Synthesis of Mannich Bases of Arylidenepyridazinones as Analgesic Agents

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Abstract □ A series of 5-arylidenepyridazin-3-ones substituted in the 2-position by an arylpiperazinoalkyl moiety (**2–16**) was synthesized and evaluated for analgesic activity. In the phenylbenzoquinone-induced writhing test, Mannich bases **2–14** were the most active compounds ($6.1 \leq ED_{50} \leq 43.0$ mg/kg, orally; ED_{50} is the half-maximal effective dose). Pyridazinones **8** and **9**, with a 3-chlorophenylpiperazinomethyl substituent, also exhibited significant anti-inflammatory and antipyretic effects. The activities in the phenylbenzoquinone-induced writhing test were subjected to a Hansch analysis, and a significant correlation with lipophilicity and Hammett's constants was obtained.

Introduction of aryl or heteroarylpiperazino groups on different pharmacophores has been of considerable interest for medicinal compounds, such as fluanisone, trazodone, buspirone, and urapidil, with neuroleptic, antidepressant, anxiolytic, and antihypertensive properties, respectively. Recently, many authors described benzoxazolinone, triazinone, and pyridazinone derivatives, including an arylpiperazino moiety, with analgesic, antidepressant, and tranquilizing activities, respectively.[1–3] In a previous paper, we reported analgesic properties of similarly substituted 4,6-diarylpyridazinones.[4]

These observations prompted us to synthesize a new series of 5-arylidene-6-methylpyridazin-3-ones substituted in the 2-position by various arylpiperazinoalkyl moieties. All the new compounds prepared were tested for their analgesic, anti-inflammatory, and antipyretic activities. The biological results were subjected to a Hansch analysis[5] to determine which structural elements were required to obtain potent analgesic activity in this series of compounds.

Experimental Section

Melting points were determined on a Kofler apparatus and are uncorrected. The IR spectra were obtained with a Beckman 4240 spectrophotometer, and proton nuclear magnetic resonance (^1H NMR) spectra were recorded on a Varian EM 360 A spectrometer, with tetramethylsilane as an internal standard. Elemental analyses (C, H, F, Cl, and N within ±0.4% of theoretical values) were performed at the Service Central d'Analyses, Centre National de la Recherche Scientifique, 69390 Vernaison, France.

Chemistry—*5-Arylidene-6-methyl-(2H,4H)-pyridazin-3-ones (1)*—The synthesis of derivative **1** was reported in previous papers.[6,7]

5-Substituted Benzylidene-6-methyl-(4H)-2-(4-arylpiperazin-1-yl)methylpyridazin-3-ones (2–13)—A solution of 35% aqueous formaldehyde (1.7 mL, 0.02 mol), pyridazinone **1** (0.02 mol), and arylpiperazine (0.02 mol) in ethanol (75 mL) was refluxed for 12 h with continuous stirring and evaporated under reduced pressure. The oily residue was triturated with diisopropyl ether, and the resulting solid was collected by filtration and recrystallized from ethanol:ethyl ether (40:60).

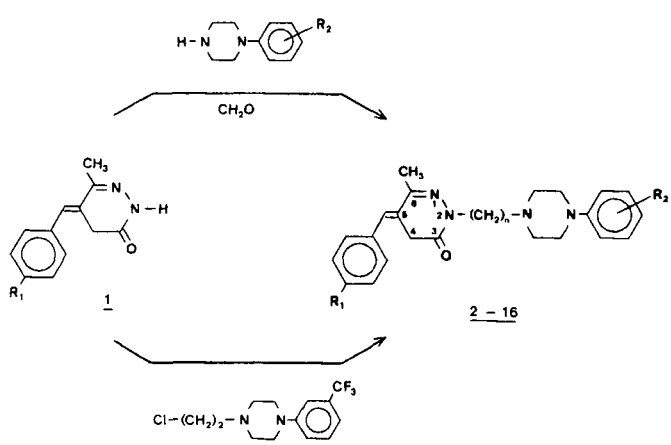
5-Substituted Benzylidene-6-methyl-(4H)-2-[2-(4-aryl-piperazin-1-yl)]ethylpyridazin-3-one Hydrochlorides (14–16)—The appropriate pyridazinone **1** (0.015 mol) was added to an ethanolic solution (40 mL) containing 0.35 g of sodium. The solution was refluxed for 1 h and evaporated to dryness. The resulting pyridazinone sodium salt was dissolved in dimethylformamide (100 mL) containing 1-(2-chloroethyl)-4-(3-trifluoromethylphenyl)piperazine (4.39 g, 0.015 mol). The solution was refluxed for 10 h with stirring, and the mineral precipitate was filtered off after cooling. The solution was then evaporated to dryness. The oily residue was dissolved in absolute ethanol (20 mL), and the ethanolic solution was saturated with gaseous hydrochloric acid. Compounds **14–16** were precipitated by addition of diethyl ether and recrystallized from 2-butanone.

For lipophilicity measurements, the corresponding bases were prepared extemporaneously from the hydrochlorides.

Pharmacology—For biological evaluation, all compounds were administered orally in a 0.5% hydroxypropyl methylcellulose aqueous suspension, a route usually and recently[8] used for pyridazinones. Iffa Credo OF1 male mice (20 g) and OFA Sprague-Dawley male rats were used.

Table I—Physical and Chemical Data for Pyridazinones 2–16

Compound	R_1	R_2	n	mp, °C	Yield, %	Formula
2	H	H	1	98	75	$C_{23}H_{26}N_4O$
3	F	H	1	120	89	$C_{23}H_{25}FN_4O$
4	CH_3	H	1	123	89	$C_{24}H_{28}N_4O$
5	H	3-CF_3	1	120	89	$C_{24}H_{25}F_3N_4O$
6	F	3-CF_3	1	100	84	$C_{24}H_{24}F_4N_4O$
7	CH_3	3-CF_3	1	114	79	$C_{25}H_{27}F_3N_4O$
8	H	3-Cl	1	86	59	$C_{23}H_{25}ClN_4O$
9	F	3-Cl	1	115	62	$C_{23}H_{24}ClFN_4O$
10	CH_3	3-Cl	1	100	76	$C_{24}H_{27}ClN_4O$
11	H	4-F	1	93	82	$C_{23}H_{25}FN_4O$
12	F	4-F	1	98	86	$C_{23}H_{24}F_2N_4O$
13	CH_3	4-F	1	121	91	$C_{24}H_{27}FN_4O$
14	H	3-CF_3	2	182	43	$C_{25}H_{27}F_3N_4O \cdot HCl$
15	F	3-CF_3	2	160	43	$C_{25}H_{26}F_4N_4O \cdot HCl$
16	CH_3	3-CF_3	2	180	44	$C_{26}H_{29}F_3N_4O \cdot HCl$



Scheme I

0022-3549/92/1100-1084$02.50/0

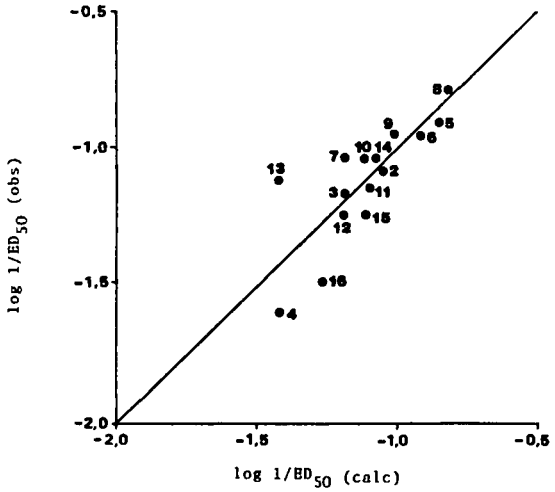


Figure 1—Correlation between calculated and observed values for data used in the analysis.

Acute Toxicity in Mice—The compounds were administered orally at doses of 200, 400, 600, 800, and 1000 mg/kg. The animals were observed for 8 days for any sign of toxicity.

Analgesic Activity—For the phenylbenzoquinone writhing test,[9,10] a 0.02% solution (ethanol:water, 5:95) of phenylbenzoquinone was administered by intraperitoneal injection to mice (groups of 10) 30 min after oral administration of the test drugs. The writhing-response frequency of each animal was counted between the 5th and the 15th min after injection of the irritant.

For the hot-plate test,[11,12] groups of 10 mice were used. Animals were placed on a copper plate maintained at a constant temperature of 56 °C. The time necessary to induce the licking reflex of the fore paws was then recorded. Two basal measurements of the pain threshold were made before drug administration. Measurements were carried out 30 min later.

Anti-inflammatory Activity—This activity was studied by the method of Winter and Risley[13] as revised by Doherty and Robinson[14] with groups of six rats weighing 100–120 g and with carrageenin as the phlogogenic agent. Edema was induced in the right hind paw by intradermal injection (0.05 mL) of a 1% carrageenin solution. The drugs were administered orally 60 min before edema induction. The volume of the inflamed paw was measured before and 3 h after

Table II—Spectral Data for Pyridazinone Derivatives 2–16

Compound	IR (KBr) Wavenumbers, cm^{-1}			^1H NMR (δ, $DMSOd_6$) Chemical Shifts, ppm[a]
	NH^+	C=O	C=N C=C	
2	—	1660	1600 1490 1450	2.25 (s, 3H, CH_3), 2.70 (m, 4H, c), 3.15 (m, 4H, d), 3.95 (s, 2H, a), 4.95 (s, 2H, b), 6.50 (s, 1H, CH=), 7.20 (m, 10H, 2Ar)
3	—	1670	1600 1510 1450	2.20 (s, 3H, CH_3), 2.70 (m, 4H, c), 3.10 (m, 4H, d), 3.70 (s, 2H, a), 4.85 (s, 2H, b), 6.45 (s, 1H, CH=), 7.10 (m, 9H, 2Ar)
4	—	1650	1590 1490 1450	2.15 (s, 3H, CH_3Ar), 2.20 (s, 3H, CH_3), 2.70 (m, 4H, c) 3.00 (m, 4H, d), 3.80 (s, 2H, a), 4.80 (s, 2H, b), 6.35 (s, 1H, CH=), 6.90 (m, 9H, 2Ar)
5	—	1660	1600 1490 1450	2.25 (s, 3H, CH_3) 2.85 (m, 4H, c), 3.25 (m, 4H, d), 3.95 (s, 2H, a), 5.00 (s, 2H, b), 6.55 (s, 1H, CH=), 7.30 (m, 9H, 2Ar)
6	—	1665	1600 1500 1450	2.30 (s, 3H, CH_3), 2.90 (m, 4H, c), 3.30 (m, 4H, d), 3.95 (s, 2H, a), 5.00 (s, 2H, b) 6.55 (s, 1H, CH=), 7.25 (m, 8H, 2Ar)
7	—	1660	1600 1500 1450	2.20 (s, 3H, CH_3Ar), 2.30 (s, 3H, CH_3), 2.80 (m, 4H, c), 3.20 (m, 4H, d), 3.85 (s, 2H, a), 4.95 (s, 2H, b), 6.50 (s, 1H, CH=), 7.25 (m, 8H, 2Ar)
8	—	1660	1600 1490 1450	2.25 (s, 3H, CH_3), 2.70 (m, 4H, c), 3.20 (m, 4H, d), 3.90 (s, 2H, a), 4.90 (s, 2H, b), 6.50 (s, 1H, CH=), 7.15 (m, 9H, 2Ar)
9	—	1660	1600 1500 1450	2.20 (s, 3H, CH_3), 2.70 (m, 4H, c), 3.20 (m, 4H, d), 3.90 (s, 2H, a), 4.90 (s, 2H, b), 6.50 (s, 1H, CH=), 7.00 (m, 8H, 2Ar)
10	—	1660	1600 1510 1450	2.20 (s, 3H, CH_3Ar), 2.30 (s, 3H, CH_3), 2.75 (m, 4H, c), 3.15 (m, 4H, d), 3.85 (s, 2H, a), 4.90 (s, 2H, b), 6.50 (s, 1H, CH=), 7.10 (m, 8H, 2Ar)
11	—	1650	1590 1500 1440	2.20 (s, 3H, CH_3), 2.80 (m, 4H, c), 3.05 (m, 4H, d), 3.90 (s, 2H, a), 4.95 (s, 2H, b), 6.50 (s, 1H, CH=), 7.15 (m, 9H, 2Ar)
12	—	1670	1600 1500 1450	2.20 (s, 3H, CH_3), 2.85 (m, 4H, c), 3.10 (m, 4H, d), 3.90 (s, 2H, a) ,4.95 (s, 2H, b), 6.50 (s, 1H, CH=), 7.20 (m, 8H, 2Ar)
13	—	1660	1600 1505 1445	2.20 (s, 3H, CH_3Ar), 2.30 (s, 3H, CH_3), 2.80 (m, 4H, c), 3.10 (m, 4H, d), 3.85 (s, 2H, a), 5.00 (s, 2H, b), 6.50 (s, 1H, CH=), 7.10 (m, 8H, 2Ar)
14	3400 2420	1660	1590 1500 1450	2.30 (s, 3H, CH_3), 3.30 (m, 4H, d), 3.50 (m, 6H, $3CH_2N^+$), 3.90 (s, 2H, a), 4.60 (m, 2H, b), 6.70 (s, 1H, CH=), 7.60 (m, 9H, 2Ar), 12.20 (br s, 1H, NH^+)
15	3410 2440	1660	1590 1500 1445	2.20 (s, 3H, CH_3), 3.30 (m, 4H, d), 3.50 (m, 6H, $3CH_2N^+$), 3.90 (s, 2H, a), 4.55 (m, 2H, b), 6.65 (s, 1H, CH=), 7.35 (m, 8H, 2Ar), 11.90 (br s, 1H, NH^+)
16	3400 2400	1660	1600 1500 1450	2.30 (s, 3H, CH_3Ar), 2.40 (s, 3H, CH_3), 3.30 (m, 4H, d), 3.60 (m, 6H, $3CH_2N^+$), 3.90 (s, 2H, a), 4.50 (m, 2H, b), 6.65 (s, 1H, CH=), 7.35 (m, 8H, 2Ar), 11.80 (br s, 1H, NH^+

[a] The letters a, b, c, and d refer to the carbon atoms in **2–16** in Scheme I.

Table III—Analgesic Activity (Expressed as ED_{50}) Determined by Phenylbenzoquinone-Induced Writhing Test (PBQ), and Hot-Plate Test (HP) and Lipophilicity (log k_w) of Pyridazinone Derivatives[a]

Compound	ED_{50}, mg/kg		log k_w
	PBQ	HP	
2	12.0 (5.1–28.0)	17.5 (8.8–35.0)	2.02
3	15.0 (7.7–29.3)	>20	2.16
4	43.0 (24.4–75.6)	>20	2.40
5	8.0 (3.5–18.1)	5.8 (2.9–11.6)	2.06
6	9.0 (4.2–19.4)	4.5 (2.6–2.8)	2.14
7	11.0 (7.5–16.0)	17.5 (14.2–21.5)	2.42
8	6.1 (5.3–7.0)	10.5 (8.2–13.4)	2.00
9	8.8 (3.1–24.6)	11.2 (10.3–12.1)	2.20
10	11.0 (7.3–16.5)	15.0 (9.4–24.0)	2.31
11	14.0 (8.4–23.4)	15.0 (12.2–18.4)	2.10
12	18.0 (12.8–25.3)	>20	2.19
13	13.5 (8.4–21.6)	>20	2.44
14	11.0 (4.9–24.6)	15.0 (12.0–18.7)	4.60[b]
15	18.0 (10.4–31.2)	>20	4.70[b]
16	31.0 (20.0–40.8)	>20	5.00[b]
Aspirin	60.0 (41.9–85.8)	—[c]	—
NAP	48.0 (34.5–66.7)	98.0 (70.0–137.2)	—
Morphine	—	2.4 (2.0–2.9)	—

[a] Drugs were administered orally; 95% confidence intervals are given in parentheses. [b] Evaluated with base compounds. [c] —, Not tested.

carrageenin injection with a Ugo Basile apparatus.

Antipyretic Activity—The method described by Rouveix[15] was used. Groups of six rats weighing 220 g were injected subcutaneously with a 20% aqueous yeast suspension in a volume of 10 mL/kg. After 16 h, the rectal temperature was measured with a Carrieri thermorapid apparatus. The temperature was also measured 4 h after administration of test drugs, and the difference from the initial values was recorded.

Sedative Activity—This activity was evaluated by a study of the spontaneous motor activity, by the method of Boissier and Simon,[16] in photoelectric activity cages (Apelex). Groups of 10 mice were used. Test drugs were administered 30 min before evaluation of spontaneous motor activity, and the number of passages were scored during a period of 10 min.

Data Analysis—The ED_{50}s (half-maximal effective doses) for analgesic activity were determined by the method of Litchfield and Wilcoxon.[17] For the other activities, all values were expressed as mean ± standard error, and the data were analyzed by the *t* test.

Lipophilicity Measurements—Lipophilicity was determined by reversed-phase high-performance liquid chromatography.[18,19] A Varian 5000 liquid chromatograph equipped with a detector operating at 254 nm was used. A Varian CDS 111L integrator was used for peak registration and calculation of retention times. A column (15 × 6 mm i.d.) prepacked with octadecyl copolymer gel (particle size, 5 μm) was used as the nonpolar stationary phase. The mobile phases were prepared volumetrically from combinations of methanol and aqueous 3-morpholinopropane sulfonic acid buffer (0.02 M, pH 7.4) in the range 50–90%. The flow rate was 1 mL/min. Isocratic capacity factors (k_i) were defined as $k_i = (t_r - t_0)/t_0$, where t_r is the retention time of the solute and t_0 is the column dead time determined with methanol as the nonretained compound. We used log k_w as the lipophilic index; log k_w was obtained by linear extrapolation of log k_i to 100% water.

Results and Discussion

Chemistry—The 5-arylidene-6-methyl-2-arylpiperazinoalkyl-3-(4*H*)-pyridazinones listed in Table I were prepared by the methods shown in Scheme 1. Reaction of formaldehyde and arylpiperazine with pyridazinones **1** afforded derivatives **2–13** via a Mannich condensation. Alkylation at the 2-N nucleophilic position of the pyridazine ring with 2-chloroethyl-4-(3-trifluoromethylphenyl)-piperazine yielded **14–16**. The structures of derivatives **2–16** were supported by elemental analysis (Table I) and spectral data (Table II).

Pharmacological Evaluation—All the prepared compounds were screened for their analgesic activity and acute toxicity. The oral LD_{50} (50% lethal dose) in mice was ~600 mg/kg for all products. Pyridazinones **2–16** exhibited significant analgesic activity in the phenylbenzoquinone-induced writhing test in mice.

Compounds **5**, **6**, **8**, and **9**, with a 3-trifluoromethyl or a 3-chlorophenylpiperazinomethyl substituent, showed potent activity (Table III). In the hot-plate test, pyridazinones **5** and **6** were the most potent derivatives, with oral ED_{50} values of 5.8 and 4.5 mg/kg, respectively. This activity is lower than that of morphine but is several times higher than that of aspirin and noramidopyrine (NAP).

The most active derivatives in both tests were run through a battery of screens and compared with aspirin, NAP, and phenylbutazone (PBZ) (Table IV). In the rat paw edema test, derivatives **5**, **6**, **8**, and **9** were approximately equipotent to aspirin or NAP at the same oral dose of 200 mg/kg. Furthermore, these four compounds were significantly effective and much more active than aspirin, NAP, and PBZ against yeast-induced fever in mice. Spontaneous motor activity in mice was used as a measure of the sedative action of **5**, **6**, **8**, and **9** in the central nervous system area. Only pyridazinones **5** and **9** provoked a very slight decrease in spontaneous motor activity at oral doses of 50 mg/kg.

To establish a quantitative structure–activity relationship, we performed a multiple linear regression analysis on all tested arylidenepyridazine derivatives. The analgesic data in the phenylbenzoquinone-induced writhing test were used for analysis. The ratios were correlated with selected physicochemical properties: the capacity factors (log k_w, 100% water) as a lipophilic parameter and Hammett's constants relative to the substituents of the two phenyl rings as an electronic parameter. The activity for the whole data set could effectively be modeled by a few parameters from the following equation, in which the standard error of the estimate for the fitted values is given in parentheses:

$$\log 1/ED_{50} = -0.95(\pm 0.24)(\log k_w/n) + 0.58(\pm 0.20)\sigma + 0.86$$

In the regression equation, the number of compounds *n* is 15, and the Hammett constant for the substituents of the only phenyl nucleus attached to the piperazinyl moiety is designated by σ. For the regression, the squared correlation coefficient (r^2) is 0.642, the residual standard deviation (s) is 0.143, and the variance ratio (F) is 10.8 ($p < 0.002$).

Data used in the analysis and log $1/ED_{50}$ values recalculated from the equation are graphically represented in Figure 1. From the equation, it can be inferred that lipophilicity displays a leading part in activity; that is, the least lipophilic compounds are the most active ones. In fact, the substituents on the arylidene moiety as well as the benzylidene group only contributed to lipophilicity. On the other hand, the presence and the nature of the substituent borne by the phenyl nucleus attached to the piperazinyl moiety seemed to be essential for potent analgesic activity.

References and Notes

1. Bermann, M. C.; Bonte, J. P.; Lesieur-Demarquilly, I.; Debaert, M.; Lesieur, D.; Leinot, M.; Benoit, J.; Labrid, C. *Eur. J. Med. Chem.* 1982, *17*, 85–88.
2. Ferrand, G.; Dumas, H.; Depin, J. C.; Chavernac, G. *Eur. J. Med. Chem.* 1987, *22*, 337–345.
3. Nakao, T.; Anami, K.; Yamamoto, Y. *Jpn. Kokai Tokyo Koho* JP01,106,868, 1989; *Chem. Abstr.* 1989, *111*, 194 797u.
4. Rubat, C.; Coudert, P.; Tronche, P.; Bastide, J.; Bastide, P.; Privat, A. M. *Chem. Pharm. Bull.* 1989, *37*, 2832–2835.
5. Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; Wiley-Interscience: New York, 1979.

Table IV—Anti-inflammatory, Antipyretic, and Sedative Activities

Compound	Increase in Paw Volume, %[a]	Rectal Temperature (°C) at the Following Times (h) after Treatment[b]:			Effect on Motor Activity at Oral Doses of[c]:	
		0	4	ΔT	25 mg/kg	50 mg/kg
5	52 ± 2	38.50 ± 0.10	33.20 ± 0.20	−5.30	−10 ± 5	−30 ± 7
6	57 ± 1	38.40 ± 0.05	32.40 ± 0.10	−6.00	−2 ± 1	−6 ± 4[d]
8	50 ± 3	38.50 ± 0.06	32.20 ± 0.20	−6.30	+2 ± 1	−3 ± 1[d]
9	70 ± 2	38.60 ± 0.08	33.30 ± 0.10	−5.30	−5 ± 3	−20 ± 6[e]
Aspirin	51 ± 10	38.60 ± 0.14	36.90 ± 0.09	−1.70	+4 ± 2	−3 ± 2[d]
NAP	62 ± 3	38.80 ± 0.04	36.00 ± 0.33	−2.80	−2 ± 1	−4 ± 2[d]
PBZ	71 ± 2	38.50 ± 0.07	35.60 ± 0.22	−2.90	−6 ± 2	−15 ± 4[f]

[a] Anti-inflammatory activity was determined by the carageenin-induced paw edema test after oral administration of compounds at 200 mg/kg, except for PBZ, which was administered orally at 100 mg/kg. All values are significantly different from control at $p < 0.001$. [b] Antipyretic activity was determined by the yeast-induced pyrexia test after oral administration of **5**, **6**, **8**, and **9** at 100 mg/kg and of aspirin, NAP, and PBZ at 200 mg/kg. ΔT is the temperature change at 4 h after drug administration. All values at 4 h after treatment are significantly different from control at $p < 0.001$. [c] Sedative activity was determined by the spontaneous motor activity test; results are expressed as percent increase (positive sign) or decrease (negative sign) compared with control. All values for the 25-mg/kg dose are not significantly different from control. [d] No significant difference from control. [e] Significantly different from control at $p < 0.01$. [f] Significantly different from control at $p < 0.05$.

6. Taoufik, J.; Couquelet, J. M.; Couquelet, J. D.; Tronche, P. *J. Heterocycl. Chem.* **1985**, *22*, 1615–1619.
7. Rubat, C.; Coudert, P.; Refouvelet, B.; Tronche, P.; Bastide, P.; Bastide, J. *Chem. Pharm. Bull.* **1990**, *38*, 3009–3013.
8. Dal Piaz, V.; Ciciani, G.; Turco, G.; Giovannoni, M. P.; Miceli, M.; Pirisino, R.; Peretti, M. *J. Pharm. Sci.* **1991**, *80*, 341–348.
9. Siegmund, E.; Cadmus, R.; Lu, G. *Proc. Soc. Exp. Biol. Med.* **1957**, *95*, 729–731.
10. Linée, P. *J. Pharmacol. (Paris)* **1972**, *3*, 513–515.
11. Woolfe, G.; McDonald, A. D. *J. Pharmacol. Exp. Ther.* **1944**, *80*, 300–307.
12. O'Callaghan, J. P.; Holtzman, S. G. *J. Pharmacol. Exp. Ther.* **1975**, *192*, 497–505.
13. Winter, C. A.; Risley, E. A. *Proc. Soc. Exp. Biol. Med.* **1962**, *3*, 544–547.
14. Doherty, M. S.; Robinson, B. V. *J. Pharmacol.* **1975**, *27*, 701–703.
15. Rouveix, B. *J. Pharmacol. (Paris)* **1980**, *11*, 133–136.
16. Boissier, J. R.; Simon, P. *Arch. Int. Pharmacodyn. Ther.* **1965**, *158*, 212–221.
17. Litchfield, J. T.; Wilcoxon, F. *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99–113.
18. El Tayar, N.; Van de Waterbeemd, H.; Testa, B. *J. Chromatogr.* **1985**, *320*, 293–304.
19. Bechalany, A.; Rothlisberger, T.; El Tayar, N.; Testa, B. *J. Chromatogr.* **1989**, *473*, 115–124.

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