(∼25 min from the pharmacokinetic study). Each rat was placed with both its forepaw on the edge of a plastic observation box, onset of catalepsy was noted if the rat remained in this position for 45 s, rats not receiving droperidol scurried away within a few seconds. A similar catalepsy endpoint has been used previously (Freye and Kuschinsky 1976). The infusion was stopped immediately, the rats quickly anaesthetised with halothane and blood (abdominal aorta) and then brain was obtained following decapitation. Droperidol was assayed in serum and brain using reversed phase liquid chromatography (Guichard et al. 1993) but with a different internal standard (diazepam). This involved solid phase extraction and UV detection at 245 nm. Droperidol and diazepam retention times were 12.8 and 8.4 min. Recovery from brain and serum was 75 ± 4%, intra- and interday assay variability was 2.0–6.8% and 6.2–12.3% and the limit of quantitation was 25 ng/mL. Droperidol doses and concentrations as a function of input rate were compared using ANOVA and post hoc Fishers LSD test, $P < 0.05$ was considered significant.

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Inhibitory activity of indolin-2-one derivatives on compound 48/80-induced histamine release from mast cells

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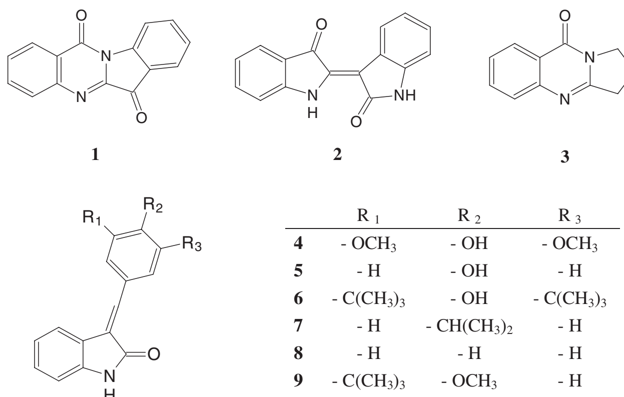
Four alkaloids, previously identified in *Isatis* species, were tested for their inhibitory effect on histamine release. Whereas tryptanthrin, indirubin and deoxyvasicinone did not inhibit histamine release, the effect of indolin-2-one exceeded that of the mast cell stabilizing drug disodium chromoglycate.

Isatis tinctoria L. (woad, family Brassicaceae) is an old indigo dye and anti-inflammatory medicinal plant (Hurry 1930). A broad-based pharmacological profiling in a panel of cell-based and mechanism-based assays involving over 20 inflammation-related targets showed that a lipophilic extract from woad leaves significantly inhibited cyclooxygenase-2, 5-lipoxygenase, inducible NO synthase, leucocytic elastase, and histamine and serotonine release from stimulated mast cells (Hamburger 2002). We identified the alkaloid tryptanthrin (**1**) as the COX-2 inhibitory principle in woad (Danz et al. 2001). The compound also strongly inhibited LTB_4 release from human granulocytes and expression of inducible NO synthase (Danz et al. 2002; Ishihara et al. 2000). *Isatis* extracts and, to a lesser extent, **1** alone, showed anti-inflammatory activity in carageenan-induced paw oedema and in TPA-induced mouse ear oedema (Recio et al. 2002), and a clinical pilot study confirmed the anti-inflammatory effect of topical application in clinically relevant skin irritation models (Heinemann et al. 2004).

In the search for woad constituents responsible for the inhibitory effect on histamine release, we adapted existing assay protocols with compound 48/80 stimulated rat mast



	R_1	R_2	R_3
4	- OCH_3	- OH	- OCH_3
5	- H	- OH	- H
6	- $C(CH_3)_3$	- OH	- $C(CH_3)_3$
7	- H	- $CH(CH_3)_2$	- H
8	- H	- H	- H
9	- $C(CH_3)_3$	- OCH_3	- H

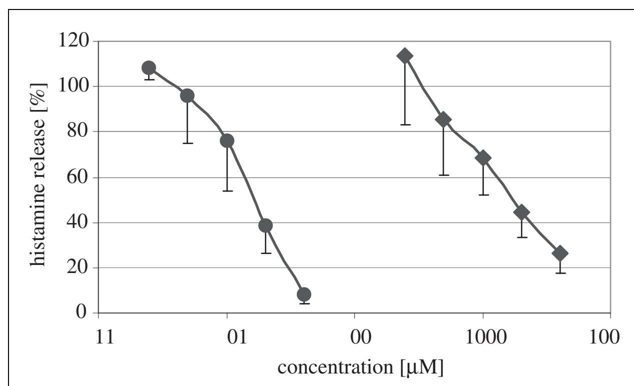


Fig.: Inhibition of compound 48/80 stimulated histamine release. Dose-inhibition curves for compound **4** (●) and disodium chromoglycate (■ positive control) are shown. Each datapoint represents a mean ± SD of 3 determinations

cells (Vogel and Vogel 1997) to the 96-well microtiter format. We then tested alkaloids **1**–**4**, which had been previously identified in *Isatis* (Hamburger 2002). Tryptanthrin (**1**), indirubin (**2**) and deoxyvasicinone (**3**) did not inhibit histamine release when tested at concentrations up to 40 μM (data not shown). Indolin-2-one **4** (Wu et al. 1997), in contrast, had a marked effect on histamine release (Fig.; IC_{50} 15 μM). The potency was higher than that of the mast cell stabilizing drug disodium chromoglycate (IC_{50} 1.5 mM). We then synthesized a series of indolin-2-ones **5**–**9** (Sun et al. 1998) which differed in the substitution pattern of the benzylidenyl moiety and tested their activity. None of the compounds inhibited histamine release at concentrations up to 40 μM.

Indolinones **5**–**9** are inhibitors of various kinases (Sun et al. 1998), and indirubin reportedly inhibits cyclin-dependent kinase (CDK) 5/P25 and glycogen synthase kinase (GSK)-3β (Hoessel et al. 1999; Leclerc et al. 2001). Although indolinone **4** had not been synthesized and tested in the kinase panel (Sun et al. 1998), the lack of activity of compounds **5**–**9**, and **2** in our screening indicates that kinase inhibition cannot be the mechanism responsible for the inhibitory activity of **4** on histamine release from mast cells.

Experimental

1. Characterisation of compounds

Identity and purity of compounds was checked by m.p., HPLC, ESI-MS, UV, IR , ^{1}H and ^{13}C NMR and was in accord with literature data (Wu et al. 1997; Sun et al. 1998; Hoessel et al. 1999; Leclerc et al. 2001; Friedländer and Roschdestwensky 1915; Molina at al. 2000).

2. Instruments

ESI-MS was performed with an API 165 with turbo ion spray interface (Applied Biosystems), connected to a Agilent 1100 HPLC system with DAD-detector. ^{1}H and ^{13}C NMR-spectra were recorded on a Bruker Avance 400. IR spectra were measured on a Specord M 82 (Carl Zeiss, Jena), and UV/VIS spectra with a Beckman DU 640 instrument. MP were determined with a Kofler block. The bioassay was analyzed with a Fluostar Galaxy plate reader (BMG LabTechnologies).

3. Synthesis

Tryptanthrin (**1**), indirubin (**2**), deoxyvasicinone (**3**) and indolin-2-ones **4**–**9** were prepared according to published procedures (Sun et al. 1998; Hoessel et al. 1999; Friedländer and Roschdestwensky 1915; Molina et al. 2000). The ratio of E to Z isomers in **4**–**9** was determined by RP HPLC (LiChrospher RP18, 5 μM, 150 × 4 mm; MeCN-H_2O 50 : 50 or 60 : 40), and was in accord with published data for **5**–**9** (Sun et al. 1998), and 81 : 19 in case of **4**. Separation of isomers was attempted by prep. RP HPLC but was unsuccessful because of slow spontaneous isomerization.

4. Bioassay

A published protocol (Vogel and Vogel 1997) was used, with some modifications. Rat peritoneal mast cells were harvested in buffer A (137 mM NaCl, 2.7 mM KCl, 5.3 mM glucose, 12 mM $NaHCO_3$, 0.3 mM NaH_2CO_3, 0.1% gelatin; pH 7.4) from the peritoneal cavity of female Wistar rats weighing 240–300 g. Cell suspensions were centrifuged (194 × g, 4 °C, 5 min). The pellet was resuspended in buffer B (130 mM NaCl, 5 mM KCl, 1.4 mM $CaCl_2$, 1 mM $MgCl_2$, 5.6 mM glucose, 10 mM HEPES, 0.1% BSA). The cell suspension ($1–2 \times 10^5$ mast cells/ml (determined with neutral red staining) were plated out into a 96-well microplate (50 μl per well) and kept at 37 °C for 10 min. Solutions of test substances were added (10 μl, in DMSO-buffer, final DMSO conc. in assay 0.5%) and cells were incubated for 5 min. Solution of stimulus (compound 48/80, final conc. 0.2 μg/ml, 10 μl) was added. Degranulation was stopped after 5 min by addition of ice-cold buffer (50 μl). After centrifugation (670 × g, 4 °C, 5 min), 50 μl of the supernatant were transferred to a black 96-well plate. Solutions were alkalized (1 N NaOH, 10 μl), derivatized (5 μl of 1% o-phthalaldehyde in MeOH), and incubated (4 min in the dark) (Shore et al. 1959). Reaction was stopped by acidification (3 N HCl, 5 μl), and analyzed by fluorometry (360 nm/460 nm). Histamine release was calculated with untreated cells (basal histamine release) as 0% and compound 48/80 treated cells as 100%.

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