Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 12 (2004) 4169–4177
Bioorganic & Medicinal Chemistry

Synthesis of new 2-arylamino-6-trifluoromethylpyridine-3-carboxylic acid derivatives and investigation of their analgesic activity

Maria T. Cocco,[a,*] Cenzo Congiu,[a] Valentina Onnis,[a] Micaela Morelli,[a] Vicente Felipo[b] and Omar Cauli[a,b]

[a]*Dipartimento di Tossicologia, Università degli Studi di Cagliari, Via Ospedale 72, Cagliari I-09124, Italy*
[b]*Fundación Valenciana de Investigaciones Biomedicas, c/Amadeo de Saboya, 4, 46010 Valencia, Spain*

Received 21 January 2004; accepted 17 May 2004
Available online 24 June 2004

Abstract—A new series of 2-arylamino-6-trifluoromethyl-3-carboxylic acid derivatives was synthesized and assayed in vivo for their analgesic properties by means of writhing test in rats. When compared to aspirin, ibuprofen and flufenamic acid some of the new compounds exhibited a comparable or improved analgesic activity and a lower ulcerogenic effect.

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a class of compounds characterized by a similar pharmacological profile encompassing anti-inflammatory, analgesic and anti-pyretic activities. They are indicated for the treatment of musculo-skeletal disorders and other syndromes involving pain.[1] Despite their well-known therapeutic efficacy, NSAIDs have the potential to cause adverse reactions, especially with regard to the digestive and the renal system.[2] The most common adverse events related to the use of NSAIDs involve the gastrointestinal (GI) tract, and include a range of disorders, from functional problems such as dyspepsia, heartburn and abdominal discomfort to GI haemorrhage, peptic ulcer and perforation. Epidemiological studies have shown that approximately 10–20% of NSAID-treated patients have gastroduodenal erosions or ulcers detected at endoscopy, and 60% of those with serious GI complications are on long-term NSAID therapy.[3] Given the vast epidemiological proportions of the problem of NSAID-induced GI damage, preclinical and clinical researchers have spent substantial efforts trying to identify the mechanisms responsible for the therapeutic and toxic effects of these drugs in order to devise strategies for effective prevention of GI toxicity.

After Vane first established a link between NSAID pharmacological activity and prostaglandin production in 1971,[4] subsequent studies have highlighted the central role of cyclo-oxygenase (COX) inhibition, with subsequent reduction of prostaglandin biosynthesis, in mediating both clinical efficacy and toxicity. In particular, NSAIDs inhibit the COX activity of prostaglandin G/H synthase, the enzyme that mediates the biotransformation of arachidonic acid into a series of prostanoids (prostaglandins and thromboxanes). These compounds display complex biological functions in several body systems and play a pivotal role in mediating inflammation and pain.[5–7]

In the early 1990s, molecular biology studies identified two COX isoenzymes, named COX-1 and COX-2, which have different molecular structures and are encoded by different genes.[8] Subsequent studies showed that COX-1 is constitutive and catalyses the production of prostaglandins involved in GI mucosal protection and other physiological activities, whereas COX-2, mostly inducible, is responsible for the production of prostaglandins that mediate inflammation, pain and fever.[6] The discovery of these two different COX isoenzymes has allowed researchers a better understanding of the varying degrees of GI toxicity associated with the use of different NSAIDs by hypothesizing that COX-2 inhibition is responsible for the therapeutic effects of these drugs, whereas the typical adverse reactions are attributable to COX-1 inhibition.[6,9] Recent

* Corresponding author. Tel.: +39-0706758645; fax: +39-0706758612; e-mail: tcocco@unica.it

0968-0896/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2004.05.025

studies have further clarified the physiological role of COX isoenzymes. COX-1 is expressed in most tissues (mainly platelets, GI tract, central nervous system (CNS) and kidney), where it mediates functions such as platelet aggregation, neurotransmission, hyperaemia, cell proliferation and differentiation.[10–12] COX-1 expression has also been detected in synovial tissue of patients with inflammatory joint disease.[13] COX-2 is induced by pro-inflammatory cytokines, mitogens and growth factors.[10] In platelet activation syndromes, COX-2 appears to increase prostacyclin production. In this respect, selective COX-2 inhibitors, which are known to decrease urinary excretion of prostacyclin metabolites, might increase the risk of thrombosis in predisposed patients.[14] Moreover, contrary to previous beliefs, COX-2 appears to be constitutively expressed in organs such as CNS, kidney, ovarian follicles and testes, where it regulates important biological functions.[6–15] However, the predominant physiological activity of COX-2 appears to be the production of prostanoids that mediate inflammation and pain. Based on the knowledge of the differential activities of the two COX isoenzymes, new NSAIDs have been developed, such as meloxicam, nimesulide, celecoxib and rofecoxib that are capable of exerting a preferential or selective inhibition of the COX-2 isoenzyme without interfering to the same extent as nonselective NSAIDs with the normal functions and mucosal protective activities mediated by COX-1.

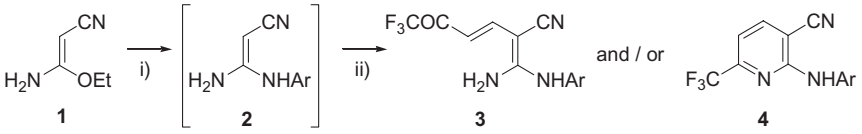
Pyridine derivatives form an important class of heterocyclic compounds and have attracted the attention of many scientists. Some pyridine compounds[16] such as derivatives of aminopyridynylmethanols and aminomethylpyridinamines have been found useful as analgesic as well as anti-inflammatory agents and for treating Alzheimer's disease.[17] The pyridine nucleus is present in niflumic acid and flunixin, two traditional NSAIDs belonging to the class of fenamates. These drugs are derivatives of N-phenyl (or heteroaryl)anthranilic acid that are employed for their analgesic, anti-inflammatory and anti-pyretic properties. Unlike most other NSAIDs, the fenamates appear also to compete with prostaglandins for binding at the prostaglandin receptor site and thus potentially antagonize the physiopathological effects of prostaglandins that have already been formed. Fenamates are used to relieve mild to moderate pain when the duration of therapy will not exceed one week. Fenamates are endowed of most of the adverse effects induced by NSAIDs, particularly gastrointestinal bleeding, ulceration and perforation. Unlike aspirin and most other NSAIDs, the fenamates, do not affect platelet aggregation and bleeding time. Substitution of the carboxylic acid functionality of several fenamates with heterocycles containing an acidic functionality provided dual inhibitors of COX and 5-lypoxygenase.[18] Furthermore recent papers show that substitution at 2-position of anthranilic acid by different substituted aryl or heteroaryl moieties markedly modulate the biological activity.[19–21] Prompted by the above observations we became interested into pyridine derivatives designed to obtain novel molecules endowed with analgesic activity as well as reduced gastrointestinal side effects. So we undertook a study on the influence of the introduction of a 6-CF_3 group on 2-aminonicotinic acid derivatives. In this paper we report the synthesis, the pharmacological and toxicological properties of 2-arylamino-6-trifluoromethylpyridine derivatives. We explored the consequence on analgesic activity of the kind and the position of substituents on arylamino moiety of 2-arylamino-6-trifluoromethylpyridine-3-carboxylic acids **5**. We also explored the effects of substitution of the carboxylic acid functionality with nitrile (compounds **4**), ester (compounds **7** and **9**) or amide (compounds **6**) moieties on selected 2-arylamino-6-trifluoromethylpyridine-3-carboxylic acids that showed the best analgesic activity.

2. Results and discussion

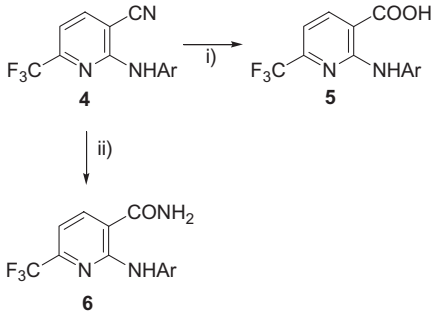
2.1. Chemistry

The preparation of 2-anilinonicotinic acid derivatives is generally achieved by condensation of a 2-halonicotinic acid (or esters) and an aniline derivative at high temperature[22–24] or in the presence of anhydrous potassium carbonate and Cu powder,[25–29] pyridine and *p*-TsOH,[30] or KI[31] as catalysts. These reactions are well described and applicable to various 6-H or 6-arylsubstituted 2-halonicotinic acids. Whereas, to the best of our knowledge, only an example of application of this reaction to methyl 6-trifluoromethyl-2-bromonicotinate has been reported.[32] The introduction of a trifluoromethylated function on a pyridine ring is not a trivial reaction, as matter of fact few approaches for the preparation of trifluoromethyl heterocycles have been developed. Among these the use of easily available trifluoromethylated building blocks has often found to be effective for introduction of a trifluoromethyl group into heterocyclic systems.[33] We have previously reported an easy and convenient method[34] for the synthesis of pyridines bearing trifluoromethyl group by reaction of trifluoroacetylvinyl ethers with 3-amino-3-dialkylaminopropenenitriles.

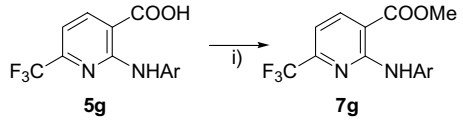
Schemes 1–4 show the synthetic approaches to obtain 6-trifluoromethylpyridine derivatives **4**–**7** and **9** (Table 1). According to Scheme 1, 2-arylamino-6-trifluoromethyl-3-pyridinecarbonitriles **4** were obtained through an one-pot, two-step reaction. 3-Amino-3-ethoxypropenenitrile **1** was first treated with the appropriate substituted arylamine in MeCN solution to give the no isolable 3-amino-3-arylaminopropenenitriles **2** and then with 1,1,1-trifluoro-4-*iso*-butoxy-3-buten-2-one. The temperature was gradually allowed to reach 60 °C. After 3 h pyridinecarbonitriles **4** are obtained in good yields through the formation of the intermediate 1,1,1-trifluoro-3,5-hexadien-2-ones **3**. Adducts **3** are the main products of the reaction between propenenitriles **2b**, **2c** and **2e**, while in all other cases pyridines **4** are exclusively obtained. The ratio of compounds **3** and **4** depended on reaction time, in fact the prolongation of reaction time up to 6 h resulted in formation of pyridines **4** as only product.



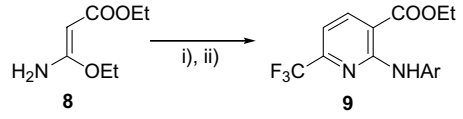
Scheme 1. Synthesis of pyridines **3**. Reagents and conditions: (i) $ArNH_2$, MeCN, rt; (ii) 1,1,1-trifluoro-4-*iso*-butoxy-3-buten-2-one, 60 °C.



Scheme 2. Synthesis of pyridines **5** and **6**. Reagents and conditions: (i) H_2SO_4, H_2O, 100 °C; (ii) 20% NaOH, 100 °C.



Scheme 3. Synthesis of pyridine **7g**. Reagents and conditions: (i) DMF–DMA, Toluene, 90 °C.



Scheme 4. Synthesis of pyridines **8g–h**. Reagents and conditions: (i) $ArNH_2$, MeCN, rt; (ii) 1,1,1-trifluoro-4-*iso*-butoxy-3-buten-2-one, 60 °C.

Cyanopyridines **4** were the starting materials for the preparation of pyridine-3-carboxylic acids **5** and pyridine-3-carboxamides **6** (Scheme 2). High yields of compounds **5** were achieved by hydrolysis of derivatives **4** in 50% aqueous sulfuric acid. Sodium hydroxide catalyzed hydrolysis of selected compounds **4** afforded pyridine-3-carboxamides **6**.

Reaction of pyridine-3-carboxylic acid **5g** with dimethylformamide dimethyl acetal (DMF–DMA), in toluene solution, afforded the corresponding methyl ester **7g** in 80% yield. (Scheme 3).

A chemistry similar to that described in the synthesis of compounds **4** was used for the preparation ethyl esters **9**.

As reported in Scheme 4, ethyl 3-amino-3-ethoxypropenoate **8** was sequentially treated with the appropriate substituted arylamine, then with 1,1,1-trifluoro-4-*iso*-butoxy-3-buten-2-one in MeCN solution to give pyridine derivatives **9**.

2.2. Pharmacology

All compounds **4–7**, **9** were screened for analgesic activity using acetic acid writhing test[35] in rats. Their activity as compared with three clinically used NSAIDs drugs namely aspirin (asa), ibuprofen (ibu) and flufenamic acid (flu) (Fig. 1).

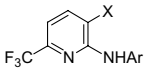
The writhing test is a model of inflammatory pain that it has long been used as a screening tool for evaluation of putative analgesic and anti-inflammatory agents.[36–38] Moreover this model has been shown to be useful in correlating the ED_{50} values of NSAID antinociception in experimental animals with those in humans.[39,40]

After ip administration all compounds **4** and **5**, except for **4a**, **4f** and **5e**, induced significant analgesic activity (at least $P < 0.05$ as compared to vehicle injected rats (Fig. 1). The percentage of inhibition of acetic acid-induced writhing (Table 1) revealed that all pyridine-3-carbonitriles **4**, except for **4a** and **4f**, at the dose of 5 mg/kg displayed a similar analgesic activity induced by aspirin, ibuprofen and flufenamic acid (Fig. 1 and Table 1). Pyridine-3-carboxylic acids **5f**, **5g** and **5h** induced a significantly higher analgesic activity as compared to the reference drugs ($P < 0.05$, Fig. 1). Acids **5a–5d** show analgesic activity comparable to that of reference compounds (Fig. 1 and Table 1). Otherwise, administration of compounds **5e**, **6**, **7** and **9** did not induced a significant analgesic activity as compared to vehicle injected rats (Fig. 1 and Table 1).

In order to assess the possible nonspecific sedative or motor effects of the investigated compounds and to distinguish analgesia from drug-induced motor changes, the motor activity after ip administration of the most active compounds was evaluated (Fig. 2). None of the tested compounds **4** and **5** induced significant motor changes (Fig. 2) indicating that they give analgesia devoid of nonspecific sedative or stimulating effects.

Successively analgesic activity (Fig. 3) after po administration of the compounds that have shown analgesic activity after ip administration (**4b**, **4c**, **4d**, **4e**, **4g**, **4h**, **5a**, **5b**, **5c**, **5d**, **5f**, **5g**, **5h**) was evaluated. After po administration, compounds **4b–e**, **4g**, **4h**, **5a–d** and **5f–h** at the dose of 10 mg/kg demonstrated noteworthy analgesic activity (at least $P < 0.05$ as compared to vehicle injected rats, Fig. 3) similar to that induced by the reference drugs.

Table 1. Percentage of inhibition of acetic acid-induced writhing test (see Pharmacology section) in rats pretreated with one of the reference drugs (aspirin, ibuprofen or flufenamic acid), one of the 2-arylamino-6-trifluoromethylpyridine derivatives or vehicle



Compound	X	Ar	ip test (5 mg/kg)% inhibition acetic acid-induced writhing	po test (10 mg/kg)% inhibition acetic acid-induced writhing
4a	CN	2-MeC_6H_4	16	—
4b	CN	3-$CF_3C_6H_4$	61	75
4c	CN	4-$CF_3C_6H_4$	63	78
4d	CN	2,6-$Me_2C_6H_3$	59	73
4e	CN	2-Me,3-ClC_6H_3	59	72
4f	CN	2-Me,4-ClC_6H_3	53	—
4g	CN	2-Me,5-ClC_6H_3	62	83
4h	CN	2-Me,6-ClC_6H_3	72	97
5a	COOH	2-MeC_6H_4	65	70
5b	COOH	3-$CF_3C_6H_4$	68	57
5c	COOH	4-$CF_3C_6H_4$	72	74
5d	COOH	2,6- $Me_2C_6H_3$	61	75
5e	COOH	2-Me,3-ClC_6H_3	40	—
5f	COOH	2-Me,4-ClC_6H_3	91	90
5g	COOH	2-Me,5-ClC_6H_3	97	94
5h	COOH	2-Me,6-ClC_6H_3	92	90
6e	$CONH_2$	2-Me,3-ClC_6H_3	31	—
6g	$CONH_2$	2-Me,5-ClC_6H_3	18	—
7g	COOMe	2-Me,5-ClC_6H_3	49	—
9g	COOEt	2-Me,5-ClC_6H_3	50	—
9h	COOEt	2-Me,6-ClC_6H_3	49	—
ibu			63	60
asa			74	70
flu			66	60
vehicle			0	0

Only compounds, which displayed analgesic activity after ip administration were subsequently tested for their analgesic activity after po administration.

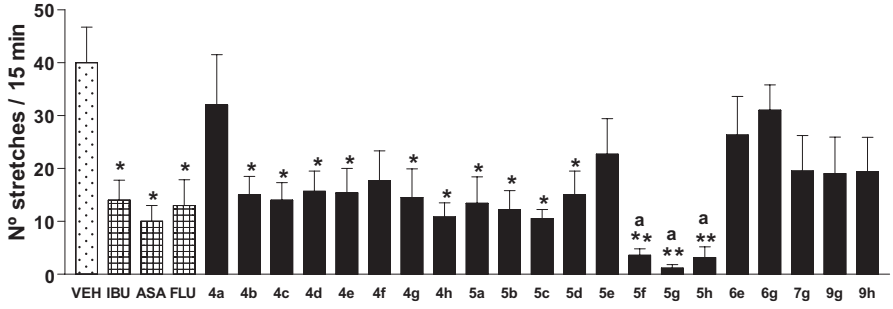


Figure 1. Stretches induced by ip acetic acid injection in rats pretreated ip (30 min before) with vehicle, one of the reference drugs or one of the experimental new compounds (5 mg/kg). *$P < 0.05$; **$P < 0.01$ as compared to vehicle pretreated rats; [a]$P < 0.05$ as compared to ibuprofen (ibu), aspirin (asa) and flufenamic acid (flu) pretreated rats.

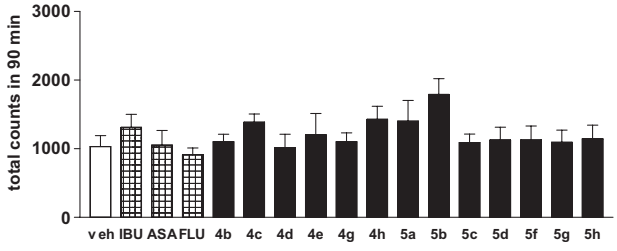


Figure 2. Locomotor activity, recorded in 90 min, starting 30 min later after ip administration of the vehicle, the reference drugs or the experimental compounds (5 mg/kg).

Particularly all tested compounds **4** display percentage of inhibition of acetic acid-induced writhing higher after po administration as compared to that of the same compounds obtained after ip administration. Furthermore compounds **4h**, **5f**, **5g** and **5h** induced even a significant higher analgesic activity as compared to that induced by the reference drugs ($P < 0.05$).

Since one of the common side effects of NSAID therapy is the gastrointestinal toxicity, the compounds that have shown analgesic activity (**4b**, **4c**, **4d**, **4e**, **4g**, **4h**, **5a**, **5b**, **5c**, **5d**, **5f**, **5g**, **5h**), were subsequently tested for their ability

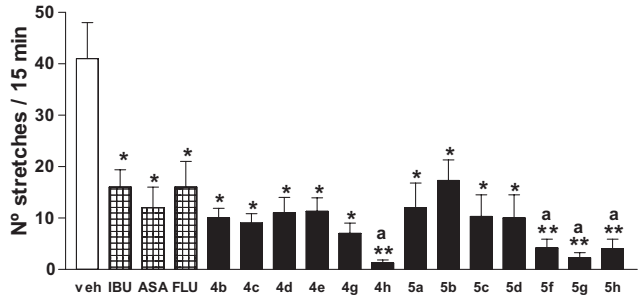


Figure 3. Stretches induced by ip acetic acid injection in rats pretreated po (60 min before) with vehicle, one of the reference drugs or one of the experimental new compounds (10 mg/kg). * $P < 0.05$, ** $P < 0.01$ as compared to vehicle pretreated rats; [a]$P < 0.05$ as compared to ibuprofen (ibu), aspirin (asa) and flufenamic acid (flu) pretreated rats.

to produce, gastric ulcers compared to those induced by the three reference drugs. The severity of gastric damage was expressed as lesion index (Table 2).

Compounds **4b–e**, **4g**, **4h**, **5a–d** and **5f–h** at the dose of 100 mg/kg induced a significantly lower (compounds **4b**, **4c**, **4e**, **5b–d** and **5f–h**, lesion index between 0.1 and 0.7, Table 2) or no (compounds **4d**, **4h** and **5a**, lesion index: 0, Table 2) ulcerogenic effect as compared to reference drugs.

Analysis of all pharmacological data indicates that analgesic activity appears to be related to some structural requirements, to the presence of a 3-carboxylic acid moiety (compounds **5**) and to 2-arylaminosubstituents. In fact the amidation of the carboxyl on 3-position is detrimental for analgesic activity. As matter of fact if we consider the most active pyridine-3-carboxylic acid **5g** (percentage of inhibition of acetic acid-induced writhing 97%) the corresponding pyridine-3-carboxamide **6g** shows a 18% percentage of inhibition of acetic acid-induced writhing. The same trend is displayed by less active compounds as **5e** (**5e**: 40%, **6e**: 31%). Similarly the esterification of 3-carboxylic function leds to the almost inactive compounds **7g** and **9g**. Independently from the kind of ester, methylic or ethylic, their values of percentage of inhibition of acetic acid-induced writhing are about the half of that showed by the parent acid **5g**. Generally substitution of 3-carboxylic group with a cyano moiety led to slight reduction in analgesic activity, except for compounds **5e** and **4e**, where this substitution have an helpful effect on the activity. The introduction of 2-methyl-5-chlorophenylamino or 2-methyl-6-chlorophenylamino functions at 2-position of showed by nitriles **4** and acids **5** is effective in inducing strong analgesic activity. The displacement of chlorine atom from 5- or 6-position to 4-position on the phenylamino ring produces variable effects: in nitriles (compound **4f**) is detrimental, while in acids the activity is maintained (compound **5f**). On the contrary displacement of chlorine atom from 5- or 6-position to 3-position does not affect activity in nitriles (compound **4e**), while it causes a drop in activity in acids (compound **5e**). When 2-methyl-6-chlorophenylamino group is replaced by a 2,6-dimethylphenylamino moiety as in **4d** and **5d** the activity is reduced.

Table 2. Gastric lesion index induced by an acute administration of the tested compounds (100 mg/kg po)

Compound	Ulcer index
ibu	37 ± 5.5
asa	58 ± 6.2
flu	4.83 ± 3
4b	0.5 ± 0.2*
4c	0.33 ± 0.2*
4d	0*
4e	0.7 ± 0.2*
4g	0.6 ± 0.2*
4h	0*
5a	0*
5b	0.1 ± 0.1*
5c	0.17 ± 0.1*
5d	0.5 ± 0.2*
5f	0.3 ± 0.15*
5g	0.17 ± 0.1*
5h	0.5 ± 0.1*
vehicle	0*

* $P < 0.01$ as compared to ibuprofen (ibu), aspirin (asa) and flufenamic acid (flu).

3. Experimental

3.1. Chemistry

Melting points were determined on a Stuart Scientific Melting point SMP1 and are uncorrected. Proton NMR spectra were recorded on a Varian Unity 300 spectrometer. The chemical shift are reported in part per million (δ, ppm) downfield from tetramethylsilane (TMS), which was used as internal standard. Infrared spectra were obtained with a Bruker Vector 22 spectrophotometer. Elemental analyses were carried out with a Carlo Erba model 1106 Elemental Analyzer and the values found were within ± 0.4% of theoretical values.

3.1.1. General procedure for the synthesis of 2-arylamino-6-trifluoromethylpyridin-3-carbonitriles 4. The appropriate arylamine (10 mmol) was added to a solution of 3-amino-3-ethoxypropenenitrile **1** (1.10 g, 10 mmol) in anhydrous acetonitrile (20 mL). The resulting solution was kept at room temperature for 4 days and then 1,1,1-trifluoro-4-*iso*-butoxy-3-buten-2-one (1.96 g, 10 mmol) was added. The resulting mixture was stirred at room temperature for 0.5 h and then refluxed for 6 h. Then solvent was removed to dryness. The resulting residue was tritured with isopropyl ether, the obtained solid was separated by filtration and recrystallized from a suitable solvent.

3.1.1.1. 2-((2-Methylphenyl)amino)-6-trifluoromethylpyridine-3-carbonitrile 4a. Yield 50%. Mp 138–140 °C (cyclohexane). ^{1}H NMR ($CDCl_3$): δ 2.34 (s, 3H, CH_3), 7.08–7.95 (m, 7H, aryl and NH). IR (Nujol) 3321, 2233,

1609, 1585 cm^{-1}. Anal. Calcd for $C_{14}H_{10}F_3N_3$: C, 60.65; H, 3.64; N, 15.16. Found: C, 60.60; H, 3.66; N, 15.20.

3.1.1.2. 2-((3-Trifluoromethylphenyl)amino)-6-trifluoromethylpyridine-3-carbonitrile 4b. Yield 24%. Mp 138–140 °C (cyclohexane). ^1H NMR ($CDCl_3$): δ 7.29–8.17 (m, 7H, aryl and NH). IR (Nujol) 3346, 2232, 1620, 1592 cm^{-1}. Anal. Calcd for $C_{14}H_7F_6N_3$: C, 50.77; H, 2.13; N, 12.69. Found: C, 50.70; H, 2.12; N, 12.71.

3.1.1.3. 2-((4-Trifluoromethylphenyl)amino)-6-trifluoromethylpyridine-3-carbonitrile 4c. Yield 58%. Mp 184–185 °C (cyclohexane). ^1H NMR ($CDCl_3$): δ 7.23–8.03 (m, 6H, aryl), 7.36 (s, 1H, NH). IR (Nujol) 3357, 2232, 1620, 1597, 1581 cm^{-1}. Anal. Calcd for $C_{14}H_7F_6N_3$: C, 50.77; H, 2.13; N, 12.69. Found: C, 50.72; H, 2.14; N, 12.66.

3.1.1.4. 2-((2,6-Dimethylphenyl)amino)-6-trifluoromethylpyridine-3-carbonitrile 4d. Yield 33%. Mp 184–185 °C (cyclohexane). ^1H NMR ($CDCl_3$): δ 2.22 (s, 6H, CH_3), 6.70 (s, 1H, NH), 7.03–7.92 (m, 5H, aryl). IR (Nujol) 3321, 2235, 1601, 1581 cm^{-1}. Anal. Calcd for $C_{15}H_{12}F_3N_3$: C, 61.85; H, 4.15; N, 14.43. Found: C, 61.80; H, 4.16; N, 14.40.

3.1.1.5. 2-((3-Chloro-2-methylphenyl)amino)-6-trifluoromethylpyridine-3-carbonitrile 4e. Yield 50%. Mp 152–154 °C (cyclohexane). ^1H NMR ($CDCl_3$): δ 2.37 (s, 3H, CH_3), 7.08 (s, 1H, NH), 7.13–7.97 (m, 5H, aryl). IR (Nujol) 3302, 2234, 1600, 1571 cm^{-1}. Anal. Calcd for $C_{14}H_9ClF_3N_3$: C, 53.95; H, 2.91; N, 13.48. Found: C, 53.90; H, 2.92; N, 13.50.

3.1.1.6. 2-((4-Chloro-2-methylphenyl)amino)-6-trifluoromethylpyridine-3-carbonitrile 4f. Yield 46%. Mp 150–152 °C (cyclohexane). ^1H NMR ($CDCl_3$): δ 2.31 (s, 3H, CH_3), 7.01 (s, 1H, NH), 7.05–7.97 (m, 5H, aryl). IR (Nujol) 3425, 2226, 1619, 1590, 1541 cm^{-1}. Anal. Calcd for $C_{14}H_9ClF_3N_3$: C, 53.95; H, 2.91; N, 13.48. Found: C, 53.99; H, 2.90; N, 13.45.

3.1.1.7. 2-((5-Chloro-2-methylphenyl)amino)-6-trifluoromethylpyridine-3-carbonitrile 4g. Yield 70%. Mp 148–150 °C (toluene). ^1H NMR ($CDCl_3$): δ 2.31 (s, 3H, CH_3), 7.01 (s, 1H, NH), 7.13–7.97 (m, 5H, aryl). IR (Nujol) 3426, 2226, 1619, 1590, 1541 cm^{-1}. Anal. Calcd for $C_{14}H_9ClF_3N_3$: C, 53.95; H, 2.91; N, 13.48. Found: C, 54.00; H, 2.90; N, 13.44.

3.1.1.8. 2-((6-Chloro-2-methylphenyl)amino)-6-trifluoromethylpyridine-3-carbonitrile 4h. Yield 83%. Mp 144–145 °C (cyclohexane). ^1H NMR ($CDCl_3$): δ 2.37 (s, 3H, CH_3), 7.08 (s, 1H, NH), 7.13–7.97 (m, 5H, aryl). IR (Nujol) 3303, 2234, 1600 cm^{-1}. Anal. Calcd for $C_{14}H_9ClF_3N_3$: C, 53.95; H, 2.91; N, 13.48. Found: C, 54.00; H, 2.90; N, 13.44.

3.1.2. General procedure for the synthesis of 2-arylamino-6-trifluoromethylpyridine-3-carboxylic acid 5. The appropriate pyridine-3-carbonitrile **4** (10 mmol) was added of 50% aqueous sulfuric acid solution (10 mL). The resulting solution was heated in boiling water bath for 6 h. After cooling the reaction mixture was diluted with water (50 mL) and the formed precipitate was separated by filtration, dried and recrystallized from a suitable solvent.

3.1.2.1. 2-((2-Methylphenyl)amino)-6-trifluoromethylpyridine-3-carboxylic acid 5a. Yield 88%. Mp 234–235 °C (toluene). ^1H NMR ($CDCl_3$): δ 2.20 (s, 3H, CH_3), 4.10 (br s, 1H, OH), 6.80-8.27 (m, 6H, aryl), 10.25 (s, 1H, NH). IR (Nujol) 3347, 1672, 1598, 1527 cm^{-1}. Anal. Calcd for $C_{14}H_{11}F_3N_2O_2$: C, 56.76; H, 3.74; N, 9.46. Found: C, 56.81; H, 3.76; N, 9.40.

3.1.2.2. 2-((3-Trifluoromethylphenyl)amino)-6-trifluoromethylpyridine-3-carboxylic acid 5b. Yield 85%. Mp 224–225 °C (toluene). ^1H NMR (DMSO-d_6): δ 4.20 (br s, 1H, OH), 7.31–8.47 (m, 6H, aryl), 10.25 (s, 1H, NH). IR (Nujol) 3321, 1686, 1612, 1592, 1550 cm^{-1}. Anal. Calcd for $C_{14}H_8F_6N_2O_2$: C, 48.01; H, 2.30; N, 8.00. Found: C, 48.07; H, 2.32; N, 7.97.

3.1.2.3. 2-((4-Trifluoromethylphenyl)amino)-6-trifluoromethylpyridine-3-carboxylic acid 5c. Yield 78%. Mp 234–235 °C (2-PrOH). ^1H NMR (DMSO-d_6): δ 6.89–8.75 (m, 6H, aryl), 10.75 (s, 1H, NH), 12.83 (br s, 1H, OH). IR (Nujol) 3212, 3170, 3136, 3106, 1678, 1637, 1623, 1604 cm^{-1}. Anal. Calcd for $C_{14}H_8F_6N_2O_2$: C, 48.01; H, 2.30; N, 8.00. Found: C, 47.97; H, 2.29; N, 8.04.

3.1.2.4. 2-((2,6-Dimethylphenyl)amino)-6-trifluoromethylpyridine-3-carboxylic acid 5d. Yield 65%. Mp 260 dec °C (toluene). ^1H NMR (DMSO-d_6): δ 2.04 (s, 6H, CH_3), 7.04–8.33 (m, 5H, aryl), 9.64 (s, 1H, NH). IR (Nujol) 3339, 1672, 1588 cm^{-1}. Anal. Calcd for $C_{15}H_{13}F_3N_2O_2$: C, 58.07; H, 4.22; N, 9.03. Found: C, 61.80; H, 4.16; N, 14.40.

3.1.2.5. 2-((3-Chloro-2-methylphenyl)amino)-6-trifluoromethylpyridine-3-carboxylic acid 5e. Yield 75%. Mp 234–235 °C (toluene). ^1H NMR (DMSO-d_6): δ 2.27(s, 3H, CH_3), 7.08–8.46 (m, 6H, aryl and NH), 11.10 (s, 1H, OH). IR (Nujol) 3337, 1668, 1617, 1580, 1541 cm^{-1}. Anal. Calcd for $C_{14}H_{10}ClF_3N_2O_2$: C, 50.85; H, 3.05; N, 8.47. Found: C, 50.90; H, 3.03; N, 8.50.

3.1.2.6. 2-((4-Chloro-2-methylphenyl)amino)-6-trifluoromethylpyridine-3-carboxylic acid 5f. Yield 90%. Mp 224–225 °C (toluene). ^1H NMR (DMSO-d_6): δ 2.24 (s, 3H, CH_3), 7.08–8.49 (m, 5H, aryl), 10.05 (s, 1H, NH). IR (Nujol) 3183, 3134, 1681, 1615, 1598, 1542 cm^{-1}. Anal. Calcd for $C_{14}H_{10}ClF_3N_2O_2$: C, 50.85; H, 3.05; N, 8.47. Found: C, 50.81; H, 3.06; N, 8.43.

3.1.2.7. 2-((5-Chloro-2-methylphenyl)amino)-6-trifluoromethylpyridine-3-carboxylic acid 5g. Yield 97%. Mp 218–220 °C (toluene). ^1H NMR ($CDCl_3$): δ 2.35 (s, 3H, CH_3), 7.19–8.43 (m, 5H, aryl), 11.17 (s, 1H, NH). IR (Nujol) 3333, 1678, 1614, 1587, 1537 cm^{-1}. Anal. Calcd

for $C_{14}H_{10}ClF_3N_2O_2$: C, 50.85; H, 3.05; N, 8.47. Found: C, 50.80; H, 3.07; N, 8.44.

3.1.2.8. 2-((6-Chloro-2-methylphenyl)amino)-6-trifluoromethylpyridine-3-carboxylic acid 5h. Yield 92%. Mp 214–215 °C (toluene). ^{1}H NMR (DMSO-d_6): δ 2.26 (s, 3H, CH_3), 7.15–8.43 (m, 6H, aryl and NH), 10.38 (s, 1H, OH). IR (Nujol) 3384, 3338, 1668, 1618, 1580, 1541 cm^{-1}. Anal. Calcd for $C_{14}H_{10}ClF_3N_2O_2$: C, 50.85; H, 3.05; N, 8.47. Found: C, 50.79; H, 3.04; N, 8.51.

3.1.3. General procedure for the synthesis of 2-arylamino-6-trifluoromethylpyridine-3-carboxamides 6. The appropriate pyridine-3-carbonitrile **4** (10 mmol) was added of 20% aqueous sodium hydroxide solution (10 mL). The resulting mixture was heated in boiling water bath for 4 h. After cooling the reaction mixture was diluted with water (50 mL) and the formed precipitate was separated by filtration, dried and recrystallized from a suitable solvent.

3.1.3.1. 2-((3-Chloro-2-methylphenyl)amino)-6-trifluoromethylpyridine-3-carboxamide 6e. Yield 86%. Mp 234–235 °C (toluene). ^{1}H NMR (DMSO-d_6): δ 2.27 (s, 3H, CH_3), 7.11–8.44 (m, 7H, aryl and NH_2), 10.43 (s, 1H, NH). IR (Nujol) 3488, 3165, 3125, 1663, 1619, 1592 cm^{-1}. Anal. Calcd for $C_{14}H_{11}ClF_3N_3O$: C, 51.00; H, 3.36; N, 12.74. Found: C, 50.96; H, 3.37; N, 12.76.

3.1.3.2. 2-((5-Chloro-2-methylphenyl)amino)-6-trifluoromethylpyridine-3-carboxamide 6g. Yield 98%. Mp 214–215 °C (toluene). ^{1}H NMR ($CDCl_3$) 2.28 (s, 3H, CH_3), 6.98 (s, 2H, NH_2), 7.00–7.93 (m, 5H, aryl), 10.51 (s, 1H, NH). IR (Nujol) 3462, 3184, 1681, 1615 cm^{-1}. Anal. Calcd for $C_{14}H_{11}ClF_3N_3O$: C, 51.00; H, 3.36; N, 12.74. Found: C, 51.06; H, 3.35; N, 12.70.

3.1.3.3. Methyl 2-((5-chloro-2-methylphenyl)amino)-6-trifluoromethylpyridine-3-carboxylate 7g. To a solution of acid **5g** (0.83 g, 2.5 mmol) in dry toluene (5 mL), DMF–DMA (0.66 mL, 5 mmol) was added. The resulting solution was heated at 90 °C for 2 h. The solvent was removed at reduced pressure obtaining ester. Yield 80%. Mp 99–100 °C (cyclohexane). ^{1}H NMR ($CDCl_3$): δ 2.34 (s, 3H, CH_3), 3.95 (s, 3H, OCH_3), 7.01–8.37 (m, 5H, aryl), 10.14 (s, 1H, NH). IR (Nujol) 1693, 1617, 1590, 1542 cm^{-1}. Anal. Calcd for $C_{15}H_{12}ClF_3N_2O_2$: C, 52.26; H, 3.51; N, 8.13. Found: C, 52.31; H, 3.50; N, 8.14.

3.1.4. General procedure for the synthesis of ethyl 2-arylamino-6-trifluoromethylpyridine-3-carboxylates 9. The appropriate arylamine (10 mmol) was added to a solution of ethyl 3-amino-3-ethoxypropenoate **8** (1.60 g, 10 mmol) in anhydrous acetonitrile (20 mL). The resulting solution was kept at room temperature for 6 days and then 1,1,1-trifluoro-4-*iso*-butoxy-3-buten-2-one (1.96 g, 10 mmol) was added. The resulting mixture was stirred at room temperature for 0.5 h and then refluxed for 3 h. Then solvent was removed to dryness and the resulting residue was treated with isopropyl ether, separated by filtration and recrystallized from a suitable solvent.

3.1.4.1. Ethyl 2-((5-chloro-2-methylphenyl)amino)-6-trifluoromethylpyridine-3-carboxylate 9g. Yield 80%. Mp 116–117 °C (hexane). ^{1}H NMR ($CDCl_3$): δ 1.44 (t, $J = 7.1$ Hz, 3H, CH_3), 2.37 (s, 3H, CH_3), 4.43 (q, $J = 7.1$ Hz, 2H, CH_2), 7.04–8.42 (m, 5H, aryl), 10.23 (s, 1H, NH). IR (Nujol) 3278, 1696, 1619, 1600, 1590 cm^{-1}. Anal. Calcd for $C_{16}H_{14}ClF_3N_2O_2$: C, 53.57; H, 3.93; N, 7.81. Found: C, 53.62; H, 3.91; N, 7.77.

3.1.4.2. Ethyl 2-((6-chloro-2-methylphenyl)amino)-6-trifluoromethylpyridine-3-carboxylate 9h. Yield 80%. Mp 90–91 °C (hexane). ^{1}H NMR ($CDCl_3$): δ 1.44 (t, $J = 7.1$ Hz, 3H, CH_3), 2.37 (s, 3H, CH_3), 4.43 (q, $J = 7.1$ Hz, 2H, CH_2), 7.04–8.42 (m, 5H, aryl), 10.23 (s, 1H, NH). IR (Nujol) 3318, 3262, 1693, 1616, 1601, 1587 cm^{-1}. Anal. Calcd for $C_{16}H_{14}ClF_3N_2O_2$: C, 53.57; H, 3.93; N, 7.81. Found: C, 53.52; H, 3.94; N, 7.84.

3.1.5. General procedure for the synthesis of 6-amino-5-cyano-6-arylamino-1,1,1-trifluoro-3,5-hexadien-2-ones 3. The appropriate arylamine (10 mmol) was added to a solution of 3-amino-3-ethoxypropenenitrile 1 (1.10 g, 10 mmol) in anhydrous acetonitrile (20 mL). The resulting solution was kept at room temperature for 4 days and then 1,1,1-trifluoro-4-*iso*-butoxy-3-buten-2-one (1.96 g, 10 mmol) was added. The resulting mixture was stirred at room temperature for 0.5 h and then refluxed for 3 h. Then solvent was removed to dryness. The resulting residue was constituted by a mixture of pyridine-3-carbonitriles **4** and 3,5-hexadienones **3**. These compounds were separated each other by treating the crude residue with EtOH, 3,5-hexadienones **3** precipitated and were separated by filtration; while pyridine-3-nitriles **4** were recovered from the ethanolic solution by removal of solvent.

3.1.5.1. 6-Amino-5-cyano-6-(3-trifluoromethylphenylamino)-1,1,1-trifluoro-3,5-hexadien-2-one 3b. Yield 40%. Mp 109–110 °C (cyclohexane). ^{1}H NMR ($CDCl_3$): δ 5.44 (s, 2H, NH_2), 7.05–8.09 (m, 6H, aryl, H-3 and H-4), 7.30 (s, 1H, NH). IR (Nujol) 3482, 3374, 3346, 2230, 1649, 1621, 1592 cm^{-1}. Anal. Calcd for $C_{14}H_9F_6N_3O$: C, 48.15; H, 2.60; N, 12.03. Found: C, 48.21; H, 2.59; N, 12.05.

3.1.5.2. 6-Amino-5-cyano-6-(4-trifluoromethylphenylamino)-1,1,1-trifluoro-3,5-hexadien-2-one 3c. Yield 32%. Mp 84–85 °C (cyclohexane). ^{1}H NMR ($CDCl_3$): δ 5.40 (s, 2H, NH_2), 5.74–7.71 (m, 6H, aryl, H-3 and H-4), 11.73 (s, 1H, NH). IR (Nujol) 3485, 3368, 2229, 1646, 1619, 1605, 1575 cm^{-1}. Anal. Calcd for $C_{14}H_9F_6N_3O$: C, 48.15; H, 2.60; N, 12.03. Found: C, 48.10; H, 2.61; N, 12.00.

3.1.5.3. 6-Amino-5-cyano-6-(3-chloro-2-methylphenylamino)-1,1,1-trifluoro-3,5-hexadien-2-one 3e. Yield 45%. Mp 114–115 °C (hexane). ^{1}H NMR ($CDCl_3$): δ

2.37, 2.46 (s, 3H, CH_3), 5.73–7.97 (m, 5H, aryl, H-3 and H-4). IR (Nujol) 3302, 2234, 1601, 1571 cm^{-1}. Anal. Calcd for $C_{14}H_{11}ClF_3N_3O$: C, 51.00; H, 3.36; N, 12.74. Found: C, 51.06; H, 3.35; N, 12.70.

3.2. Pharmacology

Pharmacological tests were conducted in adult male Sprague–Dawley rats of 250–300 g body/weight. Animal room was maintained on a 12 light/dark cycle. Food and water were freely available except for the acute ulcerogenesis test. All test compounds were suspended in a solution of methylcellulose (0.5%) and administered ip or po in a volume of 10 mL/kg body weight. All animal experimentations have been conducted in accordance with the guidelines for care and use of experimental animals of the European Communities Directive (86/609/EEC; D.L., 27.01.1992, number 116).

3.3. Writhing test

The Writhing test was performed by an ip injection of 1% aqueous acetic acid solution in a volume of 2 mL/kg body weight. Before acetic acid injection, rats were placed individually in the test cage (33 w, 56 l, 20 h, cm) and habituated for 30 min. Stretching movements consisting of arching of the back, development of tension in the abdominal muscles, elongation of the body and extension of forelimbs were counted by an experimenter unaware to the drug treatment for 15 min after the acetic acid injection. Rats received acetic acid injection 30 or 60 min after ip or po administration of the test compound, respectively. Screening of analgesic activity was performed after ip administration of compounds at the dose of 5 mg/kg. Compounds that showed comparable or greater analgesic activity than the three reference drugs (aspirin, ibuprofen and flufenamic acid) were screened at the dose of 10 mg/kg after po administration. Control rats received ip or po administration of vehicle (suspension of 0.5% methylcellulose). Ibuprofen, aspirin and flufenamic acid were used as reference drug at the same dose of the investigated compounds. The analgesic activity was expressed also, in terms of percentage of inhibition:

$$\%\ \text{Analgesic Activity} = n - n'/n\ 100$$

n = mean number of writhes of control group (vehicle-injected animals) and n' = mean number of writhes of test group.

Each experimental group consisted of eight rats.

3.4. Motor activity

Measurement of motor activity was conducted in a quiet and isolated room. Rats received an ip injection of the investigated compounds, one of the three reference drugs at the dose of 5 mg/kg or vehicle. 30 min later rats were put individually in cages equipped with two pairs of infrared photocell emitters and detectors situated along the long axis of each cage (Opto-Varimex, Columbus Instruments, Columbus, Ohio). Interruption of a photocell beam was detected by a counter that recorded the total number of interruptions caused by rat's movements during the test. Motor activity was recorded for 90 min. Each experimental group consisted of six rats.

3.5. Acute ulcerogenesis

The ability to produce gastric damage was evaluated according to previously reported method.[41–43] Ulcerogenic activity was evaluated after po administration of the test compound or one of the three reference drugs at the dose of 100 mg/kg. Control rats received po administration of vehicle (suspension of 0.5% methylcellulose). Food but not water was removed 24 h before administration of the test compound. Eight hours after po administration of the test compound, rats were anaesthetized with chloral hydrate and the stomach was extracted and dipped in 1% formaldehyde solution for about 15 min and then cut out along its great curvature. The number and the length of ulcers were detected using a microscope. The severity of the gastric lesion was measured along its greatest length (1 mm = rating of 1, 1–2 mm = rating of 2, >2 mm = rating according to their length in mm). The overall total of length was designated as the 'ulcer index'. Each experimental group consisted of six rats.

3.6. Statistics

Mean and standard error of the mean (SEM) of writhes, locomotor activity values, ulcers index were calculated for each experimental group. Significant differences were evaluated by one-way analysis of variance followed Dunnett's multiple comparison test.

References and notes

1. Brooks, P. M.; Day, R. O. *N. Engl. J. Med.* **1991**, *324*, 1716–1725.
2. Wallace, J. L. *Gastroenterology* **1997**, *112*, 1000–1016.
3. Laine, L. *Gastroenterology* **2001**, *120*, 594–606.
4. Vane, J. R. *Nature* **1971**, *231*, 232–235.
5. Malmberg, A. B.; Yaksh, T. L. *J. Pharmacol. Exp. Ther.* **1992**, *263*, 136–146.
6. Vane, J. R.; Bakhle, Y. S.; Botting, R. M. *Annu. Rev. Pharmacol. Toxicol.* **1998**, *38*, 97–120.
7. Tilley, S. L.; Coffman, T. M.; Koller, B. H. *J. Clin. Invest.* **2001**, *108*, 15–23.
8. Xie, W. L.; Chipman, J. G.; Robertson, D. L.; Erikson, R. L.; Simmons, D. L. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 2692–2696.
9. Dequeker, J.; Degner, F. *Inflamm. Res.* **2001**, *50*(Suppl. 1), S3–S4.
10. Dubois, R. N.; Abramson, S. B.; Crofford, L.; Gupta, R. A.; Simon, L. S.; Van De Putte, L. B.; Lipsky, P. E. *FASEB J.* **1998**, *12*, 1063–1073.
11. Jouzeau, J. Y.; Terlain, B.; Abid, A.; Nedelec, E.; Netter, P. *Drugs* **1997**, *53*, 563–582.

12. O'Banion, M. K. *Crit. Rev. Neurobiol.* **1999**, *13*, 45–82.
13. Siegle, I.; Klein, T.; Backman, J. T.; Saal, J. G.; Nusing, R. M.; Fritz, P. *Arthritis Rheum.* **1998**, *41*, 122–129.
14. Fitzgerald, G. A.; Patrono, C. *N. Engl. J. Med.* **2001**, *345*, 433–442.
15. Wallace, J. L. *Am. J. Med.* **1999**, *107*(Suppl 6A), S7–S11.
16. Sondhi, S. M.; Singhal, N.; Johar, M.; Reddy, B. S. N.; Lown, J. W. *Curr. Med. Chem.* **2002**, *9*, 1045–1074.
17. Effland, R. C.; Klein, J. T. Eur. Pat. Appl. EP 453,222, 1991.
18. Boschelli, D. H.; Connor, D. T.; Bornemeier, D. A.; Dyer, R. D.; Kennedy, J. A.; Kuipers, P. J.; Okonkwo, G. C.; Schrier, D. J.; Wright, C. D. *J. Med. Chem.* **1993**, *36*, 1802–1810.
19. Kumar, A.; Jaju, B. P.; Sinha, J. N. *Ind. J. Pharm. Sci.* **1990**, *1*, 257–260.
20. Goel, B.; Ram, T.; Tyagi, R.; Bansal, E.; Kumar, A.; Mukherjee, D.; Sinha, J. N. *Eur. J. Med. Chem.* **1999**, *34*, 265–269.
21. Sharma, S.; Srivastava, V. K.; Kumar, A. *Eur. J. Med. Chem.* **2002**, *37*, 689–697.
22. Kermak, W. O.; Weatherhead, A. P. *J. Chem. Soc.* **1942**, 726.
23. Stampa, A. Ger. Offen. 2,409,260; *Chem. Abstr*. **1975**, *82*, 156112s.
24. Jaouhari, R.; Quinn, P. *Heterocycles* **1994**, *38*, 2243–2246.
25. Sherlock, M. H. US Patent, US 3,839,344; *Chem. Abstr*. **1975**, *82*, 16705n.
26. Nantka-Namirski, P.; Kaczmarek, L. *Acta Polym. Pharm.* **1978**, *35*, 393–402; Nantka-Namirski, P.; Kaczmarek, L. *Chem. Abstr.* 1979, *90*, 203821z.
27. Nantka-Namirski, P.; Kaczmarek, L. *Acta. Polym. Pharm.* **1978**, *35*, 509–513; Nantka-Namirski, P.; Kaczmarek, L. *Chem. Abstr.* **1979**, *91*, 123609s.
28. Reisch, J.; Mester, I.; El-Moghazy Aly, S. M. *J. Chem. Soc., Perkin. Trans. 1* **1983**, 219–223.
29. Monge, A.; Narro, S.; Martinez-Crespo, F. J.; Lopez de Cerain, A.; Hamilton, E.; Barker, A. J. *Eur. J. Med. Chem.* **1994**, *29*, 441–445.
30. Ting, P. C.; Kaminski, J. J.; Sherlock, M. H.; Tom, W. C.; Lee, J. F.; Bryant, R. W.; Watnick, A. S.; McPhail, A. T. *J. Med. Chem.* **1990**, *33*, 2697–2706.
31. Laboratories U.P.S.A., Neth. Appl. 6,414,717; *Chem. Abstr*. **1966**, *64*, 712h.
32. Coppo, F. T.; Fawzi, M. M. *J. Heterocycl. Chem.* **1998**, *35*, 499–501.
33. Differding, E.; Frick, W.; Lang, R. W.; Martin, P.; Schimt, C.; Veenstra, S.; Greute, H. *Bull. Soc. Chim. Belg.* **1990**, *99*, 647.
34. Cocco, M. T.; Congiu, C.; Onnis, V. *J. Heterocycl. Chem.* **1995**, *32*, 543–545.
35. Koster, R.; Anderson, M.; De Beer, E. J. *Fed. Proc.* **1959**, *18*, 412.
36. Collier, H. O.; Dinneen, L. C.; Johnson, C. A.; Schneider, C. *Br. J. Pharmacol.* **1968**, *32*, 295–310.
37. Ikeda, Y.; Ueno, A.; Naraba, H.; Oh-ishi, S. *Life Sci.* **2001**, *69*, 2911–2919.
38. McCormack, K. *Drugs* **1994**, *47*(Suppl 5), 28–47.
39. Pong, S. F.; Demuth, S. M.; Kinney, C. M.; Deegan, P. *Arch. Int. Pharmacodyn. Ther.* **1985**, *273*, 212–220.
40. Dubinsky, B.; Gebre-Mariam, S.; Capetola, R. J.; Rosenthale, M. E. *Agents Actions* **1987**, *20*, 50–60.
41. Verna, M.; Sinha, J. N.; Guijrati, V. R. *Pharmacol. Res. Commun.* **1981**, *13*, 967–969.
42. Suleyman, H.; Akcay, F.; Altinkaynak, K. *Pharmacol. Res.* **2002**, *45*, 155–158.
43. Cocco, M. T.; Congiu, C.; Onnis, V.; Morelli, M.; Cauli, O. *Eur. J. Med. Chem.* **2003**, *38*, 513–518.