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Original article

Anti-inflammatory, analgesic and antipyretic 4,6-disubstituted 3-cyano-2-aminopyridines

Fedele Manna[a]*, Franco Chimenti[a], Adriana Bolasco[a], Bruna Bizzarri[a], Walter Filippelli[b], Amelia Filippelli[b], Luigi Gagliardi[c]

[a]*Dipartimento di Studi di Chimica e Tecnologia delle Sostanze Biologicamente Attive, Università di Roma 'La Sapienza', P. le Aldo Moro 5, 00185 Rome, Italy*
[b]*Istituto di Farmacologia e Tossicologia, Facoltà di Medicina e Chirurgia, II Università di Napoli, Via Costantinopoli 16, 80138 Naples, Italy*
[c]*Laboratorio di Chimica del Farmaco, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy*

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Abstract – 4,6-diaryl and 4,6-aryl-indolyl substituted 3-cyano-2-aminopyridines were synthesized and submitted to evaluation for their anti-inflammatory, analgesic and antipyretic activity. The electronegativity of the substituents and their displacement on the 4- or 6-aryl ring of the 4,6-diaryl-3-cyano-2-aminopyridine nucleus (**3a**–**q**) influenced the anti-inflammatory activity which was higher in the presence of electron-realising groups. The introduction of the indol-3-yl substituent in the 4-position of the 3-cyano-2-aminopyridine nucleus (**6a**–**x**) increased the anti-inflammatory and analgesic power, but there was no evidence of the relationship among the electronic characteristic of the substituents, their displacement on the 6-phenyl ring and the activity. Conversely, the displacement of the 2-hydroxyphenyl group in the 4-position (**4a**–**e**) and of the indol-3-yl group in the 6-position (**8h**–**w**) decreased the anti-inflammatory activity. All derivatives did not show any significative antipyretic activity. © Elsevier, Paris

anti-inflammatory activity / analgesic activity / antipyretic activity / 4,6-diarylsubstituted 3-cyano-2-aminopyridine synthesis / 4,6-aryl-indolylsubstituted 3-cyano-2-aminopyridine synthesis

1. Introduction

4,6-disubstituted 3-cyano-2-aminopyridines were not much studied: only some 4,6-diaryl or 4,6-aryl-heterocyclyl-3-cyano-2-aminopyridines were tested for antimicrobial activity and were found to be active [1, 2]. Furthermore, 6-indolyl-3-cyano-2-aminopyridines were synthesized but not pharmacologically tested [3]. In a previous paper [4], we reported the synthesis of 6-(2-hydroxyphenyl)-4-(2,4-dimethoxyphenyl)-3-cyano-2-aminopyridine **3w'** and of the corresponding 4-(2,4-dichlorophenyl) derivative **3u'**, which were ten times less active than indomethacin.

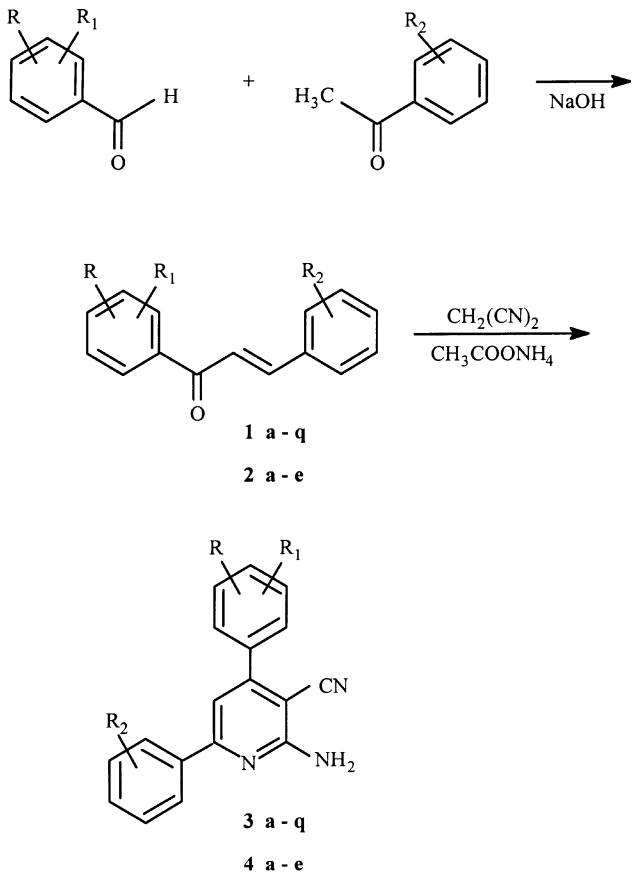
These results led us to study a new series of 3-cyano-2-aminopyridine derivatives and their structure–activity relationship. The indolic nucleus was also inserted in the 3-cyano-2-aminopyridine structure on account of its presence in the most potent anti-inflammatory derivatives, including indomethacin.

In this work we report the synthesis of 4,6-diaryl and 4,6-aryl-indolyl substituted 3-cyano-2-aminopyridines and the evaluation of their anti-inflammatory, analgesic and antipyretic activity.

2. Chemistry

The 6-(2-hydroxyphenyl)-4-(R,R'-phenyl)-3-cyano-2-aminopyridines (**3a**–**q**) and 6-(R,R'-phenyl)-4-(2-hydroxyphenyl)-3-cyano-2-aminopyridines (**4a**–**e**) were synthesized as previously reported [4] by reaction of substituted 1,3-diaryl-2-propen-1-ones with malononitrile. 1,3-diaryl-2-propen-1-ones were obtained by condensation of *o*-hydroxyacetophenone with substituted

*Correspondence and reprints



1 a - q : R_2 = 2-OH

2 a - e : R = 2-OH; R_1 = H

3 a - q : R_2 =2-OH

4 a - e : R =2-OH; R_1 = H

Figure 1. 6-(2-hydroxyphenyl)-4-(R,R'-phenyl)-3-cyano-2-aminopyridines (**3a–q**) and 6-(R-phenyl)-4-(2-hydroxyphenyl)-3-cyano-2-aminopyridines (**4a–e**).

benzaldehydes (**1a–q**) and of *o*-hydroxyphenylcarboxaldehyde with substituted acetophenones (**2a–e**) *(figure 1) (tables I* and *II)*.

The IR spectra of **3a–q** and **4a–e** showed bands at 3400–3200 cm^{-1} (NH_2), 2220–2210 cm^{-1} (CN) and 1640–1580 cm^{-1} (C=N–). The NMR spectra showed a broad singlet at ppm 13.6–13.4 attributed to the hydroxylic proton and a multiplet of the aromatic and aminic protons at ppm 8.1–6.7.

The 6-(R,R'-phenyl)-4-(indol-3-yl)-3-cyano-2-aminopyridines (**6a–x**) were synthesized by reaction of 3-(indol-3yl)-1-(R,R'-phenyl)-2-propen-1-ones (**5a–x**) with malononitrile in the presence of ammonium acetate in EtOH *(figure 2) (table III)*. The low yields were probably due to the lone pair present on the indolic nitrogen atom that decreased the electrophilic character of the acceptor in the Michael addition. The indolic chalcones (**5a–x**) were obtained by condensation of indol-3-carboxyaldehyde with substituted acetophenones in presence of piperidine as catalyst in EtOH.

The IR spectra of **6a–x** showed multiple bands at 3500–3300 cm^{-1} (NH_2), at 2220–2210 cm^{-1} (CN) and at 1640–1580 cm^{-1} (C=N–). The NMR spectra showed a singlet at ppm 11.9–11.7 attributable to the NH proton and multiplets for the aromatic, indolic and aminic protons in the region ppm 9.2–6.7. In most cases, the signals of the aminic protons were superimposed to that of the aromatic protons.

The 6-(indol-3-yl)-4-(R,R'-phenyl)-3-cyano-2-aminopyridines (**8h–w**) were synthesized by reaction of

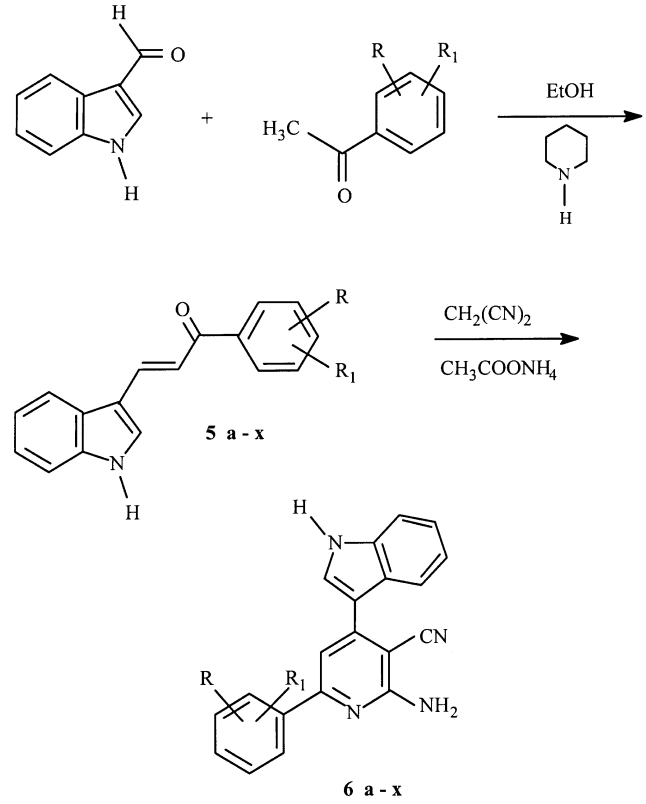
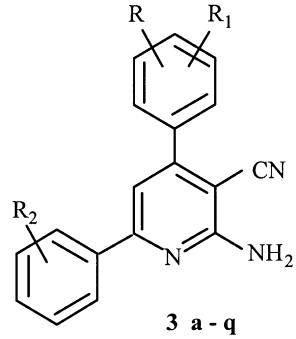


Figure 2. 6-(R,R'-phenyl)-4-(indol-3-yl)-3-cyano-2-aminopyridines (**6a–x**).

Table I. Physical constants for the 6-(2-hydroxyphenyl)-4-(R,R'-phenyl)-3-cyano-2-aminopyridines (**3a–q).**



3 a - q

4 a - e

Compound	R	R_1	Yield (%)	M.p. (°C)	Formula
3a	H	2-Cl	28	235	$C_{18}H_{12}N_3OCl$
3b	H	3-Cl	26	257–260	$C_{18}H_{12}N_3OCl$
3c	H	4-Cl	34	270	$C_{18}H_{12}N_3OCl$
3d	H	2-Br	15	243–245	$C_{18}H_{12}N_3OBr$
3e	H	3-Br	28	265	$C_{18}H_{12}N_3OBr$
3f	H	4-Br	38	285	$C_{18}H_{12}N_3OBr$
3g	H	2-CH_3	33	260	$C_{19}H_{15}N_3O$
3h	H	3-CH_3	35	275–277	$C_{19}H_{15}N_3O$
3i	H	4-CH_3	47	253–256	$C_{19}H_{15}N_3O$
3j	H	2-OCH_3	29	203	$C_{19}H_{15}N_3O_2$
3k	H	3-OCH_3	37	209–210	$C_{19}H_{15}N_3O_2$
3l	H	4-OCH_3	39	263–265	$C_{19}H_{15}N_3O_2$
3m	H	3-NO_2	49	225–227	$C_{18}H_{12}N_4O_3$
3n	H	4-NO_2	51	220–221	$C_{18}H_{12}N_4O_3$
3o	H	4-$N(CH_3)_2$	11	177–179	$C_{18}H_{17}N_4O$
3p	2-OCH_3	3-OCH_3	22	265–268	$C_{20}H_{17}N_3O_3$
3q	2-COH_3	5-OCH_3	43	228–230	$C_{20}H_{17}N_3O_3$

3-(R,R'-phenyl)-1-(indol-3-yl)-2-propen-1-ones (**7h**–**w**) with malononitrile in the presence of ammonium acetate *(figure 3) (table IV)*. The **7h**–**w** compounds were obtained by condensation of 3-acetylindole with substituted benzaldehydes.

Table II. Physical constants for the 6-(R-phenyl)-4-(2-hydroxyphenyl)-3-cyano-2-aminopyridines (**4a–e).**

Compound	R_2	Yield (%)	M.p. (°C)	Formula
4a	2-OCH_3	30	> 350	$C_{19}H_{15}N_3O_2$
4b	3-OCH_3	32	> 350	$C_{19}H_{15}N_3O_2$
4c	4-OCH_3	26	> 350	$C_{19}H_{15}N_3O_2$
4d	2-CH_3	31	> 350	$C_{19}H_{15}N_3O$
4e	4-CH_3	33	> 350	$C_{19}H_{15}N_3O$

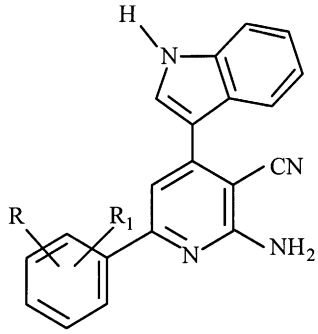
The IR spectra of **8h**–**w** showed bands at 3500–3300 cm^{-1} (NH_2), 2220–2210 cm^{-1} (CN) and 1640–1580 cm^{-1} (C=N–). The NMR spectra showed signals of the NH proton at ppm 11.9 and of the aromatic, indolic and aminic protons at ppm 8.0–6.7.

3. Pharmacology

All derivatives were investigated with respect to their anti-inflammatory, analgesic and antipyretic activity, at dose of 50 mg/kg corresponding to their ED_{50} values.

The (4-methyl)-, (3-methoxy)- and (2,4-dimethoxy)-4-phenyl derivatives (**3i, 3k** and **3w'** respectively), characterized by electron-releasing or alkyl substituents, were the most potent anti-inflammatory compounds of this series showing an activity approximately ten times lower than that of indomethacin *(table V)*. Strong electron-withdrawing substituents (Cl or NO_2) decreased the

Table III. Physical constants for the 6-(R,R'-phenyl)-4-(indol-3-yl)-3-cyano-2-aminopyridines (**6a–x).**



6 a - x

Compound	R	R_1	Yield (%)	M.p. (°C)	Formula
6a	H	2-Cl	9	226	$C_{20}H_{13}N_4Cl$
6b	H	3-Cl	10	271	$C_{20}H_{13}N_4Cl$
6c	H	4-Cl	8	276	$C_{20}H_{13}N_4Cl$
6d	H	2-Br	74	223–25	$C_{20}H_{13}N_4Br$
6e	H	3-Br	9	280	$C_{20}H_{13}N_4Br$
6f	H	4-Br	8	> 280	$C_{20}H_{13}N_4Br$
6g	H	2-CH_3	10	220–224	$C_{21}H_{16}N_4$
6h	H	3-CH_3	8	235–238	$C_{21}H_{16}N_4$
6i	H	4-CH_3	17	240–245	$C_{21}H_{16}N_4$
6j	H	2-OCH_3	88	222–225	$C_{21}H_{16}N_4O$
6k	H	3-OCH_3	25	235	$C_{21}H_{16}N_4O$
6l	H	4-OCH_3	13	215–218	$C_{21}H_{16}N_4O$
6r	H	2-NO_2	9	210	$C_{20}H_{13}N_5O_2$
6m	H	3-NO_2	16	254	$C_{20}H_{13}N_5O_2$
6n	H	4-NO_2	15	280	$C_{20}H_{13}N_5O_2$
6s	H	2-OH	10	270–273	$C_{20}H_{14}N_4O$
6t	H	4-OH	36	206–209	$C_{20}H_{14}N_4O$
6u	2-Cl	4-Cl	24	216–218	$C_{20}H_{13}N_4Cl_2$
6v	2-Cl	5-Cl	30	208–210	$C_{20}H_{13}N_4Cl_2$
6w	2-OCH_3	4-OCH_3	7	220–223	$C_{22}H_{18}N_4O_2$
6x	3-OCH_3	4-OCH_3	20	263–266	$C_{22}H_{18}N_4O_2$

potency, (3-chloro) and (4-chloro)-4-phenyl derivatives (**3b, 3c**) being almost inactive, the bromine derivatives **3d, 3e** showed a transient activity, as 2,5-dimethoxy **3q.**

The displacement of the 2-hydroxyphenyl substituent in the 4-position of the 3-cyano-2-aminopyridine (**4a**–**e**) produced a decrease of potency and a transient activity *(table VI)*. All compounds **3** and **4** did not show significant analgesic and antipyretic activities.

The replacement of the aryl in the 4-position with a β-indolic group gave rise to compounds **6,** characterized by a better anti-inflammatory activity as compared to that of the corresponding **3** *(table VII)*, however, the influence of the 6-phenyl substituents on the structure/activity relationship was not so clear. Thus, while their electronic characteristic did not influence the anti-inflammatory activity, their position significantly influenced the potency, the most potent derivatives being the 6-(3-phenyl) monosubstituted compounds **6b, 6h, 6m** and the disubstituted ones **6t**–**u.** Furthermore, many derivatives showed a transient activity (**6f, 6j**–**k, 6r, 6v**). The displacement of the indolic group from 4 to 6 position of the 3-cyano-2-aminopyridine nucleus (**8h, 8w**) decreased the anti-inflammatory activity *(table VIII)*.

Compounds **6h, 6m** and **6x** also showed a good analgesic activity which was ten times lower than indomethacin *(table IX)*, while the **8** derivatives were nearly inactive. Furthermore, the **6** and **8** compounds had a very low antipyretic properties.

The new 3-cyano-2-aminopyridine derivatives (**3**, **6**) generally showed a more potent anti-inflammatory activity in comparison with the corresponding 2-(1H)-pyridone derivatives synthesized in our previous

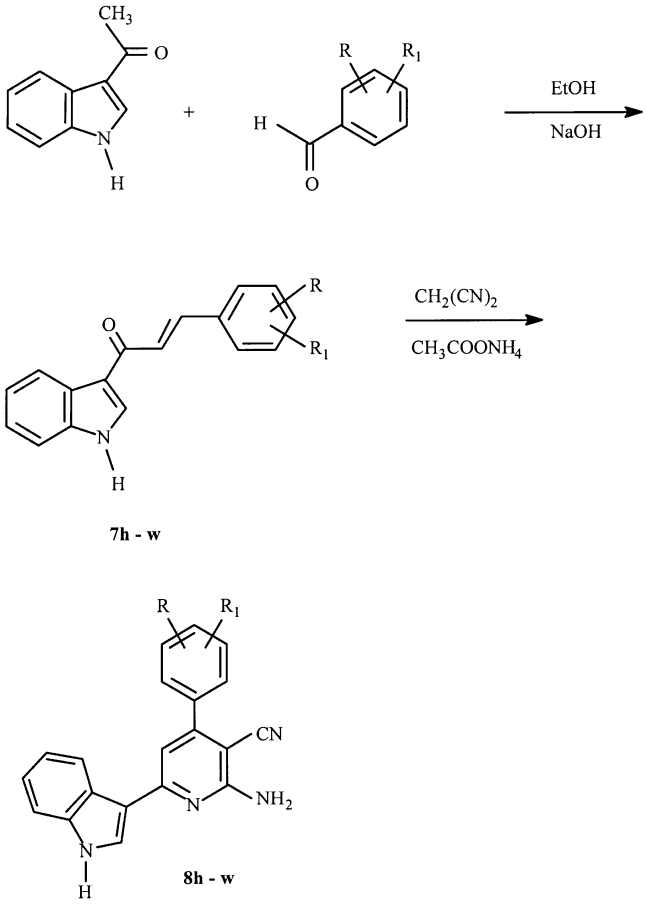


Figure 3. 6-(indol-3-yl)-4-(R,R'-phenyl)-3-cyano-2-aminopyridines (**8h–w**).

search [4]. Furthermore, the activity of 3-cyano-2-aminopyridine derivatives (**3**, **6**) was increased by electron-releasing substituents on the 4-aryl group and on the 6-aryl group respectively, while the activity of 4,6-diaryl-3-cyano-2-(1H)-pyridones was markedly increased by electron-withdrawing substituents.

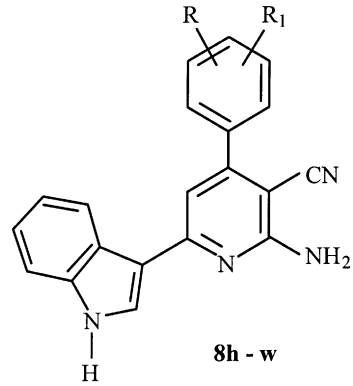
4. Conclusions

The presence of amine group in the 2-position of the pyridine ring gave rise to an increment of the anti-inflammatory activity and affected also the pharmacological answer to the influence of the substituents on 4 or 6-aryl rings in comparison with the corresponding 4,6-diaryl-3-cyano-2-(1H)-pyridones.

The electronegativity of the substituents and their displacement on the 4-aryl ring of the 4,6-diaryl-3-cyano-2-aminopyridines **3a**–**q** influenced the anti-inflammatory activity which was higher in the presence of electron-releasing groups. Furthermore, a decrease of potency was observed when the 2-hydroxyphenyl group was inserted in the 4-position of the 3-cyano-2-aminopyridine nucleus **4a**–**e**. All **3** and **4** derivatives did not show any interesting analgesic and antipyretic activities.

The introduction of the indol-3-yl group in the 4-position of the 3-cyano-2-aminopyridine nucleus gave rise to derivatives **6a**–**x** which exhibited better anti-inflammatory and analgesic properties, but there was no evidence of the relationship between the electronic nature of the substituents, their displacement on the 6-phenyl ring and the activity. In addition, the introduction of the

Table IV. Physical constants of the 6-(indol-3-yl)-4-(R,R'-phenyl)-3-cyano-2-aminopyridines (**8h–w).**



Compound	R	R_1	Yield (%)	M.p. (°C)	Formula
8h	H	3-CH_3	7	203–205	$C_{21}H_{16}N_4$
8w	2-OCH_3	4-OCH_3	6	208–210	$C_{22}H_{19}N_4O_2$

Table V. Anti-inflammatory activity of the 6-(2-hydroxyphenyl)-4-(R,R'-phenyl)-3-cyano-2-aminopyridines (**3a–q).**

Compound	mg/kg/os	Volume of edema (mL ± S.E.)			
		0	1 h [a]	2 h	4 h
Controls	Carrag. 1 % Sol.	0.8 ± 0.1	1.2 ± 0.1 (+50)	1.2 ± 0.1 (+50)	1.5 ± 0.1 (+87)
Indomethacin	5	1.0 ± 0.1	1.2 ± 0.1 (–50)	1.2 ± 0.1 (–50)	1.2 ± 0.1 (–71)
3a	50	0.9 ± 0.1	1.2 ± 0.1 (–25)	1.2 ± 0.1 (–25)	1.4 ± 0.1 (–28)
3b	50	0.9 ± 0.1	1.2 ± 0.1 (–25)	1.3 ± 0.1 (0)	1.6 ± 0.1 (0)
3c	50	1.1 ± 0.1	1.5 ± 0.1 (0)	1.5 ± 0.1 (0)	1.7 ± 0.1 (–14)
3d	50	1.0 ± 0.1	1.2 ± 0.1 (–50)	1.3 ± 0.1 (–25)	1.6 ± 0.1 (–14)
3e	50	0.7 ± 0.1	0.9 ± 0.1 (–50)	1.0 ± 0.1 (–25)	1.3 ± 0.1 (–28)
3f	50	0.8 ± 0.1	1.1 ± 0.1 (–25)	1.2 ± 0.1 (0)	1.3 ± 0.1 (–28)
3g	50	1.0 ± 0.1	1.3 ± 0.1 (–25)	1.4 ± 0.1 (0)	1.5 ± 0.1 (–28)
3h	50	1.1 ± 0.1	1.3 ± 0.1 (–50)	1.4 ± 0.1 (–25)	1.5 ± 0.1 (–43)
3i	50	1.1 ± 0.1	1.3 ± 0.1 (–50)	1.3 ± 0.1 (–50)	1.4 ± 0.1 (–57)
3j	50	0.9 ± 0.1	1.2 ± 0.1 (–25)	1.2 ± 0.1 (–25)	1.3 ± 0.1 (–43)
3k	50	1.0 ± 0.1	1.2 ± 0.1 (–50)	1.2 ± 0.1 (–50)	1.3 ± 0.1 (–57)
3l	50	1.1 ± 0.1	1.4 ± 0.1 (–25)	1.5 ± 0.1 (0)	1.6 ± 0.1 (–28)
3m	50	0.8 ± 0.1	1.1 ± 0.1 (–25)	1.2 ± 0.1 (0)	1.4 ± 0.1 (–14)
3n	50	1.1 ± 0.1	1.4 ± 0.1 (–25)	1.5 ± 0.1 (0)	1.6 ± 0.1 (–28)
3o	50	0.9 ± 0.1	1.2 ± 0.1 (–25)	1.3 ± 0.1 (0)	1.5 ± 0.1 (–14)
3p	50	0.9 ± 0.1	1.2 ± 0.1 (–25)	1.2 ± 0.1 (–25)	1.5 ± 0.1 (–14)
3q	50	1.2 ± 0.1	1.4 ± 0.1 (–50)	1.5 ± 0.1 (–25)	1.8 ± 0.1 (–14)
Controls [b]	Carrag. 1 % sol.	0.7 ± 0.1	1.2 ± 0.1 (+ 71.4)	1.4 ± 0.1 (+100.0)	1.8 ± 0.1 (+157.1)
Indomethacin [b]	5	1.2 ± 0.1	1.3 ± 0.1 (–63.1)	1.5 ± 0.2 (–75.0)	1.5 ± 0.1 (–132.1)
3w' [b]	50	0.9 ± 0.1	1.2 ± 0.1 (–38.1)	1.3 ± 0.1 (–55.6)	1.4 ± 0.1 (–101.6)
3u' [b]	50	0.8 ± 0.1	1.0 ± 0.2 (–46.0)	1.2 ± 0.1 (–50.0)	1.3 ± 0.1 (–94.6)

[a] Times from the administration; [b] published data [4]; percentages (in parantheses) indicate a variation of edema volume calculated in comparison with control values.

indol-3-yl group in the 6-position of the 3-cyano-2-aminopyridine nucleus markedly decreased potency (**8h**–**w**). Furthermore, compounds **6** and **8** did not display a significant antipyretic activity.

Table VI. Anti-inflammatory activity of the 6-(R-phenyl)-4-(2-hydroxyphenyl)-3-cyano-2-aminopyridines (**4a–e).**

Compound	mg/kg/os	Volume of edema (mL ± S.E.)			
		0	1 h [a]	2 h	4 h
Controls	Carrag. 1 % Sol.	0.8 ± 0.1	1.3 ± 0.1 (+62)	1.5 ± 0.1 (+87)	1.7 ± 0.1 (+112)
Indomethacin	5	1.0 ± 0.1	1.2 ± 0.1 (–60)	1.2 ± 0.1 (–71)	1.2 ± 0.1 (–78)
4a	50	0.8 ± 0.1	1.1 ± 0.1 (–60)	1.2 ± 0.1 (–43)	1.4 ± 0.1 (–44)
4b	50	0.9 ± 0.1	1.1 ± 0.1 (–60)	1.2 ± 0.1 (–57)	1.3 ± 0.1 (–55)
4c	50	0.7 ± 0.1	0.9 ± 0.1 (–60)	1.0 ± 0.1 (–57)	1.2 + 0.1 (–44)
4d	50	1.1 ± 0.1	1.4 ± 0.1 (–40)	1.4 ± 0.1 (–57)	1.6 ± 0.1 (–44)
4e	50	1.0 ± 0.1	1.2 ± 0.1 (–60)	1.3 ± 0.1 (–57)	1.5 ± (0.1) (–44)

[a] Times from the administration; percentages (in parantheses) indicate a variation of edema volume calculated in comparison with control values.

Table VII. Anti-inflammatory activity of the 6-(R,R'-phenyl)-4-(indol-3-yl)-3-cyano-2-aminopyridines (**6a–x).**

Compound	mg/kg/os	Volume of edema (mL ± S.E.)			
		0	1 h [a]	2 h	4 h
Controls	Carrag. 1 % Sol.	0.9 ± 0.1	1.3 ± 0.1 (+44)	1.5 ± 0.2 (+66)	1.6 ± 0.2 (+78)
Indomethacin	5	0.9 ± 0.1	1.1 ± 0.1 (–50)	1.1 ± 0.1 (–67)	1.1 ± 0.1 (–71)
6a	50	0.8 ± 0.1	1.0 ± 0.1 (–25)	1.2 ± 0.1 (–30)	1.4 ± 0.1 (–14)
6b	50	0.8 ± 0.1	1.0 ± 0.1 (–50)	1.0 ± 0.1 (–50)	1.1 ± 0.1 (–57)
6c	50	0.9 ± 0.1	1.1 ± 0.1 (–50)	1.2 ± 0.1 (–50)	1.4 ± 0.1 (–29)
6d	50	0.9 ± 0.1	1.2 ± 0.1 (–25)	1.2 ± 0.1 (–50)	1.4 ± 0.1 (–28)
6e	50	0.9 ± 0.1	1.2 ± 0.1 (–25)	1.3 ± 0.1 (–33)	1.6 ± 0.1 (0)
6f	50	0.7 ± 0.1	0.9 ± 0.1 (–50)	1.0 ± 0.1 (–50)	1.2 ± 0.1 (–28)
6g	50	0.9 ± 0.1	1.2 ± 0.1 (–25)	1.3 ± 0.1 (–33)	1.5 ± 0.1 (–14)
6h	50	1.0 ± 0.1	1.3 ± 0.1 (–25)	1.3 ± 0.1 (–50)	1.3 ± 0.1 (–57)
6i	50	0.8 ± 0.1	1.1 ± 0.1 (–25)	1.1 ± 0.1 (–50)	1.3 ± 0.1 (–28)
6j	50	1.1 ± 0.1	1.3 ± 0.1 (–50)	1.4 ± 0.1 (–33)	1.5 ± 0.1 (–43)
6k	50	0.7 ± 0.1	0.9 ± 0.1 (–50)	1.0 ± 0.1 (–50)	1.2 ± 0.1 (–28)
6l	50	0.8 ± 0.1	1.1 ± 0.1 (–25)	1.2 ± 0.1 (–33)	1.4 ± 0.1 (–14)
6r	50	0.7 ± 0.1	0.9 ± 0.1 (–50)	1.0 ± 0.1 (–50)	1.2 ± 0.1 (–28)
6m	50	0.8 ± 0.1	1.0 ± 0.1 (–50)	1.1 ± 0.1 (–50)	1.1 ± 0.1 (–57)
6n	50	0.9 ± 0.1	1.1 ± 0.1 (–25)	1.2 ± 0.1 (–50)	1.3 ± 0.1 (–43)
6s	50	1.0 ± 0.1	1.4 ± 0.1 (–25)	1.5 ± 0.1 (–33)	1.5 ± 0.1 (–43)
6t	50	1.0 ± 0.1	1.2 ± 0.1 (–50)	1.3 ± 0.1 (–50)	1.4 ± 0.1 (–43)
6u	50	0.8 ± 0.1	1.1 ± 0.1 (–25)	1.2 ± 0.1 (–33)	1.3 ± 0.1 (–28)
6v	50	1.1 ± 0.1	1.4 ± 0.1 (–50)	1.5 ± 0.1 (–50)	1.7 ± 0.1 (–14)
6w	50	0.9 ± 0.1	1.1 ± 0.1 (–25)	1.1 ± 0.1 (–50)	1.1 ± 0.1 (–57)
6x	50	1.0 ± 0.1	1.3 ± 0.1 (–25)	1.3 ± 0.1 (–50)	1.3 ± 0.1 (–57)

[a] Times from the administration; percentages (in parantheses) indicate a variation of edema volume calculated in comparison with control values.

5. Experimental protocols

5.1. Chemical synthesis

Melting points were determined in open glass capillaries with a Büchi 510 apparatus and are uncorrected. The IR spectra were recorded on a Perkin Elmer 218 B instrument, NMR spectra on a Varian EM-390 instrument, using TMS as internal standard, and were listed in *table X*. Results of elemental analysis were within ±0.4% of the theoretical values.

5.1.1. General procedure for compounds **3a**–**q***: 6-(2-hydroxyphenyl)-4-(R,R'-phenyl)-3-cyano-2-aminopyridines*

The compounds **3a**–**q** were synthesized according to the method described in [4]. IR (KBr) cm^{-1}: 3400–3200 (NH_2), 2200–2210 (CN), 1640–1580 (C=N–). ^{1}H-NMR (DMSO-d_6) ppm: 13.6–13.4 (s, 1H, OH); 8.0–6.7 (m, 10 or 11H, aromatic and aminic protons).

Table VIII. Anti-inflammatory activity of the 6-(indol-3-yl)-4-(R,R'-phenyl)-3-cyano-2-aminopyridines (**8h–w).**

Compound	mg/kg/os	Volume of edema (mL ± S.E.)			
		0	1 h [a]	2 h	4 h
Controls	Carrag. 1 % Sol.	0.9 ± 0.1	1.3 ± 0.1 (+44)	1.5 ± 0.1 (+67)	1.7 ± 0.1 (+89)
Indomethacin	5	1.0 ± 0.1	1.2 ± 0.1 (–50)	1.2 ± 0.1 (–67)	1.2 ± 0.1 (–75)
8h	50	1.2 ± 0.1	1.5 ± 0.1 (–25)	1.6 ± 0.1 (–33)	1.8 ± 0.1 (–25)
8w	50	0.8 ± 0.1	1.1 ± 0.1 (–25)	1.2 ± 0.1 (–33)	1.4 ± 0.1 (–25)

[a] Times from the administration; percentages (in parantheses) indicate a variation of edema volume calculated in comparison with control values.

Table IX. Analgesic activity of the 6-(R,R'-phenyl)-4-(indol-3-yl)-3-cyano-2-aminopyridines (**6a–x).**

Compound	mg/kg/os	Mean no. of writhes ± S.E. within % decrease compared with the	
		25 min after treatment	control group
Controls	Acetic acid	51.2 ± 10.8	
Indomethacin	5	26.3 ± 7.6	–48.6
6a	50	37.1 ± 1	–27.7
6b	50	42.5 ± 10.6	–16.9
6c	50	41.09 ± 9.3	–19.7
6d	50	45.6 ± 3.5	–10.9
6e	50	50.2 ± 3.5	–1.95
6f	50	46.4 ± 9.1	– 9.3
6g	50	33.5 ± 9.2	–34.5
6h	50	27.6 ± 9.9	–46.4
6i	50	35.4 ± 6.8	–30.8
6j	50	35.8 ± 6.5	–30.1
6k	50	26.5 ± 11.2	–48.2
6l	50	37.1 ± 9.4	–27.5
6r	50	37.5 ± 8.4	–26.7
6m	50	22.1 ± 12	–56.8
6n	50	31.3 ± 9.8	–38.8
6s	50	37.4 ± 9.8	–26.9
6t	50	45.4 ± 7.6	–11.3
6u	50	34.6 ± 7.6	–32.4
6v	50	37.5 ± 8.1	–26.7
6w	50	39.6 + 5.4	–22.6
6x	50	26.2 ± 8.1	–47.6

5.1.2. General procedure for compounds **2a**–**e***: 3-(R,R'-phenyl)-1-(2-hydroxyphenyl)-2-propen-1-ones*

0.1 mol of suitable acetophenone, dissolved in 30 mL of EtOH, in the presence of 30% NaOH (15 mL), were added dropwise to ice-cooled salicylic aldehyde (0.1 mol). The reaction mixture was allowed to stand for 24 h at room temperature, then neutralized with 15% HCl. The resulting solid was washed with petroleum

Table X. (a) ^{1}H-NMR ppm data of the 6-(2-hydroxyphenyl)-4-(R,R'-phenyl)-3-cyano-2-aminopyridines (**3a–q**), 6-(R,R'-phenyl)-4-(2-hydroxyphenyl)-3-cyano-2-aminopyridines (**4a–e**), 6-(R,R'-phenyl)-4-(indol-3-yl)-3-cyano-2-aminopyridines (**6a–x**), 6-(indol-3-yl)-4-(R,R'-phenyl)-3-cyano-2-aminopyridines (**8h–w**). (b) IR (KBr) cm^{-1} data of the 6-(2-hydroxyphenyl)-4-(R,R'-phenyl)-3-cyano-2-aminopyridines (**3a–q**), 6-(R,R'-phenyl)-4-(2-hydroxyphenyl)-3-cyano-2-aminopyridines (**4a–e**), 6-(R,R'-phenyl)-4-(indol-3-yl)-3-cyano-2-aminopyridines (**6a–x**), 6-(indol-3-yl)-4-(R,R'-phenyl)-3-cyano-2-aminopyridines (**8h–w).**

	OH	Ar, Ar', NH_2	NH indolic	Ar, Indolic, NH_2
3a–q	13.6–13.4 singlet	8.1–6.7 multiplet		
4a–e	13.6–13.4 singlet	8.1–6.7 multiplet		
6a–x			11.9–11.7 singlet	9.2–6.7 multiplet
8h–w			11.9 singlet	8.0–6.7 multiplet

Table X. (b) IR (KBr) cm^{-1} data of the 6-(2-hydroxyphenyl)-4-(R,R'-phenyl)-3-cyano-2-aminopyridines (**3a–q**), 6-(R,R'-phenyl)-4-(2-hydroxyphenyl)-3-cyano-2-aminopyridines (**4a–e**), 6-(R,R'-phenyl)-4-(indol-3-yl)-3-cyano-2-aminopyridines (**6a–x**), 6-(indol-3-yl)-4-(R,R'-phenyl)-3-cyano-2-aminopyridines (**8h–w).**

	NH_2	CN	C=N–
3a–q	3400–3200	2220–2210	1640–1580
4a–e	3400–3200	2220–2210	1640–1580
6a–x	3500–3300	2220–2210	1640–1580
8h–w	3500–3300	2220–2210	1640–1580

ether, then crystallized from 1:1 toluene/cyclohexane. Physical and spectroscopic characteristics of these compounds were comparable with known data [5, 6, 7, 8, 9, 10, 11, 12].

5.1.3. General procedure for compounds **4a**–**e***: 6-(R,R'-phenyl)-4-(2-hydroxyphenyl)-3-cyano-2-aminopyridines*

A solution of chalcone **2** (0.022 mol), malononitrile (0.044 mol) and ammonium acetate (0.0176 mol) in 80 mL of EtOH was refluxed under stirring, at 110 °C for 24 h. The solid separated was collected by filtration and washed with warm EtOH and acetone. IR, (KBr) cm^{-1}: 3400–3200 (NH_2), 2220–2210 (CN) and 1640–1580 (C=N–). ^{1}H-NMR (DMSO-d_6) ppm: 13.6–13.4 (s, 1H, OH); 8.1–6.8 (m, 10 or 11H, aromatic and aminic protons).

5.1.4. General procedure for compounds **5a**–**x***: 3-(indol-3-yl)-1-(R,R'-phenyl)-2-propen-1-ones*

0.11 mol of indol-3-carboxyaldehyde and 0.11 mol of suitable acetophenone, in the presence of 0.6 mL of piperidine, were dissolved in 80 mL of anhydrous EtOH and refluxed for 24 h at 110 °C under N_2, with stirring. The reaction mixture was poured into ice and neutralized with diluted acetic acid; the solid was filtered and crystallized from EtOH. IR (KBr) cm^{-1}: 3100–3000 (NH), 1645–1630 (C=O) and 1590–1560 (CH=CH). ^{1}H-NMR (DMF-d_7) ppm: 12–11 (s, 1H, NH), 9–8.2 (m, 4 or 5H, aromatic protons), 8–7.5 (2d, 2H, CH=CH, J = 18 Hz) and 7.9–7.2 (m, 5H, indolic protons).

5.1.5. General procedure for compounds **6a**–**x***: 6-(R,R'-phenyl)-4-(indol-3-yl)-3-cyano-2-aminopyridines*

A solution of indolic chalcone **5** (0.056 mol), malononitrile (0.056 mol) and ammonium acetate (0.38mol) in 80 mL of anhydrous EtOH was refluxed at 110 °C, with stirring, for 24 h. The resulting solid was collected by filtration, washed with water and purified by repeated washing with warm EtOH. IR (KBr) cm^{-1}: 3500–3300 (NH_2), 2220–2210 (CN) and 1640–1580 (C=N–). ^{1}H-NMR (DMSO-d_6) ppm: 11.9–11.7 (s, 1H, NH) and 9.2–6.7 (m, 11 or 12H, aromatic, indolic and aminic protons).

5.1.6. General procedure for compounds **7h**–**w***: 3-(R,R'-phenyl)-1-(indol-3-yl)-2-propen-1-ones*

0.07 mol of substituted benzaldehyde in 80 mL of EtOH were added to 0.07 mol of acetylindole, in the presence of 10% NaOH and were allowed to stand at room temperature for 24 h, with stirring. The reaction mixture was neutralized with 15% HCl; the resulting solid was filtered and crystallized from EtOH. IR (KBr) cm^{-1}: 3100–3000 (NH), 1645–1630 (C=O) and 1590–1560 (CH=CH). ^{1}H-NMR (DMSO-d_6) ppm: 11.9 (s, 1H, NH), 8.4–8.2 (m, 4 or 5H, aromatic protons), 8–7.6 (2d, 2H, CH=CH, J = 18 Hz) and 7.8–7.1 (m, 5H, indolic protons).

5.1.7. General procedure for compounds **8h**–**w***: 6-(indol-3-yl)-4-(R,R'-phenyl)-3-cyano-2-aminopyridines*

0.011 mol of chalcone **5,** 0.022 mol of malononitrile and 0.088 mol of ammonium acetate were dissolved in 80 mL of EtOH and refluxed at 110 °C, with stirring, for 24 h. The resulting solid was washed with warm EtOH and acetone. IR (KBr) cm^{-1}: 3500–3300 (NH_2), 2220–2210 (CN) and 1640–1580 (C=N). ^{1}H-NMR (DMSO-d_6) ppm: 11.9 (s, 1H, NH) and 8.0–6.7 (m, 11 or 12H, aromatic, aminic and indolic protons).

5.2. Pharmacological evaluation

5.2.1. Anti-inflammatory activity

Anti-inflammatory activity was evaluated by the paw edema test, using carrageenin (1%) on albino rats of both sexes (pregnant rats excluded) weighing 180–250 g; each group comprised five animals. The compounds were administered by oral route (gavage) 30 min before the carrageenin, at a dose of 50 mg/kg. The volume of the rat's paw was measured 1, 2 and 4 h after the administration of carrageenin, and indomethacin was used as reference compound (5 mg/kg).

The reported values were the average of five determinations ± S.E. and the percentage of activity was calculated in comparison with controls. The significance was calculated by Student's *t*-test for coupled values *(tables V, VI, VII* and *VIII)*.

5.2.2. Analgesic activity

Analgesic activity was determined by means of acetic acid test (writhing test) [13] carried out on *Mus musculus*. Each group was composed of five animals of both sexes, pregnant females excluded (body weight 18–25 g). The compounds were administered orally at a dose of 50 mg/kg and indomethacin was used as reference compound (5 mg/kg).

The animals were injected intraperitoneally with 0.25 mL /mouse of 0.5% aqueous acetic acid solution and writhes were counted during the subsequent 25 min. An oral dose of a test compound was administered 30 min before the injection. The mean number of writhes for each experimental group ± S.E. and percent decrease compared with the control group (five mice not treated with test compounds) were calculated. Results are given in *table IX*.

5.2.3. Antipyretic activity

Antipyretic activity was determined by measuring the variation in the body temperature in albino rats of both sexes (pregnant rats excluded) weighing 180–200 g. Fever was induced by a 10% solution of brewer's yeast (10 mL /kg) subcutaneously administered. Each group was composed of five animals.

The reported values were the average of five determinations ± S.E. and the percentage of activity was calculated in comparison with basal values. The significance was calculated by the Student's *t*-test for coupled values.

The compounds tested were administered by oral route at a dose of 50 mg/kg and indomethacin was used as reference compound (5 mg/kg).

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