

Superoxide Respiratory Burst Inhibitory Activity of *Bis*-Schiff Bases of Isatins

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Summary: *Bis*-Schiff bases **1-27** were synthesized and evaluated for their anti-inflammatory activity and possible cytotoxicity. Compounds **1-27** showed a varying degree of respiratory burst inhibitory activity with IC₅₀ values between 242.97 - 652.12 μM. Compound **4** (IC₅₀ = 242.97 ± 2.24 μM) showed an excellent activity, when compared with standard indomethacine (IC₅₀ = 271.12 ± 1.12 μM). Compounds **9, 16, 18, 19, 24,** and **26** showed varying degree of activity, while remaining compounds were found to be inactive.

Keywords: Isatins, *Bis*-Schiff bases, respiratory burst inhibition, anti-inflammatory activity, immunomodulating activity, cytotoxicity

Introduction

Bis-Schiff bases possess diverse biological properties, including anticancer [1], antimicrobial [2], and anti-herbicide activities [2-4]. A variety of heterocyclic Schiff bases were reported to possess cytotoxic [5], antiepileptic [6], anti-proliferative [7], and anticancer properties [8]. A variety of Schiff bases [9-12] have also been reported for antimicrobial [13-17], and anticancer activities [18, 19]. Diversified bioactivities are related to Schiff bases of isatin, like analgesic [20], anticonvulsant [21], antidepressant [22], anti-inflammatory [23], antimicrobial, CNS effects [24], and potentiating pentobarbitone induce narcosis [25].

Present research work describes the *in vitro* anti-inflammatory activity through ROS inhibitory potential of compounds **1-27** in continuation of our studies on biologically active heterocycles [26].

Inflammation is a complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants [27]. Reactive oxygen species (ROS) are formed subsequent to the assembly and activation of the phagocyte-specific enzyme, NADPH oxidase. This process is initiated by the production of superoxide anion O₂⁻ during a 'respiratory burst' of non-mitochondrial oxygen uptake by an NADPH oxidase system, play a key role in immune response and inflammatory cascade. Inhibition of large quantities release of highly toxic ROS is pathological

conditions is an approach to treat chronic inflammation and to modulate immune response in immune compromised patients.

Most of the inflammatory disorders are treated with NSAIDs, *e.g.* aspirin. However, the main side effect of aspirin is gastric ulceration. There has been continuing interest in the discovery of novel anti-inflammatory agents.

In the current study, we used the water-soluble tetrazolium salt (WST-1) to measure the superoxide production by neutrophils, activated by opsonized zymosan which induces phagocytic activation. This technique is more sensitive and reliable than other available techniques to measure the superoxide scavenging properties as an indirect evaluation of anti-inflammatory potential [28].

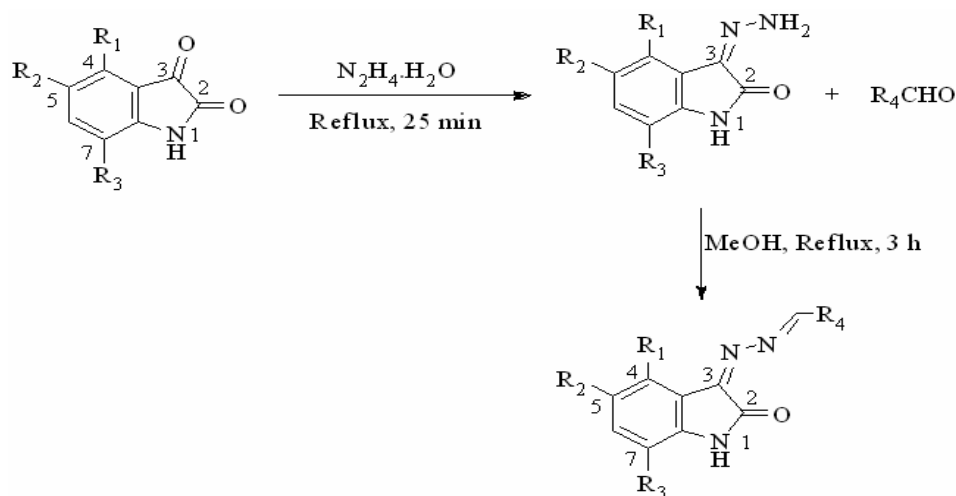
As series of *bis*-Schiff bases **1-27** have been evaluated by using above-mentioned assay.

Results and Discussion

Chemistry

Bis-Schiff bases **1-27** were synthesized in high yield from isatins by reacting with hydrazine hydrate and the Schiff bases so obtained were reacted with corresponding aromatic aldehydes (Scheme-1).

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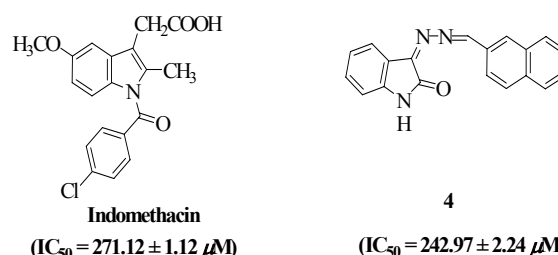
Scheme-1: Synthesis of *bis*-Schiff bases from isatins.

Generally, isatins were dissolved in hydrazine hydrate and refluxed. The reaction completed in 25 min to afford Schiff bases. Schiff bases so obtained were refluxed with corresponding aromatic aldehydes in equimolar amount in methanol for 3 hours. The completion of reaction was judged by TLC. It was cooled and filtered; the solid so obtained was washed with methanol and dried in a vacuum desiccator to give *bis*-Schiff bases **1-27** in high yields. The solid *bis*-Schiff bases were recrystallized from methanol to afford pure crystalline compounds with sharp melting points. The structures of synthetic compounds **1-27** were elucidated by using different spectroscopic techniques, like ^1H NMR, EI, IR, and UV spectroscopy. All the compounds gave satisfactory elemental analysis.

Anti-inflammatory Activity

Previously we have reported antiglycating and antioxidant activities of *bis*-Schiff bases of isatins [26]. Due to structure similarity of indomethacin, a commercially available NSAID, and *bis*-Schiff bases of isatins **1-27**, we decided to evaluate the anti-inflammatory activity of these compounds. *Bis*-Schiff bases of isatins **1-27** were re-synthesized and screened for anti-inflammatory activity through measuring their effect on superoxide production according to literature protocol [28]. Compounds **4**, **9**, **16**, **18**, **19**, **24** and **26** showed varying degree of anti-inflammatory activities, when compared with the standard indomethacin ($\text{IC}_{50} = 271.12 \pm 1.12 \mu\text{M}$) (Table-1). Compound **4** ($\text{IC}_{50} = 242.97 \pm 2.24 \mu\text{M}$) showed better activity than the standard indomethacin while compounds **9** and **19**

demonstrated anti-inflammatory potential with IC_{50} values of 470.11 ± 2.42 and $429.97 \pm 3.42 \mu\text{M}$, however, compounds **18**, **24** and **26** showed IC_{50} values 492.71 ± 6.12 , 495.12 ± 2.02 , and $519.12 \pm 1.93 \mu\text{M}$, respectively. All other compounds showed less than 50% inhibitions were not screened for their IC_{50} values and considered to be as inactive.



A comparison of the activity of compound **4** and indomethacin, compound **4** appear more active than indomethacin. It also indicates that the indole skeleton is prerequisite for anti-inflammatory activity, along with substitutions at specific position of the indole moiety. Comparison of the structure and activity of various members of the series does not provide any clear understanding of the structural feature responsible of activity. However, complete inactivity of compound **3** which has similar structure like compounds **4** and **9** except two chloro substituents at C-4 and C-7 of indole moiety seems to be due to Cl substituent. Two fold differences in activities of compounds **4** and **9** are apparently due to different naphthyl linkages. In general nature of R_4 seems to have a greater influence on the activity as compared to other substituents. For better understanding of SAR, larger libraries of *bis*-Schiff base derivatives will be required.

Table-1: *In vitro* anti-inflammatory activity of compounds 1-27.

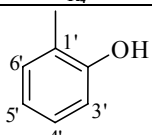
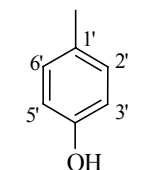
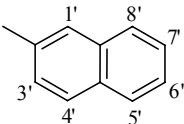
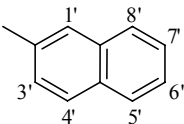
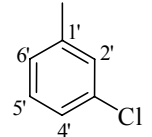
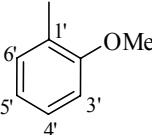
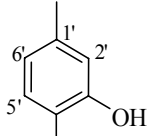
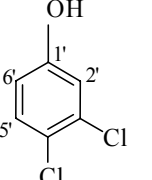
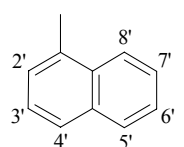
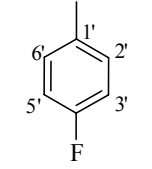
Compounds	R ₁	R ₂	R ₃	R ₄	IC ₅₀ ± ^a SEM (μM)
1	H	H	H		^c N. A.
2	H	H	H		^c N. A.
3	Cl	H	Cl		^c N. A.
4	H	H	H		242.97 ± 2.24
5	H	H	H		^c N. A.
6	H	H	H		^c N. A.
7	H	H	H		^c N. A.
8	H	H	H		^c N. A.
9	H	H	H		470.11 ± 2.42
10	H	H	H		^c N. A.

Table-1: Continued...

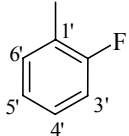
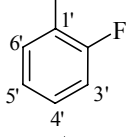
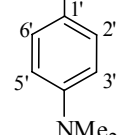
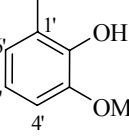
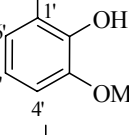
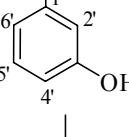
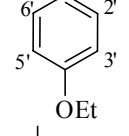
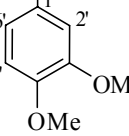
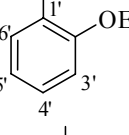
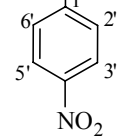
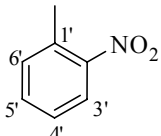
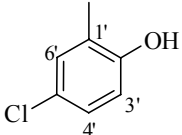
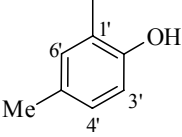
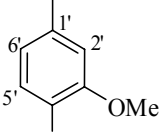
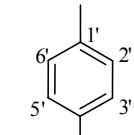
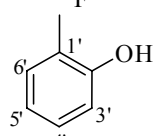
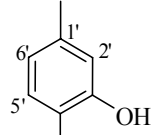
11	H	H	H		^a N. A.
12	Cl	H	Cl		^a N. A.
13	H	H	H		^a N. A.
14	H	H	H		^a N. A.
15	Cl	H	Cl		^a N. A.
16	H	H	H		652.12 ± 2.19
17	H	H	H		^a N. A.
18	Cl	H	Cl		492.71 ± 6.12
19	H	H	H		429.97 ± 3.42
20	H	H	H		^a N. A.

Table-2: Continued...

21	H	H	H		^c N. A.
22	H	H	H		^c N. A.
23	H	H	H		^c N. A.
24	H	Cl	H		495.12 ± 2.02
25	H	Cl	H		^c N. A.
26	H	Cl	H		519.12 ± 1.93
27	H	Cl	H		^c N. A.
^a Indomethacin					271.12 ± 1.12

^aSEM is the standard error of the mean, ^bIndomethacin standard for anti-inflammatory activity, ^cN. A. Not Active

Preliminary, it is found that compound **4** can serve as lead for the development of more effective anti-inflammatory agents.

Cytotoxic Bioassay

Brine shrimp lethality assay (*Artemia salina*) has indicative value for the evaluation of toxicity. During this experiment, etoposide (LD₅₀ = 0.178 µg/ml) was used as standard drug, while derivatives **1-27** showed no cytotoxicity against *Artemia salina*.

Material

During the biological testing, absorbance was measured on a SpectraMax 340 microplate

reader (Molecular Devices, CA, USA). WST-1 (Dojindo Laboratories, Kumamoto, Japan), zymosan A (Sigma Chemicals, St Louis, MO, USA) was used.

Experimental

Isolation of Human Neutrophils

Heparinized fresh venous blood was drawn from healthy volunteers and neutrophils were isolated by the method of Siddiqui *et al* [29].

Respiratory burst assay

Anti-inflammatory activity of the test compounds was determined by using a modified assay [29]. This *in vitro* assay was based on the

reduction of highly water-soluble tetrazolium salt (WST-1) in the presence of activated neutrophils. Respiratory burst inhibitory activity was determined in a total volume of 200 μ L MHS (pH 7.4) containing 1×10^6 neutrophils/mL, 250 μ M WST-1 and various concentrations of test compounds. The control contained buffer, neutrophils and WST-1. All compounds were equilibrated at 37 °C and the reaction was initiated by adding opsonized zymosan A (15 mg/mL), which was prepared by mixing with human blood serum, followed by centrifugation at 3000 rpm and the pellet was re-suspended in PBS buffer. Absorbance was measured at 450 nm. Indomethacin was used as positive control which is widely used as non-steroidal anti-inflammatory drug (NSAID). Values of IC₅₀ were calculated by comparison with the DMSO as the blank and expressed as the percent inhibition of superoxide anions (ROS) produced. The percent respiratory inhibitory activity by the samples was determined against a DMSO blank and calculated using the following formula:

$$\% \text{ Inhibition} = 100 \left\{ \frac{\text{OD test compound}}{\text{OD control}} \times 100 \right\}$$

IC₅₀ values were determined by using EZ-FIT Windows-based software.

Conclusion

In conclusion compound **4** may serve as lead compound and its more closely related synthetically modified molecule may emerged as more effective anti-inflammatory agents.

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