Synthesis and Antiinflammatory Evaluation of 9-Anilinoacridine and 9-Phenoxyacridine Derivatives

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Mast cells, neutrophils, and macrophages are important inflammatory cells that have been implicated in the pathogenesis of acute and chronic inflammatory diseases. To explore a novel antiinflammatory agent, we have synthesized two types of acridines, 9-anilinoacridine and 9-phenoxyacridine derivatives, for evaluation on the grounds that acridine is a versatile heterocycle possessing a wide variety of biological properties. The title compounds were synthesized by reaction of 9-chloroacridine with appropriate Ar−NH_2 and Ar−OH, and their antiinflammatory activities on inhibitory effects on the activation of mast cells, neutrophils, and macrophages were studied. Three acridine derivatives **4**, **10**, and **11** were proved to be more potent than the reference inhibitor mepacrine for the inhibition of rat peritoneal mast cell degranulation with similar IC_{50} values (16−21 μM). Compound **3** also showed potent inhibitory activity (IC_{50} = 8.2 and 4.4 μM, respectively) for the secretion of lysosomal enzyme and β-glucuronidase from neutrophils. Moreover, compounds **5** and **9** were shown to be efficacious inhibitors of TNF-α production in macrophage-like cell lines RAW 264.7. Compounds **2** and **12** were the potent inhibitors of TNF-α production in murine microglial cell lines N9. To further explore the cytotoxic properties of these acridine derivatives, (*E*)-**12** was selected for NCI's in vitro disease-oriented tumor cells screen. The results indicated that this compound had no significant cytotoxicity with a mean GI_{50} of 58.0 μM. These results indicated that the antiinflammatory effects of acridine derivatives were mediated, at least in part, through the suppression of chemical mediators released from mast cells, neutrophils, and macrophages and that these compounds have the potential to be novel antiinflammatory agents with no significant cytotoxicity.

Introduction

Mast cells play an important role in anaphylaxis and inflammation. A variety of inflammatory mediators are secreted from the mast cell during cell activation. Elevated secretory product levels, which result in edema, bronchoconstriction, and leukocyte infiltration,[1] have been observed in a number of inflammatory diseases. Phagocytes are also important participants in the host defense against microbial infection and are implicated in acute and chronic inflammatory diseases. Activated neutrophils release lysosomal enzymes that are capable of proteolytic disruption of healthy tissue in a number of disease states such as pulmonary emphysema, rheumatoid arthritis, arteriosclerosis, and glomerulonephritis.[2,3] Macrophages interact with other immune cells and serve as central regulators of specific immune response.[4] Following the activation of macrophages, tumor necrosis factor-α (TNF-α) was generated, which mediated a wide variety of pathologic states such as lethal septic shock, rheumatoid arthritis, and cachexia.[5] Microglia, the resident macrophage of the brain, is implicated in the pathogenesis of inflammation and neurodegenerative diseases in the central nervous system.[6,7] Thus, a therapeutic agent that inhibits the activation of inflammatory cells and the following release of inflammatory mediators may be useful for the treatment of these inflammatory conditions.

9-Aminoacridine has been used clinically as an antiseptic drug. This tricyclic heterocycle may interact with DNA through intercalation, thus disrupting DNA replication.[8,9] A large number of its derivatives have been prepared and evaluated for biological activities.[10−12] Two notable examples are mepacrine (quinacrine), the acridine derivative to be clinically used as an antimalarial drug that also acts as a calmodulin inhibitor to suppress the histamine secretion process in the mast cell,[13,14] and amsacrine (*m*-AMSA),[15,16] an antileukemic agent.[17−19] Certain 9-thioacridines have also been synthesized as inhibitors of trypanothione reductase from *Trypanosoma cruzi*, the causative agent of Chagas' disease.[20] Because of the biological versatility of acridine, we decided to synthesize its 9-anilino and 9-phenoxy derivatives and to evaluate their antiinflammatory activities. The cytotoxicity profile on mammalian cancer cells is also described.

Chemistry

9-Anilinoacridines **2**−**5** and 9-phenoxyacridines **6**−**12** were prepared as described in Scheme 1. Reaction of 9-chloroacridine (**1**, R = H) with 4-aminoacetophenone afforded 1-[4-(acridin-9-ylamino)phenyl]ethanone hydrochloride (**2**), which was then reacted with hydroxylamine or *O*-methylhydroxylamine to give (*E*)-9-[4-(1-

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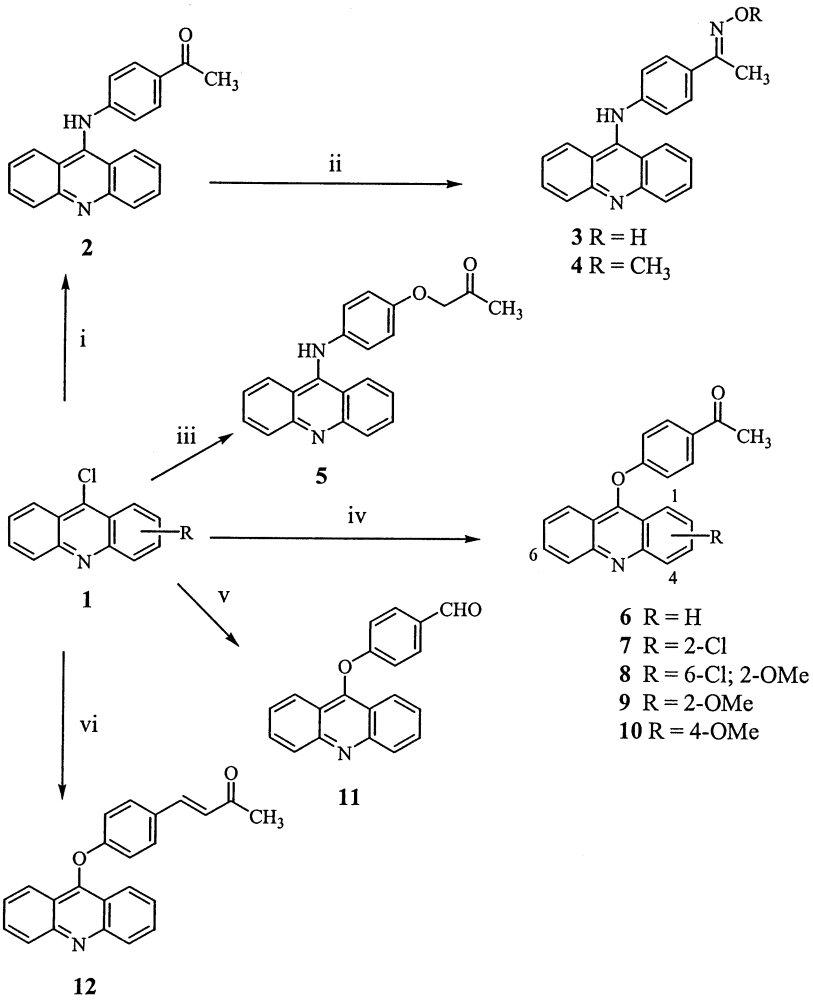
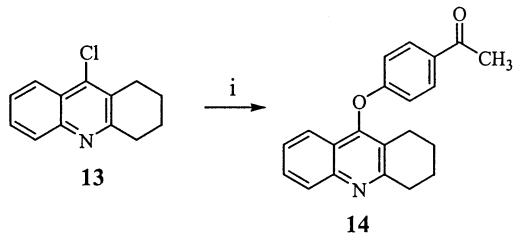
Scheme 1[a]



[a] Reagents: (i) 1-(4-aminophenyl)ethanone in 2 BuOH; (ii) NH_2OH or NH_2OCH_3 in EtOH; (iii) *N*-[4-(2-oxopropoxy)phenyl]acetamide in 2-BuOH; (iv) 4-hyroxyactophenone, K_2CO_3, acetone in a sealed bomb; (v) 4-hydroxybenzaldehyde, K_2CO_3, THF in a sealed bomb; (vi) 4-hydroxybenzaldehyde, K_2CO_3, acetone in a sealed bomb.

Scheme 2[a]



[a] Reagents: (i) 4-hyroxyacetophenone, K_2CO_3, THF in a sealed tube.

hydroxyiminoethyl)anilino]acridine hydrochloride (**3**) or its methoxy congener **4**, respectively. The configuration of the oxime moiety was determined by through-space nuclear Overhauser effect spectroscopy (NOESY), which revealed coupling connectivity to CH_3 protons. Accordingly, 9-[4-(2-oxopropoxy)anilino]acridine hydrochloride (**5**) was obtained from **1** and 1-(4-aminophenoxy)propan-2-one.[21]

9-(4-Acetylphenoxy)acridine (**6**) was obtained by heating a mixture of **1**, 4-hydroxyacetophenone, K_2CO_3, and acetone in a sealed tube. Accordingly, compounds **7**, **8**, **9**, and **10** were prepared from 2,9-dichloroacridine, 6,9-dichloro-2-methoxyacridine, 9-chloro-2-methoxyacridine, and 9-chloro-4-methoxyacridine, respectively, under the same reaction conditions. Reaction of 9-chloroacridine, 4-hydroxybenzaldehyde, K_2CO_3, and THF in a sealed tube afforded 9-(4-formylphenoxy)acridine (**11**). However, when the reaction was run in acetone instead of THF, (*E*)-9-[4-(3-oxo-but-1-enyl)phenoxy]acridine (**12**), the product of aldol condensation, was isolated as the sole product. 1-[4-(1,2,3,4-Tetrahydroacridin-9-yloxy)-phenyl]ethanone (**14**), the tetrahydro congener of **6**, was also synthesized from 9-chlorotetrahydracridine (**13**)[22] under the same reaction conditions (Scheme 2).

Biological Results and Discussion

Biological evaluation of the compounds in this series initially involved measuring inhibitory potencies against the activation of inflammatory cells, mast cells, and neutrophils, which are recognized to participate in acute inflammation. Measurement of cell degranulation was used as the screening model to determine if compounds were effective inhibitors. Then the inhibitory activity of compounds on chronic inflammation was evaluated by examination of cytokines released from macrophages. The percentage inhibition was calculated as the ratio of mediator release in the test-compound-treated cell suspension to the DMSO-treated cell suspension. For those compounds with activity at the screening dose, a

Table 1. IC_{50} Values of Acridine Derivatives against Mast Cell Degranulation

	IC_{50}[a] (μM)	
compound	histamine	β-glucuronidase
2	>30 (15.7 ± 1.5%)*	>30 (34.7 ± 4.2%)**
3	>30 (23.3 ± 6.6%)**	>30 (29.5 ± 7.6%)
4	20.8 ± 0.1	21.0 ± 0.4
5	>30.0 (19.2 ± 2.4%)**	>30.0 (1.3 ± 9.5%)
6	>30.0 (43.8 ± 3.0%)**	>30.0 (44.6 ± 2.9%)**
7	>30.0 (14.4 ± 2.2%)	>30.0 (29.4 ± 0.5%)**
8	>30.0 (7.2 ± 3.7%)	>30.0 (26.4 ± 2.9%)*
9	>30.0 (9.4 ± 0.6%)	>30.0 (25.0 ± 0.4%)*
10	16.0 ± 0.6	19.5 ± 1.6
11	21.5 ± 0.3	20.7 ± 0.7
12	>30.0 (−10.5 ± 1.5%)	>30.0 (7.7 ± 5.6%)
14	>30.0 (5.7 ± 1.0%)	>30.0 (13.3 ± 0.7%)
mepacrine	31.2 ± 0.7	28.8 ± 3.6

[a] Values are means ± SE of at least three separate experiments. When 50% inhibition could not be reached at the highest concentration, the percentage of inhibition is given in parentheses. (*) $P < 0.05$; (**) $P < 0.01$.

Table 2. IC_{50} Values of Acridine Derivatives against Neutrophil Degranulation

	IC_{50}[a] (μM)	
compound	lysozyme	β-glucuronidase
2	23.7 ± 0.6	>30.0 (26.7± 0.6%)*
3	8.2 ± 0.2	4.4 ± 0.1
4	>30.0 (15.4 ± 11.8%)	>30.0 (46.9 ± 0.4%)**
5	>30.0 (21.6 ± 5.4%)	>30.0 (35.8 ± 4.5%)**
6	18.7 ± 1.0	>30.0 (42.0 ± 3.3%)**
7	>30.0 (−16.4 ± 5.3%)	>30.0 (14.8 ± 6.9%)
8	>30.0 (−12.8 ± 9.7%)	>30.0 (10.4 ± 2.4%)
9	18.3 ± 0.8	12.9 ± 0.6
10	21.3 ± 1.5	>30.0 (35.2 ± 8.0%)**
11	>30.0 (44.8 ± 3.1%)**	>30.0 (49.9 ± 3.9%)**
12	>30.0 (10.3 ± 11.3%)	>30.0 (32.4 ± 1.5%)**
14	>30.0 (32.4 ± 5.3%)*	>30.0 (42.1 ± 2.7%)**
trifluoperazine	10.1 ± 1.1	12.8 ± 0.4

[a] Values are means ± SE of at least three separate experiments. When 50% inhibition could not be reached at the highest concentration, the percentage of inhibition is given in parentheses. (*) $P < 0.05$; (**) $P < 0.01$.

dose−response curve was obtained for analysis of the IC_{50} value. Test compounds were evaluated and compared with reference inhibitor.

Mast Cell Degranulation. Mast cells are dominated by the presence of electron-dense granules throughout the cytoplasm. Rodent mast cell granules appear to be of uniform density and contain preformed secretory granule components such as histamine, serotonin, heparin, chemotactic peptides, and protease.[1] In the present study, assessment of inhibitory efficacy with respect to mast cell degranulation was performed by measuring the content of histamine and β-glucuronidase in the supernatant. Cells stimulated with 10 μg/mL of compound 48/80, the low-molecular-weight polymer of *p*-methoxy-*N*-methylphenylamine, which is the prototype and most thoroughly studied histamine liberator,[23] released about 76.6% and 16.6% of histamine and β-glucuronidase, respectively, of the initial cellular content. As shown in Table 1, compounds **4**, **10**, **11** had very similar IC_{50} values in both assays and were proved to be more potent than the reference inhibitor, mepacrine. These results also indicated that the *N*-methoxy substituent at the side chain of 9-anilinoacridines is more favorable than the *N*-hydroxy group (**4** vs **3**) and unsubstituted ketone (**4** vs **2**). For the 9-phenoxyacridines, 9-(4-formylphenoxy)acridine (**11**) exhibited a significant inhibition of mast cell activation while its 4′-acetyl counterpart **6** showed only marginal potency. With the exception of **10**, which bears a 4-methoxy substituent at the C-4 position, all the substituted derivatives **7**−**9** and the tetrahydro counterpart **14** demonstrated less than 30% inhibition at 30 μM. Both compounds **5** and **12** were inactive in this assay, suggesting that the distance between acridine and the ketone group also plays an important role.

Neutrophil Degranulation. Neutrophils possess a large number of cytoplasmic granules. These appear to be of two main types, azurophil and specific granules, which contain lysosomal hydrolase, peroxidase, lysozyme, and cationic protein.[24] Activation of neutrophils with 1 μM formylmethionylleucylphenylalanine (fMLP) in the presence of cytochalasin B (5 μg/mL) evoked the release of 21.2% and 19.8% of lysozyme and β-glucuronidase, respectively, of the initial cellular content. Compound **3**, with an IC_{50} value of 8.2 and 4.4 μM, respectively, was the most potent inhibitor in these assays and more potent than the calmodulin inhibitor trifluoperazine, which inhibits the degradation and superoxide anion generation in noutrophils[25,26] However, its *N*-methoxy derivative **4** and the ketone precursor **2** were less active than trifluoperazine. Compound **6** showed moderate activities, with an IC_{50} value of 18.7 μM against lysozyme release and 42% inhibition ($P < 0.01$) of β-glucuronidase release at 30 μM. A comparable potency was observed for its 4-methoxy derivative **10**, while its 2-methoxy derivative **9** was especially active against the release of β-glucuronidase with an IC_{50} value of 12.9 μM. All other analogues in Table 2 demonstrated diminished activities. The results also indicated that the 9-phenoxyacridine skeleton is more favorable than 9-anilinoacridine (**6** vs **2**).

TNF-α Release. TNF-α, an early cytokine produced by activated macrophages, plays an essential role in pathological inflammatory reactions. The steroid antiinflammatory drug dexamethasone, which strongly impedes translational derepression, is a very potent inhibitor of TNF-α formation.[27] None of compounds **2**−**14** had IC_{50} values similar to that of dexamethasone in the inhibition of TNF-α formation. However, compound **2** showed great potency in N9 cells. Moreover, compound **5** and **12** proved to be potent inhibitors in RAW 264.7 and N9 cells, respectively, giving IC_{50} values less than 10 μM, whereas compound **9** shows moderate potency for the inhibition of TNF-α formation (Table 3).

To further explore the cytotoxic properties of these acridine derivatives, compound **12** was selected for evaluation in vitro against 60 human cancer cell lines derived from nine cancer cell types. Dose−response curves for each cell line were measured with five different drug concentrations, and the molar concentration causing 50% cell growth inhibition (GI_{50}), total cell growth inhibition (TGI, 0% growth), and 50% cell death (LC_{50}, −50% growth) compared with the control was calculated.[28] The mean values over all cell lines tested are the following: $GI_{50} = 58.0$ μM; TGI > 100 μM; LC_{50} > 100 μM. The results also showed a selective growth inhibiting activity against UO-31 (a renal cancer cell) with a GI_{50} value of 0.26 μM compared with a GI_{50} value of >20.0 μM for other individual cell lines.

Table 3. IC_{50} Values of Acridine Derivatives on TNF-α Formation

compound	IC_{50}[a] (μM) RAW 264.7	N9
2	>10.0 (47.2 ± 3.1%)**	0.8 ± 0.3
3	>10.0 (44.9 ± 2.0%)**	>3.0 (40.1 ± 3.1%)**
4	>30.0 (7.2 ± 0.8%)	>3.0 (39.3 ± 1.2%)**
5	8.2 ± 1.4	>1.0 (6.6 ± 5.6%)
6	>30.0 (45.6 ± 6.1%)**	>3.0 (23.9 ± 3.6%)*
7	>30.0 (4.6 ± 5.0%)	>30.0 (12.3 ± 5.6%)
8	>30.0 (33.1 ± 0.6%)**	>10.0 (45.9 ± 1.8%)**
9	19.3 ± 0.8	>3.0 (36.1 ± 2.4%)**
10	>30.0 (34.3 ± 4.2%)**	>10.0 (19.7 ± 1.4%)
11	>30.0 (17.2 ± 3.3%)*	>3.0 (19.9 ± 3.8%)*
12	>30.0 (−15.7 ± 4.1%)	8.7 ± 0.3
14	>30.0 (48.9 ± 2.9%)**	>3.0 (−3.5 ± 8.7%)
dexamethasone	0.42 ± 0.12	0.074 ± 0.009

[a] Values are means ± SE of at least three separate experiments. When 50% inhibition could not be reached at the highest concentration, the percentage of inhibition is given in parentheses. (*) $P < 0.05$; (**) $P < 0.01$.

Conclusion

These results indicated that the antiinflammatory effects of acridine derivatives were mediated, at least in part, through the suppression of chemical mediators released from mast cells, neutrophils, and macrophages and that these compounds have the potential to be novel antiinflammatory agents with no significant cytotoxicity.

Experimental Section

General. Melting points were determined on an Electrothermal IA9100 melting point apparatus and are uncorrected. UV spectra (λ_{max} in nm) were recorded in spectroscopic grade MeOH on a Shimadzu UV-160A UV−vis spectrophotometer. Nuclear magnetic resonance (^1H and ^{13}C) spectra were recorded on a Varian-Unity 400 spectrometer. Chemical shifts were expressed in parts per million (δ) with tetramethylsilane (TMS) as an internal standard. Thin-layer chromatography was performed on silica gel 60 F-254 plates purchased from E. Merck and Co. The elemental analyses were performed at the Instrument Center of National Science Council at National Cheng-Kung University and National Chung-Hsing University using Heraeus CHN−O Rapid EA, and all values are within ±0.4% of the theoretical compositions.

1-[4-(Acridin-9-ylamino)phenyl]ethanone Hydrochloride (2). 4-Aminoacetophenone (4.06 g, 30 mmol) was refluxed with 9-chloroacridine hydrochloride (6.41 g, 25.6 mmol) in 2-butanol (80 mL) for 3 h (TLC monitoring). The reaction mixture was allowed to cool to room temperature and then was poured into ice water (150 mL). A precipitate formed, which was filtered off with suction, washed with water, and crystallized from MeOH to yield **2** (7.93 g, 89%) as an orange powder: mp 259 °C (dec); UV λ nm (MeOH) (log ε) 242 (4.55), 264 (4.51), 418 (4.05); ^1H NMR (DMSO-d_6) δ 2.60 (s, 3H), 7.52 (m, 4H), 8.05 (m, 4H), 8.22 (d, 2H, J = 8.4 Hz), 8.32 (d, 2H, J = 8.8 Hz), 11.69 (br s, 1H), 15.36 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 26.56, 115.11, 119.46, 122.60, 124.38, 125.74, 129.79, 130.41, 133.98, 135.54, 140.15, 154.60, 196.61. Anal. Calcd for $C_{21}H_{17}ClN_2O·0.33H_2O$: C, 71.10; H, 5.02; N, 7.90. Found: C, 71.03; H, 5.01; N, 7.88.

***(E)*-Acridin-9-yl-[4-(1-hydroxyiminoethyl)phenyl]ammonium Chloride (3).** To a suspension of **2** (0.35 g, 1 mmol) in methanol (50 mL) was added hydroxylamine hydrochloride (0.10 g, 1.5 mmol) and sodium acetate (0.12 g, 1.5 mmol). The reaction mixture was stirred at room temperature for 24 h (TLC monitoring), then concentrated in vacuo to give a solid that was washed by MeOH (20 mL) and purified by flash column chromatography (silica gel, with acetone−MeOH (10:1) as the eluent) to give an orange solid (0.31 g, 85%): mp 280 °C (dec); UV λ nm (MeOH) (log ε) 212 (4.31), 244 (4.52), 263 (4.56), 436 (4.01); ^1H NMR (DMSO-d_6) δ 2.20 (s, 3H), 7.44 (m, 2H), 7.49 (m, 2H), 7.78 (m, 2H), 8.02 (m, 2H), 8.09 (d, 2H, J = 8.0 Hz), 8.28 (d, 2H, J = 8.4 Hz), 11.54 (br s, 1H), 14.67 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 11.33, 114.01, 119.25, 123.89, 123.97, 125.73, 126.77, 135.29, 135.39, 140.07, 141.34, 152.12, 154.93. Anal. Calcd for $C_{21}H_{18}ClN_3O·0.33H_2O$: C, 68.20; H, 5.09; N, 11.36. Found: C, 68.18; H, 4.89; N, 11.16.

***(E)*-Acridin-9-yl-[4-(1-methoxyiminoethyl)phenyl]ammonium Chloride (4).** To a suspension of **2** (0.709 g, 2 mmol) in methanol (30 mL) was added 40% aqueous *O*-methylhydroxylamine hydrochloride (0.33 g, 4 mmol). The reaction mixture was stirred at room temperature for 6 h (TLC monitoring), then concentrated in vacuo to give a solid that was recrystallized from MeOH to give a yellow solid (0.69 g, 91%): mp 299 °C (dec); UV λ nm (MeOH) (log ε) 213 (4.33), 245 (4.52), 264 (4.59), 435 (4.05); ^1H NMR (DMSO-d_6) δ 2.22 (s, 3H), 3.94 (s, 3H), 7.47 (m, 4H), 7.78 (m, 2H), 8.02 (m, 2H), 8.10 (d, 2H, J = 8.4 Hz), 8.27 (d, 2H, J = 8.8 Hz), 11.57 (br s, 1H), 14.78 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 12.12, 61.69, 114.21, 119.33, 123.85, 123.99, 125.73, 127.18, 134.09, 135.38, 140.12, 141.39, 153.27, 154.91. Anal. Calcd for $C_{22}H_{20}ClN_3O$: C, 69.93; H, 5.33; N, 11.12. Found: C, 69.86; H, 5.37; N, 11.01.

9-[4-(2-Oxopropoxy)anilino]acridine Hydrochloride (5). *N*-[4-(2-Oxopropoxy)phenyl]acetamide (4.56 g, 22 mmol) was refluxed with 9-chloroacridine hydrochloride (5.50 g, 22 mmol) in 2-butanol (30 mL) for 6 h (TLC monitoring). The reaction mixture was allowed to cool to room temperature and then was poured into ice water (50 mL). A precipitate formed, which was filtered off with suction, washed with water, and dried in vacuo to afford **5** (5.38 g, 64%) as red powder: mp 139−141 °C; UV λ nm (MeOH) (log ε) 243 (4.54), 263 (4.54), 435 (4.01); ^1H NMR (DMSO-d_6) δ 2.19 (s, 3H), 4.92 (s, 2H), 7.06 (m, 2H), 7.42 (m, 3H), 7.98 (m, 2H), 8.09 (d, 2H, J = 8.4 Hz), 8.23 (d, 2H, J = 8.8 Hz), 11.54 (br s, 1H), 14.69 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 26.19, 72.29, 113.16, 115.73, 119.07, 123.50, 125.61, 126.29, 133.53, 135.09, 139.99, 155.31, 157.05, 203.64. Anal. Calcd for $C_{22}H_{19}ClN_2O_2·H_2O$: C, 66.58; H, 5.33; N, 7.06. Found: C, 66.54; H, 5.48; N, 7.02.

9-(4-Acetylphenoxy)acridine (6). A mixture of 9-chloroacridine hydrochloride (2.50 g, 10 mmol), 4-hydroxyacetophenone (1.36 g, 10 mmol), and K_2CO_3 (2.07 g, 15 mmol) in acetone (100 mL) was heated at 150 °C for 20 h in a steel bomb. It was cooled, filtered, concentrated, washed well with water, and crystallized from EtOH to yield **6** (3.07 g, 98%) as a pale-brown powder: mp 138−141 °C; UV λ nm (MeOH) (log ε) 215 (4.19), 247 (4.72), 356 (3.79); ^1H NMR (DMSO-d_6) δ 2.52 (s, 3H), 7.00 (m, 2H), 7.62 (m, 2H), 7.90 (m, 2H), 7.95 (m, 2H), 8.01 (d, 2H, J = 8.8 Hz), 8.27 (d, 2H, J = 8.8 Hz); ^{13}C NMR (DMSO-d_6) δ 26.53, 115.38, 119.26, 121.81, 126.84, 129.54, 131.03, 131.86, 149.90, 153.52, 162.30, 196.30. Anal. Calcd for $C_{21}H_{15}NO_2$: C, 80.49; H, 4.82; N, 4.47. Found: C, 80.42; H, 4.96; N, 4.47.

1-[4-(2-Chloroacridin-9-yloxy)phenyl]ethanone (7). Compound **7** was prepared according to the synthesis of **6**, using 2,9-dichloroacridine hydrochloride instead of 9-chloroacridine hydrochloride (70% yield): mp 210−211 °C; UV λ nm (MeOH) (log ε) 214 (4.32), 250 (4.93), 361 (3.98), 384 (3.73); ^1H NMR (DMSO-d_6) δ 2.56 (s, 3H), 6.91 (m, 2H), 7.53 (m, 1H), 7.92 (m, 6H), 8.38 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 26.46, 115.51, 120.10, 120.38, 120.73, 122.33, 127.23, 128.82, 130.42, 131.10, 131.91, 132.57, 132.85, 132.99, 147.45, 149.38, 154.57, 162.39, 196.37. Anal. Calcd for $C_{21}H_{14}ClNO_2$: C, 72.52; H, 4.06; N, 4.03. Found: C, 72.59; H, 4.11; N, 3.95.

1-[4-(6-Chloro2-methoxyacridin-9-yloxy)phenyl]ethanone (8). Compound **8** was prepared according to the synthesis of **6**, using 6,9-dichloro-2-methoxyacridine instead of 9-chloroacridine hydrochloride (58% yield): mp 143−144 °C; UV λ nm (MeOH) (log ε) 216 (4.28), 259 (4.93), 335 (3.72), 351 (3.86), 382 (3.79), 401 (3.79); ^1H NMR (DMSO-d_6) δ 2.54 (s, 3H), 3.80 (s, 3H), 6.89 (m, 2H), 7.04 (d, 1H, J = 2.8 Hz), 7.36 (dd, 1H, J = 9.2, 2.0 Hz), 7.47 (dd, 1H, J = 9.4, 2.8 Hz), 7.86 (d, 1H, J = 8.8 Hz), 7.92 (m, 2H), 8.11 (d, 1H, J = 9.4 Hz), 8.20 (d, 1H, J = 1.8 Hz); ^{13}C NMR (DMSO-d_6) δ 26.34, 55.52, 97.08, 115.34, 118.34, 120.72, 123.08, 126.55, 127.58, 128.17, 130.92, 131.29, 132.14, 135.47, 148.33, 148.39, 152.28, 157.76, 162.20, 196.35.

Anal. Calcd for $C_{22}H_{16}ClNO_3$: C, 69.94; H, 4.27; N, 3.71. Found: C, 69.55; H, 4.37; N, 3.63.

1-[4-(2-Methoxyacridin-9-yloxy)phenyl]ethanone (9). Compound **9** was prepared according to the synthesis of **6**, using 9-chloro-2-methoxyacridine instead of 9-chloroacridine hydrochloride (69% yield): mp 176−177 °C; UV λ nm (MeOH) (log ϵ) 215 (4.22), 254 (4.85), 335 (3.61), 351 (3.81), 375 (3.74), 395 (3.73); ^{1}H NMR (DMSO-d_6) δ 2.54 (s, 3H), 3.82 (s, 3H), 6.91 (m, 2H), 7.11 (d, 1H, J = 2.8 Hz), 7.48 (m, 2H), 7.75 (m, 1H), 7.92 (m, 3H), 8.20 (d, 1H, J = 9.4 Hz), 8.28 (d, 1H, J = 8.8 Hz); ^{13}C NMR (DMSO-d_6) δ 26.38, 55.56, 97.11, 115.43, 120.04, 120.78, 121.68, 126.33, 126.49, 129.39, 129.76, 130.94, 131.07, 132.07, 147.35, 148.24, 152.42, 157.69, 162.40, 196.48. Anal. Calcd for $C_{22}H_{17}NO_3$: C, 76.95; H, 4.99; N, 4.08. Found: C, 76.98; H, 5.08; N, 3.99.

1-[4-(4-Methoxyacridin-9-yloxy)phenyl]ethanone (10). Compound **10** was prepared according to the synthesis of **6**, using 9-chloro-4-methoxyacridine instead of 9-chloroacridine hydrochloride (58% yield): mp 159−160 °C; UV λ nm (MeOH) (log ϵ) 212 (4.35), 254 (4.84), 357 (3.66), 384 (3.78); ^{1}H NMR (DMSO-d_6) δ 2.53 (s, 3H), 4.18 (s, 3H), 6.87 (m, 2H), 7.07 (dd, 1H, J = 7.6, 1.0 Hz), 7.39 (dd, 1H, J = 8.8, 7.6 Hz), 7.50 (m, 1H), 7.58 (dd, 1H, J = 8.8, 1.0 Hz), 7.79 (m, 1H), 7.90 (m, 2H), 8.01 (m, 1H), 8.46 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 26.34, 56.25, 107.04, 113.81, 115.34, 120.15, 120.98, 121.83, 126.35, 126.67, 130.39 (2C), 130.86, 131.96, 143.41, 149.24, 154.04, 155.35, 162.67, 196.43. Anal. Calcd for $C_{22}H_{17}NO_3$: C, 76.95; H, 4.99; N, 4.08. Found: C, 76.80; H, 5.09; N, 4.12.

9-(4-Formylphenoxy)acridine (11). A mixture of 9-chloroacridine hydrochloride (3.75 g, 15 mmol), 4-hydroxybenzaldehyde (1.83 g, 15 mmol), and K_2CO_3 (2.76 g, 20 mmol) in dry THF (150 mL) was heated at 150 °C for 20 h in a steel bomb. It was cooled, filtered, concentrated, washed well with water, and crystallized from EtOH to yield **11** (4.26 g, 84%) as a pale-brown powder: mp 181−183 °C dec; UV λ nm (MeOH) (log ϵ) 215 (4.20), 250 (4.86), 357 (3.77); ^{1}H NMR (DMSO-d_6) δ 7.10 (m, 2H), 7.63 (m, 2H), 7.92 (m, 4H), 8.02 (d, 2H, J = 8.8 Hz), 8.28 (d, 2H, J = 8.8 Hz), 9.92 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 116.00, 119.15, 121.72, 126.86, 129.51, 131.00, 131.43, 132.24, 149.87, 153.31, 163.16, 191.37. Anal. Calcd for $C_{20}H_{13}NO_2$: C, 80.25; H, 4.38; N, 4.68. Found: C, 80.22; H, 4.46; N, 4.63.

***(E)*-9-[4-(3-Oxo-but-1-enyl)phenoxy]acridine (12).** Compound **12** was prepared according to the synthesis of **11**, using acetone as solvent (95% yield): mp 227 °C (dec); UV λ nm (MeOH) (log ϵ) 213 (4.37), 250 (4.68), 324 (4.22), 379 (3.87), 397 (3.85); ^{1}H NMR (DMSO-d_6) δ 2.30 (s, 3H), 6.69 (d, 1H, J = 16.4 Hz), 6.95 (m, 2H), 7.58 (d, 1H, J = 16.4 Hz), 7.62 (m, 2H), 7.69 (m, 2H), 7.91 (m, 2H), 8.03 (d, 2H, J = 8.8 Hz), 8.26 (d, 2H, J = 9.2 Hz); ^{13}C NMR (DMSO-d_6) δ 27.17, 115.91, 119.35, 121.89, 126.35, 126.66, 129.29, 129.47, 130.60, 130.94, 142.25, 149.88, 153.80, 160.51, 197.89. Anal. Calcd for $C_{23}H_{17}NO_2$: C, 81.40; H, 5.05; N, 4.13. Found: C, 81.36; H, 5.24; N, 4.18.

1-[4-(1,2,3,4-Tetrahydroacridin-9-yloxy)phenyl]ethanone (14). Compound **14** was prepared according to the synthesis of **6**, using 9-chloro-1,2,3,4-tetrahydroacridine instead of 9-chloroacridine hydrochloride (54% yield): mp 144−145 °C; UV λ nm (MeOH) (log ϵ) 229 (4.62), 264 (4.23), 320 (3.72); ^{1}H NMR (DMSO-d_6) δ 1.89 (m, 4H), 2.55 (s, 3H), 2.73 (t, 2H, J = 6.4 Hz), 3.19 (t, 2H, J = 6.4 Hz), 6.85 (m, 2H), 7.40 (m, 1H), 7.65 (m, 2H), 7.91 (m, 2H), 8.07 (d, 1H, J = 8.4 Hz); ^{13}C NMR (DMSO-d_6) δ 22.02, 22.70, 23.41, 26.35, 33.68, 114.98, 121.42, 121.72, 122.95, 126.14, 128.39, 129.47, 130.88, 131.81, 147.76, 154.28, 161.08, 161.18, 196.44. Anal. Calcd for $C_{21}H_{19}NO_2$: C, 79.47; H, 6.03; N, 4.41. Found: C, 79.32; H, 6.09; N, 4.41.

Biological Evaluation. 1. Mast Cell Degranulation. Peritoneal mast cells were isolated as previously described.[29] Briefly, heparinized Tyrode's solution was injected into the peritoneal cavity of exsanguinated rat (Sprague Dawley). After abdominal massage, the cells in the peritoneal fluid were harvested and then separated through 38% bovine serum albumin. After being washed, cells were resuspended in Tyrode's solution with 0.1% bovine serum albumin at 1×10^6 cells/mL. The cell suspension was preincubated with DMSO or test compounds at 37 °C for 5 min. Fifteen minutes after the addition of compound 48/80 (10 μg/mL),[30] β-glucuronidase (1 mM phenolphthalein-β-D-glucuronide in 0.1 M acetic acid buffer, pH 4.5, as substrate; absorbance was monitored at 550 nm after alkalization)[31] and histamine (condensation with 0.2% *o*-phthaldialdehyde in pH 12.5; fluorescence was monitored at 350/450 nm after acidification)[29] in the supernatant were determined. The total content was measured after treatment of the cell suspension with Triton X-100. The percentage release was calculated.

2. Neutrophil Degranulation. Rat neutrophils were isolated as previously described.[32] Briefly, rat blood was collected from the abdominal aorta and neutrophils were purified by dextran sedimentation, hypotonic lysis of erythrocytes, and centrifugation through Ficoll-Hypaque. After being washed, cells were suspended in Hanks' balanced salt solution (Gibco BRL, NY) at 1×10^7 cells/mL. The cell suspension was preincubated with DMSO or test compounds at 37 °C for 10 min in the presence of cytochalasin B (5 μg/mL). Forty-five minutes after the addition of 1 μM fMLP, β-glucuronidase[30] and lysozyme (0.02% *Micrococcus lysodeikticus* in 0.06 M phosphate buffer, pH 6.2, as substrate; absorbance was monitored at 450 nm) in the supernatant were determined.[33] The total content was measured after treatment of the cell suspension with Triton X-100. The percentage release was calculated.

3. TNF-α Release. Mouse macrophage-like cell lines RAW 264.7 (ATCC, MD) was plated in 96-well tissue-culture plates in DMEM (Gibco BRL, NY) supplemented with 10% fetal calf serum, 100 U/mL of penicillin, and 100 μg/mL of streptomycin at 2×10^5 cells per well. Murine microglial cell lines N9 (provided by Dr. P. Ricciardi-Castagnoli, CNR Cellular & Molecular Pharmacology Center, Italy)[34] were plated in 96-well tissue-culture plates in IMDM (Gibco BRL, NY) supplemented with 5% fetal calf serum and antibiotics at 8×10^4 cells per well. Cells were pretreated with DMSO or test compound at 37 °C for 1 h before stimulation with 1 μg/mL lipopolysaccharide (LPS) (for RAW 264.7) or 10 ng/mL LPS plus 10 U/mL interferon-γ (IFN-γ) (for N9) for 24 h. Then the medium was collected for TNF-α determination by using the TNF-α ELISA kit (Genzyme, MA).

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