# Reactive Oxygen Species (ROS) Generation Inhibited by Aporphine and Phenanthrene Alkaloids Semi-Synthesized from Natural Boldine

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**Four phenanthrene and one aporphine alkaloids semi-synthesized from boldine were evaluated for their inhibitory effect on reactive oxygen species (ROS) generation. ROS generation by neutrophils stimulated with *N*-formyl-methionyl-leucyl-phenylalanine was inhibited in a concentration dependent manner. Alkaloids exerted similar inhibitory effect in the hypoxanthine–xanthine oxidase system than in stimulated neutrophils, which could be attributed to a direct ROS scavenging activity. None of the alkaloids assayed had any effect on xanthine oxidase activity. Therefore the synthesized alkaloids might constitute an alternative therapy in inflammation disorders in which ROS generation is involved.**

**Key words** phenanthrene alkaloid; aporphine alkaloid; semi-synthesis; antioxidant activity

A generalized inflammatory response is associated to the pathogenesis of various circulatory and neurodegenerative disorders such as thrombosis, atherosclerosis[1] and Alzheimer's disease,[2] which is now thought to have an inflammatory base.[3] The development of antiinflammatory agents which prevent the accumulation of leucocytes, platelet activation or lost of function of the vessel wall, could constitute new therapeutic strategies for the control of these and others inflammatory diseases.

Interestingly aporphines and phenanthrene alkaloids have been shown to exert a strong antiplatelet and vasorelaxing effects.[4,5] In this context, the aporphine boldine can inhibit the aggregation of rabbit platelets induced by arachidonic acid and collagen but it did not affect to the platelet aggregation induced by platelet activating factor (PAF), thrombin or a tromboxane analog U46619.[6] Conversely, its corresponding phenanthrene alkaloid secoboldine, in addition to arachidonic acid and collagen, it can also inhibit the platelet aggregation induced by PAF or U46619.

Furthermore, some aporphine and phenanthrene alkaloids have been shown to inhibit PAF binding to its receptor.[7] In this study it was suggested that the piperidine ring (ring B) of the aporphine skeleton may not be important for PAF antagonisms from the established structure–activity relationship.

On the other hand, studies investigating the potential vasorelaxing activity of these type of alkaloids demonstrated that phenanthrene alkaloids with a tertiary amine or *N*-oxide and two methoxy groups or a methylenedioxy group at C-3 and C-4 (ring A), were the most potent vasorelaxing agent. In contrast, when the substituent group at C-6 and C-7 in ring C are both methoxy groups vasorelaxing action was reduced.[4]

Therefore, the aim of the present study is to synthesize phenanthrene alkaloids with secondary, tertiary or quaternary amine from boldine as starting material with oxygenated substituents in C-3 and C-4 (ring A) and with or without both methoxy groups at C-6, C-7 (ring C). Despite this class of compounds may inhibit platelet aggregation or behave as vasorelaxing agents, little is known about their antioxidant properties. Since ROS are involved in both platelet aggregation and vessel wall reactivity,[8,9] and we have recently found that phenanthrene alkaloids can exert antiinflammatory activity through inhibition of ROS generation,[10] we have evaluated the possible antioxidant activity of these compounds by two different methods. Thus, they may have a potential use as antiinflammatory agents.

## Experimental

**Reagents** All chemicals are of the highest commercially available purity. Boldine, BSA, DMSO, f-MLP, gelatin, glucose, hypoxanthine, luminol, microperoxidase and xanthine oxidase were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other reagents are purchased from Aldrich-Chemie (Steinheim, Germany).

**General Experimental Procedure** Melting points were determined on a Fisher-John apparatus and are uncorrected. UV spectra were taken on a Shimadzu 2101 UV/Vis spectrophotometer in MeOH solution. MS were performed using a VG Auto Spec Fisons Spectrometer. NMR measurements (data reported in $\delta$) were run on a Varian Unity-400 instrument. The chemical shift are referenced to solvent signals at 7.25 and 77.0 ppm respectively. Multiplicities of $^{13}$C-NMR resonances were determined by distortionless enhancement by polarization transfer (DEPT). Standard pulse sequences were employed for magnitude COSY (correlated spectroscopy). HMQC (heteronuclear multiple quantum coherence) and HMBC (heteronuclear multiple bond connectivity) experiments to establish correlations were carried out. Thin-layer chromatography (TLC) was run on Merck precoated silica gel $F_{254}$ type 60 plates with detection by UV light and Munnier's reagent.

**Synthesized** Compound **1**: A mixture of boldine (1 g, 3.05 mmol) and 2 M aqueous ammonium acetate (7.5 ml) and ethanol (7.5 ml) was refluxed in a bath at 103 °C during 48 h. After it was cooled at room temperature a white amorphous powder was precipitated (887.9 mg). The solid was filtered under vacuum and washed with ethanol to give **1**[11] as a white crystal, yield 88.8%; mp 163—165 °C. UV (MeOH): $\lambda_{max}$ (log $\varepsilon$)=265 (1.64), 320 (0.35) nm. $^1$H-NMR (DMSO-$d_6$) $\delta$: 2.38 (3H, s, N–$CH_3$), 2.84 (2H, m, $CH_{2\alpha}$), 3.13 (2H, m, $CH_{2\beta}$), 3.79 (3H, s, $OCH_3$-4), 3.93 (3H, s, $OCH_3$-6), 7.07 (1H, s, H-2), 7.19 (1H, s, H-8), 7.44 (1H, d, $J$=8.8 Hz, H-9), 7.69 (1H, d, $J$=8.8 Hz, H-10), 9.04 (1H, s, H-5). $^{13}$C-NMR (DMSO-$d_6$) $\delta$: 32.04 ($CH_{2\beta}$), 34.84 ($NCH_3$), 51.65 ($CH_{2\alpha}$), 55.30 ($OCH_3$, C-6), 59.16 ($OCH_3$, C-4), 108.50 (C-5), 111.57 (C-8), 117.96 (C-2), 120.61 (C-10), 122.68 (C-9), 123.30 (C-5a), 123.82 (C-4a), 124.37 (C-10a), 128.22 (C-8a), 132.43 (C-1), 142.23 (C-4), 146.60 (C-7), 147.70 (C-6), 148.12 (C-3). [HMQC data were used to assign proton and carbon correlations] HR-EI-MS: $m/z$=327.146326 calc. mass 327.147058 $C_{19}H_{21}NO_4$: EI-MS: $m/z$ (rel. int.)=327 (20), 284 (100), 269 (8.2), 240 (10), 84 (6.5), 58 (2.8).

Compound **2**: To a solution of **1** (100 mg, 0.305 mmol) in a mixture of methanol (5 ml) and acetonitrile (7.5 ml) was added $CH_3I$ (0.019 ml, 0.305 mmol). The resulting mixture was stirred overnight at room tempera-

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ture. At the same time a solution of **1** (100 mg, 0.305 mmol) in the above conditions was treated during 48 h with an excess of $CH_3I$ (0.5 ml, 8.034 mmol). In both experiments compound **2** after crystalization with acetone was obtained as yellow needles, combined yield 95%; mp 237—239 °C. UV (MeOH): $\lambda_{max}$ (log $\varepsilon$)=265 (1.57), 320 (0.33) nm. $^1$H-NMR (DMSO-$d_6$) $\delta$: 3.22 (9H, s, 3×N–$CH_3$), 3.44 (2H, m, $CH_{2\alpha}$), 3.57 (2H, m, $CH_{2\beta}$), 3.79 (3H, s, $OCH_3$-4), 3.95 (3H, s, $OCH_3$-6), 7.17 (1H, s, H-2), 7.23 (1H, s, H-8), 7.50 (1H, d, $J$=9.2 Hz, H-9), 7.68 (1H, d, $J$=9.2 Hz, H-10), 9.04 (1H, s, H-5). $^{13}$C-NMR (DMSO-$d_6$) $\delta$: 25.99 ($CH_{2\beta}$), 52.29 ($NCH_3$), 55.32 ($OCH_3$, C-6), 59.29 ($OCH_3$, C-4), 65.32 ($CH_{2\alpha}$), 108.39 (C-5), 111.62 (C-8), 118.40 (C-2), 120.20 (C-10), 122.54 (C-5a), 123.84 (C-4a), 123.95 (C-9), 124.47 (C-10a), 128.21 (C-8a), 129.13 (C-1), 142.86 (C-4), 146.60 (C-7), 147.85 (C-6), 148.17 (C-3). EI-MS 356 $C_{19}H_{21}NO_4$ $m/z$ (rel. int.)=341 (5), 327 (17), 285 (17), 284 (100), 269 (51), 268 (11), 240 (11), 127 (15), 58 (44).

Compound **3**: The compound **3** was prepared according to the method described for **2** by employing a solution of boldine (100 mg, 0.305 mmol) and $CH_3I$ (0.019 ml, 0.305 mmol) to afford compound **3**[12] as a white crystal, yield 98%; mp 168—170 °C. UV (MeOH): $\lambda_{max}$ (log $\varepsilon$)=284.9 (0.93), 305 (1.02) nm. $^1$H-NMR (DMSO-$d_6$) $\delta$: 2.79 (1H, dd, $J$=14.2, 13.2 Hz, H-7a), 2.90 (1H, m, H-4a), 2.94 (3H, s, N–$CH_3$), 3.13 (1H, m, H-4b), 3.21 (1H, dd, $J$=13.2, 3.6 Hz, H-7b), 3.35 (3H, s, N–$CH_3$), 3.61 (3H, s, $OCH_3$-1), 3.66 (1H, m, H-5a), 3.75 (1H, m, H-5b), 3.77 (3H, s, $OCH_3$-10), 4.55 (1H, dd, $J$=14.2, 3.6 Hz, H-6a), 6.66 (1H, s, H-3), 6,79 (1H, s, H-8), 7.84 (1H, s, H-11). $^{13}$C-NMR (DMSO-$d_6$) $\delta$: 23.03 (C-4), 28.09 (C-7), 42.99—52.97 (2×$NCH_3$, C-6), 55.74 ($OCH_3$, C-10), 59.54 ($OCH_3$, C-1), 60.18 (C-5), 68.02 (C-6a), 111.88 (C-11), 114.24 (C-3), 115.23 (C-8), 118.09 (C-3a), 121.76 (C-7a), 124.94 (C-1a), 125.89 (C-11a), 143.81 (C-1), 146.50 (C-9), 146.73 (C-10), 150.91 (C-2). [HMQC data were used to assign correlations and HMBC for quaternary carbons]. EI-MS 342 $C_{20}H_{24}NO_4$ $m/z$ (rel. int.)=341 (11), 283 (3), 128 (6), 59 (3), 58 (100).

Compound **4**: A solution of **3** (53 mg, 0.155 mmol) in 1 M aqueous ammonium acetate (0.4 ml) and ethanol (0.4 ml) was refluxed in a bath at 104 °C for 24 h. The ethanol was evaporated under vacuum and the aqueous layer was extracted with dichloromethane. The combined organic extracts were dried over anhydrous $Na_2SO_4$ and the solvent was evaporated to give **4**[13,14] as a yellow crystal, combined yield 50 mg (94%); mp 227—229 °C. UV (MeOH): $\lambda_{max}$ (log $\varepsilon$)=265 (1.10), 320 (0.25) nm. $^1$H-NMR (DMSO-$d_6$) $\delta$: 2.46 (6H, s, 2×N–$CH_3$), 2.78 (2H, m, $CH_{2\alpha}$), 3.16 (2H, m, $CH_{2\beta}$), 3.79 (3H, s, $OCH_3$-4), 3.95 (3H, s, $OCH_3$-6), 7.09 (1H, s, H-2), 7.19 (1H, s, H-8), 7.42 (1H, d, $J$=9.2 Hz, H-9), 7.66 (1H, d, $J$=9.2 Hz, H-10), 9.05 (1H, s, H-5). $^{13}$C-NMR (DMSO-$d_6$) $\delta$: 29.81 ($CH_{2\beta}$), 44.04 (2×$NCH_3$), 55.26 ($OCH_3$, C-6), 59.13 ($OCH_3$, C-4), 59.20 ($CH_{2\alpha}$), 108.45 (C-5), 111.54 (C-8), 117.95 (C-2), 120.39 (C-10), 122.69 (C-5a), 123.42 (C-9), 123.76 (C-4a), 124.36 (C-10a), 128.17 (C-8a), 131.97 (C-1), 142.27 (C-4), 146.53 (C-7), 147.68 (C-6), 148.05 (C-3). HR-EI-MS: $m/z$=341.161528 calc. mass 341.162708 $C_{20}H_{23}NO_4$. EI-MS: $m/z$ (rel. int.)=341 (50), 283 (15), 240 (12), 58 (100).

Compound **5**: This compounds was prepared in three diferents conditions. $K_2CO_3$ (200 mg, 1.449 mmol) was added to three solutions of boldine (100 mg, 0.305 mmol) in acetonitrile (7.5 ml) and methanol (5 ml). After addition of $K_2CO_3$ was completed the solutions were treated with 2 equivalents (0.038 ml, 0.610 mmol), 3 equivalents (0.057 ml, 0.915 mmol) or an excess of $CH_3I$ (0.1 ml, 1.607 mmol). The mixtures were stirred at room temperature for 48 h. The methanol/acetonitrile was evaporated under vacuum and extracted with dichloromethane. The combined organic extracts were purified by addition of hexane to give in all reactions compound **5**[15] as a yellow crystal, combined yield 75%; mp 361—363 °C. UV (MeOH): $\lambda_{max}$ (log $\varepsilon$)=265 (0.85), 310 (0.24) nm. $^1$H-NMR (DMSO-$d_6$) $\delta$: 3.26 (9H, s, 3×N–$CH_3$), 3.56 (2H, m, $CH_{2\alpha}$), 3.63 (2H, m, $CH_{2\beta}$), 3.79 (3H, s, $OCH_3$-4), 3.95 (3H, s, $OCH_3$-6), 7.47 (1H, s, H-2), 7.53 (1H, s, H-8), 7.70 (1H, d, $J$=9.2 Hz, H-9), 7.79 (1H, d, $J$=9.2 Hz, H-10), 9.11 (1H, s, H-5). $^{13}$C-NMR (DMSO-$d_6$) $\delta$: 26.19 ($CH_{2\beta}$), 52.31 (3×$NCH_3$), 55.22—59.52 (4×$OCH_3$), 65.24 ($CH_{2\alpha}$), 108.31 (C-5), 108.40 (C-2), 115.27 (C-8), 120.16 (C-10), 125.24 (C-9). EI-MS 384 $C_{23}H_{30}NO_4$ $m/z$ (rel. int.)=324(11), 277 (12), 58 (100).

**Measurement of ROS Generation from Human PMNs** The formation of ROS by human PMNs was assessed by luminol-enhanced chemiluminescence with a modified method.[16] Assay was carried out in opaque 96 well plates, $10^5$ cells per well were suspended in an assay volume of 180 $\mu$l of Krebs-HEPES buffer containing glucose 5.6 mM, bovine serum albumin (BSA) 0.05% (w/v), microperoxidase 2 $\mu$M with gelatine 0.1% pH 7.4, alone or in combination with alkaloids (final concentration in 200 $\mu$l, 100—0.01 $\mu$M) for 30 min at 37 °C. All the assays were performed in duplicate. Plates were placed in a Wallac 1420 Victor$^2$ Multilabel Counter. Then 20 $\mu$l of luminol 5 $\mu$M, $CaCl_2$ 1 mM and $N$-formyl-methionyl-leucyl-phenylalanine (f-MLP) 100 nM was added sequentially to each well, except in the blank group. Experiments with the appropriate DMSO concentration were also carried out (1—0.01%). Chemiluminescence was recorded at 4 s intervals over a 100 s period per well and the area under the curve (*AUC*'s) was integrated. Drug-induced reduction was expressed as % inhibition. Inhibitory concentration 50% ($IC_{50}$) values were then calculated from the concentration–inhibition curves by non-linear regression analysis.

**Measurement of ROS Generation by Hypoxanthine–Xanthine Oxidase** The ROS was generated by hypoxanthine–xanthine oxidase system and detected by luminol-enhanced chemiluminescence using a modified method.[17] Assay was carried out in opaque 96 well plates. One hundred and eighty microliters of Krebs–HEPES buffer containing hypoxanthine 0.1 mM, glucose 5.6 mM, gelatine 0.1% pH 7.4, alone or in combination with synthesized alkaloids (final concentration in 200 $\mu$l, 100—0.01 $\mu$M) were added to the wells for 5 min at 37 °C. All the assays were performed in duplicate. Plates were placed in a Wallac 1420 Victor$^2$ Multilabel Counter. Then 20 $\mu$l of luminol 5 $\mu$M, $CaCl_2$ 1 mM and xanthine oxidase 0.02 u/ml was added sequentially to each well, except in the blank group. Experiments with the appropriate DMSO concentration were also carried out (1—0.01%). Chemiluminescence was recorded at 4 s intervals over a 100 s period per well and the area under the curve (*AUC*'s) was integrated. Drug-induced reduction was expressed as % inhibition. Inhibitory concentration 50% ($IC_{50}$) values were then calculated from the concentration–inhibition curves by non-linear regression analysis.

**Measurement of the Effect on Xanthine Oxidase Activity by Isoxantopterine Formation from Pterine** A direct inhibitory effect on xanthine oxidase activity was tested by measuring isoxantopterine formation from pterine by fluorimetry (excitation wavelength at 345 nm and emission wavelength at 390 nm) following the previously described method.[18]

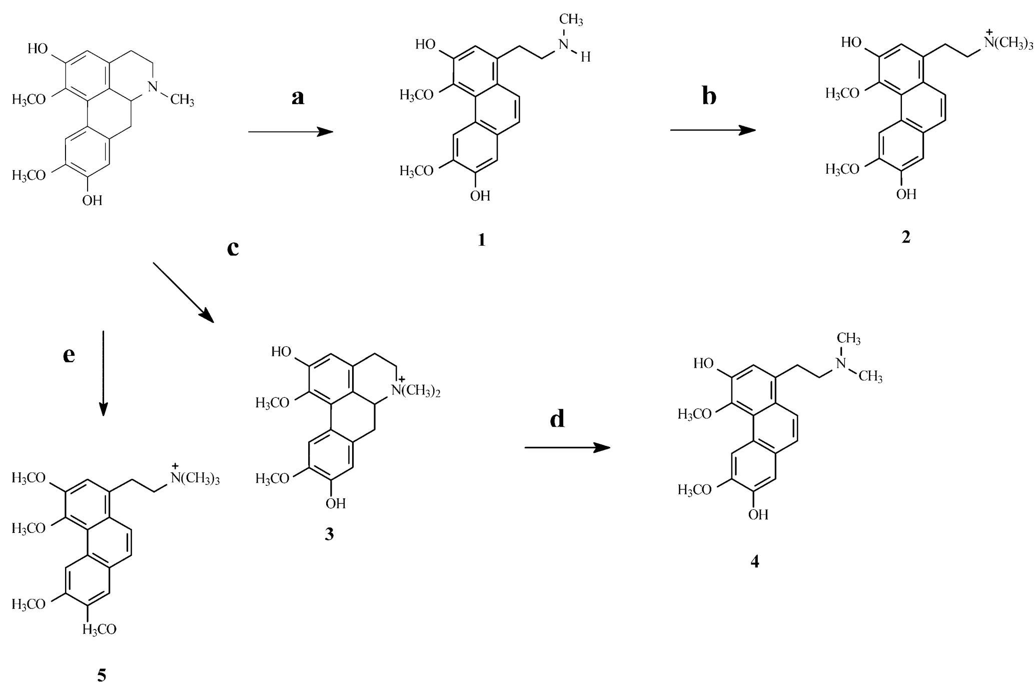
**Statistical Analysis** The $IC_{50}$ values were calculated from non-linear regression by a software of Prisma 3.0 (Graph Pad Software, San Diego, California, U.S.A.). All values are shown as mean±S.E.M. The difference between two values was determined by use of unpaired Student's $t$-test. The differences were considered statistically significant if the $p$-value was less than 0.05.

## Results and Discussion

The synthesis of the compounds **1**—**5** was performed starting from boldine, according to the pathways shown in Chart 1. A variety of synthetic methods to obtain secoboldine **1**, have been reported in the literature, ranging from Von Braun reaction of boldine by treatment with BrCN in $CHCl_3$ under reflux, followed by alkaline hydrolysis,[11] photolysis[19] and *via* solvolysis of boldine with $NH_4OAc$ in EtOH under reflux.[20,21] Among these reactions, we have selected this last strategy since this method seemed to be a straightforward one-pot reaction. In this method the participation of a phenolic group and the temperature control were essential for satisfactory yields. After several trials the bath temperature of 103—104 °C was the best range to obtain our purpose. At bath temperature under 98 °C the reaction did not take place. Boldine was refluxed 48 h in a bath at 103 °C with aqueous $NH_4OAc$ and EtOH (1 : 1) to give after crystallization compound **1**[22] in 89% yield.

Modification of the methylamino-dimethylene side chain of **1** was performed to reduce lipophilicity by treating with methyl iodide in excess at room temperature during 48 h. The crystallization as needles with acetone led us analytically pure product **2** for biological assays.

The phenanthrene compound boldine methine **4**, was provided as shown in Chart 1 in two steps. First, the $N$-methylboldine **3**,[13] was synthesized using a simple reaction in which the starting boldine was stirred overnight at room temperature with an excess of methyl iodide (98% yield) in a mixture of $CH_3CN$–MeOH (7.5 : 5). The ring openning of the piperidinium ring in compound **3** with aqueous ammonium,




(a) $NH_4AcO$, EtOH; (b) MeOH, $CH_3CN$, $CH_3I$; (c) MeOH, $CH_3CN$, $CH_3I$; (d) $NH_4AcO$, EtOH; (e) MeOH, $CH_3CN$, $K_2CO_3$, $CH_3I$.

Chart 1

Table 1. $IC_{50}$ of the Alkaloids Tested for ROS Generation by f-MLP Stimulated Human Neutrophils or Hypoxanthine–Xanthine Oxidase System

| Alkaloid | f-MLP stimulated human neutrophils $IC_{50}$ ($\mu$M) | Hypoxanthine–xanthine oxidase system $IC_{50}$ ($\mu$M) |
|---|---|---|
| Ascorbic acid | 99.60±0.2 | 167.80±13.5 |
| Boldine | 1.39±0.18** | 0.27±0.17** |
| **1** | 0.50±0.13** | 0.16±0.07** |
| **2** | 0.62±0.22** | 0.15±0.09** |
| **3** | 1.27±0.10** | 0.47±0.11** |
| **4** | 0.15±0.07** | 0.23±0.08** |
| **5** | 18.83±0.35** | 6.12±0.10** |

Statistical significance from positive control ** $p<0.01$

as described above, was carried out in the final step. The crude was concentrated and extracted with dichloromethane. Boldine methine[14] was obtained in 94% yield.

Finally, the treatment of boldine at room temperature, using 3, 4 equivalents or an excess of methyl iodide and an excess of $K_2CO_3$, gave in all the experiments the 1-phenanthrene ethanaminium 3,4,6,7-tetramethoxy-*N*-trimethyl iodide **5**.[15]

The effect of aporphine and phenanthrene alkaloids on free radical generation was investigated by two different methods: measurement of ROS generation in stimulated human PMNs[16] and measurement of ROS scavenging activity in a enzymatic system.[17] In the first test, all the alkaloids tested inhibited in a concentration-dependent manner f-MLP induced ROS generation in human PMNs although with different potency. The order of potency, based on the calculated $IC_{50}$ values (Table 1) showed that the phenanthrene alkaloid with phenolic groups at C-3, C-7 (compounds **1**, **2**, **4**) were more potent than their corresponding equivalents with aporphine skeleton. Phenanthrene alkaloids without phenolic group displayed lower activity than even though those with

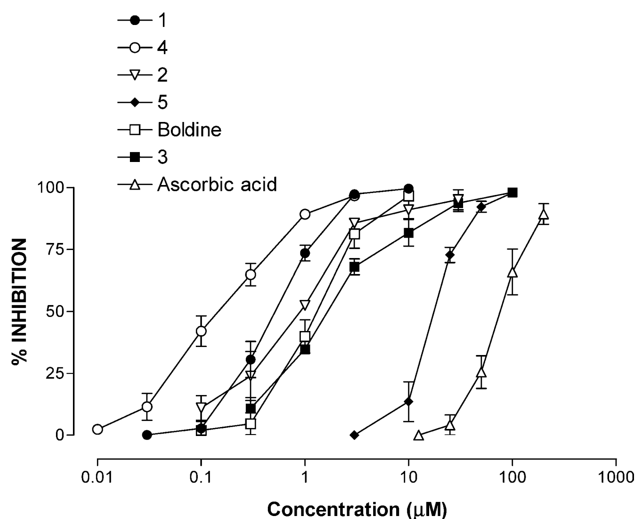



Fig. 1. Effect of Alkaloids on ROS Generation in f-MLP Stimulated PMNs

PMNs were incubated in the absence or presence of alkaloids (100—0.01 $\mu$M) at 37 °C as described in Experimental. Thirty minutes later f-MLP 100 nM was added to each well and chemiluminiscence recorded at 4 s intervals over a 100 s period. Results are expressed as percentage inhibition of ROS generation in PMNs stimulated with f-MLP. Results are represented as mean±S.E.M. of $n=5$—7 preparations.

aporphine skeleton (Fig. 1). All the alkaloids assayed were more potent than ascorbic acid ($IC_{50}$ 99.6 $\mu$M), the reference drug employed in this test. Since most of these compounds may exert this action, as occurs with other types of compounds such $\alpha$-tocopherol or ascorbic acid, through their free radical scavenging activity, they were tested on a cell free ROS generation system. All the alkaloids assayed were capable of scavenging ROS generated by the hypoxanthine–xanthine oxidase system in a concentration-dependent manner (Fig. 2). The order of potency based on the calculated $IC_{50}$ values (Table 1) revealed that all the synthesized compounds

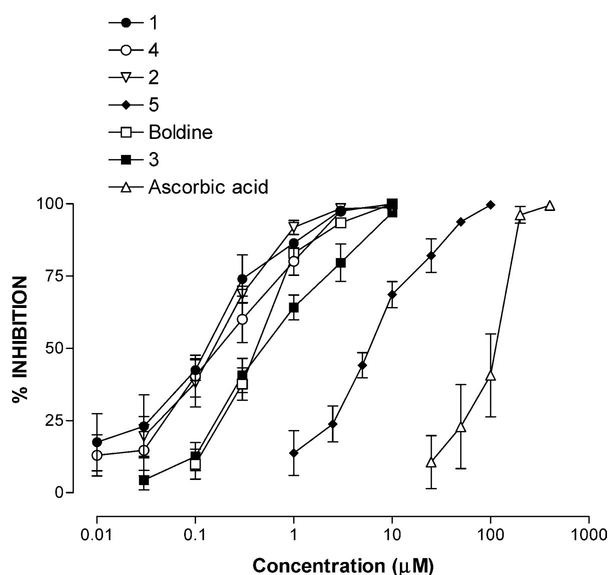



Fig. 2. Effect of Alkaloids on ROS Generation by Hypoxanthine–Xanthine Oxidase System

Hypoxanthine was incubated in the absence or presence of alkaloids (100—0.01 $\mu$M) at 37 °C as described in Experimental. Five minutes later xanthine oxidase was added to each well and chemiluminiscence recorded at 4 s intervals over a 100 s period. Results are expressed as percentage inhibition of ROS generation in hypoxanthine–xanthine oxidase system. Results are represented as mean±S.E.M. of $n$=5—6 preparations.

Table 2. Effect of Alkaloids on Isoxantopterine Formation from Pterine by Measuring Enzyme Activity ($\mu$mol/min)

| | | | |
|---|---|---|---|
| Control | 4.827±0.247 | Control | 3.928±0.648 |
| Boldine | 5.001±0.320 | **2** | 3.820±0.300 |
| **1** | 4.858±0.072 | **3** | 4.280±0.130 |
| **4** | 5.160±0.110 | **5** | 3.770±0.520 |

Data are means±S.E.M. No statistical significance $p<0.05$ from control was observed.

were more active than the classic antioxidant ascorbic acid ($IC_{50}$ 167.8 $\mu$M), employed as control in this test. In this assay, a similar profile of structure–activity relationship to that encountered in ROS generated in f-MLP stimulated neutrophils was observed. Indeed, phenanthrene alkaloids with phenolic substituents were the most active, followed by the group of the aporphine alkaloids with phenolic groups and phenanthrene alkaloid without phenolic groups.

To confirm that aporphine and phenantrene alkaloids exert ROS scavenging activity and that this effect was not due to xanthine oxidase inhibition, another set of experiments were carried out to investigated such possibility. None of the alkaloids assayed affected xanthine oxidase activity (Table 2), indicating a clear ROS scavenging activity of these compounds. Therefore a potent new class of phenanthrene alkaloids with ROS scavenger activity have been synthesized. The preliminary studies of structure–activity relationship with these class of compounds revealed that the presence of phenolic groups is essential for a strong antioxidant activity and show that with the same substituents the alkaloids with phenanthrene skeleton are more active than their respective alkaloids with aporphine skeleton. In this sense phenanthrene alkaloids may become promising candidates for the development of antiinflammatory agents ought to their potent ROS scavenging activity.

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