BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1994 Sep; 15 (5): 385—388

Inhibition of [*methyl*-^{3}H]diazepam binding to rat brain membranes *in vitro* by dinatin and skrofulein[1]

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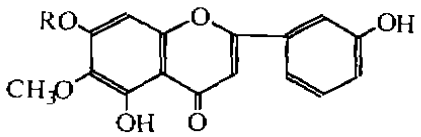
ABSTRACT Two flavones, 4', 5, 7-trihydroxy-6-methoxy flavone (dinatin) and 4', 5-dihydroxy-6, 7-dimethoxy flavone (skrofulein), were extracted from *Artemisia herba alba* L. Dinatin and skrofulein inhibited the binding of [*methyl*-^{3}H]diazepam to rat brain membranes *in vitro* with IC_{50} of 1.3 and 23 μmol·L^{-1}, respectively. The GABA-ratios (the ratio of IC_{50} values in the absence/presence of GABA in the binding assay) were 1.1 and 1.2 for dinatin and skrofulein, respectively. Both flavones induced a slight increase in [^{35}S]TBPS binding. The data suggest that the flavones are antagonists or partial agonists of benzodiazepine receptors.

KEY WORDS *Artemisia herba alba* L; flavones; dinatin; skrofulein; GABA-A receptors; brain; cell membrane

Benzodiazepine tranquilizers exert their pharmacological action by modulating the efficacy of the inhibitory neurotransmitter (GABA) at GABA/benzodiazepines-chloride channel complex in the brain. 1, 4-Benzodiazepines bind with high affinity to binding sites within the complex.

Our strategy for the discovery of benzodiazepine receptor ligands with a new chemical structure is to analyze medicinal plants for receptor active compounds using radio-receptor assays (RRA). The active compounds are subsequently isolated and identified. Here we describe the isolation of 4', 5, 7-trihydroxy-*L*-methoxy flavone (dinatin) and 4', 5-dihydroxy-6, 7-dimethoxy flavone (skrofulein) from *Artemisia herba alba* L. We have characterized the effects of the flavones on the benzodiazepine binding sites in the rat brain *in vitro*.



R=H Dinatin
(4',5,7-trihydroxy-6-methoxy flavone)
R=CH_3 Skrofulein
(4',5-dihydroxy-6,7-dimethoxy flavone)

MATERIALS AND METHODS

Materials *Artemisia herba alba* L was supplied by the Department of Pharmacology, Faculty of Pharmacy, Cairo University, Egypt. [*Methyl*-^{3}H]diazepam (3.15 TBq·mol^{-1}) and [^{35}S]TBPS (butyl bicyclophosphoro[*tertiary*-^{35}S]thionate, 3.41 TBq·mol^{-1}) were from New England Nuclear, Du Pont, USA. All HPLC solvents (HPLC grade) were from Rathburn Chemicals, Scotland.

Isolation of active compounds Batches of

Received 1993-09-23 Accepted 1994-06-27
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Artemisia herba alba L were extracted by cold percolation with ethanol, followed by petroleum ether and ethyl acetate. The ethylacetate fraction (20 mg) was redissolved in 80 % water — 20 % acetonitril (vol·vol) and chromatographed on a preparative Bondapak C-18 reverse-phase column (19 mm×150 mm), flow 4 ml·min^{-1}. Aliquots of 2-min fractions were tested for activity in RRA and active fractions pooled into fraction A and B with retention times 34—36 min and 40—41 min, respectively. For the second step of HPLC separation, fraction A and B were dissolved in 0.5 ml solvent (75 % water—25 % acetonitril) and applied to a C-18 ODS (OCTADECYLSILAN). reverse-phase column (3.9 mm×150 mm), flow 1 ml·min^{-1}. Two substances were collected showing absorption peaks (210 nm) with retention times at 10 min and 22 min for A and B, respectively. Fraction A and B were pooled from the second step of HPLC and applied to the same column using the same flow as the second HPLC step (70 % water — 30 % acetonitril, isocratic). The purified compounds A and B were eluted as single peaks at 5 min and 12 min retention time from the third HPLC step.

Brain membrane preparations Brain tissue from Wistar rats (weighing 200±*s* 20 g) was prepared for binding studies[1,2]. Cortex, cerebellum, and hippocampus were homogenized in Tris-citrate buffer (50 mmol·L^{-1}, pH 7.1) and centrifuged at 30 000×*g* for 10 min. The pellet was washed twice and the final pellet was resuspended in Tris-citrate buffer at the concentration of 2 mg original tissue·ml^{-1}. One ml aliquots of membranes were used for [*methyl*-^{3}H]diazepam (0.8 nmol·L^{-1}) binding. Nonspecific binding was obtained in the presence of midszolam (10 μmol·L^{-1}). [^{35}S]TBPS binding to three times-washed membrane preparations was done according to Nielsen *et al*[1].

The concentration of the compounds causing 50 % inhibition (IC_{50}) of [*methyl*-^{3}H]diazepam specific binding was determined with a series of concentrations[2,3], and IC_{50} values were expressed with 95 % confidence limits.

RESULTS AND DISCUSSION

Compounds A and B were identified by proton nuclear magnetic resonance (NMR) spectroscopy (compound A in $CDCl_3$, and compound B in CD_3OD, in both cases at 300 MHz with tetramethylsilane as internal standard), and by mass spectrometry (electronimpact ionization at 70 eV). When comparing our data with published data of corresponding flavones, we conclude that compounds A and B are 4', 5, 7-trihydroxy-6-methoxy flavone (dinatin) and 4', 5-dihydroxy-6, 7-dimethoxy flavone (skrofulein), respectively.

Displacement of [*methyl*-^{3}H] diazepam binding to membranes from cortex, cerebellum and hippocampus showed no differences in IC_{50} values and Hill coefficients between the brain regions (Tab 1).

The GABA-ratio is defined as the ratio of IC_{50} values in the abscence and presence of GABA (10 μmol·L^{-1}) in the binding assay. The IC_{50} values were estimated to be 1.3 (0.9 — 1.7) μmol·L^{-1} (without GABA) and 1.2 (0.9 — 1.5) μmol·L^{-1} (with GABA) for dinatin and 28.0 (24.0—32.0) and 23.1 (15.1 — 32.8) μmol·L^{-1} for skrofulein (n=3), respectively. Dinatin and skrofulein showed GABA-ratios of 1.12 and 1.21, respectively. Both dinatin and skrofulein inhibited the binding of [*methyl*-^{3}H]diazepam to cortical

Tab 1. Inhibition of dinatin and skrofulein on [^{3}H]diazepam binding in rat brain *in vitro*. n=3 rats.

	Dinatin		Skrofulein	
	IC_{50} (μmol·L^{-1})	Hill coefficient	IC_{50} (μmol·L^{-1})	Hill coefficient
Cortex	1.3 (0.9 — 1.7)	0.97 (0.95 — 0.99)	28.0 (24.0 — 32.0)	0.96 (0.87 — 1.15)
Cerebellum	1.4 (0.8 — 2.0)	0.96 (0.92 — 1.00)	27.9 (25.1 — 31.2)	0.86 (0.85 — 0.87)
Hippocampus	2.1 (0.9 — 3.3)	0.96 (0.92 — 1.00)	24.6 (22.9 — 27.4)	0.95 (0.93 — 0.97)

membranes *in vitro* with IC_{50} values of 1.3 and 22.7 μmol · L^{-1}, respectively[3] (Fig 1). Scatchard plot analysis showed a mixed competitive and noncompetitive inhibition by both compounds of [*methyl*-^{3}H]diazepam binding to cortical, cerebellar or hippocampal membranes[3].

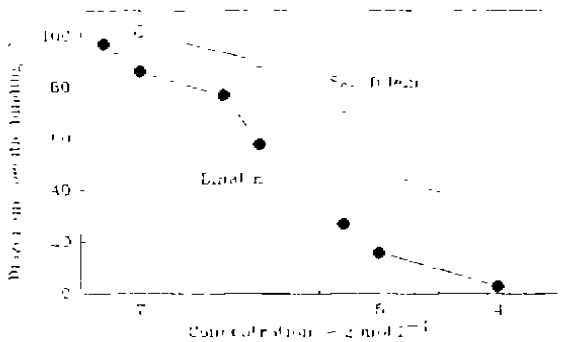

Fig 1. Inhibition of [*methyl*-^{3}H]diazepam (0.8 nmol · L^{-1}) specific binding to rat cerebellar membranes by dinatin and skrofulein.

Both compounds induced a slight increase in the on-rate of [^{35}S]TBPS binding to membranes from cortex (Fig 2).

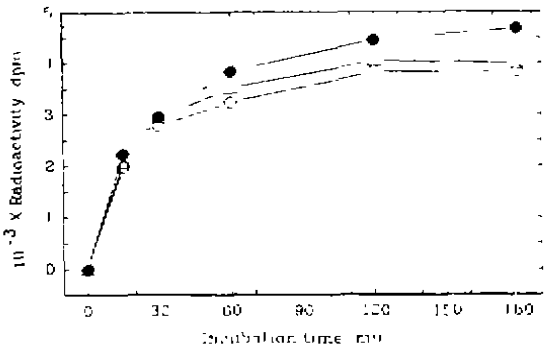

Fig 2. Specific binding of [^{35}S]TBPS (0.9 nmol · L^{-1}, NaCl 1 mol · L^{-1}, 25 ℃) to rat cortex membranes: binding (○); dinatin 100 μmol · L^{-1} (●) and skrofulein 10 μmol · L^{-1} (△).

The flavones dinatin and skrofulein were the first compounds isolated from *Artemisia herba alba* L showing affinity to brain benzodiazepine receptor *in vitro*. The flavones did not differentiate between BZ_1 and BZ_2 subtypes of benzodiazepine receptors, since both flavones showed similar IC_{50} values on inhibition of [*methyl*-^{3}H]diazepam binding to cerebellar and hippocampal membranes. A GABA-ratio slightly higher in one than the other suggested that dinatin and skrofulein were antagonists or weak partial agonists at the benzodiazepine receptors. This was further substantiated by the slight increase in on-rate of [^{35}S]TBPS specific binding by the two compounds (Fig 2). Benzodiazepine receptor ligands modulated the on-rate of [^{35}S]TBPS binding according to their pharmacological profile: benzodiazepine receptor agonists increased the on-rate; antagonists had on effect and inversed agonists decreased the on-rate of [^{35}S]TBPS binding[2,4].

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毛地黄黄酮和玄参黄酮抑制[^{3}H]地西泮和体外大鼠脑膜的结合

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94,15(5)
385-388
R965.2

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摘要 从阿拉伯艾蒿提取到两种苯并二氮杂䓬受体的配基，毛地黄黄酮和玄参黄酮. 两种化合物在体外可抑制[^{3}H]地西泮和大鼠皮层细胞膜的结合，IC_{50}值分别为1.3 μmol·L^{-1}和23 μmol·L^{-1}. 两种化合物GABA比分别为1.1和1.2，都可少量增加[^{35}S]TBPS的结合，提示这种化合物是苯并二氮杂䓬受体的拮抗剂或部分激动剂.

关键词 阿拉伯艾蒿；黄酮类；毛地黄黄酮；玄参黄酮；γ-氨基丁酸-A受体；脑；细胞膜

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1994 Sep; 15 (5): 388—391

Effects of 3'-angeloyloxy-4'-acetoxy-3', 4'-dihydroseselin on myocardial dysfunction after a brief ischemia in anesthetized dogs

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ABSTRACT The effect of a 30-min infusion of 3'-angeloyloxy-4'-acetoxy-3', 4'-dihydroseselin (Pd-Ia), a coumarin isolated from *Peucedanum praeruptorum* Dunn 0.15 mg·kg^{-1}·min^{-1} on regional myocardial dysfunction was examined in 16 anesthetized open-chest dogs subjected to a 15-min occlusion of the left anterior descending coronary artery followed by a 3-h reperfusion. Segment lengths of left ventricular wall were measured with an ultrasonic micrometer. The control caused a decrease in the % of segment shortening (SS %) throughout the reperfusion period (n=8), while Pd-Ia ameliorated segment function immediately after reperfusion and restored the SS % to 41±51 % of the baseline value (n=8, P<0.05 *vs* control) 5 min after reperfusion without any significant changes in cardiohemodynamics. The improvement of myocardial function induced by Pd-Ia was maintained at least 3 h after reperfusion. These findings revealed that Pd-Ia had a cardioprotective action in stunned myocardium.

KEY WORDS coumarins; myocardial reperfusion injury; hemodynamics; ultrasonography

Our previous studies showed that the crude extract of the root of a Chinese traditional herb bai-hua qian-hu (*Peucedanum praeruptorum* Dunn, BQ) increased the coronary blood flow in isolated rabbit hearts and anesthetized cats[1]. The racemate of 3'-angeloyloxy-4'-acetoxy-3', 4'-dihydroseselin[2]

Received 1993-12-06 Accepted 1994-06-01
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