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Laboratory note

Synthesis and antinociceptive properties of new structurally planned imidazo[1,2-*a*]pyridine 3-acylarylhydrazone derivatives†

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Abstract – This paper describes recent results of a research program aiming at the synthesis and pharmacological evaluation of new *N*-heterocyclic functionalized acylarylhydrazone compounds, belonging to 3-acyl-(2-methyl-imidazo[1,2-*a*]pyridinyl)-arylhydrazone **2** series. These compounds were structurally planned applying classical ring bioisosterism strategies on previously described 4-acyl-(*N*-phenylpyrazolyl)-arylhydrazone **1**, which presented important analgesic properties, in order to identify the pharmacophore contribution of the acylarylhydrazone moiety and investigate the structure–activity relationship (SAR) in these series. The results herein disclosed indicate that this strategy of molecular modification gave rise to a new series of analgesic and anti-inflammatory agents, where the activity seems to be more dependent on the nature of the *para*-substituent at the pharmacophore acylarylhydrazone (AAH) moiety, than the *N*-heterocyclic acyl-ring pattern. © Elsevier, Paris

imidazo[1,2-*a*]pyridine / acylarylhydrazone / 2-methyl-imidazo[1,2-*a*]pyridine 3-acylarylhydrazone derivative / analgesic activity / anti-inflammatory activity

1. Introduction

The therapeutic benefits of inhibitors of the arachidonic acid (AA) cascade enzymes have well been established for a number of pathological conditions that involve inflammation, bronchial asthma, allergy and thromboembolic diseases [1]. These therapeutical effects are based on the understanding that the products of AA metabolism play an important role in the pathogenesis of these disorders. In fact, while a number of mechanisms have been proposed to explain the pharmacological effects of non-steroidal anti-inflammatory drugs (NSAID'S), it is believed that inhibition of icosanoid biosynthesis, at COX or PGHS level, is one of greatest importance [2]. The discovery of the 5-LO pathway of arachidonic acid (AA) metabolism, as well the recognition of leukotrienes (LTs) act by stimulating the degranulation, aggregation chemotaxis and chemokinesis of polimorphonuclear (PMN) leukocytes, enhancing the cell–cell recognition to initiate neutrophil and eosinophil aggregation and calcium mobilization, favoring the release of degradation enzymes. These findings indicated the obvious therapeutic benefit of the COX/5-LO inhibition for the treatment of chronic inflammatory states [3]. The involvement of icosanoids and other agents, as mediators of pain after noxious stimuli can explain the analgesic properties of AAC enzymes inhibitors in relieving the moderate pain [4].

In the course of a research program aiming at synthesis and pharmacological evaluation of new bioactive compounds, structurally planned to acting at arachidonic acid cascade (AAC) enzymes level, we described previously the analgesic and platelet anti-aggregatory profile of the pyrazolic 4-acylarylhydrazone derivatives **1a,b**. Compound **1a** displayed significant platelet-antiaggregating properties at 100 μM in the arachidonic acid induced model [5], whereas the derivative **1b** showed a potency 11-fold greater than dypirone as analgesic agent p.o., at the same molar concentration [6].

These results conduct us to design new structurally related derivatives, where the nature of the *N*-hetero-

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cyclic ring present in the lead compound **1** was modified assuming that the acylarylhydrazone (AAH) group and the *para*-substituent in the aryl moiety would be an important pharmacophore to analgesic activity [7, 8]. Thus, we described in this paper the synthesis and antinociceptive profile of a new series of functionalized imidazo[1,2-*a*]pyridine 3-acylarylhydrazone derivatives **2a–i**. The structure of these new derivatives **2a–i** was rationally designed applying the bioisosterism concept [9] on compound **1**, represented by *ortho*-ring fusion of the phenyl ring placed at N-1 of pyrazole ring in **1** which was moved to C-5 in the imidazo[1,2-*a*]pyridine nucleus present in **2** rising the new heterocyclic system. This structural modification represents a classical bioisosteric replacement of aromatic nitrogen atoms for the CH group as shown in *figure 1*. Using this rational basis, we can evaluate the eventual lipophilic contribution of the electron-π number present in the heterocyclic moiety, i.e. 6-π in **1** versus 10-π electrons in **2**, in the anti-inflammatory and analgesic activity of the new series of compounds **2a–i**. Furthermore, the structural modification promoted in **2** with respect to the *N*-phenylpyrazole **1** series, adopted here as lead-series, introduced a steric liberation of the corresponding N-1 lone-pair, hindered in the lead-series **1** (*figure 1*). In fact, Albuquerque et al. [10] have shown, from previous work of these laboratories, that *N*-phenyl pyrazole derivatives, similar to **1**, presents an important steric hindrance at N-2 due the *quasi*-plane relationship between both aromatic rings of the phenylpyrazole system. Thus, we considered these structural modifications as a strategy to effect the optimization of the previously described *N*-phenyl-pyrazole **1** series.

The anticipated planar nature of the imidazo[1,2-*a*]-pyridine system, in the new derivatives **2**, was briefly investigated by performing a molecular mechanics analysis [11]. For instance, using the Hamiltonian AM1 in the MOPAC 7.0 program as tools, we are able to observe an *s-cis* like preferred conformation to the AAH framework being coplanar with the heteroaromatic nitrogen-containing ring (*figure 2*).

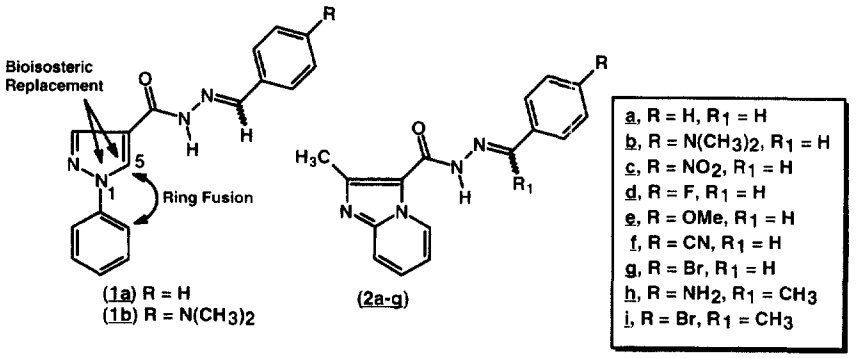


Figure 1.

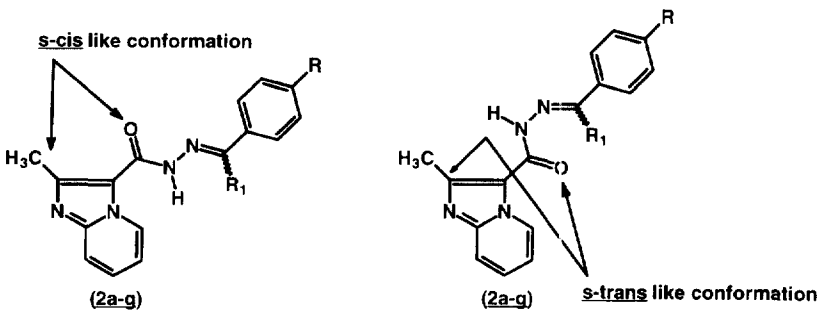


Figure 2.

2. Chemistry

Among several obvious synthetic routes to derivatives **2**, we decided to explore in our synthetic approach the acylhydrazine derivative **7** as a key intermediate. This compound could be transformed in **2** using classical functional group interconversion, i.e. $CONHNH_2 \rightarrow CONHN{=}CHAr$. Thus, the target compounds **2** could be prepared by acid-catalyzed condensation of **7** with corresponding aromatic aldehydes or acetophenones. The compound **7** was synthesized, in high overall yield, by the synthetic sequence despicted in the *figure 3*. The regioselective condensation of 2-aminopyridine **3** with the adequate ethyl 2-chloro-acetoacetate **4** in absolute ethanol, at reflux, furnished the functionalized imidazo[1,2-*a*]pyridine derivative **6**, in 93% yield [12–15] (*figure 3*). This condensation process occurs by initial nucleophilic displacement of the halogen atom by the heteroatom of the pyridine ring, forming the resonance-stabilized pyridinium salt intermediate **5**, which suffers a facile ring closure mediated by imine–enamine formation, via selective attack of the amino group on the ketone carbonyl function [12] (*figure 3*).

With an efficient synthetic method to access the ethyl ester **6**, the next step in the planned route was to promote a nucleophilic substitution reaction employing hydrazine hydrate. Treatment of a methanolic solution of the ester **6** with hydrazine hydrate at reflux gave the desired acylhydrazine derivative **7** as a white solid, in 70% yield.

Finally, the new target compounds **2a–i** were obtained, in good yields, by condensing **7** with the corresponding aromatic aldehydes or acetophenones in ethanol, using hydrochloric acid as catalyst, as illustrated in *table I*.

The next step in this work was to determinate the relative configuration of the N=C bond in acylarylhydrazone derivatives **2**, in order to assure the diastereomeric ratio, essential to the understanding of the biological results. Careful analysis of the ^1H NMR spectra of **2a–i** (*table II*) allowed to determine that the imino hydrogen signals, due to the major diastereomer, is upfielded by 0.2–0.3 ppm compared to the minor one, indicating the (*Z*)-configuration (*table II*). These results are in agreement with previous results disclosed by Karabatsos for the relative configuration of hydrazones and related compounds [16–18].

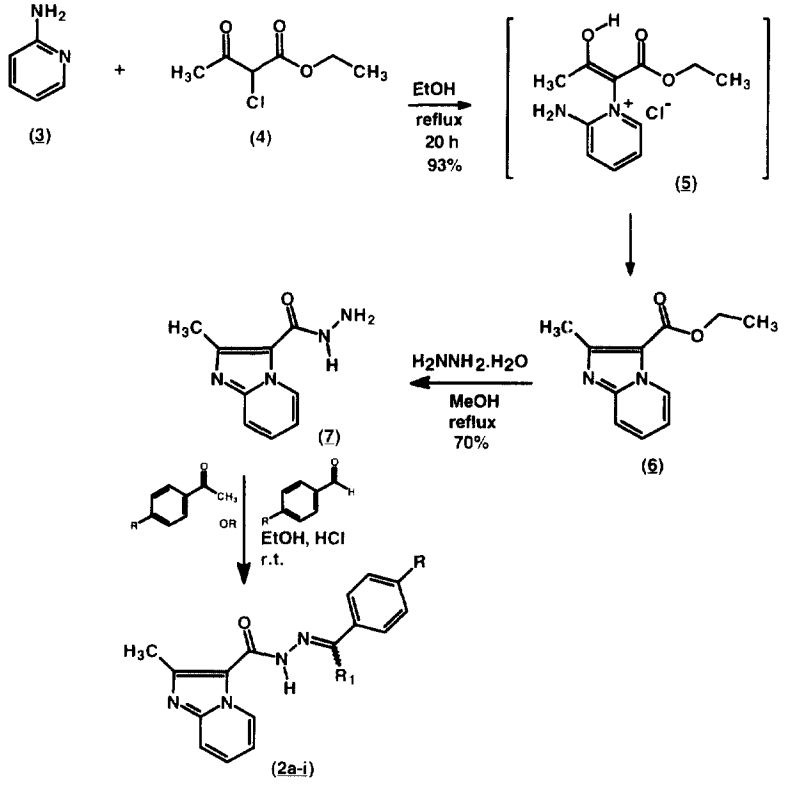
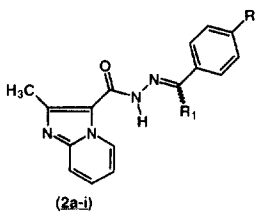


Figure 3.

Table I. 2-Methyl-3-acyl-arylhydrazone imidazo[1,2-*a*]pyridine derivatives **2a–i**.



(2a-i)

Compound	R	R_1	Molecular formula	Yield (%)	Molecular weight	M.p. (°C)
2a	H	H	$C_{16}H_{14}N_4O$	79	278	178
2b	$N(CH_3)_2$	H	$C_{18}H_{19}N_5O$	99	321	136
2c	NO_2	H	$C_{16}H_{13}N_5O_3$	92	323	281
2d	F	H	$C_{16}H_{13}FN_4O$	84	296	246
2e	OCH_3	H	$C_{17}H_{16}N_4O_2$	94	308	185
2f	CN	H	$C_{17}H_{14}N_5O$	88	303	274
2g	Br	H	$C_{16}H_{13}BrN_4O$	82	357	266
2h	NH_2	CH_3	$C_{17}H_{17}N_5O$	49	307	213
2i	Br	CH_3	$C_{17}H_{15}BrN_4O$	78	371	240

Table II. Determination of relative configuration of imidazo[1,2-*a*]pyridine acylarylhydrazones **2a–g** by assignment of imino-hydrogen chemical shift and relative integration in ^{1}H NMR spectra at 200 MHz (DMSO-d_6).

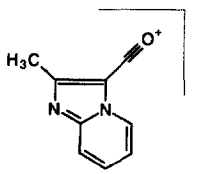
Compound	δ (ppm) (*Z*)-diastereomer	δ (ppm) (*E*)-diastereomer	Δδ (ppm)	Relative proportion (*Z*):(*E*)
2a	8.37	8.72	0.35	2.8:1
2b	8.17	8.48	0.31	17.4:1
2c	8.50	8.68	0.18	5.4:1
2d	8.46	8.73	0.27	9.0:1
2e	8.52	8.63	0.11	6.9:1
2f	8.39	8.68	0.29	18.1:1
2g	8.32[a]	–	–	–

[a]Only one diastereomer was detected.

In *table III* are described the ^{13}C NMR chemical shift assignments to the carbon atom in this series of new derivatives **2a–i**. The attributions of carbon chemical shifts in compounds **2** were proposed by applying special NMR techniques as APT, DEPT and HETEROCOSY. Curiously we were not able to detect any important signal due the minor diastereomeric imine-benzylic carbon atom which occurs in the major isomer at the range of δ 144.1–151.5 ppm.

The mass spectrometry analysis of this series of derivatives **2** indicated the fragment with *m/z* = 159, attributed to the acylium ion described below (*figure 4*), as the most important electron impact scission pathway.

All new AAH compounds described herein were completely structurally characterized by ^{1}H and ^{13}C NMR (*table III*) and by other common spectroscopic methods (see the Experimental section) and subsequently submitted to pharmacological evaluation.



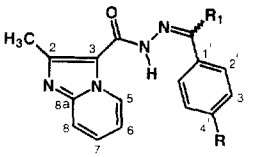
fragment m/z = 159

Figure 4.

3. Results and discussion

All new imidazo[1,2-*a*]pyridinyl-3-acylarylhydrazone compounds **2a–i** were initially evaluated for analgesic activity using the acetic acid-induced mice abdominal constrictions test, p.o. [19]. The results for analgesic activity as the percent of abdominal constriction inhibition are shown in *table IV*. The

Table III. ^{13}C nuclear magnetic spectra at 50 MHz of compounds **2**.[a]



(2a-g) R_1 = H
(2h-i) R_1 = CH_3

	Compound[d]								
	2a	**2b**	**2c**	**2d**	**2e**	**2f**	**2g**[c]	**2h**[c]	**2i**[c]
C-2	133.8	126.6	128.7	130.2	129.5	129.5	126.3	127.0	115.3
C-3	134.1	127.1	140.0	130.6	129.6	130.0	126.7	127.0	122.5
C-5	127.0	121.5	123.0	126.5	126.3	126.9	122.7	124.9	126.7
C-6	114.0	113.0	112.2	112.6	113.4	111.9	112.4	112.9	113.0
C-7	127.6	129.5	127.1	128.8	127.3	138.7	132.1	126.5	131.2
C-8	115.3	116.2	115.5	146.2	114.4	116.2	115.7	116.1	116.1
C-8a	144.3	145.3	145.2	146.2	141.8	146.9	146.2	145.1	146.8
CH_3	14.9	15.7	14.9	15.3	12.9	15.7	15.1	15.8	15.8
C=O	161.5	–[b]	159.0	160.4	160.7	159.3	158.8	–[b]	159.6
CR_1–Ar	144.1	151.5	143.8	145.4	147.4	144.5	145.2	150.2	151.1
C_1'	146.9	115.3	146.5	146.2	140.7	127.1	130.5	115.5	137.1
C_2'	128.9	128.7	126.5	126.8	128.0	127.4	128.2	127.5	127.0
C_3'	128.5	111.8	126.1	115.2	114.0	132.6	131.3	113.1	128.1
C_4'	130.1	145.9	147.7	159.3	157.3	113.0	133.2	146.1	122.6
Other	–	39.7 (NCH_3)	–	–	55.1 (OCH_3)	118.4 (C≡N)	–	13.8 (CH_3)	13.9 (CH_3)

[a]Ca. 30 mg of the compound in 0.7 mL of DMSO-d_6; [b]not detected; [c]recorded at 75 MHz; [d]only the most abundant signal, referent to the major diastereomer, was included.

analysis of these values allowed to evidence us that 4'-bromophenylacylhydrazone **2g**, 4'-dimethylaminophenylacylhydrazone **2b** and 4'-aminophenylacylhydrazone **2h** were the more active compounds of this series, where the first derivative **2g** was the most active one. The classical electronic effects of the *para*-bromo substituent, due to the inductive electron-attracting properties, could explain the more important analgesic profile of **2g**. Replacement of *para*-bromine (Hammett σ_p + 0.23) by *para*-fluorine (Hammett σ_p + 0.06) atom in the phenyl ring of **2** was found to have a deleterious effect on the analgesic potency, as can be seen by comparing **2d** (30.3%) with **2g** (45.3%).

Additionally, the 4'-aminophenylacylhydrazone derivatives **2b** (38.8%) and **2h** (39.5%) showed also a significative analgesic profile, in agreement with previous works [6–8]. In fact, we were able to observe in different heteroarylacylarylhydrazone derivatives (e.g. **8**, see *figure 5*) [7] a very important analgesic profile. The known ability of these substituents to promote hydrogen-bonding interactions with a bio-receptor could be an important element to explain the observed analgesic activity in these compounds. On the other hand, the analgesic activity observed to derivative **2** appears to be insensitive to the nature of the heterodouble bond in the hydrazone framework, at least in the *para*-aminophenylacylhydrazone derivatives **2b** and **2h**. However, the introduction of a methyl group in the N=C double bond of the hydrazone moiety reduces the analgesic activity in the *para*-bromophenyl compound, as can be seen by comparing the *para*-bromophenyl analog **2g** with **2i**.

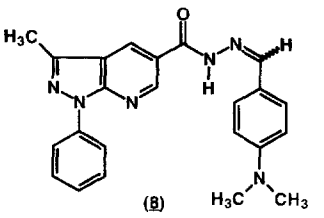


Figure 5.

Table IV. Effect of imidazo[1,2-*a*]pyridinyl acylarylhydrazone derivatives **2a–i**, dipyrone and indomethacin in the inhibition of abdominal constrictions induced by acetic acid (0.6%, i.p.) in mice.

Compound	Dose[a] (μmol/kg)	*n*[b]	Constriction number	Inhibition (%)[c]
Control	–	32	74.4 ± 4.2	–
Vehicle control (arabic gum 5%)	–	14	72.9 ± 3.7	2.0 n.s.
Dipyrone	100	10	36.9 ± 3.2	49.7[d]
Indomethacin	100	14	37.1 ± 3.4	49.1[d]
2a	100	7	69.1 ± 3.8	–5.2 n.s.
2b	100	10	44.6 ± 4.1	38.8[d]
2c	100	12	64.2 ± 6.2	11.9 n.s.
2d	100	13	50.8 ± 4.1	30.3[d]
2e	100	11	51.1 ± 3.0	29.9[d]
2f	100	13	50.1 ± 3.6	31.3[d]
2g	100	10	39.9 ± 4.0	45.3[d]
2h	100	13	44.1 ± 4.0	39.5[d]
2i	100	8	68.0 ± 5.6	18.9 n.s.

[a]All compounds were administered p.o.; [b]*n* = number of animals; [c]percentage of inhibition obtained by comparison with the vehicle control group; [d]$p < 0.05$ (Student's *t*-test). Results are expressed as mean ± SEM.

These results could indicate that the presence of the methyl group in **2i** serves to block the imine framework of the AAH moiety in the bioreceptor recognition, contributing to reduce the analgesic activity.

For instance, the close analgesic profile observed for compound **2g**, relative to dipyrone and indomethacin, used as standard, indicated that this derivative represents an important new analgesic lead compound.

We therefore turned our attention to the next step in the pharmacological evaluation of these 4'-*para*-substituted-phenyl-3-acylhydrazone derivatives **2** to the anti-inflammatory profile. The anti-inflammatory activity was investigated using the carrageenan-induced rat paw edema (CIRPE) test [20] with indomethacin and phenidone as standard. We decided to examine the bioavailability of these compounds studying the time-course effect in the anti-inflammatory activity for the three more analgesic derivatives **2b,g,h**, varying the administration route, i.e., p.o. against i.p. The results for anti-inflammatory activity, as the percent of inhibition CIRPE, are shown in *tables V* and *VI*.

Unfortunately, in vivo inhibition of carrageenan-induced edema by these compounds was not favorable when we used p.o. administration at the molar concentration indicated in *table V*. However, the compounds **2g,h** demonstrated good in vivo i.p. administrated anti-inflammatory activity where the 4'-bromophenylacylhydrazone **2g** compound was the most active one. While this compound has a significant anti-inflammatory activity after 1 h, it was much more active then phenidone, used as standard, after 4 h, indicating a better bioavailability profile. Replacing the *para*-bromo atom by the *para*-amino group in the phenyl fragment introduces a more metabolic-soft unit [21, 22, 23] that could explain the loss of anti-inflammatory activity presented by **2h** after 1 h (44.1%), which seems to be time-dependent, falling to

Table V. Time-course effect of p.o. administration of imidazo[1,2-*a*]pyridinyl acylarylhydrazone derivatives **2a–i**, indomethacin and phenidone in the inhibition of carrageenan-induced rat paw edema.

Compound	Time (h)[a]	*n*[b]	Dose (μmol/kg)[c]	Volume variation (μL)[d]	Inhibition (%)[e]
Vehicle control (arabic gum 5%)	1	12	–	221.7 ± 26.4	–
	2	12	–	326.5 ± 30.1	–
	3	12	–	362.8 ± 35.8	–
	4	12	–	398.0 ± 38.7	–
Indomethacin	1	5	100	49.2 ± 9.6	77.8[f]
	2	5	100	87.1 ± 22.1	73.3[f]
	3	5	100	113.6 ± 23.2	68.7[f]
	4	5	100	79.5 ± 33.6	80.0[f]
Phenidone	1	5	617	57.6 ± 19.2	74.0[f]
	2	5	617	82.7 ± 21.7	74.7[f]
	3	5	617	143.9 ± 22.8	60.3[f]
	4	5	617	176.3 ± 28.7	55.7[f]
2b	1	10	100	171.1 ± 24.3	22.8 n.s.
	2	10	100	316.9 ± 39.7	2.9 n.s.
	3	10	100	403.6 ± 40.8	–11.2 n.s.
	4	9	100	406.0 ± 43.7	–2.0 n.s.
2g	1	10	100	174.9 ± 31.4	21.1 n.s.
	2	10	100	324.2 ± 31.1	0.7 n.s.
	3	10	100	408.6 ± 30.4	–12.6 n.s.
	4	10	100	378.7 ± 23.6	4.8 n.s.
2h	1	8	100	180.7 ± 59.8	18.5 n.s.
	2	8	100	344.6 ± 82.5	–5.5 n.s.
	3	7	100	411.1 ± 56.0	–13.3 n.s.
	4	7	100	397.4 ± 52.2	0.1 n.s.

[a]Time after carrageenan injection (0.1 mg/paw); [b]*n* = number of animals; [c]all compounds were administered p.o.; [d]volume variation is the difference between the volumes of carrageenan-treated paw and salina-treated paw; [e]percentage of inhibition obtained by comparison with the vehicle control group; [f]$p < 0.05$ (Student's *t*-test). Results are expressed as mean ± SEM.

Table VI. Effect of i.p. administration of imidazo[1,2-*a*]pyridinyl acylarylhydrazone derivatives (IZ 12, IZ 17 and IZ 18), indomethacin and phenidone in the inhibition of carrageenan-induced rat paw edema.

Compound	Time (h)[a]	*n*[b]	Dose (μmol/kg)[c]	Volume variation (μL)[d]	Inhibition (%)[e]
Vehicle control (arabic gum 5%)	1	9	–	178.8 ± 43.1	–
	2	9	–	284.4 ± 49.2	–
	3	9	–	323.9 ± 50.6	–
	4	9	–	322.8 ± 36.1	–
Indomethacin	1	4	100	47.7 ± 16.5	73.3[f]
	2	4	100	85.9 ± 16.5	69.8[f]
	3	4	100	62.0 ± 34.3	80.8[f]
	4	3	100	114.5 ± 22.1	64.5[f]
Phenidone	1	5	308.5	40.7 ± 15.9	77.2[f]
	2	5	308.5	137.0 ± 30.2	51.8[f]
	3	5	308.5	292.6 ± 37.2	9.7 n.s.
	4	5	308.5	304.2 ± 23.4	5.8 n.s.
2b	1	5	100	142.9 ± 37.8	20.1 n.s.
	2	5	100	225.6 ± 57.3	26.1 n.s.
	3	5	100	278.2 ± 62.2	14.1 n.s.
	4	5	100	312.0 ± 45.9	3.3 n.s.
2g	1	5	100	99.8 ± 11.2	44.2 n.s.
	2	5	100	134.4 ± 16.0	52.8[f]
	3	5	100	161.2 ± 9.8	50.2[f]
	4	5	100	176.6 ± 25.3	45.3[f]
2h	1	4	100	100.0 ± 30.5	44.1 n.s.
	2	4	100	209.5 ± 23.3	26.3 n.s.
	3	4	100	271.4 ± 28.4	16.2 n.s.
	4	4	100	308.6 ± 12.1	4.4 n.s.

[a]Time after carrageenan injection (0.1 mg/paw); [b]*n* = number of animals; [c]all compounds were administered i.p.; [d]volume variation is the difference between the volumes of carrageenan-treated paw and saline-treated paw; [e]percentage of inhibition obtained by comparison with the vehicle control group; [f]$p < 0.05$ (Student's *t*-test). Results are expressed as mean ± SEM.

only 4.4% after 4 h in this last derivative. Since in vivo activity depends on highly complex physiological interactions, we are not able, at this moment, to rationalize all of these pharmacological results. However, the good and persistent anti-inflammatory activity displayed by *para*-bromophenyl derivative **2g**, at the i.p. administered carrageenan-induced paw edema test, seems to indicate that this new compound is an important representative of a new class of anti-inflammatory and analgesic acylarylhydrazone derivatives.

4. Experimental protocols

4.1. Chemistry

Melting points were determined with a Thomas-Hoover apparatus and are uncorrected. Proton magnetic resonance (^{1}H NMR), unless otherwise stated, was determined in deuterated dimethylsulfoxide containing ca. 1% tetramethylsilane as an internal standard with Brucker AC 200, Bruker DRX 300 spectrometers at 200 MHz and 300 MHz respectively. Splitting patterns are as follows: s, singlet; d, doublet; t, triplet; q, quartet; td, triple doublet; ddd, double double doublet; br, broad; m, multiplet. Carbon magnetic resonance (^{13}C NMR) was determined with the same spectrometers described above at 50 MHz and 75 MHz respectively, using deuterated dimethylsulfoxide as internal standard. Infrared (IR) spectra were obtained with a Nicolet-550 Magna spectrophotometer by using potassium bromide plates. The mass spectra (MS) were obtained by electron impact (70 eV) with a GC/VG Micromass 12 spectrometer.

The progress of all reactions was monitored by TLC performed on 2.0 cm x 6.0 cm aluminum sheets precoated with silica gel 60 (HF-254, Merck) to a thickness of 0.25 mm. The developed chromatograms were viewed under ultraviolet light at 254 nm. For column chromatography Merck silica gel (70–230 mesh) was used. The usual work-up means that the organic extracts prior to concentration, under reduced pressure, were treated with a saturated aqueous sodium chloride solution, referred to as brine, dried over anhydrous sodium sulfate and filtered.

4.1.1. Ethyl 2-Methyl-imidazo[1,2-a]pyridine-3-carboxylate **6**

A solution of 1 g (10.6 mmol) of 2-aminopyridine **3** and 1.26 mL (1.6 g; 9.6 mmol) of ethyl 2-chloro-acetoacetate **4** in 50 mL of absolute ethanol was refluxed by 20 h. Then, the solvent was removed under reduced pressure and the brown oily residue was extracted with 50 mL of dichloromethane. The organic layer was washed with cold water (3 x 20 mL) and submitted at usual work-up to give 1.84 g (93%) of the ester **5**, as a brownish powder, m.p. 42–44 °C. ^{1}H NMR (200 MHz): δ 1.45 (t, 3H, O=C–O–CH_2–CH_3, J = 6.7 Hz), 2.72 (s, 3H, Im-CH_3), 4.44 (q, 2H, O=C–O–CH_2–CH_3, J = 6.7 Hz), 6.98 (dt, 1H, H-6, J_{ax} = 7.2 and J_{bx} = 1.6 Hz), 7.36 (ddd, 1H, H-7, J_{ax} = 9.2 and J_{bx} = 6.8 and J_{cx} =1.2 Hz), 7.61 (dt, 1H, H-8, J_{ax} = 8.8 and J_{bx} = 1.6 Hz), 9.32 (dt, 1H, H-5, J_{ax} = 7.0 and J_{bx} = 1.1 Hz) ppm; IR (KBr) cm^{-1}: 1683 (ν C=O), 1557 (ν C=C), 1520, 1501 (ν C=N), 1223 (ν C–O) and 762 (δ N=C–H); MS (*m/z*): 204 (M$^{+\bullet}$, 76%), 176% (34%), 159 (79%), 132 (100%), 90 (23%), 78 (24%).

4.1.2. 3-Acyl-(2-methyl-imidazo[1,2-a]pyridinyl)-hydrazine **7**

A mixture of 2 g (9.8 mmol) of the ethyl ester derivative **6** and 9.8 mL of 80% hydrazine hydrate in 35 mL of absolute ethanol was stirred at reflux for 20 h. At the end of reaction (observed by TLC) the product **7** was isolated by filtration, in 70% yield, as a white solid, after concentration of reaction mixture under reduced pressure and cold water addition (ca. 20 mL), m.p. 183 °C. ^{1}H NMR (200 MHz): δ 2.71 (s, 3H, Im-CH_3), 4.15 (br, 2H, NH–NH_2), 6.95 (dt, 1H, H-6, J_{ax} = 7.0 and J_{bx} = 1.0 Hz), 7.03 (br, 1H, NH–NH_2), 7.36 (ddd, 1H, H-7, J_{ax} = 8.9 and J_{bx} = 7.0 and J_{cx} = 1.1 Hz), 7.60 (dt, 1H, H-8, J_{ax} = 9.0 and J_{bx} = 1.1 Hz), 9.37 (dt, 1H, H-5, J_{ax} = 7.0 and J_{bx} = 1.0 Hz) ppm; IR (KBr) cm^{-1}: 3290, 3218 (ν N–H), 1626 (ν C=O), 1522, 1495 (ν C=N), 761 (δ C=C–H) and 744 (δ N=C–H); MS (*m/z*): 190 (M$^{+\bullet}$, 17%), 159 (100%), 131 (7%), 90 (22%), 78 (24%); Anal. (C, H, N) $C_9H_{10}N_4O$.

4.1.3. General procedure for the preparation of the imidazo-[1,2-a]pyridine 3-acylarylhydrazone derivatives **2a–i**

A mixture of 0.19 g (1 mmol) of acylhydrazine **7** and 1 mmol of the corresponding benzaldehyde or acetophenone derivative in 10 mL of absolute ethanol was stirred at room temperature, in the presence of two drops of chloridric acid as catalyst. The end of the reaction was observed by TLC, and the hydrazones **2a–i** were isolated by concentration of the reaction mixture under reduced pressure, followed by neutralization with a 10% aqueous solution of sodium bicarbonate. Then, the resulting precipitate was filtered out, washed with 10 mL water and air-dried affording the target compounds **2a–i** as described below.

4.1.3.1. Benzylidene-2-methyl-imidazo[1,2–a]pyridin-3-carbo-hydrazide **2a**

The title compound was obtained as a white powder, in 79% yield, by condensing **7** with benzaldehyde, as described above, mp 178 °C. ^{1}H NMR (200 MHz): δ 2.60 (s, 3H, Im-CH_3), 7.00 (t, 1H, H-6, J_{ax} = 7.1 Hz), 7.39 (t, 1H, H-7, J_{bx} = 6.9 Hz), 7.57 (d, 1H, H-8, J_{ax} = 8.9 Hz), 7.89 (d, 2H, H-2', J = 8.7 Hz), 8.05–8.30 (m, 2H, H-3'), 8.50 (s, 1H, CH (*Z*)-diastereomer), 8.68 (s, CH (*E*)-diastereomer), 8.88 (d, 1H, H-5, J_{ax} = 6.9 Hz), 11.75 (br, D_2O exchangeable, 1H, NH) ppm; IR (KBr) cm^{-1}: 3296, 3206 (ν N–H), 1624 (ν C=O), 1600 (ν C=N), 1550 (ν C=C–H), 1494 (ν C=N–Ph), 767 (δ C=C–H) and 693 (δ N=C–H); MS (*m/z*): 278 (M$^{+\bullet}$, 1%), 159 (6%), 131 (100%), 89 (10%), 77 (59%); Anal. (C, H, N) $C_{16}H_{14}N_4O$.

4.1.3.2. (4'-Dimethylaminobenzylidene)-2-methyl-imidazo[1,2-a]-pyridin-3-carbohydrazide **2b**

The title compound was obtained as a yellow powder, in 99% yield, by condensing **7** with 4-dimethylaminobenz-aldehyde, as described above, mp 136 °C. ^{1}H NMR (200 MHz): δ 2.55 (s, 3H, Im-CH_3), 2.95 (s, 6H, N(CH_3)$_2$), 6.73 (d, 2H, H-3', J = 8.2 Hz), 7.02 (t, 1H, H-6, J_{ax} = 7.0 Hz), 7.33–7.67 (m, 4H, H-7, H-8 and H-2'), 8.17 (s, 1H, CH (*Z*)-diastereomer), 8.48 (s, CH (*E*)-diastereomer), 8.86 (m, 1H, H-5), 11.25 (br, D_2O exchangeable, 1H, NH) ppm; IR (KBr) cm^{-1}: 3394, 3161 (ν N–H), 1637 (ν C=O), 1598 (ν C=N), 1526 (ν C=C–H), 1495 (ν C=N–Ph), 754 (δ N=C–H); MS (*m/z*): 321 (M+, 26%), 175 (20%), 159 (100%), 132 (30%), 90 (35%), 78 (25%); Anal (C, H, N) $C_{18}H_{19}N_5O$.

4.1.3.3. (4'-Nitrobenzylidene)-2-methyl-imidazo[1,2-a]pyridin-3-carbohydrazide **2c**

The title compound was obtained as a yellow powder, in 92% yield, by condensing **7** with 4-nitrobenzaldehyde, as described above, mp 281 °C. ^{1}H NMR (200 MHz): δ 2.60 (s, 3H, Im-CH_3), 7.00 (t, 1H, H-6, J = 7.1 Hz), 7.39 (t, 1H, H-7, J_{ax} = 6.9 Hz), 7.57 (d, 1H, H-8, J = 8.9 Hz), 7.89 (d, 2H, H-2', J = 8.7 Hz), 8.05–8.30 (m, 2H, H-3'), 8.50 (s, 1H, CH (*Z*)-diastereomer), 8.68 (s, CH (*E*)-diastereomer), 8.88 (d, 1H, H-5, J = 6.9 Hz) ppm; IR (KBr) cm^{-1}: 3195 (ν N–H), 1633 (ν C=O), 1611 (ν C=N), 1590 (ν C=C–H), 1545 (ν C=N–Ph), 1517, 1341 (ν N–O), 764 (δ N=C–H); MS (*m/z*): 323 (M$^{+\bullet}$, 7%), 175 (4%), 159 (100%), 90 (18%), 78 (14%); Anal. (C, H, N) $C_{16}H_{13}N_5O_3$.

4.1.3.4. (4'-Fluorobenzylidene)-2-methyl-imidazo[1,2-a]pyri-din-3-carbohydrazide **2d**

The title compound was obtained as a white powder, in 84% yield, by condensing **7** with 4'-fluorobenzaldehyde, as described above, mp 246 °C; ^{1}H NMR (200 MHz): δ 2.70 (s, 3H, Ar-CH_3), 7.11 (t, 1H, H-6, J = 6.7 Hz), 7.33 (t, 2H, H-3', J = 8.8 Hz), 7.50 (t, 1H, H-7, J_{ax} = 6.9 Hz), 7.68 (d, 1H, H-8, J = 8.9 Hz), 8.01 (t, 2H, H-2', J = 8.9 Hz), 8.46 (s, 1H, CH (*Z*)-diastereomer), 8.73 (s, CH (*E*)-diastereomer), 8.97 (d, 1H, H-5, J = 6.9 Hz), 11.36 (br, D_2O exchangeable, 1H, NH) ppm; IR (KBr) cm^{-1}: 3203 (ν N–H), 1634 (ν C=O), 1606 (ν C=N), 1546 (ν C=N–Ph), 1232 (ν C–F), 752 (δ N=C–H); MS (*m/z*): 296 (M$^{+\bullet}$, 13%), 175 (16%), 159 (100%), 90 (22%), 78 (11%); Anal. (C, H, N) $C_{16}H_{13}N_4OF$.

4.1.3.5. (4'-Methoxybenzylidene)-2-methyl-imidazo[1,2-a]pyri-din-3-carbohydrazide **2e**

The title compound was obtained as a white powder, in 94% yield, by condensing **7** with 4'-methoxybenzaldehyde, as described above, mp 185 °C; ^{1}H NMR (200 MHz): δ 2.76 (s, 3H, Im-CH_3), 3.92 (s, 3H, OCH_3), 7.08 (d, 1H, H-6, J = 6.8 Hz), 7.20 (d, 2H, H-3', J = 8.7 Hz), 7.38 (t, 1H, H-7, J_{ax} = 6.8 Hz), 7.69 (d, 1H, H-8, J = 6.8 Hz), 7.94 (d, 2H, H-2', J = 8.8 Hz), 8.52 (s, 1H, CH (*Z*)-diastereomer), 8.63 (s, CH (*E*)-diastereomer), 9.04 (d, 1H, H-5, J = 6.8 Hz), 9.98 (br, D_2O exchangeable, 1H, NH) ppm; IR (KBr) cm^{-1}: 3207 (ν N–H), 1636 (ν C=O), 1604 (ν C=N), 1544, 1508 (ν C=N–Ph), 1257 (ν C–O), 764 (δ N=C–H); MS (*m/z*): 323 (M$^{+\bullet}$, 0%), 308 (10%), 175 (30%), 159 (100%), 90 (31%), 78 (17%); Anal. (C, H, N) $C_{17}H_{16}N_4O_2$.

4.1.3.6. (4'-Cyanobenzylidene)-2-methyl-imidazo[1,2-a]pyri-din-3-carbohydrazide **2f**

The title compound was obtained as a white powder, in 88% yield, by condensing **7** with 4'-cyanobenzaldehyde, as described above, mp 274 °C; ^{1}H NMR (200 MHz): δ 2.57 (s, 3H,

Im-CH_3), 7.03 (t, 1H, H-6, J = 6.8 Hz), 7.41 (t, 1H, H-7, J = 8.4 Hz), 7.59 (d, 1H, H-8, J_{ax} = 8.9 Hz), 7.84 (s, 4H, H-2' and H-3'), 8.39 (s, 1H, C*H* (*Z*)-diastereomer), 8.68 (s, C*H* (*E*)-diastereomer), 8.88 (d, 1H, H-5, J = 6.9 Hz), 11.50 (br, D_2O exchangeable, 1H, N*H*) ppm; IR (KBr) cm^{-1}: 3192 (ν N–H), 2225 (ν C≡N), 1633 (ν C=O), 1601 (ν C=N), 1546 (ν C=N–Ph), 760 (δ N=C–H); MS (*m/z*): 303 ($M^{+\bullet}$, 16%), 175 (7%), 159 (100%), 90 (13%), 78 (5%); Anal. (C, H, N) $C_{17}H_{13}N_5O$.

4.1.3.7. (4'-Bromobenzylidene)-2-methyl-imidazo[1,2-a]pyridin-3-carbohydrazide ***2g***

The title compound was obtained as a white powder, in 82% yield, by condensing **7** with 4'-bromobenzaldehyde, as described above, mp 266 °C; ^1H NMR (300 MHz): δ 2.59 (s, 3H, Im-CH_3), 7.16 (t, 1H, H-6, J = 6.9 Hz), 7.37 (t, 1H, H-7, J = 8.5 Hz), 7.60–7.75 (m, 5H, H-8, H-2' and H-3'), 8.32 (s, 1H, C*H* (*Z*)-diastereomer), 8.92 (d, 1H, H-5, J = 6.8 Hz), 11.73 (br, D_2O exchangeable, 1H, N*H*) ppm; IR (KBr) cm^{-1}: 3189 (ν N–H), 1632 (ν C=O), 1603 (ν C=N), 1548 (ν C=N–Ar), 750 (δ N=C–H), 514 (ν C–Br); MS (*m/z*): 357 ($M^{+\bullet}$, 2%), 356 ($M^{+\bullet}$ – 1, 3%), 175 (17%), 159 (100%), 90 (28%), 78 (13%); Anal. (C, H, N) $C_{16}H_{13}N_4OBr$.

4.1.3.8. (Methyl-4'-aminobenzylidene)-2-methyl-imidazo[1,2-a]-pyridin-3-carbohydrazide ***2h***

The title compound was obtained as a yellow powder, in 49% yield, by condensing **7** with 4'-aminoacetophenone, as described above, mp 213 °C; ^1H NMR (300 MHz): δ 2.26 (s, 3H, N=C–CH_3), 2.49 (s, 3H, Im-CH_3), 5.47 (s, 2H, NH_2), 6.54 (d, 2H, C-3', J = 8.6 Hz), 7.04 (dt, 1H, H-6, J_{ax} = 6.8 and J_{bx} = 1.2 Hz), 7.35–7.45 (m, 2H, H-7 and H-8), 7.59 (d, 2H, H-2', J = 8.0 Hz), 7.61 (dt, 1H, H-5, J_{ax} = 8.9 and J_{bx} = 1 Hz), 10.30 (br, D_2O exchangeable, 1H, N*H*) ppm; IR (KBr) cm^{-1}: 3429, 3339, 3220 (ν N–H), 1645 (ν C=O), 1613 (ν C=N), 1535 (ν C=N–Ph), 768 (δ N=C–CH_3); MS (*m/z*): 307 ($M^{+\bullet}$, 27%), 159 (100%), 133 (16%), 90 (15%), 78 (8%); Anal. (C, H, N) $C_{17}H_{17}N_5O$.

4.1.3.9. (Methyl-4'-Bromobenzylidene)-2-methyl-imidazo[1,2-a]-pyridin-3-carbohydrazide ***2i***

The title compound was obtained as a white powder, in 78% yield, by condensing **7** with 4'-bromoacetophenone, as described above, mp 240 °C; ^1H NMR (300 MHz): δ 2.36 (s, 3H, N=C–CH_3), 2.57 (s, 3H, Im-CH_3), 7.04 (t, 1H, H-6, J = 6.8 Hz), 7.42 (t, 1H, H-7, J = 7.8 Hz), 7.70–7.80 (m, 3H, H-8 and H-2'), 7.72 (d, 2H, H-3', J = 8.5 Hz), 8.93 (d, 1H, H-5, J = 6.7 Hz), 10.47 (br, D_2O exchangeable, 1H, N*H*) ppm; IR (KBr) cm^{-1}: 3199 (ν N–H), 1646 (ν C=O), 1601 (ν C=N), 1518 (ν C=N–Ar), 746 (δ N=C–CH_3), 559 (ν C–Br); MS (*m/z*): 370 ($M^{+\bullet}$, 2%), 370 ($M^{+\bullet}$ – 1, 10%), 159 (100%), 132 (8%), 90 (12%), 78 (7%); Anal. (C, H, N) $C_{17}H_{15}N_4OBr$.

4.2. Pharmacology

4.2.1. Analgesic activity

The analgesic activity was determined in vivo by the abdominal constriction test induced by acetic acid 0.6% (0.1 mL/10 g) in mice [19]. Albino mice of both sexes (18–23 g) were used. Compounds were administered orally (100 μmol/kg; 0.1 mL/20 g) as a suspension in 5% arabic gum in saline (vehicle). Indomethacin (100 μmol/kg) and dipyrone (100 μmol/kg) were used as standard drugs in the same conditions. Acetic acid solution was administered i.p. one hour after administration of imidazo[1,2-*a*]pyridine compounds **2**. Ten minutes following i.p. acetic acid injection, the number of constrictions per animal was recorded for 20 min. Control animals received an equal volume of the vehicle. Analgesic activity was expressed as the percentage of inhibition of constrictions when compared with the vehicle control group (*table IV*)

4.2.2. Anti-inflammatory activity

The anti-inflammatory activity was determined in vivo using the carrageenan-induced rat paw edema test accordly to Ferreira [20]. Fasted albino rats of both sexes (150–200 g) were used. Compounds were administered orally and intraperitoneally (100 μmol/kg; 0.1 mL/20 g) as a suspension in 5% arabic gum in saline (vehicle). Control animals received an equal volume of the vehicle. One hour after, the animals were then injected with either 0.1 mL of 1% carrageenan solution in saline (0.1 mg/paw) and sterile saline (NaCl 0.9%), into the subplantar surface of one of the hind paws respectively. The paw volumes were measured using a glass plethysmometer coupled to a peristaltic pump, each hour until 4 h after the subplantar injection. The edema was calculated as the volume variation by the volume difference between the carrageenan- and saline-treated paw. Indomethacin (100 μmol/kg) and phenidone (617 μmol/kg, p.o. and 308.5 μmol/kg, i.p.) were used as standard drugs in the same conditions. Anti-inflammatory activity was expressed as the percentage of inhibition of the edema when compared with the vehicle control group (*tables V* and *VI*).

4.2.3. Statistics

Results are expressed as the mean ± SEM of n animals per group. The data were statistically analyzed by the Student's t-test for a significance level of $p < 0.05$.

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References and notes

[1] Davies P., Bailey P.J., Goldenberg M., Ford-Hutchinson, A.W., Ann. Rev. Immunol. 2 (1984) 335.
[2] Vane J.R., Nature New Biology 231 (1971) 232.
[3] Higgs A.G., Vane J.R., Br. Med. Bull. 39 (1983) 265.
[4] Dray A., Urban L., Dickenson A., Trends Pharmacol. Sci. 15 (1994) 190.
[5] Soler O., M.Sc. Thesis, Faculdade de Farmácia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, 1994.
[6] Matheus M.E., Oliveira L.F., Freitas A.C.C., Carvalho A.M.A.S.P., Barreiro E.J., Braz. J. Med. Biol. Res. 24 (1991) 1219.
[7] Dias L.R.S., Alvim M.J.F., Freitas A.C.C., Barreiro E.J., Miranda A.L.P., Pharm. Acta Helv. 69 (1994) 163.
[8] Gaston M.J., Dias L.R.S., Freitas A.C.C., Barreiro E.J., Miranda A.L.P., Pharm. Acta Helv. 71 (1996) 213.
[9] Patani G.A., LaVoie E.J., Chem. Rev. 96 (1996) 3147.
[10] Albuquerque M.G., M.Sc. Thesis, Instituto de Química/LASSBio Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, 1994.

[11] Ifas D., Alencastro R.B., Barreiro E.J., unpublished results.
[12] Elliott A.J., Guzik H., Soler J.R., J. Heterocyclic Chem. 19 (1982) 1437.
[13] Kaminski J.J., Bristol J.A., Puchalski C., Lovey R.G., Elliott A.J., Guzik H., Solomon D.M., Conn D.J., Domalsky M.S., Wong S.-C., Gold E.H., Long J.F., Chiu P.J.S., Steinberg M., McPhail A.T., J. Med. Chem. 28 (1985) 876.
[14] Kaminski J.J., Hilbert J.M., Pramanik B.N., Solomon D.M., Conn D.J., Rizvi R.K., Elliott A.J., Guzik H., Lovey R.G., Domalsky M.S., Wong S-C., Puchalski C., Gold E.H., Long J.F., Chiu P.J.S., McPhail A.T., J. Med. Chem. 30 (1987) 2031.
[15] Kaminski J.J., Puchalski C., Solomon D.M., Rizvi R.K., Conn D.J., Elliott A.J., Lovey R.G., Guzik H., Chiu P.J.S., Long J.F., McPhail A.T., J. Med. Chem. 32 (1989) 1686.
[16] Karabatsos G.J., Graham J.D., Vanne F.M., J. Am. Chem. Soc. 84 (1962) 753.
[17] Karabatsos G.J., Taller R.A., J. Am. Chem. Soc. 85 (1963) 3624.
[18] Karabatsos G.J., Shapiro B.L., Vanne F.M., Fleming J.S., Ratka J.S., J. Am. Chem. Soc. 85 (1963) 2785.
[19] Whittle B.A., Brit. J. Pharmacol. Chemother. 22 (1964) 246.
[20] Ferreira S.H., J. Pharm. Pharmacol. 31 (1979) 648.
[21] Bodor N., Trends Pharmacol. Sci. 3 (1982) 53.
[22] For an example of the metabolism of aromatic amine drugs, see: Maurich V., De Amici M., De Micheli C., Rossato P., Eur. J. Med. Chem. 30 (1995) 757.
[23] For an example of the metabolism studies in *para*-substituted hydrazone derivatives, see: Komurcu S.G., Rollas S., Ulgen M., Gorrod J.W., Cevikbas A., Boll. Chim. Farm. 134 (1995) 375.