

Laboratory note

Synthesis of ibuprofen heterocyclic amides and investigation of their analgesic and toxicological properties

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Received 12 November 2002; received in revised form 31 January 2003; accepted 5 March 2003

Abstract

A series of amides of ibuprofen with heteroaromatic amines was synthesized and assayed *in vivo* for their analgesic properties by means of writhing test in rats. When compared to parent ibuprofen some of the new amides exhibited a comparable or improved analgesic activity and a lower ulcerogenic effect.

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Keywords: Ibuprofen amides; Analgesic activity; Ulcerogenic activity; Aminopyridine; Aminopyrimidine

1. Introduction

Traditional anti-inflammatory drugs (NSAIDs) are used in treatment of mild to moderate pain and as an adjunct to opioids in the management of moderate to severe pain [1]. The clinical effects of NSAIDs are based on the inhibition of the enzyme cyclooxygenase (COX), which catalyses the rate limiting step in the formation of prostanoids, prostaglandins (PGs) and thromboxane A₂ (TxA₂) [2,3]. PGs are ubiquitous compounds that mediate a variety of physiologic and pathologic processes. Under normal physiologic conditions, PGs play an essential homeostatic role in cytoprotection of gastric mucosa, hemostasis, renal function, gestation and parturition [4–6].

The production of PGs is induced at sites of inflammation, where they are involved in propagation of inflammation, pain and fever. Some of these pathologic effects may be mediated by increased synthesis of PGs within the peripheral and central nervous system. Inhibition of PGs production alleviates most of the pathologic effects associated with inflammation, but it also interferes with the physiologic role of these molecules. Consequently, long-term therapy with traditional NSAIDs is frequently limited by their adverse

effects, particularly those caused by gastrointestinal bleeding, ulceration and perforation [7–9], inhibition of TxA₂ synthesis in platelet, nephrotoxicity. The discover of novel NSAIDs that have high efficacy as well as slight side effects represents one of the main objectives in current pain research.

Ibuprofen, namely 2-(4-isobutylphenyl) propionic acid is a traditional NSAID that belongs to arylpropionic acid family and that is largely employed for its analgesic, anti-inflammatory and anti-pyretic properties. Ibuprofen is available under prescription, primarily for the treatment of inflammatory and painful disorders including rheumatoid arthritis, ankylosing spondylitis, osteoarthritis, acute gouty arthritis, postoperative pain, postpartum pain and soft tissue injuries, generally at doses up to 3200 mg day⁻¹. Ibuprofen is also available as a non-prescription drug, primarily for the treatment of symptoms of pain and fever including headache, migraine, rheumatic pain, muscular pain, backache, neuralgia, dysmenorrhoea, dental pain and colds and flu, generally at doses up to 1200 mg day⁻¹ [10]. Similar to other prototypical NSAIDs, ibuprofen can cause gastric mucosal damage, which may result in ulceration and/or bleeding [9]. Several patents can be found on ibuprofen derivatives, its association and its salts with various amines [11–21] and it is well known that derivatives of arylpropionic acids as esters and amides may retain the activity of the parent acids and decrease their gastrointestinal toxicity [22,23]. There is strong evidence that inhibition of COX-1 rather than inhibition

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of COX-2 underlies this gastrointestinal toxicity [24]. There are in fact two isoforms of the COX [25,26]: the constitutive COX-1 that is detectable in most normal tissue, including human stomach, kidney and platelets [25] and COX-2 that generally is rapidly induced in response to inflammatory stimuli by endotoxin, mitogens and cytokines [27,28]. Ibuprofen, like other prototypical NSAIDs, inhibits both COX-1 and COX-2. Among numerous studies dedicated to amides of arylpropionic acids, only a few have been devoted to amides of ibuprofen with heteroaromatic amines [29,30]. On the other hand pyridine and pyrimidine rings occur in a great variety of substances that play a vital role in biological processes. Furthermore antibacterial [31–36], cholinergic [37,38] and analgesic [39–44] properties have been described for several molecules that contain these nuclei. Prompted by the above observations we became interested into heteroaromatic ibuprofen amides designed to obtain novel NSAIDs that could retain the analgesic activity of the parent compound as well as reduced gastrointestinal side effects. So we undertook a study on the influence of the type and position of substituents on heterocyclic moiety of ibuprofen amides and in this paper we report the synthesis, the pharmacological and toxicological properties of a new series of ibuprofen amides.

2. Chemistry

The two methods utilized for the synthesis of *N*-(pyridyl or pyrimidyl)-2-(4-isobutyl-phenyl)propionamides **1–11** are outlined in Fig. 1 and both utilized commercially available ibuprofen and heterocyclic amines as starting materials. Ibuprofen was treated with thionyl chloride to generate acid chloride [45], this, after purification by distillation, was reacted with an appropriately substituted amine in the presence of potassium carbonate to yield the desired amides (Method A). Since this synthetic procedure gave some troubles in purification step of the target amides, due to

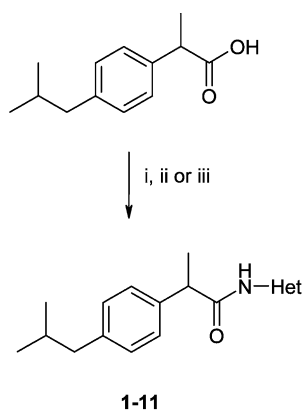


Fig. 1. Synthetic pathways to ibuprofen amides **1–11**. i: SOCl_2 , reflux; ii: Het-NH_2 , K_2CO_3 , benzene, reflux; iii: Het-NH_2 , CDI, CH_2Cl_2 , reflux.

partial decomposition of starting amines, propionamides **1–11** were also synthesized starting directly from ibuprofen and the appropriate amine in the presence of 1,1-carbonyldiimidazole (Method B). This last synthetic pathway is milder, utilizes less toxic reagents and gives better yields respect to method A.

3. Pharmacology

All compounds **1–11** were screened for analgesic activity using the test of acetic acid-induced writhing in the rats [46] which is a sensitive and predictive animal model for analgesic drugs as shown by the good correlation between values obtained in rats and analgesic doses in humans [47]. In order to avoid wasting of rats for inactive compounds, we employed a two-step activity screening model. In the first step, rats received intraperitoneal (i.p.) acetic acid injection 30 min after i.p. administration of the test compound (as shown in Fig. 2). The most active compounds **2**, **5**, **6**, **9** and **10**, that showed an analgesic activity improved or comparable to ibuprofen, were selected for secondary evaluation in which oral bioavailability was assessed. This second step allows us to assess if they are orally active since a favourite property of new drugs is to be effective after per os (p.o.) administration. In the second step analgesic activity of the test compound was measured 60 min after p.o. administration (results are shown in Fig. 3). These compounds were also tested for their ulcerogenic activity [48,49] as listed in Table 3. The severity of gastric lesions was measured 8 h after p.o. administration of the test compound and the results expressed as lesion index.

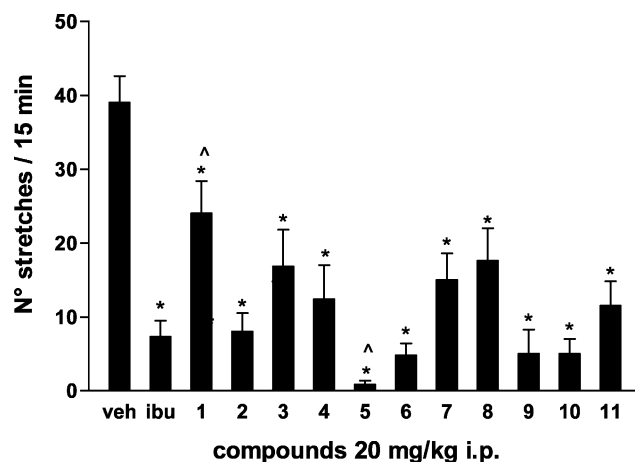


Fig. 2. Analgesic activity of amides **1–11** in the acetic acid-induced writhing test. Mean \pm standard error of the mean (SEM) of number of stretches recorded in 15 min after acetic acid injection (see Method). Thirty minutes before acetic acid injection, rats received an i.p. injection of vehicle, ibuprofen or one of the amides **1–11** at the dose of 20 mg kg^{-1} . $N=8$ each group. Significance was evaluated by Newman–Keuls post hoc test. (*) $P < 0.05$ as compared to vehicle-pretreated rats; (^) $P < 0.05$ as compared to ibuprofen-pretreated rats.

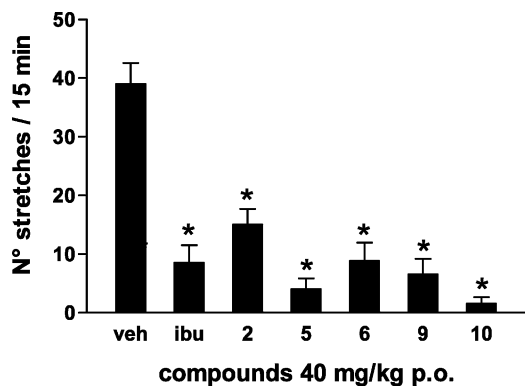


Fig. 3. Analgesic activity of amides **2**, **5**, **6**, **9** and **10** in the acetic acid-induced writhing test after p.o. administration. Mean \pm S.E.M. of number of stretches recorded in 15 min after acetic acid injection (see Method). Sixty minutes before acetic acid injection, rats received a p.o. administration of vehicle, ibuprofen or one of the amides **2**, **5**, **6**, **9** and **10** at the dose of 40 mg kg⁻¹. $N=8$ each group. Significance was evaluated by Newman–Keuls post hoc test. (*) $P < 0.05$ as compared to vehicle-pretreated rats.

4. Results and discussion

Analgesic properties of amides **1–11** have been compared with those of the parent acid. Compounds equally or more potent than ibuprofen were further investigated to isolate those with highest analgesic activity and reduced toxicity. After i.p. administration all compounds **1–11** showed significant analgesic activity ($P < 0.05$) as compared with vehicle injected rats (Fig. 2). *N*-(Pyridin-2-yl)propionamide **1** and *N*-(6-methylpyridin-2-yl) propionamide **5** showed significantly higher and lower number of writhes, respectively, as compared to ibuprofen ($P < 0.05$, Fig. 2). The percentage of inhibition of acetic acid-induced writhing (Table 1) revealed that compounds **2**, **5**, **6**, **9** and **10** at the dose of 20 mg kg⁻¹ displayed a greater (compounds **5**, **6**, **9** and **10**) or similar (compound **2**) analgesic activity as compared to parent ibuprofen. After p.o. administration, compounds **2**, **5**, **6**, **9** and **10** at the dose of 40 mg kg⁻¹ demonstrated noteworthy analgesic activity ($P < 0.05$) as compared to vehicle injected animals indicating that they possess a good oral bioavailability (Fig. 3). Compounds **2**, **5**, **6**, **9** and **10** at the dose of 100 mg kg⁻¹ induced a significantly lower (compounds **5** and **6**, lesion index: 0.5 and 3, respectively, Table 3) or no (compounds **2**, **9** and **10**, lesion index: 0, Table 3) ulcerogenic effect as compared to the parent ibuprofen. There are two main components of gastric damaging properties of NSAIDs, an irritating effect dependent upon direct contact with the mucosa, related to the presence of acidic groups in the molecules, and a systemic effect attributable to the inhibition of PGs synthesis [23,50,51]. Both components contribute to the ulcerogenic activity of ibuprofen [50]. Each of these mechanisms has a different time course for the injury induction, and the acute toxicity should be principally influenced by the former component. So we can hypothesise that reduced

toxicity of ibuprofen amides could be partly due to the amidation of the free carboxylic group of ibuprofen, with the consequent reduction of the topical irritating action. Another tentative justification for the low ulcerogenicity of these compounds could be a consequence of their inhibitory effects on COX-2 rather than COX-1. Recent reports show that this is the case of a series of ester and amide derivatives of arylacetic and fenamic acids [52,53].

Analysis of all pharmacological data shows that the best activity is presented by *N*-(6-methylpyridin-2-yl)-propionamide **5**, as matter of fact this derivative showed a 98% inhibition of acetic acid-induced writhing associated with very low ulcerogenic effects. High analgesic activity was also obtained when the *N*-pyridin-2-yl moiety is 3-methylsubstituted as in compound **6**. In contrast, shift of methyl group in 4- or 5-position (amides **3** and **4**) led to a drop in the analgesic activity. The introduction of another methyl group on *N*-pyridin-2-yl ring seemed to not improve the analgesic properties of these molecules, while lack of substituents on pyridin-2-yl ring is detrimental for activity. Good activity is also shown by *N*-pyridin-3-yl-propionamides, above all, when a chlorine atom is introduced in 2-position of heterocyclic moiety as in compound **9**. This last compound exhibited analgesic activity and no ulcerogenic effects similar to *N*-(pyrimidin-2-yl)-2-(4-isobuty-phenyl)propionamide **10**; conversely the introduction of methyl groups on *N*-pyrimidin-2-yl moiety was associated to a weaker analgesic activity.

In conclusion the transformation of ibuprofen into definite heterocyclic amides does not alter the analgesic activity. Some of the amides described in the present study exhibited reduced gastric ulcerogenicity when compared with ibuprofen. Some of amides described herein might be potential candidates as safer analgesic drugs.

5. Experimental protocols

5.1. Chemistry

Melting points were determined on a Stuart Scientific Melting point SMP1 and are uncorrected (Table 1). IR spectra were recorded on Nujol mulls between salt plates, unless otherwise indicated, in Bruker Vector 22 spectrophotometer (Table 2). ¹H-NMR spectra were recorded, in CDCl₃ solution, on a Varian Unity 300 spectrometer. The chemical shift are reported in part per million (δ , ppm) downfield from tetramethylsilane, which was used as internal standard (Table 2). Elemental analyses were carried out with a Fisons EA 1108 elemental analyzer. Analytical TLC were carried out on Merck 0.2 mm precoated silica gel aluminium sheets (60 F-254) eluting with light petroleum–ethyl ether (2:1). Analyses of C, H, N were within $\pm 0.4\%$ of the

Table 1
Yields, physical data and analgesic activity (20 mg kg⁻¹ i.p.) of amides **1–11**

Comp.	Het.	Yield (%)		M.p. (recryst. solvent)	Formula (F.W.)	% inhibition acetic acid-induced writhing
		Method A	Method B			
1	pyridin-2-yl	71	80	103–104 (Hexane)	C ₁₈ H ₂₂ N ₂ O (282.38)	38
2	pyridin-3-yl	68	77	120–121 (Cyclohexane)	C ₁₈ H ₂₂ N ₂ O (282.38)	80
3	4-Me-pyridin-2-yl	75	90	69–70 (Hexane)	C ₁₉ H ₂₄ N ₂ O (294.21)	57
4	5-Me-pyridin-2-yl	72	88	91–92 (Hexane)	C ₁₉ H ₂₄ N ₂ O (294.21)	68
5	6-Me-pyridin-2-yl	78	98	87–88 (Hexane)	C ₁₉ H ₂₄ N ₂ O (294.21)	98
6	3-Me-pyridin-2-yl	74	97	93–94 (Hexane)	C ₁₉ H ₂₄ N ₂ O (294.21)	88
7	4,6-Me ₂ -pyridin-2-yl	80	95	79–80 ^a (Hexane)	C ₂₀ H ₂₆ N ₂ O (310.20)	62
8	4-Cl-pyridin-2-yl	82	95	89–90 (Benzene)	C ₁₈ H ₂₁ ClN ₂ O (316.82)	55
9	2-Cl-pyridin-2-yl	80	98	Oil	C ₁₈ H ₂₁ ClN ₂ O (316.82)	87
10	Pyrimidin-2-yl	82	98	Oil	C ₁₇ H ₂₁ N ₃ O (283.38)	87
11	4,6-Me ₂ -pyrimidin-2-yl	75	95	93–94 (Hexane)	C ₁₉ H ₂₅ N ₃ O (310.42)	70
Vehicle						0
Ibuprofen						80

^a Lit. 69–70 °C [30].

theoretical values. 2-(4-Isobutyl-phenyl)propionyl chloride was prepared as previously described [45].

5.2. General procedures for synthesis of amides (**1–11**)

5.2.1. Method A

To a stirred mixture of the appropriate amine (0.02 mol) and potassium carbonate (5.52 g, 0.04 mol) in anhydrous benzene (60 mL) ibuprofen acid chloride [45] (4.5 g, 0.02 mol) was added drop wise. After heating at reflux for 96 h the solvent was evaporated and the residue was taken up with CHCl₃ and washed with water. The organic layer was dried over sodium sulfate, filtered, and concentrated in vacuo. The resultant residue was purified by column chromatography on silica gel60 (230–400 Mesh, Merck), eluting with light petroleum–ethyl ether (2:1) to give amides **1–11** (Tables 1 and 2).

5.2.2. Method B

1,1'-Carbonyldiimidazole (3.89 g, 0.024 mol) was added to a solution of ibuprofen (4.13 g, 0.02 mol) in 20 mL of dichloromethane. After the reaction mixture was stirred at r.t. for 30 min the appropriate amine (0.02 mol) was added and the reaction mixture was heated at reflux until disappearance of starting material detected by analytical TLC (72 h). After cooling the dichloromethane solution was washed consecutively with water, saturated sodium hydrogen carbonate and water. The organic layer was dried over sodium sulfate, filtered, and concentrated in vacuo. The resultant residue was purified as described in method A.

5.3. Pharmacology

Pharmacological tests were conducted in adult male Sprague–Dawley rats weighing 250–300 g. 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 i.p. or p.o. in a volume of 10 mL kg⁻¹ 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).

5.3.1. Writhing test

The writhing test [46] was performed by an i.p. injection of 1% aqueous acetic acid solution in a volume of 2 mL kg⁻¹ 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 i.p. or p.o. administration of the test compound, respectively. Screening of analgesic activity was performed after i.p. administration of compounds at the dose of 20 mg kg⁻¹. In order to avoid wasting of animals, amides that displayed a similar or higher analgesic activity than ibuprofen were administered at the dose of 40 mg kg⁻¹ p.o. and then acetic acid writhing test performed. Control rats received i.p. or p.o administration of vehicle (suspension of 0.5% methylcellulose). Ibuprofen was used as refer-

Table 2
IR and ¹H-NMR data of compounds **1–11**

Comp.	IR (ν, cm ⁻¹)	¹ H-NMR (δ, ppm)
1	3266, 3197, 1666, 1591	0.87 (d, <i>J</i> = 6.7 Hz, 6H, CH ₃), 1.55 (d, <i>J</i> = 7.1 Hz, 3H, CH ₃), 1.82 (hept, <i>J</i> = 6.7 Hz, 1H, CH), 2.42 (d, <i>J</i> = 7.1 Hz, 2H, CH ₂), 3.67 (q, <i>J</i> = 7.1 Hz, 1H, CH), 6.95, 7.64, 8.17 (m, 4H, Py), 7.10, 7.20 (d, <i>J</i> = 8.0 Hz, 4H, Ar), 7.84 (s, 1H, NH)
2	3037, 1695, 1606, 1585	0.86 (d, <i>J</i> = 6.7 Hz, 6H, CH ₃), 1.54 (d, <i>J</i> = 7.1 Hz, 3H, CH ₃), 1.82 (hept, <i>J</i> = 6.7 Hz, 1H, CH), 2.43 (d, <i>J</i> = 7.1 Hz, 2H, CH ₂), 3.69 (q, <i>J</i> = 7.1 Hz, 1H, CH), 7.16, 8.08, 8.22, 8.36 (m, 4H, Py), 7.09, 7.21 (d, <i>J</i> = 8.0 Hz, 4H, Ar), 7.66 (s, 1H, NH)
3	3274, 3148, 1668, 1613, 1568	0.86 (d, <i>J</i> = 6.7 Hz, 6H, CH ₃), 1.54 (d, <i>J</i> = 7.1 Hz, 3H, CH ₃), 1.82 (hept, <i>J</i> = 6.7 Hz, 1H, CH), 2.30 (s, 3H, CH ₃), 2.42 (d, <i>J</i> = 7.1 Hz, 2H, CH ₂), 3.68 (q, <i>J</i> = 7.1 Hz, 1H, CH), 6.78, 7.98, 8.07 (m, 3H, Py), 7.23, 7.28 (d, <i>J</i> = 8.0 Hz, 4H, Ar), 8.23 (s, 1H, NH)
4	3275, 3053, 1690, 1668, 1613, 1567	0.86 (d, <i>J</i> = 6.7 Hz, 6H, CH ₃), 1.54 (d, <i>J</i> = 7.1 Hz, 3H, CH ₃), 1.80 (hept, <i>J</i> = 6.7 Hz, 1H, CH), 2.31 (s, 3H, CH ₃), 2.41 (d, <i>J</i> = 7.1 Hz, 2H, CH ₂), 3.66 (q, <i>J</i> = 7.1 Hz, 1H, CH), 6.79, 8.03, 8.18 (m, 3H, Py), 7.09, 7.21 (d, <i>J</i> = 8.0 Hz, 4H, Ar), 7.99 (s, 1H, NH)
5	3301, 3253, 3087, 3048, 1672, 1631, 1568	0.89 (d, <i>J</i> = 6.3 Hz, 6H, CH ₃), 1.51 (d, <i>J</i> = 7.1 Hz, 3H, CH ₃), 1.84 (hept, <i>J</i> = 6.3 Hz, 1H, CH), 2.10 (s, 3H, CH ₃), 2.43 (d, <i>J</i> = 7.1 Hz, 2H, CH ₂), 3.70 (q, <i>J</i> = 7.1 Hz, 1H, CH), 6.20 (s, 1H, NH), 6.57, 7.32, 7.75 (m, 3H, Py), 7.09, 7.28 (d, <i>J</i> = 8.0 Hz, 4H, Ar)
6	3281, 1666, 1601, 1578, 1538	0.89 (d, <i>J</i> = 6.7 Hz, 6H, CH ₃), 1.51 (d, <i>J</i> = 7.1 Hz, 3H, CH ₃), 1.84 (hept, <i>J</i> = 6.7 Hz, 1H, CH), 2.36 (s, 3H, CH ₃), 2.43 (d, <i>J</i> = 7.1 Hz, 2H, CH ₂), 3.69 (q, <i>J</i> = 7.1 Hz, 1H, CH), 5.80 (s, 1H, NH), 6.30, 6.42, 7.35 (m, 3H, Py), 7.09, 7.28 (d, <i>J</i> = 8.0 Hz, 4H, Ar)
7	3204, 3051, 1702, 1688, 1616, 1569	0.87 (d, <i>J</i> = 4.9 Hz, 6H, CH ₃), 1.54 (d, <i>J</i> = 5.5 Hz, 3H, CH ₃), 1.82 (hept, <i>J</i> = 4.9 Hz, 1H, CH), 2.27 (s, 3H, CH ₃), 2.32 (s, 3H, CH ₃), 2.43 (d, <i>J</i> = 5.5 Hz, 2H, CH ₂), 3.63 (q, <i>J</i> = 5.5 Hz, 1H, CH), 6.66, 7.87 (s, 2H, Py), 7.09, 7.22 (d, <i>J</i> = 8.0 Hz, 4H, Ar), 7.70 (s, 1H, NH)
8	3279, 3025, 1668, 1587, 1571	0.87 (d, <i>J</i> = 6.6 Hz, 6H, CH ₃), 1.64 (d, <i>J</i> = 7.0 Hz, 3H, CH ₃), 1.86 (hept, <i>J</i> = 6.6 Hz, 1H, CH), 2.48 (d, <i>J</i> = 7.0 Hz, 2H, CH ₂), 3.78 (q, <i>J</i> = 7.0 Hz, 1H, CH), 7.20, 7.28 (m, 4H, Ar), 7.60, 8.08, 8.18 (m, 3H, Py), 7.83 (s, 1H, NH)
9	3373, 1697, 1582 ^a	0.90 (d, <i>J</i> = 6.7 Hz, 6H, CH ₃), 1.54 (d, <i>J</i> = 7.1 Hz, 3H, CH ₃), 1.82 (hept, <i>J</i> = 6.7 Hz, 1H, CH), 2.42 (d, <i>J</i> = 7.1 Hz, 2H, CH ₂), 3.67 (q, <i>J</i> = 7.1 Hz, 1H, CH), 7.10, 7.20 (d, <i>J</i> = 8.0 Hz, 4H, Ar), 7.04, 8.03, 8.72 (m, 3H, Py), 7.62 (s, 1H, NH)
10	3240, 1731, 1579 ^a	0.90 (d, <i>J</i> = 6.7 Hz, 6H, CH ₃), 1.52 (d, <i>J</i> = 7.1 Hz, 3H, CH ₃), 1.83 (hept, <i>J</i> = 6.7 Hz, 1H, CH), 2.44 (d, <i>J</i> = 7.1 Hz, 2H, CH ₂), 3.72 (q, <i>J</i> = 7.1 Hz, 1H, CH), 7.11, 7.24 (d, <i>J</i> = 7.9 Hz, 4H, Ar), 7.16, 8.81 (m, 3H, Pym), 7.80 (s, 1H, NH)
11	3260, 3195, 1689, 1601, 1564	0.86 (d, <i>J</i> = 5.0 Hz, 6H, CH ₃), 1.54 (d, <i>J</i> = 5.5 Hz, 3H, CH ₃), 1.81 (m, 1H, CH), 2.37 (s, 6H, CH ₃), 2.42 (d, <i>J</i> = 5.5 Hz, 2H, CH ₂), 4.04 (m, 1H, CH), 6.67 (s, 1H, Pym), 7.08, 7.24 (m, 4H, Ar), 7.78 (s, 1H, NH)

^a Neat.Table 3
Ulcerogenic effect of amides **2, 5, 6, 9, 10**

Compounds	Lesion index (mm)
Vehicle	0
Ibuprofen	36.75 ± 5
2	0 *
5	0.5 ± 0.2 *
6	3 ± 1 *
9	0 *
10	0 *

Significance was evaluated by Newman–Keuls post hoc test. *N* = 6 per group.* *P* < 0.01 as compared to ibuprofen lesion index.

ence drug. The analgesic activity was expressed also, in terms of percentage of inhibition:

$$\% \text{ analgesic activity} = n - n' / n \times 100$$

where *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.

5.3.2. Acute ulcerogenesis

Acute ulcerogenesis test was done according to Verna et al. and Suleyman et al. [48,49]. Ulcerogenic activity was evaluated after p.o. administration of the test compound or ibuprofen at the dose of 100 mg kg⁻¹. Control rats received p.o. 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 p.o. 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.

5.3.3. Statistics

Mean and mean standard error (S.E.M.) of writhes and index ulcers were calculated for each experimental

group. Significant differences were evaluated by Neu-
mann-Keuls post-hoc test.

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