II and III).

The initiation and duration of the antinociceptive activity varied between compounds. Based on our observations, compound **10**, **11** and **12** were the most active ones in both tests among the compounds. The antinociceptive activity of the compounds **10**, **11** and **12** started at 30 minutes and continued up to 150 minutes in the hotplate test. We may speculate that the methyl substituent may lead to an increase in the analgesic activity of the pyrazole ring and also prolongation of the analgesic activity. Halogen substitution of the phenyl ring (**10**, **11**, **12**) also seems to be an important factor and among the

Table III. Tail-flick latencies of the compounds.

Time (min)	saline	morphine	Comp. 1	Comp. 2	Comp. 3	Comp. 4	Comp. 5	Comp. 6	Comp. 7	Comp. 8	Comp. 9	Comp.10	Com.11	Comp. 12
0	0.83 ± 0.05	0.74 ± 0.04	0.79 ± 0.03	0.82 ± 0.01	0.84 ± 0.04	0.80 ± 0.02	0.83 ± 0.04	0.80 ± 0.02	0.82 ± 0.03	0.80 ± 0.03	0.73 ± 0.02	0.79 ± 0.0	0.80 ± 0.03	0.81 ± 0.05
30	0.840 ± 0.05	4.880 ± 0.35***,$^{+++}$	0.86 ± 0.04	1.44 ± 0.13^{++}	1.16 ± 0.2^{+}	2.78 ± 0.30**,$^{+++}$	0.84 ± 0.05	0.80 ± 0.03	1.05 ± 0.02**,$^{+}$	2.71 ± 0.43**,$^{+++}$	0.69 ± 0.04	1.42 ± 0.23*,$^{++}$	1.76 ± 0.13*,$^{++}$	2.98 ± 0.25***,$^{++}$
60	0.86 ± 0.06	2.41 ± 0.25***,$^{+++}$	0.77 ± 0.04	1.89 ± 0.27***,$^{++}$	0.96 ± 0.1	2.45 ± 0.16***	0.87 ± 0.07	0.84 ± 0.04	0.77 ± 0.03	1.83 ± 0.40^{++}	0.75 ± 0.04	1.48 ± 0.15*,$^{++}$	1.59 ± 0.16*, +	2.65 ± 0.19***$^{+++}$
90	0.91 ± 0.91	1.78 ± 0.36*,$^{+}$	0.70 ± 0.04	1.21 ± 0.08	0.94 ± 0.01	1.76 ± 0.31*	0.87 ± 0.1	0.80 ± 0.04	0.76 ± 0.04	2.18 ± 0.64*,$^{++}$	0.76 ± 0.07	1.35 ± 0.13*, +	1.29 ± 0.13*	1.36 ± 0.13*
120	0.79 ± 0.05	1.27 ± 0.22^{+}	0.73 ± 0.03	1.33 ± 0.12^{+}	0.78 ± 0.03	1.90 ± 0.24**,$^{++}$	0.79 ± 0.04	0.93 ± 0.13	0.77 ± 0.02	1.13 ± 0.12	0.77 ± 0.02	1.29 ± 0.17*, +	1.06 ± 0.19*	1.31 ± 0.15^{+}

The results were expressed as mean ± SEM. Repeated measures of ANOVA were performed for statistical analysis ($n = 8$ for each group). 0 minutes refer to the baseline values. *:$p < 0.05$, **$p < 0.01$, ***: $p < 0.001$ when compared with its own baseline value or control (saline) group (+: $p < 0.05$).

halogen-substituted compounds the bromide derivative (**10**) had the longest duration of analgesic activity. Also the fluoride derivative (**12)** exhibited a remarkable activity.

The incidence of gastrointestinal adverse effects limit the use of analgesic-anti-inflammatory drugs in many patients. The new class of COX-2 inhibitors are thought be promising; a common property is that they contain a pyrazole nucleus. Celecoxib and deracoxib are already used for the treatment of pain and inflammation. Moreover, there are various reports which have demonstrated the analgesic, antiinflammatory and antipyretic effects of pyrazole derivatives. In the present study our results have demonstrated the potent analgesic effects of these pyrazole derivatives. On the other hand, our findings are limited, since they depend on the preliminary results of a single dose administration. We plan to conduct experiments with inflammation and lipopolysaccharide-induced fever models to investigate the other pharmacological activities. Moreover, further studies need to be carried out to highlight other aspects, such as dose-response effects and toxicological studies or side effect profile of these compounds.

Acknowledgements

This study was supported by The Research Fund of Marmara University (project number SAG-YY-028/131102 and SAG-BGS-270605-0132).

References

[1] Penning TD, Talley JJ, Bertenshaw SR, Carter JS, Collins PW, Docter S, Graneto MJ, Lee LF, Malecha JW, Miyashiro JM, Rogers RS, Rogier DJ, Yu SS, Anderson GD, Burton EG, Cogburn JN, Gregory SA, Koboldt CM, Perkins WE, Seibert K, Veenhuizen AW, Zhang YY, Isakson PC. Synthesis and biological evaluation of the 1,5-diarylpyrazole class of cyclooxygenase-2 inhibitors: Identification of 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide (SC-58635, celecoxib). J Med Chem 1997;40: 1347–1365.

[2] Cheng H, Lundy DeMello KM, Li J, Sakya SM, Ando K, Kawamura K, Kato T, Rafka RJ, Jaynes BH, Ziegler CB, Stevens R, Lund LA, Mann DW, Kilroy C, Haven ML, Nimz EL, Dutra JK, Li C, Minich ML, Kolosko NL, Petras C, Silvia AM, Seibel SB. Synthesis and SAR of heteroaryl-phenyl-substituted pyrazole derivatives as highly selective and potent canine COX-2 inhibitors. Bioorg Med Chem Lett 2006;16(8): 2076–2080.

[3] Sakya SM, DeMello KM, Minich ML, Rast B, Shavnya A, Rafka RJ, Koss DA, Cheng Li J, Jaynes BH, Ziegler CB, Mann DW, Petras CF, Seibel SB, Silvia AM, George DM, Lund LA, Denis S. St, Hickman A, Haven ML. Lynch MP. 5-Heteroatom substituted pyrazoles as canine COX-2 inhibitors. Part 1: Structure-activity relationship studies of 5-alkylamino pyrazoles and discovery of a potent, selective, and orally active analog. Bioorg Med Chem Lett 2006;16(2):288–292.

[4] Oruc EE, Kocyigit-Kaymakcioglu B, Oral B, Altunbas-Toklu HZ, Kabasakal L, Rollas S. Synthesis of some novel azo derivatives of 3,5-dimethyl-1-(2-hydroxyethyl)pyrazole as

potent analgesic agents. Arch Pharm (Weinheim) 2006; 339(5):267–272.

[5] Thomas BF, Francisco ME, Seltzman HH, Thomas JB, Fix SE, Schulz AK, Gilliam AF, Pertwee RG, Stevenson LA. Synthesis of long-chain amide analogs of the cannabinoid CB1 receptor antagonist N-(piperidinyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (SR141716) with unique binding selectivities and pharmacological activities. Bioorg Med Chem Lett 2005;13(18):5463–5474.

[6] Sukuroglu M, Caliskan-Ergun B, Unlu S, Sahin MF, Kupeli E, Yesilada E, Banoglu E. Synthesis, analgesic, and anti-inflammatory activities of [6-(3,5-dimethyl-4-chloropyrazole-1-yl)-3(2H)-pyridazinon-2-yl]acetamides. Arch Pharm Res 2005;28(5):509–517.

[7] Singh SK, Saibaba V, Rao KS, Reddy PG, Daga PR, Rajjak SA, Misra P, Rao YK. Synthesis and SAR/3D-QSAR studies on the COX-2 inhibitory activity of 1,5-diarylpyrazoles to validate the modified pharmacophore. Eur J Med Chem 2005; 40(10):977–990.

[8] Bekhit AA, Ashour HM, Guemei AA. Novel pyrazole derivatives as potential promising anti-inflammatory antimicrobial agents. Arch Pharm (Weinheim) 2005;338(4): 167–174.

[9] Bekhit AA, Abdel-Rahman HM, Guemei AA. Synthesis and biological evaluation of some hydroxypyrazole derivatives as anti-inflammatory-antimicrobial agents. Arch Pharm (Weinheim) 2006;339(2):81–87.

[10] Bekhit AA, Fahmy HT. Design and synthesis of some substituted 1H-pyrazolyl-oxazolidines or 1H-pyrazolyl-thiazolidines as anti-inflammatory-antimicrobial agents. Arch Pharm (Weinheim) 2003;336(2):111–118.

[11] Menozzi G, Mosti L, Schenone P. N-substituted 4-carboxy-1-phenyl-1H-pyrazole-5-propanamides with antiinflammatory, analgesic, antipyretic and platelet antiaggregating activities. Farmaco 1993;48(4):539–549.

[12] Bruno O, Ranise A, Bondavalli F, Schenone S, D'Amico M, Falciani M, Vacca C, Filippelli A. 3,5-Diphenyl-1H-pyrazole derivatives XII. N-substituted 4-amino-1-(2-hydroxy- or 2-alkylaminoethyl)-3,5-diphenyl-1H-pyrazoles with local anesthetic, analgesic and platelet antiaggregating activities. Farmaco 1994;49(9):533–540.

[13] Bruno O, Schenone S, Ranise A, Bondavalli F, Falcone G, Flippelli W, Marabese I, Motula G. N-arylsubstituted-1-aryl-3,4-diphenyl-5-pyrazole amines with analgesic and antiarrhythmic properties. Farmaco 1997;52(10):615–618.

[14] Wustrow DJ, Capiris T, Rubin R, Knobelsdorf JA, Akunne H, Davis MD, MacKenzie R, Pugsley TA, Zoski KT, Heffner TG, Wise LD. Pyrazolo [1,5-a]pyrimidine CRF-1 receptor antagonists. Bioorg Med Chem Lett 1998;16:2067–2070.

[15] Lankau HJ, Menzer M, Rostock A, Arnold T, Rundfeldt C, Unverferth K. 3-Amino- and 5-aminopyrazoles with anticonvulsant activity. Arch Pharm Pharm Med Chem 1999; 332:219–221.

[16] Lankau HJ, Menzer M, Rostock A, Arnold T, Rundfeldt C, Unverferth K. Synthesis and anticonvulsant activity of new 4-aminopyrazoles and 5-aminopyrazol-3-ones. Pharmazie 1999; 54(9):705–706.

[17] Kucukguzel SG, Rollas S, Erdeniz H, Kiraz M, Ekinci AC, Vidin A. Synthesis, characterization and pharmacological properties of some 4-arylhydrazono-2-pyrazoline-5-one derivatives obtained from heterocyclic amines. Eur J Med Chem 2000;35(7–8):761–771.

[18] Kaymakcioglu BK, Rollas S, Korcegez E, Aricioglu F. Synthesis and biological evaluation of new N-substituted-N′-(3,5-di/1,3,5-trimethylpyrazole-4-yl)thiourea/urea derivatives. Eur J Pharm Sci 2005;26:97–103.

[19] Koçyiğit-Kaymakçıoğlu B, Rollas S. Synthesis, characterization and evalution of antituberculosis activity of some hydrazones. Farmaco 2001;57:595–599.

[20] El-Emary TI, Bakhite EA. Synthesis and biological screening of new 1,3-diphenylpyrazoles with different heterocyclic moieties at position-4. Pharmazie 1999;54(2):106–111.

[21] Genin MJ, Biles C, Keiser BJ, Poppe SM, Swaney SM, Tarpley WG, Yagi Y, Romero DL. Novel 1,5-diphenylpyrazole nonnucleoside HIV-1 reverse transcriptase inhibitors with enhanced activity versus the delavirdine-resistant P236L mutant: Lead identification and SAR of 3- and 4-substituted derivatives. J Med Chem 2000;43(5):1034–1040.

[22] Daidone G, Maggio B, Plesci S, Raffa D, Musiu C, Milia C, Perra G, Marongiu ME. Antimicrobial and antineoplastic activities of new 4-diazopyrazole derivatives. Eur J Med Chem 1998;33:375–382.

[23] Bekhit AA, Abdel-Aziem T. Design, synthesis and biological evaluation of some pyrazole derivatives as anti-inflammatory-antimicrobial agents. Bioorg Med Chem Lett 2004;12(8): 1935–1945.

[24] Sridhar R, Perumal PT, Etti S, Shanmugam G, Ponnuswamy MN, Prabavathy VR, Mathivanan N. Design, synthesis and anti-microbial activity of 1H-pyrazole carboxylates. Bioorg Med Chem Lett 2004;14(24):6035–6040.

[25] Yalçın I, Koçyiğit-Kaymakçıoğlu B, Ören I, Şener E, Temiz Ö, Akın A, Altanlar N. Synthesis and microbiological activity of some novel N-(2-hydroxyl-5-substitutedphenyl)benzacetamides, phenoxyacetamides and thiophenoxy-acetamides as the possible metabolites of antimicrobial active benzoxazoles. Farmaco 1997;52(11):685–689.

[26] Eddy NB, Leimbach CE. Synthetic analgesics II. Dithienylbutenyl- and dithienylbutylamines. J Pharmacol Exp Ther 1953;107:385–393.

[27] Janssen PAJ, Neimegeers CJE, Donny JGH. The inhibitory effect of fentanyl and other morphine-like analgesics on the warm water induced tail withdrawl reflex in rats. Arzneimittel Forsh 1963;13:502–507.