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Butyrylcholinesterase, lipoxygenase inhibiting and antifungal alkaloids from isatis tinctoria

IJAZ AHMAD[1] & ITRAT FATIMA[2]

[1]*Medicinal Botanic Centre, PCSIR Laboratories Complex Peshawar, N.W.F.P, Pakistan, and* [2]*International Center for Chemical Sciences, HEJ Research Institute of Chemistry, University of Karachi, Karachi -75270, Pakistan*

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Abstract

Phytochemical investigations on the alkloidal fraction of the whole plant of the *Isatis tinctoria* led to the isolation of the alkaloids **1–6**. Compounds **3**, **2** were found to be potent butyrylcholinesterase and lipoxygenase enzymes inhibitors in a concentration-dependent manner with the IC_{50} values 16.3 ± 0.06 and $19.7 \pm 0.03\,\mu M$ against BChE and 30.6 ± 0.02 and $33.7 \pm 0.05\,\mu M$ against LOX, respectively. The compounds (**1–6**) showed significant antifungal activity against *Trichophyton schoen leinii*, *Aspergillus niger, Candida albicans, Trichophyton simii*, and *Macrophomina phaseolina*.

Keywords: *Isatis tinctoria, brassicaseae, alkaloids, butyrylcholinesterase lipoxygenase inhibition, antifungal*

Introduction

The genus *Isatis*, belonging to the family Brassicaseae, comprises 50 species mainly distributed in Irano-Turanian region. In Pakistan it is represented by seven species [1]. *Isatis tinctoria* is used in Chinese folk and modern medicine [2]. "Ban-Lan-Gen" is one of the most commonly used traditional Chinese medicines for antipyretic, anti-inflammatory, antiviral and detoxifying purposes. Its original source was considered to be the dried roots of three plants, *Isatis indigotica*, *Isatis tinctoria* and *Strobilanthes cusia* [3,4]. In a recent nationwide investigation, the roots of *Isatis indigotica* have been identified as the main source of "Ban-Lan-Gen" and recorded in Chinese Pharmacopoeia (1990 edn) [5]. The ethano pharmacological importance of the genus *Isatis* prompted us to investigate the chemical constituents of *Isatis tinctoria*, which is an annual or biennial herb, found in northern part of Pakistan. Our previous work on *Isatis costata* has resulted oxindole alkaloids [6,7].

An ethanolic extract of *Isatis tinctoria* resulted showed significant antifungal and inhibitory activity against the butyrylcholinesterase and lipoxygenase enzymes which prompted us to conduct phytochemical studies on this plant. As a result six alkaloids, 2-[cyano(3-indolyl)methylene]-3-indolone (**1**) [8], epiglucoisatisin (**2**) [9], 3′-hydroxyepiglucoisatisin (**3**) [9], sulfoglucobrassicin (**4**) [10], isatan A (**5**) [11] and isatan B (**6**) [11] were isolated (Figure 1)

Cholinesterases are implicated as key biological players in Alzheimer's disease (AD), which makes them logical targets for inhibitory therapeutics. It has been found that butyrylcholinesterase (BChE, horse-serum EC 3.1.1.8) inhibition may help in the treatment of Alzheimer's disease (AD) and related dementias [12]. Thus the search for new cholinesterase inhibitors appears to be a promising approach to develop potential drugs for the treatment of AD.

Lipoxygenase (LOX, EC 1.13.11.12) are key enzymes in the biosynthesis of variety of bioregulatory compounds such as hydroxyeicosatetraenoic acids (HETEs), leukotrienes, lipoxins and hepoxylines [13]. It has been found that these LOX products play a role in a variety of disorders such as bronchial asthma, inflammation [14] and tumor angiogenesis [15].

Correspondence: Dr. I. Ahmad, Medicinal Botanic Centre, PCSIR Laboratories Complex Peshawar, N.W.F.P, Pakistan. E-mail: drijaz_chem@yahoo.com

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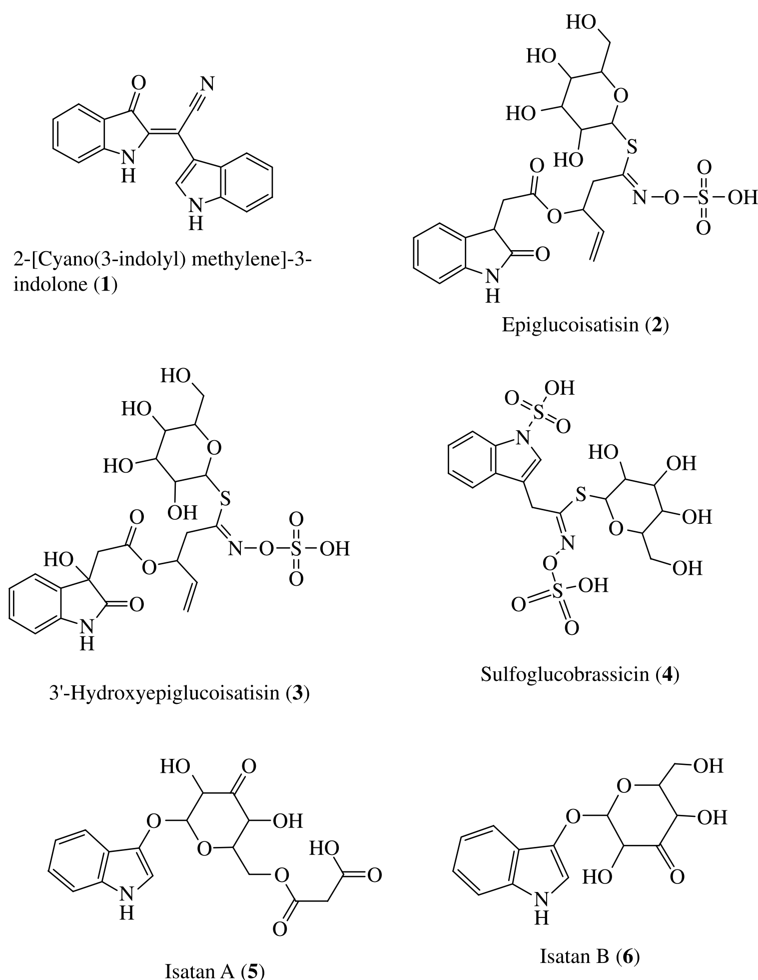

Figure 1. Structures of compounds 1–6.

LOXs are therefore potential target for the rational drug design and discovery based on the inhibition mechanism of inhibitors for the treatment of bronchial asthma, inflammation, cancer and autoimmune diseases.

In the current study we have described the butyrylcholinesterase & lipoxygenase inhibitory and antifungal activities of the alkaloids (**1–6**) which were isolated from *Isatis tinctoria* and although the structures of the compounds were published previously not their BChE/LOX and antifungal activities. All of these compounds (**1–6**) were found inactive against acetylcholinesterase (AChE, Electric-eel EC 3.1.1.7).

Materials and methods

In vitro cholinesterase inhibition assay

Butyrylcholinesterase (BChE; horse-serum E.C 3.1.1.8), butyrylthiocholine chloride, 5,5'-dithiobis [2-nitrobenzoic acid] (DTNB), and galanthamine were purchased from Sigma (St. Louis, MO, USA). Buffer and other chemicals were of analytical grade. BChE activity-inhibiting activities were measured by a slightly modified spectrophotometric method developed by Ellman *et al.* [16]. Butyrylthiocholine chloride were used as substrates to assay BChE activity. DTNB was used for the measurement of cholinesterase activity. Sodium phosphate buffer (pH 8.0; 140 μL of 100 mM soln.), DTNB (10 μL), test-compound soln. (20 μL), and BChE soln. (20 μL) were mixed and incubated for 15 min at 25°C. The reaction was initiated by addition of butyrylthiocholine (10 μL). The hydrolysis of butyrylthiocholine was monitored by the formation of the yellow 2-nitro-5-sulfidobenzenecarboxylate anion, as the result of the reaction of DTNB with released, thiocholine at a wavelength of 412 nm (15 min). Test compounds and the control were dissolved in EtOH. All the inhibition studies were performed in 96-well micro-titer-plates using *SpectraMax 340* (Molecular Devices, CA, USA).

According to Ellman *et al.* [16] since the extinction coefficient of the yellow anion is known, the rate of the enzymatic reaction was determined by the following equation:

$$\text{Rate (mols/L/min)} = \frac{\text{Change in absorbanc/ min}}{13,600}$$

Table I. *In vitro* quantitative inhibition of butyrylcholinesterase and lipoxygenase enzymes by compounds **1–6**.

Serial No.	Name of Compound	BChE $IC_{50} \pm SEM^{a}[\mu M]$	LOX $IC_{50} \pm SEM^{a}[\mu M]$
1	2-[Cyano(3-indolyl)methylene]-3-indolone	23.5 ± 0.02	39.1 ± 0.03
2	Epiglucoisatisin	19.7 ± 0.03	33.7 ± 0.05
3	3′-Hydroxyepiglucoisatisin	16.3 ± 0.06	30.6 ± 0.02
4	Sulfoglucobrassicin	24.8 ± 0.05	41.9 ± 0.01
5	Isatan A	43.4 ± 0.02	49.3 ± 0.04
6	Isatan B	47.6 ± 0.04	53.8 ± 0.06
7	Galanthamine[b]	8.5 ± 0.01	ND
8	Baicalein[c]	ND	22.0 ± 0.05

[a] Standard mean error of five determinations; [b] positive control used in BChE inhibiting assay; [c] positive control used in LOX assays. ND = not done.

In vitro lipoxygenase inhibition assay

LOX inhibiting activity was measured by modifying the spectrophotometric method developed by Tappel [17]. LOX (1.13.11.12, type I-B, Soybean) and linoleic acid was purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade. Sodium phosphate (160 μL 100 mM) buffer (pH 8.0), 10 μL of test compound solution and 20 μL of lipoxygenase solution were mixed and incubated for 10 min at 25°C. The reaction was then initiated by the addition of 10 μL linoleic acid (substrate) solution, with the formation of (9*Z*, 11*E*)-(13*S*)-13-hydroperoxyoctadeca-9,11-dienoate, the change of absorbance at 234 nm was followed for 10 min. Test compounds and the control were dissolved in MeOH. All the kinetic experiments were performed in 96-well microtitre plates by using *SpectraMax 384plus* (Molecular Devices, USA).

Determination of IC_{50} values

The concentrations of the test compounds that inhibited the hydrolysis of substrates (butyrylthiocholine and linoleic acid) by 50% (IC_{50}) were determined by monitoring the effect of various concentrations of these compounds in the activity assays. The IC_{50} values were then calculated using the EZ-Fit Enzyme Kinetics program (*Perrella Scientific Inc., Amherst*, USA).

Bioassays

The antifungal bioassay was performed on human, animal and plant pathogens. The crude extract, compounds **1–6** and the standard drugs (each at a concentration of 400 μg/mL of Sabourd Dextose Agar) were subjected to antifungal activity assays against *Trichophyton schoen leinii* ATCC 22775, *Aspergillus niger* ATCC 1015, *Pseudallescheria boydri* ATCC 44330, *Candida albicans* ATCC 10231, *Microsporum canis* ATCC 36299, *Trichophyton mentagrophytes* ATCC 28185, *Trichophyton simii* ATCC 25923, *Fusarium solan* ATCC 36031, *Macrophomina phaseolina* ATCC 53789, *Rhizoctonia solani* ATCC 76131, according to the established protocol [18].

Table II. *In vitro* fungicidal bioassay of crude extract and alkaloids 1–6*.

		Inhibition (%)							
Name of fungus	Inhibition (%) by of crude extract	1	2	3	4	5	6	Standard drugs	Inhibition (%) of Standard drugs
Trichophyton schoen leinii	65.9	60.0	69.7	71.5	61.2	50.2	46.9	Miconazole	90
								Ketoconazole	90
Aspergillus niger	52.3	59.7	68.8	75.0	59.1	47.7	40.0	Amphotericin-B	100
Pseudallescheria boydri	31.6	53.9	62.5	60.2	54.5	40.5	37.7	Miconazole	90
								Ketoconazole	90
Candida albicans	50.4	69.2	77.3	79.1	70.5	56.3	49.1	Nystatin	90
Microsporum canis	29.9	19.1	27.9	29.5	20.5	14.0	11.4	Miconazole	100
								Ketoconazole	100
Trichophyton mentagrophytes	50.7	50.0	61.7	60.2	50.9	30.1	21.0	Miconazole	100
								Ketoconazole	100
Trichophyton simii	73.1	71.0	85.5	87.2	70.4	50.1	44.8	Miconazole	100
Fusarium solani var. lycopersici (tomato)	10	10.0	15.3	17.0	8.9	3.7	2.9	Benlate	100
Macrophomina phaseolina	61.0	59.2	79.5	81.3	56.7	40.6	30.9	Benlate	100
								Nabam	
Rhizoctonia solani	57.8	48.6	57.3	59.1	54.7	34.2	27.9	Benlate	100

*400 μg/mL.

Results and discussion

The ethanolic extract of *Isatis costata* was partitioned between EtOAc and water. Alkaloids liberated from the aqueous fraction with 10% NH_4OH were extracted out with CH_2Cl_2.Column chromatography of CH_2Cl_2 fraction provided the alkaloids (**1–6**).

Butyrylcholinesterase inhibitory activities of alkaloids 1–6

Compounds **3** and **2** showed potent inhibitory potential against BChE with IC_{50} values (16.3 ± 0.06) and (19.7 ± 0.03) μM, respectively. While compounds **1**, **4**, **5** and **6** displayed significant inhibitory activity against BChE (Table I), whereas the standard inhibitor of BChE (galanthamine) have IC_{50} value of (8.5 ± 0.1) μM.

Lipoxygenase inhibitory activities of alkaloids 1–6

3′-Hydroxyepiglucoisatisin (**3**) and Epiglucoisatisin (**2**), showed promising inhibitory activity against LOX (IC_{50} values 30.6 ± 0.02 and 33.7 ± 0.05 μM) compared to baicalein used as positive control (IC_{50} value 22.0 ± 0.5 μM). On the other hand, 2-[cyano(3-indolyl)methylene]-3-indolone (**1**), sulfoglucobrassicin (**4**), isatan A (**5**) and isatan B (**6**) displayed moderate inhibitory potential against LOX (Table I).

Antifungal activity

The antifungal activities of compounds **1–6** were determined by the agar tube dilution method and significant activity was observed against *Trichophyton schoen leinii*, *Aspergillus niger, Candida albicans, Trichophyton simii*, *Macrophomina phaseolina*; moderate activity against *Pseudallescheria boydri, Trichophyton mentagrophytes*, *Rhizoctonia solani*, and weak activity against *Microsporum canis* and *Fusarium solani* (Table II).

Conclusion

In conclusion, our search for BChE, LOX inhibitory and antifungal constituents from *Isatis tinctoria* has resulted in the isolation of alkaloids **1–6**, as potential agents in the treatment inflammation, asthama, aging, tumor, angiogenesis, cancer and Alzheimer's disease. However, further *in vivo* study would help in exploring the pharmacological properties of these compounds.

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