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Synthesis of xanthoxyline derivatives with antinociceptive and antioedematogenic activities

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Introduction

We have previously shown that 2-hydroxy-4,6-dimethoxyacetophenone, xanthoxyline **1**, isolated from the leaves and stems of *Sebastiania schottiana* (Euphorbiaceae), produces concentration-dependent, non-competitive and reversible antagonistic effect against acetylcholine, histamine and oxytocin-mediated contractions of the smooth muscles in vitro. Xanthoxyline **1** also inhibited in a graded and reversible manner, the neurogenic contractions induced by electrical stimulation of strips of guinea-pig ileum, guinea-pig urinary bladder and rat left atrium [1–3]. Recently, we have synthesized several xanthoxyline derivatives and demonstrated that some of them exhibit about 20-fold higher potency than xanthoxyline itself in antagonizing acetylcholine-mediated contraction in the guinea-pig isolated ileum [4–6].

In the current series of experiments, we have extended these studies and have evaluated the antinociceptive and antioedematogenic actions, by analyzing the structure–activity relationships of several xanthoxyline derivatives. For comparison, in this study we have also included the effects of some well-known nonsteroidal antiinflammatory drugs.

Results and discussion

Chemistry

The synthesis of xanthoxyline derivatives was carried out by the conventional or modified methods (scheme 1), and all compounds were characterized by their physical and spectroscopic data. It is interesting to discuss some special aspects of these syntheses.

All attempts to obtain 2-hydroxy-4,6-dimethoxy phenacyl bromide, an analogue of 4-bromophenacyl bromide, a compound previously reported as an inhibitor of the biosynthesis or actions of the leukotrienes [7], were unsuccessful. Thus, treatment of xanthoxyline with several brominating agents, such as AcOH/Br_2, $CHCl_3$/Br_2, AcOH/AcONa/Br_2 or NBS, as previously described [8] for bromination of the methyl group, in order to obtain the corresponding α-bromoketone produced mainly regioisomer **9**, according to spectroscopic and physical data [9]. The use of copper(II) bromide, reported as a selective brominating agent for ketones [10], also gave similar results. Compound **9** was obtained in the best yield with AcOH/Br_2. On the other hand, the use of MeOH/NaOH/Br_2 as brominating agent led directly to compound **10**. This fact may be explained by the formation of the phenoxy group which strongly activates positions 3 and 5 of the aromatic ring. Attempts to introduce one chlorine atom in position 3 of xanthoxyline, using the method described previously for obtaining of chlorine gas [11], furnished a complex mixture of products. The reaction between xanthoxyline and 4-bromophenacyl bromide did not give the expected product, ie, the 2-phenacetyloxy derivative. This product seems to be very unstable, resulting in the new compound **5**, after intramolecular aldolic condensation and further dehydration. The structural confirmation of this compound was obtained from its spectroscopic data. The analysis of IR shows the presence of only one carbonyl group, whereas the ^{1}H-NMR indicates the absence of -CH_2- and ^{13}C (APT) confirms the presence of C=C and one carbonyl group

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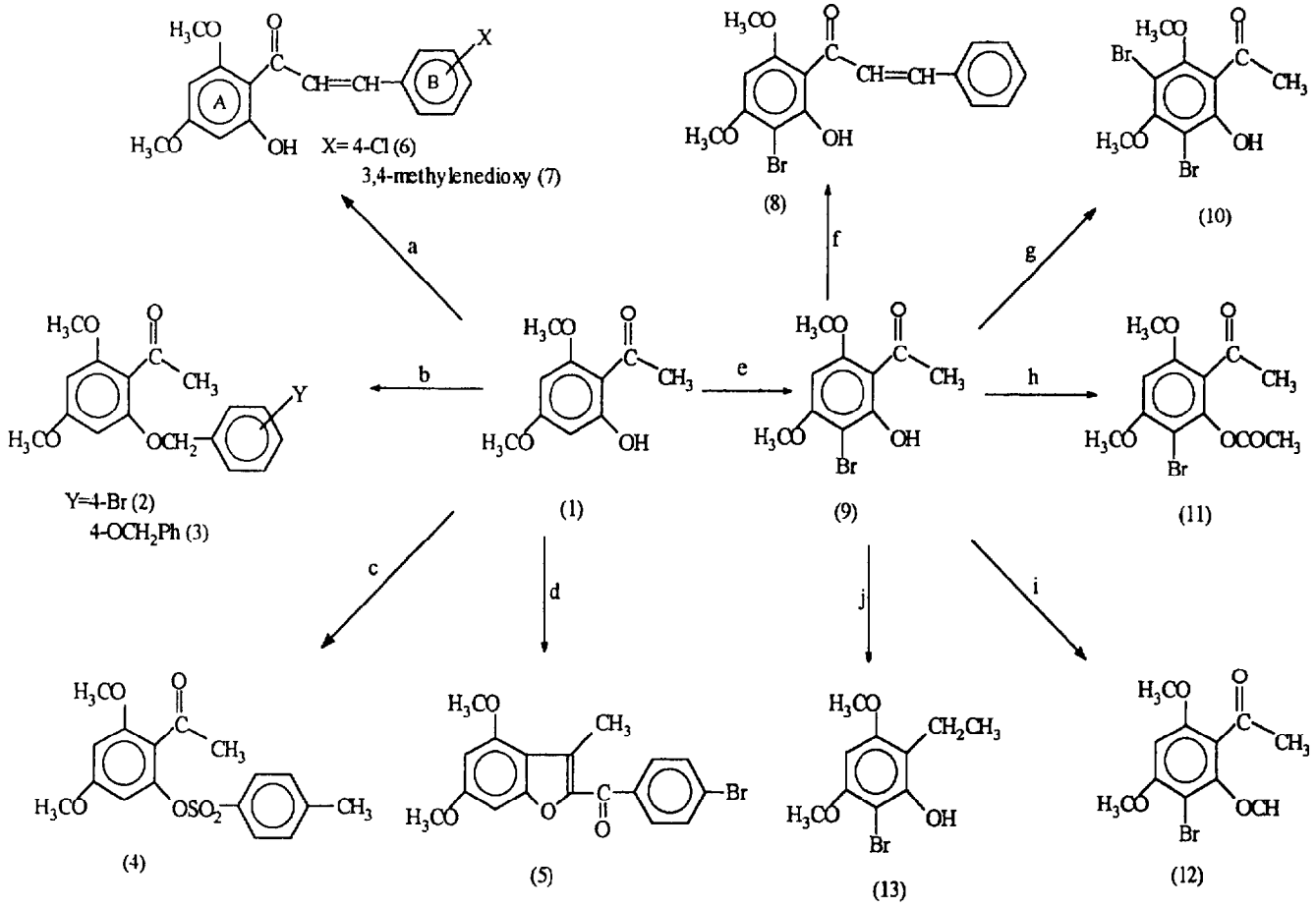

Scheme 1. Reagents: a) CHO-Ph-X, NaOH/EtOH; b) $BrCH_2Ph$-X, NaOH/MeOH/DMF, 90–100 °C; c) TsCl, NaOH/IsoprOH, 90–100 °C; d) 4-$BrPhCOCH_2Br$, NaOH/MeOH/DMF, 90–100 °C; e) Br_2/OHAc; f) CHOPh, NaOH/EtOH; g) $Br_2/CHCl_3$; h) Ac_2O/Py, 90–100 °C; i) Me_2SO_4, NaOH/Me_2CO; j) HCl/Zn, MeOH.

as well as all other absorptions, in accordance with structure **5**. To our knowledge, benzofuran analogues have not yet been synthesized by this method. Burgstahler and Worden [12] have obtained other benzofurans in two steps using salicyaldehyde, chloroacetic acid and additional cyclization, with a mixture of acetic anhydride, sodium acetate and glacial acetic acid. However, this class of compounds occurs naturally in several plants, and their chemical and biological aspects have already been reported [13].

All other reactions afforded the expected products. Satisfactory yields were found for all the compounds.

Pharmacology

The results summarized in the table I show that xanthoxyline **1**, up to 306 μmol/kg (60 mg/kg ip), given 30 min prior, exhibited partial antinociceptive effect when assessed against acetic-acid-induced abdominal constriction in mice, being essentially inactive against both phases of the formalin-induced licking. However, most of the xanthoxyline derivatives showed marked antinociception against both acetic-acid- and formalin-induced nociception. Thus, compound **5** and, to a lesser extent, compound **3**, revealed potent analgesic effect against acetic-acid-induced writhing response in mice, with mean ID_{50} values of 1.3 and 27 μmol/kg, respectively. When compared with aspirin and acetoaminophen, compounds **3** and **5** were about 5- and 100-fold more active, respectively. Interestingly, compound **5** given intraperitoneally, but not aspirin and acetoaminophen, dose-dependently prevented the neurogenic effects (early phase) and like the standard drugs the inflammatory (late phase) effects of the formalin-induced licking with mean ID_{50} values of 36

and 8 μmol/kg, respectively. In relation to the late phase of the formalin response, compounds **3** and **5** were about 4- and 15-fold more active than aspirin and acetoaminophen, respectively. These results suggest that the benzofuran analogue **5** may interfere with some mediators involved in the neurogenic pain response [14–17].

Similarly, the results of table I show that the *p*-bromobenzyloxy derivative **2**, caused dose-related inhibition against both phase of the formalin-induced licking, being about two-fold less potent than aspirin and acetoaminophen, respectively. In addition, compound **2** also produced graded inhibition against acetic-acid-induced writhing response, exhibiting similar activity to those shown by aspirin and acetoaminophen. However, its analogue *p*-benzyloxy **3** was about three- and sixfold more active than the standard drugs in inhibiting acetic acid and the late phase of the formalin test, respectively (table I). On the other hand, the chalcone derivative **8** caused dose-related inhibition of acetic-acid-induced writhing, but it was completely inactive against both phases of the formalin-induced licking (table I). Interestingly, the chalcone **7**, without the bromine atom in ring A, but containing the methylenedioxy group in positions 3 and 4 of ring B (see scheme 1), produced graded inhibition of acetic acid, being equipotent with aspirin and acetoaminophen and against both phases of formalin-induced algesic responses, being about twofold less potent than standard drugs. The introduction of tosyl group produced compound **4**, which also is about 2.5-fold more active than aspirin and acetoaminophen in the writhing test. Compound **4** did not exhibit antinociception in the formalin test, but other substituent groups can be introduced in the aromatic ring of the phenyl moiety in the attempt to increase the analgesic activity. Of interest are the results showing that the monobrominated xanthoxyline **9** caused dose-related antinociception against acetic-acid-induced writhing and the late phase of formalin-mediated pain response, being about 1.5- and 1.6-fold more active than aspirin and acetoaminophen, respectively, in both models. In marked contrast, dibrominated xanthoxyline **10** was completely void of antinociception against both models employed. Thus, the introduction of one, but not two atoms of bromine into the xanthoxyline molecule is able to produce a more potent analgesic compound against inflammatory pain, but not against neurogenic pain.

In view of the interesting analgesic effect of compound **9**, three other derivatives were synthesized, compounds **11**, **12** and **13**. Among them, only compound **12** exhibited antinociception against acetic-acid-induced pain, but it was about threefold less potent than **9**, and was inactive against both phases of the formalin-induced licking (table I). In addition, neither of the xanthoxyline derivatives interfered with formalin-induced paw oedema. It is interesting to

Table I. Comparison of the antinociceptive effect of some xanthoxyline derivatives with non-steroidal antiinflammatory drugs.

Compound	*Writhing test*		*Formalin test*			
	ID_{50} (μmol/kg, ip)	*MI[a] (%)*	*First phase ID_{50} (μmol/kg, ip)*	*MI[a] (%)*	*Second phase ID_{50} (μmol/kg, ip)*	*MI[a] (%)*
1	>306	23 ± 2	Inactive	NT	Inactive	NT
2	102 (30–225)[b]	98 ± 1	810 (679–960)	40 ± 4	274 (233–324)	80 ± 5
3	27 (22–34)	98 ± 1	>76	33 ± 5	33 (24–45)	99 ± 2
4	49 (40–61)	67 ± 1	Inactive	NT	>86	25 ± 6
5	1.3 (0.8–2)	91 ± 4	36 (12–107)	63 ± 4	8 (5–13)	88 ± 6
6	>188	30 ± 5	Inactive	NT	Inactive	NT
7	137 (128–146)	98 ± 1	549 (366–828)	68 ± 4	273 (235–316)	89 ± 5
8	18 (11–32)	61 ± 2	Inactive	NT	Inactive	NT
9	81 (58–113)	100	>109	17 ± 4	74 (39–139)	54 ± 5
10	Inactive	NT	>170	16 ± 2	Inactive	NT
11	Inactive	NT	>189	14 ± 3	Inactive	NT
12	248 (238–258)	62 ± 1	Inactive	NT	Inactive	NT
13	>115	42 ± 2	Inactive	NT	Inactive	NT
AA	125 (104–250)	88 ± 1	Inactive	NT	120 (90–161)	85 ± 5
Aspirin	133 (73–243)	83 ± 1.4	Inactive	NT	123 (77–209)	88 ± 3

AA = acetoaminophen; [a]maximal inhibition; [b]95% confidence limit; NT = not tested. Each group represents the mean of four to six animals.

observe that some compounds that exhibited potent antinociceptive activity, such as **4**, **5** and **9**, were devoid of antispasmodic activity against acetylcholine-induced contraction of guinea-pig ileum [4, 5], suggesting that they act by different mechanisms of action.

Table II shows the antioedematogenic activity for the xanthoxyline derivatives in the rat by the method described previously [18]. It should be noted that xanthoxyline **1** and also compounds **5**, **8**, **9** and **11**, revealed important antioedematogenic action, inhibiting the formation of paw oedema caused by dextran. While xanthoxyline presented a maximal inhibition (MI) of 36% (2 h), the monobromated compound **9** exhibited an MI of 30% (1 h), and the dibrominated compound **10** caused an MI of 41% (2 h). These results indicate that the introduction of bromine atoms into aromatic ring of xanthoxyline, in contrast with that observed in analgesic activity, does not significantly affect its antioedematogenic properties when tested against dextran-induced paw oedema. However, the acetylation and the methylation of **9** led to compounds **11** and **12** which are less active.

On the other hand, chalcones **7** and **8** showed a significant antioedematogenic effect, with MIs of 59% (1 h) and 58% (1 h), respectively. Similar results were found for the benzofuran analogue **5**, which presented an MI of 54% (1 h). It is important to note that compounds **5** and **8** reduce the paw oedema formation caused by dextran by 46 and 47%, respectively, at 4 h, indicating a long duration of action.

In the paw oedema caused by carrageenan, xanthoxyline **1** showed weak effects, with an MI of 26% (1 h), whereas the benzyloxy derivatives **2** and **3** were more active, with MIs of 35% (1 h) and 66% (2 h), respectively. However, compound **5** presented an MI of 45% (1 h), being slightly less active when compared with dextran-induced oedema formation. Whereas chalcone **8** presented an MI of 43% (1 h), chalcone **7**, in marked contrast, potentiated the carrageenan-induced paw oedema. Monobrominated xanthoxyline **9** displayed moderate antioedematogenic activity, with an MI of 30% (1 h). In marked contrast, dibrominated xanthoxyline **10** showed only weak effect, with an MI of 17% (1 h). Furthermore, the acetylation of **9** produced compound **11**, which did not significantly affect the carrageenan-induced paw oedema, whereas the methylation and reduction of the carbonyl group, in order to obtain **11** and **13**, respectively, decreased the antioedematogenic effect against carrageenan-induced oedema formation. As shown in table II, some of xanthoxyline derivatives tested, such as compounds **3**, **5** and **8**, exhibited marked antioedematogenic properties, being able to strongly inhibit the paw oedema induced by carrageenan, with MIs, in the time of 1 h, similar to the well-known antiinflammatory drugs, such as indomethacin and ibuprophen.

Considering the marked antioedematogenic activity of some xanthoxyline derivatives against dextran and carrageenan-induced paw oedema, the most active compounds were chosen and were evaluated against bradykinin-induced paw oedema. The results, summa-

Table II. Percent of inhibition of oedema formation in the rat paw induced by carrageenan, dextran or bradykinin by xanthoxyline derivatives (30 mg/kg, ip).

Compound	*Carrageenan (300 µg/paw)*			*Dextran (100 µg/paw)*			*Bradykinin (3 nmol/paw)*		
	1 h	*2 h*	*4 h*	*1 h*	*2 h*	*4 h*	*0.5 h*	*1 h*	*2 h*
1	25*	26*	0.6	11	36**	13	49***	51***	37**
2	35***	25*	22*	3	4	–10	NT	NT	NT
3	57***	66***	39**	29*	26*	17	NT	NT	NT
4	NT	NT	NT	NT	NT	NT	NT	NT	NT
5	45***	33**	–6	54***	54***	46***	56***	55***	44***
6	–2	–2	–4	28*	24*	23	NT	NT	NT
7	–18	–9	12	59***	58***	21	2	12	20
8	43***	8	21	58***	54***	47***	36**	33**	31**
9	30**	24	21	32**	30**	21	37**	33**	33**
10	17	–6	–2	38***	41***	27**	5	5	24
11	29*	23	23	33**	22	–12	39***	50***	53***
12	26*	20	5	16	15	1	NT	NT	NT
13	8	9	–13	26*	36**	31**	NT	NT	NT
IND	43***	53***	53***	NT	NT	NT	NT	NT	NT
IBU	45***	51***	19	NT	NT	NT	NT	NT	NT

IND: indomethacin; IBU: ibuprophen; NT: not tested; negative values indicate potentialization of oedema. Each value represents the mean of four to six animals. Values differ significantly from respective control (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

rized in table II, show that xanthoxyline itself and compounds **5** and **11**, strongly prevented bradykinin-induced oedema formation, with MIs of 56% (0.5 h) and 53% (2 h), respectively. The antioedematogenic activity demonstrated by some xanthoxyline derivatives, may explain, at least in part, their antinociceptive activities.

Conclusion

The results obtained in the current study demonstrate that some xanthoxyline derivatives produced potent antinociception when analysed against the neurogenic and inflammatory models of nociception. Modification of the hydroxyl group by the reaction with 4-bromophenacyl bromide, afforded an unexpected product **5**, which was the most active compound tested. This compound presented high activity in both models of pain employed. It was able to attenuate both phases of the formalin-induced licking, being about 15-fold more active than aspirin and acetaminophen in relation to the second phase of pain. The mechanism by which compound **5** produces antinociception remains unclear. Its antinociceptive effect was insensitive to naloxone, suggesting the lack of involvement of endogenous opioid, and this effect does not involve interaction with L-arginine, nitric oxide pathway, activation of α_1-adrenoceptors or $GABA_B$ receptors (GABA = γ-aminobutyric acid), but involves, at least in part, the serotoninergic pathway [19].

Xanthoxyline **1** and some of its derivatives also showed considerable antioedematogenic properties, being capable of inhibiting the paw oedema formation induced by dextran, carrageenan and bradykinin. Compounds **3**, **5** and **8** presented antioedematogenic activities, with similar efficacy to indomethacin and ibuprophen when evaluated by the method of carrageenan-induced paw oedema.

In summary, with the use of natural product xanthoxyline **1** as model, it was possible to produce several derivatives which showed potent and relatively long-lasting antinociception and antioedematogenic properties. Therefore, such xanthoxyline derivatives may constitute important potential antinociceptive non-opioid compounds, which could be useful for the management of neurogenic pain.

Experimental protocols

Chemistry

Melting points were determined with a Microquimica AP-300 apparatus and are uncorrected. IR spectra were recorded with a Perkin-Elmer 720 spectrometer, on a Varian XL 60, or on a Brucker 200 MHz. The ^{13}C-NMR for compounds **5** and **9** was recorded on a Varian VXR-75 in $CDCl_3$ as solvent at 25 °C (Cq: quaternary carbon). Elementary analyses were obtained on a Perkin Elmer 2400. Percentages of C and H were in agreement with the product formula (within ± 0.4% of theoretical values). The purity of the synthesized substances was checked by thin-layer chromatography (TLC) using Sigma silica precoated plastic plates, 200 μm in thickness, with several solvent systems of different polarity. Spots were visualized by short-wave UV light and iodine vapour. The solvents and reagents were purified in the usual manner. Xanthoxyline **1** was isolated from hexanic extract of *Sebastiania schottiana* as previously described [2, 3].

Synthesis of xanthoxyline derivatives

2-(4-Bromobenzyloxy)-4,6-dimethoxyacetophenone ***2***
Xanthoxyline **1** (0.2 g, 1.02 mmol), NaOH (0.041 g, 1.05 mmol), MeOH (2 mL), DMF (10 mL) and 4-bromobenzyl bromide (0.26 g, 1.05 mmol) were refluxed for 1.5 h. The mixture was cooled at room temperature and poured into water (70 mL). The precipitate was recrystallized from ethanol to give **2**. Yield = 78% (0.26 g); mp = 109 °C; IR (KBr): 1690 ($COCH_3$) cm^{-1}; ^{1}H NMR ($CDCl_3$, 60 MHz): δ: 2.50 (s, 3H, $COCH_3$), 3.88 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 5.15 (s, 2H, OCH_2Ph), 6.29 (d, 1H, Ar), 6.31 (d, 1H, Ar), 7.35–7.75 (m, 4H, Ar). Anal calcd for $C_{17}H_{17}BrO_4$ (365.16).

2-(4-Benzyloxybenzyloxy)-4,6-dimethoxyacetophenone ***3***
Compound **3** was prepared in a manner similar to that described for **2**. Yield = 40%, 0.16 g; mp = 88 °C; IR (KBr): 1698 ($COCH_3$) cm^{-1}; ^{1}H NMR ($CDCl_3$, 60 MHz), δ 2.60 (s, 3H, $COCH_3$), 3.90 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 5.25 (s, 2H, OCH_2Ph), 5.32 (s, 2H, OCH_2Ph), 6.40 (d, 1H, Ar), 6.50 (d, 1H, Ar), 7.15–7.75 (2m, 9H, 2Ar). Anal calcd for $C_{24}H_{24}O_5$ (392.38).

2-(4-Toluenesulphonyl)-4,6-dimethoxyacetophenone ***4***
Obtained as previously described [6].

2-(4-Bromobenzoyl)-4,6-dimethoxy-3-methylbenzofuran ***5***
Xanthoxyline **1** (0.2 g, 1.02 mmol), NaOH (0.05 g, 1.25 mmol), MeOH (5 mL), DMF (20 mL) and 4-bromophenacyl bromide (0.29 g, 1.04 mmol) were refluxed for 1.5 h. The mixture was poured into cool water and allowed to stand for several hours. The precipitate was recrystallized from EtOH to give **5**. Yield = 47.5% (0.179 g); mp = 185 °C; IR (KBr): 1630 (COPh) cm^{-1}; ^{1}H-NMR ($CDCl_3$, 60 MHz): δ 2.90 (s, 3H, CH_3), 3.97 (s, 3H, OCH_3), 4.00 (s, 3H, OCH_3), 6.30 (d, 1H, Ar), 6.60 (d, 1H, Ar), 7.50–8.00 (m, 4H, Ar); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ 12.0 (CH_3), 55.8 (2 × OCH_3), 87.5 (CH), 94.9 (CH), 127.0 (C_q), 129.7 (C_q), 131.1 (CH), 131.4 (CH), 137.1 (C_q), 156.8 (C_q), 157.0 (C_q), 162.5 (C_q), 183.6 (C=O). Anal calcd for $C_{18}H_{17}BrO_5$ (318.70).

1-(4,6-Dimethoxy-2-hydroxyphenyl)-3-(4-chlorophenyl)-2-propen-1-one ***6***
The methodology employed was previously described by Guider et al [20] for the synthesis of chalcones. Thus, a mixture of xanthoxyline (0.18 g, 0.92 mmol), EtOH (15 mL), NaOH (0.1 g, 2.5 mmol, with a minimum of H_2O) and 4-chlorobenzaldehyde (0.13 g, 0.95 mmol) was kept at room temperature for several hours. The crude product, isolated by acidification of the cool diluted solution, was recrystallized from CCl_4 to give **6**. Yield = 70.5% (0.20 g); mp = 167 °C; IR (KBr): 3450 (OH), 1639 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 60 MHz): δ 3.85 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 6.25 (d, 1H, Ar),

6.28 (d, 1H, Ar), 7.65 (d, 1H, Ar), 7.75 (d, 2H, Ar), 7.90 (s, 2H, CH=CH), 16.60 (s, 1H, OH). Anal calcd for $C_{17}H_{15}ClO_4$ (318.70).

1-(4,6-Dimethoxy-2-hydroxyphenyl)-3-(4-3,4-methylenedioxyphenyl)-2-propen-1-one 7
Compound **7** was prepared as compound **6**, but was recrystallized from hexane/ethyl acetate 3:1. Yield = 88.5% (0.26 g); mp = 160 °C; IR (KBr): 3400 (OH), 1625 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 3.90 (s, 3H, OCH_3), 4.05 (s, 3H, OCH_3), 6.25 (d, 1H, Ar), 6.30 (s, 2H, O-CH_2-O), 6.40 (d, 1H, Ar), 7.00–7.50 (m, 3H, Ar), 7.95 (s, 2H, CH=CH), 15.00 (s, 1H, OH). Anal calcd for $C_{18}H_{16}O_6$ (328.25).

1-(3-Bromo-4,6-dimethoxy-2-hydroxyphenyl)-3-phenyl)-2-propen-1-one 8
Monobrominated xanthoxyline **9** (0.2 g, 0.73 mmol) obtained according to the description below, was dissolved in a solution containing EtOH (15 mL) and NaOH (0.1 g, 2.5 mmol, with a minimum of H_2O). After the addition of benzaldehyde (0.08 g, 2.5 mmol), the mixture was kept at room temperature over night and was poured into cool diluted AcOH. The precipitate was recrystallized from hexane/ethyl acetate to give **8**. Yield = 72.5% (0.19 g); mp = 183 °C; IR (KBr): 3450 (OH), 1650 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 3.97 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 6.06 (s, 1H, Ar), 7.25–7.60 (m, 5H, Ar), 7.83 (s, 2H, CH=CH), 14.78 (s, 1H, OH). Anal calcd for $C_{17}H_{15}BrO_4$ (363.15).

2-Hydroxy-3-bromo-4,6-dimethoxyacetophenone 9
Xanthoxyline **1** (0.2 g, 1.02 mmol) was dissolved in glacial AcOH (10 mL), and the solution was left in an ice-bath for 5 min, and a solution of Br_2 (0.3 g) in AcOH was added slowly until the red colour became permanent, indicating the excess of bromine. After 30 min, the mixture was poured into cooled water and the precipitate was recrystallized from EtOH to give **9**. Yield = 94.5% (0.263 g); mp = 185 °C, lit [21] mp = 186–187 °C; IR (KBr): 3450 (OH), 1613 ($COCH_3$) cm^{-1}; ^1H-NMR ($CDCl_3$, 60 MHz): δ 2.75 (s, 3H, $COCH_3$), 4.10 (s, H, OCH_3), 4.15 (s, 3H, OCH_3), 6.30 (s, 1H, Ar), 12.15 (s, 1H, OH); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ 33.0 (CH_3), 55.7 (OCH_3), 56.3 (OCH_3), 86.6 (C_q), 91.3 (CH), 106.3 (C_q), 161.8 (C_q), 162.4 (C_q), 162.6 (C_q), 203.3 (C=O). Anal calcd for $C_{10}H_{11}BrO_4$ (275.05).

2-Hydroxy-3,5-dibromo-4,6-dimethoxyacetophenone 10
Monobrominated xanthoxyline **9** (0.2 g, 0.73 mmol) was dissolved in $CHCl_3$ and Br_2 (0.3 g) in $CHCl_3$ was added slowly, until the red colour became permanent. The mixture was kept at room temperature for several hours, and after evaporation of the solvent the solid was recrystallized from hexane to give **10**. Yield = 46% (0.12 g); mp = 109 °C, lit [21] mp = 111 °C; IR (KBr): 3450 (OH), 1614 ($COCH_3$) cm^{-1}; ^1H NMR ($CDCl_3$, 60 MHz): δ 2.75 (s, 3H, $COCH_3$), 3.87 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 13.50 (s, 1H, OH). Anal calcd for $C_{10}H_{10}Br_2O_4$ (353.94).

2-Acetoxy-3-bromo-4,6-dimethoxyacetophenone 11
Compound **9** (0.2 g, 0.73 mmol) was acetylated in the usual manner with pyridine (2 mL) and acetic anhydride (2 mL). The solution was heated at 80–90 °C and poured into cool water. The resulting solid was recrystallized from MeOH to give **11**. Yield = 78% (0.189 g); mp = 148 °C; IR (KBr): 1755 ($COOCH_3$), 1670 ($COCH_3$) cm^{-1}; ^1H NMR ($CDCl_3$, 60 MHz): 2.32 (s, 3H, $COCH_3$), 2.50 (s, 3H, $COOCH_3$), 3.95 (s, 3H, OCH_3), 4.00 (s, 3H, OCH_3), 6.55 (s, 1H, Ar). Anal calcd for $C_{12}H_{13}BrO_5$ (318.15).

2,4,6-Trimethoxy-3-bromoacetophenone 12
Compound **9** (0.2 g, 0.73 mmol) was dissolved in a solution containing acetone (15 mL), NaOH (0.05 g, 1.25 mmol in 1 mL H_2O) and $(CH_3)_2SO_4$ (0.1 mL, 0.73 mmol). The mixture was allowed to stand for 24 h. After evaporation of solvent, the solid was recrystallized from hexane to give **12**. Yield = 81% (0.17 g); mp = 71 °C; IR (KBr): 1685 ($COCH_3$) cm^{-1}; ^1H NMR ($CDCl_3$, 60 MHz): δ 2.35 (s, 3H, $COCH_3$), 3.80 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 6.15 (s, 1H, Ar). Anal calcd for $C_{11}H_{13}BrO_4$ (289.07).

2-Ethyl-3,5-dimethoxy-6-bromophenol 13
Compound **9** (0.3 g, 1.09 mmol) was dissolved in MeOH (5 mL), and a mixture of amalgamated mossy zinc (1.5 g), H_2O (3 mL), EtOH (5 mL) and HCl (3 mL) was added to it. The mixture was refluxed for 2 h, filtered and kept for 3 days in the freezer. The resulting precipitate was recrystallized from EtOH 50% to give **13**. Yield = 46% (0.13 g); mp = 71 °C; IR (KBr): 3500 (OH) cm^{-1}; ^1H-NMR ($CDCl_3$, 60 MHz): δ 1.00 (t, 3H, CH_3), 2.65 (q 2H, CH_2), 3.85 (s, 2H, OCH_3), 3.90 (s, 3H, OCH_3), 5.80 (s, 1H, OH), 6.25 (s, 1H, Ar). Anal calcd for $C_{10}H_{13}BrO_3$ (261.07).

Pharmacology

Abdominal constriction response caused by intraperitoneal injection of acetic acid
Male Swiss mice (25–30 g) were kept in a temperature-controlled environment (23 ± 2 °C) with a 12 h light–dark cycle. Food and water were freely available. The abdominal constriction induced by intraperitoneal injection of acetic acid (0.6%), which consisted of a contraction of the abdominal muscle together with a stretching of hind limbs, was carried out according to the procedures previously described [22]. Animals were pretreated with the xanthoxyline derivatives (0.3–100 mg/kg) 30 min before ip injection of acetic acid. Control animals received similar volume of 0.9% NaCl solution (10 mL/kg). All experiments were carried out at 20–22 °C. After challenge, pairs of mice were placed in separate boxes, and the number of abdominal constrictions was cumulatively counted over a period of 20 min. Antinociception was expressed as the reduction of the number of abdominal constrictions between control animals and mice pretreated with xanthoxyline derivatives or standard drugs.

Formalin-induced pain
Male Swiss mice, 25–30 g, were used. The procedure was similar to that described previously [14, 15, 23]. Animals from the same strain were slightly anaesthetized with ether, except when used to analyse the first phase of formalin-induced pain, and 20 µl 2.5% formalin (0.92% formaldehyde) made up in phosphate-buffer solution was injected under the paw surface of the right hindpaw. Two mice (control and treated) were observed simultaneously from 0 to 30 min following formalin injection. The amount of time spent licking the injected paw was considered as indicative of pain. The initial nociceptive scores normally peaked 5 min after formalin injection (early phase) and 15–30 min after formalin injection (late phase), representing the tonic and inflammatory pain responses, respectively [14]. Animals were treated with xanthoxyline derivatives intraperitoneally (1–200 mg/kg), 30 min before formalin injection, respectively. Following intraplantar injection of formalin, the animals were immediately placed into a glass cylinder of 20 cm in diameter, and the time spent licking the injected paw

(second phase of formalin test) was determined. To investigate whether the antinociceptive activity of xanthoxyline derivatives in formalin-induced pain was associated with antioedematogenic activity, we measured the paw oedema by comparing the difference in weight of the formalin-treated paw and the weight of the control paw (treated with PBS). For this purpose, animals were sacrificed 30 min after formalin injection by cervical dislocation, and the paw was cut at the knee joint and weighed on an analytical balance.

Measurement of rat paw odema

Experiments were conducted using non-fasted male Wistar rats (14–200 g) kept in a room controlled for temperature (22 ± 2 °C) and illumination (12 h on, 12 h off). Under ether anaesthesia, animals received 0.1 mL intraplantar injections in one hindpaw of phosphate-buffered saline (PBS; composition mmol/L: NaCl 137; KCl 2.7 and phosphate buffer 10) containing carrageenan (300 µg/paw), dextran (100 µg/paw) or bradykinin (BK, 3 nmol/paw). The contralateral paw received 0.1 mL PBS and was used as a control. In experiments with bradykinin, animals were pretreated with captopril (5 mg/kg, sc) [15] 1 h prior to any given experiment to prevent BK degradation [18]. Oedema was measured by use of a plethysmometer (Ugo, Basile) at several time-points after injection of inflammatory mediators or the irritants. Oedema was expressed in millilitres as the difference between the test and control paws.

The xanthoxyline derivatives were given intraperitoneally (30 mg/kg) 30 min prior to testing. Separate groups of animals were treated intraperitoneally with indomethacin (5 mg/kg) or ibuprophen (30 mg/kg) which were used as positive controls.

Statistical analysis

Results are presented as mean ± se mean, except the mean ID_{50} values (ie, the dose of drugs or compounds reducing the algesic responses by 50% relative to control value) which are reported as geometric means accompanied by their respective 95% confidence limits. The statistical significance between groups was analysed by variance followed by Dunnett's multiple comparison test. *P*-values less than 0.05 were considered as indicative of significance. The ID_{50} values were determined by graphical interpolation from individual experiments.

Drugs

The following drugs were used: aspirin, acetoaminophen, carrageenan, bradykinin, dextran, indomethacin, captopril, PBS, and ibuprophen (all from Sigma Chemical, Saint Louis, USA). Formalin and acetic acid (Merck, Darmstadt, Germany). The xanthoxyline derivatives, aspirin and acetaminophen were dissolved in Tween 80 (Merck, AG, Darmstad, Germany), plus 0.9% of NaCl solution and carboxymethylcellulose 0.5% plus 0.9% of NaCl solution, respectively. The final concentration of Tween and ethanol did not exceed 5% and did not cause any effect per se.

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