PRELIMINARY SCREENING OF BIOLOGICAL ACTIVITIES OF SOME NEW SCHIFF BASES OF ISATINS

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Abstract

Schiff bases of isatin/substituted isatins were prepared by reacting with 4-aminoantipyrine. The chemical structures were confirmed by [1]HMR, IR spectral data and elemental analysis. The synthesized compounds were tested *in vitro* against a number of microorganisms in order to assess their antimicrobial and antifungal activity. The synthesized compounds were screened for their antioxidant activity, ascorbic acid being used as standard. All the investigated compounds were moderately active against Gram positive bacteria and they have a significant antioxidant activity.

Rezumat

Prin condensarea isatinei şi a unor derivaţi substituiţi ai isatinei cu 4-amino-antipirina s-au obţinut o serie de compuşi a căror structură a fost confirmată de spectrele H-RMN, IR şi datele analizei elementale cantitative. Derivaţii sintetizaţi au fost investigaţi în ceea ce priveşte acţiunea antioxidantă şi activitatea antimicrobiană, rezultatele evidenţiind o acţiune moderată faţă de bacterii Gram positive şi o semnificativă acţiune antioxidantă.

Keywords: Schiff bases, isatin, biological activity

Introduction

Isatin, known as 1H-indole-2,3-dione, and its derivatives are important molecules due their antibacterial, antifungal, antiviral, and anti-inflammatory activities [14]. In addition, isatins are synthetically versatile substrates, they are useful for the synthesis of a large variety of heterocyclic compounds. Similarly, 4-aminoantipyrine itself has a antipyretic, analgesic and antiinflamatory action but because of its toxicity, it has not come into extensive use. Schiff bases are characterized by the –N=CH- (imine group) which is important for their biological activities: antimicrobial, antifungal, antitumor and herbicidal [3,7].

In view of biological importance of these two moieties, it was planned to synthesize a new series of isatin containing 4-aminoantipyrine ring and to evaluate them for their antimicrobial and antioxidant activity.

Materials and Methods

Elemental analysis was performed on an Elemental Exeter Analytical CE 440 Analyzer. The IR spectra were recorded on a FTIR Shimadzu Prestige 8400s spectrophotometer. 1H-NMR spectra were recorded on a Bruker Avance DRX- 400 spectrometer at 400 MHz in dimethyl sulfoxide (DMSO) – d_6 using tetramethylsilane (TMS) as an internal reference. Melting points were determined using an electro thermal Melting Point apparatus and were uncorrected. The purity of the new synthesized derivatives was checked by *thin-layer chromatography (TLC) and was* carried out on precoated Silica Gel 60F254 plates using a *dichloromethane - methanol 9:1 system.*

Reversed phase *thin-layer chromatography* was performed on silica gel plates impregnated with 5% (v/v) liquid paraffin in light petroleum ether. Reversed-phase thin-layer chromatographic parameters, R_M values were determined from corresponding retention factors, R_f values using the equation $R_M = \log [(1/R_f) -1]$ [5,11].

Procedure for the Preparation of Isatin, 5-methyl-isatin and 7-methyl-isatin. Sulphuric acid was warmed in a flask and powdered appropriate isonitrosoacetanilide was added at such a rate so as to maintain the temperature between 60-70 ^{0}C. After the addition of isonitroso compound was completed, the temperature was raised to 80 ^{0}C and maintained to this value for 10 minutes. Then, the mixture was cooled and poured on crushed ice. After 30 minutes, the solid was filtered, dried and purified.

1-methyl-isatin was prepared using the method proposed by Bhardwaj [4].

General Procedure for the Preparation of the Schiff bases. 0.01 mols isatin /1-, 5- or 7-substituted isatins and 0.01 mols 4-aminoantipyrine were dissolved in ethanol containing a few drops of acetic acid and heated on a steam bath for 2 hours. After standing at room temperature, the crystalline products were separated by filtration, dried and recrystallised from ethanol: water [15].

DPPH (2,2-diphenyl-1-picrylhydrazyl**)** radical scavenging activity [2, 6, 16]. Solutions of tested substances 2mM were mixed with a methanolic solution of DPPH 0.1 mM. The mixture was shaken and the

absorbance was measured at 517 nm after one hour. Ascorbic acid was used as standard.

Scavenging effect (%) = (Absorbance of control-Absorbance of test)/Absorbance of control) X100

Reducing power. The reducing power was determined according to the method of Oyaizu [9]. Solutions of investigated substances prepared in DMSO were mixed with 2.5 mL phosphate buffer (pH=6.6) and potassium ferricyanide 1% (2.5 mL). The mixture was incubated at 50 ^{0}C for 20 minutes and 2.5 mL trichloroacetic acid was added. 2.5 mL from this was mixed with water (5 mL) and ferric chloride 0.1% (1 mL). The absorbance was measured at 700 nm after 15 min.

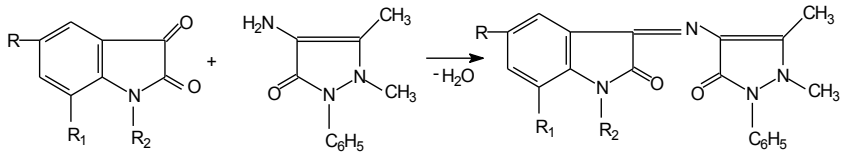
The pharmacokinetic parameters were performed using Molinsipration WebMe Editor 3.0 and ChemDraw Ultra 8.

Test for Antibacterial and Antifungal Activity [8]. The disk diffusion test was performed using Mueller Hinton (Oxoid) for bacteria and Sabouraud agar for fungal. Microorganisms test: *Staphylococcus aureus* ATCC 25923, *Sarcina lutea* ATCC 9341, *Bacillus cereus* ATCC 14579, *Bacillus subtilis*, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa*, *Candida albicans* ATCC 10231. The bacterial and fungal strains were incubated over night at 30 ^{0}C and from each microbial culture was prepared a suspension with the same density as the 0.5 standard from the Mac Farland turbidimetric scale. The suspensions of microorganisms were incorporated in Muller-Hinton medium, melted and cooled afterwards to 50 ^{0}C in a 1/10 ratio. After homogenization, we placed 25 mL of this mixture in the Petri plates with a diameter of 9 cm. On the surface of each plate, we put (after solidification) paper circles impregnated with 10 μL from the DMSO solutions (100 mg/mL) of the tested compounds.

The inhibition level (%) = 100x (diameter of the inhibition zone / 90)

Results and Discussion

The synthetic pathway followed for the preparation of the new compounds is shown in Figure 1.



1a-1f

Figure 1

General procedure for the synthesis of Schiff bases **1a-1f**

5-Chloro-isatin, 5-nitro-isatin were purchased from Sigma Aldrich and 4-amino-antipyrin was purchased from Fluka.

The others isatin and its substituted derivatives were obtained by cyclization of the appropriate isonitrosoacetanilide. In the next stage, isatin /1-, 5- or 7-substituted isatins were condensed with 4-aminoantipyrine, giving the Schiff bases (1a-1f) (Table I).

Table I

The structure of the synthesized compounds

Compound	R	R_1	R_2
1a	-H	-H	-H
1b	$-CH_3$	-H	-H
1c	-H	$-CH_3$	-H
1d	-Cl	-H	-H
1e	$-NO_2$	-H	-H
1f	-H	-H	$-CH_3$

The new synthesized compounds were characterized by elemental analysis, IR spectroscopy and ^{1}HMR spectra. All the elemental and spectral data were in accordance with the proposed structures.

3-[1-phenyl, 2,3-dimethyl-5-oxo-4-amino-pyrazol-4-yl]-iminoisatin (1a): orange powder, yield: 50%. m.p. 163-164 °C; Rf=0.238; Anal.Calcd. for $C_{19}H_{16}O_2N_4$: C, 68.67; H, 4.81; N, 16.86. Found: C, 68.51; H, 4.75; N, 16.80. IR (KBr, cm^{-1}): 1643 (C=N), 1732 (C=O), 3380 (NH).

3-[1-phenyl,2,3-dimethyl-5-oxo-4-amino-pyrazol-4-yl]-5-methyl-iminoisatin (1b): orange powder, yield: 72%. m.p. 154-156°C; Rf=0.267; Anal. Calcd for $C_{20}H_{18}O_2N_4$: C, 69.36; H, 5.2; N, 16.18. Found: C, 69.05; H, 4.98; N, 16.16. IR (KBr, cm^{-1}): 1624 (C=N), 1727 (C=O), 2930 (CH_3), 3200 (NH).

3-[1-phenyl,2,3-dimethyl-5-oxo-4-amino-pyrazol-4-yl]-7-methyl-iminoisatin (1c): orange powder, yield: 49%. m.p. 241-242°C; Rf=0.252; Anal. Calcd for $C_{20}H_{18}O_2N_4$: C, 69.36; H, 5.2; N, 16.18. Found: C, 69.32; H, 5.00; N, 16.16. IR (KBr, cm^{-1}): 1602, 1623 (C=N), 1730 (C=O), 2918 (CH_3), 3188 (NH).

3-[1-phenyl,2,3-dimethyl-5-oxo-4-amino-pyrazol-4-yl]-5-chloro-iminoisatin (1d): orange powder, yield: 49%. m.p. 256-257°C; Rf=0.257; Anal. Calcd for $C_{19}H_{15}O_2N_4Cl$: C, 62.21; H, 4.09; N, 15.27. Found: C, 62.2; H, 4.01; N, 15.15. IR (KBr, cm^{-1}): 702 (C-Cl), 1641 (C=N), 1728(C=O), 3434 (NH). ^{1}HMR (200 MHz, DMSO-d_6, δ /ppm): 2.42(3H, s), 3.30 (3H, s), 6.88-6.86 (1H, d), 7.43-7.36 (5H, m), 7.59-7.55 (2H), 10.85 (1H, s).

3-[1-phenyl,2,3-dimethyl-5-oxo-4-amino-pyrazol-4-yl]-5-nitro-iminoisatin (1e): orange powder, yield: 25%. m.p. 302°C; Rf=0.242; Anal. Calcd for $C_{19}H_{15}O_4N_5$: C, 60.47; H, 3.97; N, 18.56. Found: C, 60.38; H, 3.91; N, 18.5. IR (KBr, cm^{-1}): 1417, 1500 (NO_2), 1622 (C=N), 1735 (C=O), 3431 (NH). ^{1}HMR (200 MHz, DMSO-d_6, δ /ppm): 2.50(3H, s), 3.42 (3H, s), 7.06-7.03 (1H, t), 7.44-7.42 (2H, d), 7.48-7.46 (2H, d), 7.61-7.57 (2H), 7.99 (1H, s), 8.28-8.25 (1H, d).

1-methyl,3-[1-phenyl,2,3-dimethyl-5-oxo-4-amino-pyrazol-4-yl]-iminoisatin (1f): orange powder, yield: 50%. m.p. 218-220°C; Rf=0.222; Anal. Calcd for $C_{20}H_{18}O_2N_4$: C, 69.36; H, 5.2; N, 16.18. Found: C, 69.88; H, 5.37; N, 16.81. IR (KBr, cm^{-1}): 1650 (C=N), 1735 (C=O), 2920 (NH).

The ability to release a hydrogen atom or an electron is reduced compared to control, 0.1 mM ascorbic acid, except for the 1c substance, that in the investigated concentration of 2 mM has a greater DPPH scavenger activity (96.29%) (Figure 2).

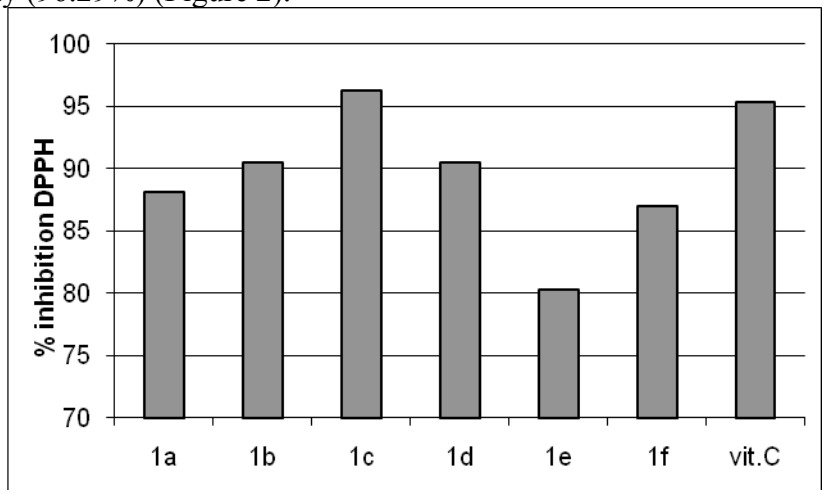


Figure 2
Scavenging effect of 1a-1f compounds and vitamin C on DPPH radical (as inhibition percentage)

The other compounds exhibited DPPH scavenger activity in the following order:

1c > 1d > 1b > 1a > 1f > 1e.

In order to assess the reducing power of the synthesized substances, the Fe^{3+} to Fe^{2+} transformation was evaluated in the presence of the 1a-f substances in solutions of concentration 0.5 mM. The reducing power was compared with the reducing power of some ascorbic acid solutions, that were used as standard. The increase of absorbance correlates directly with

the reducing power and this parameter may represent a significant indicator of the antioxidant potential. The observed data on the reducing power is given in figure 3.

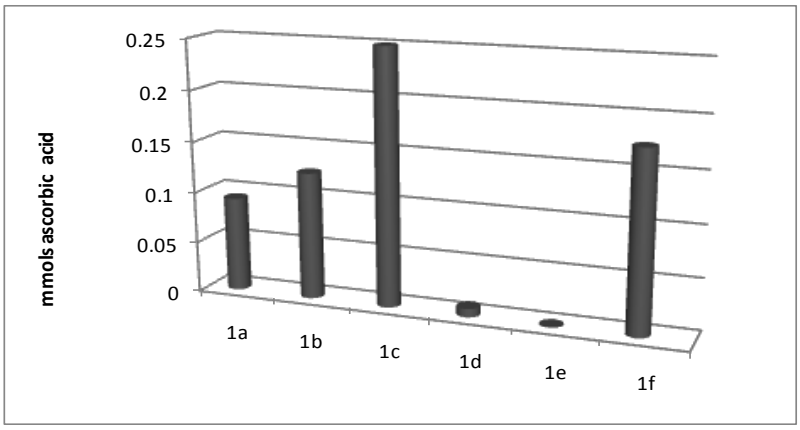


Figure 3
Reducing power of investigated substances compared with that of some ascorbic acid solutions

The analysis of the obtained data have shown that at identical concentrations, the investigated substances have a different reducing power. In the N-unsubstituted compounds, the compound 1c has the best reducing action, and it is followed in this respect by the methylate derivative in 5 position. The compounds substituted in position 5 for electron attracting groups have a weak activity (1d, 1e).

The substitution of the nitrogen atom with a methyl group, favourably influenced the reducing power, and this was evident in the 1f compound that had a reducing power superior to the unmethylated at nitrogen derivative 1a.

The weakest inhibitory activity was observed in the 1d and 1e compounds probably due to electron withdrawing nature of the substituent.

The antibacterial activity of the synthesized compounds was evaluated in comparison with ampicillin (Amp.) and chloramphenicol (Chl.), that were used as standards.

The results of the antimicrobial screening of the synthesized compounds showed that all compounds exhibited a moderate activity against Gram positive bacteria and *Bacillus sp*. (Table II).

All the investigated compounds were inactive against Gram negative and *Candida albicans*, which indicates that the cellular membrane of Gram positive bacteria is more permeable for the tested compounds.

Table II

The diameters (mm) of the inhibition zone of tested microorganisms

Compound		*S. aureus* ATCC 25923	*Sarcina lutea ATCC 9341*	*Bacillus cereus ATCC 14579*	*B. subtilis*	*E. coli* ATCC 25922	*C. albicans* ATCC 10231
1a	D(mm)	16	21	15	15	0	0
	% inhibition level	17.77	23.33	16.66	16.66	-	-
1b	D(mm)	20	20	11	20	0	0
	% inhibition level	22.22	22.22	12.22	22.22	-	-
1c	D(mm)	21	22	14	13	0	0
	% inhibition level	23.33	24.44	15.55	14.44	-	-
1d	D(mm)	21	22	13	12	0	0
	% inhibition level	23.33	24.44	14.44	13.33	-	-
1e	D(mm)	20	21	13	12	0	0
	% inhibition level	22.22	23.33	14.44	13.33	-	-
1f	D(mm)	15	21	0	18	0	0
	% inhibition level	16.66	23.33	-	20	-	-
Ampicillin (Amp.)	D(mm)	27	40	12	15	15	-
	% inhibition level	30	44.44	13.33	16.66	16.66	-
Chloramphenicol (Chl.)	D(mm)	27	40	30	30	28	-
	% inhibition level	30	44.44	33.33	33.33	31.11	-
Nystatin	D(mm)	-	-	-	-	-	30
	% inhibition level	-	-	-	-	-	33.33

In order to predict ADME properties of molecules, a computational study was performed by determination of lipophilicity, PSA (polar surface area), HF (heat of formation), energies of the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) and other Lipinski parameters (nON = number of hydrogen acceptor, nOHNH = number of hydrogen donor) (Table III) [1,13,17].

Table III

Main quantum –chemical properties

Parameter	Substance					
	1a	**1b**	**1c**	**1d**	**1e**	**1f**
logP	2.627	3.051	3.027	3.281	2.562	2.694
R_M	0.505	0.438	0.471	0.460	0.494	0.54
3V (Å)	293.5	310.066	310.066	307.041	316.839	310.447
E Lumo	-1.0955	-1.0767	-1.0795	-1.235	-1.6848	-1.1888
E Humo	-7.9632	-7.9558	-7.9507	-7.9879	-8.0947	-7.7319
HF	238.82	206.71	206.71	211.61	66.24	200.96
PSA	72.165	72.165	72.165	72.165	316.839	61.308
nON	6	6	6	6	9	6
nOHNH	1	1	1	1	1	0

High logP values imply high solubility and good penetration of lipid membranes, together with low solubility in aqueous phases and hence the inability for the molecule to be transported through the body [10,12].

As observed in the study, clogP values are consistent with data described by the Lipinski`s rule, since they are smaller than 5, which suggests a good membrane penetrability.
Very high PSA values contribute to a low bioavailability of the molecule. This parameter is a sum of surfaces of polar atoms (usually oxygen, nitrogen and attached hydrogen) in a molecule. It is a very good predictor of drug transport properties such as intestinal absorption, bioavailability, blood brain barrier penetration etc. PSA of the derivatives 1a-1f has o convenable value (below the 160 Å limit), except the compound 1e (PSA=316.8396).

The investigated compounds are relatively small molecules according to their molecular volume data.

The energies of HOMO orbitals are between 7.7319 and 8.0947, depending on the nature of the substituent.
Also other drug-like properties (nON and nOHNH) for structures 1a-1f were estimated by freely available prediction toolkit Molinspiration. Number of hydrogen bond acceptors (O and N atoms) and number of hydrogen bond donors (NH and OH) in the tested compounds were found to be within Lipinski's limit i.e. less than 10 and 5 respectively.

Conclusions

In conclusion, some new Schiff bases derived from isatin were synthesized and have been subjected to *in vitro* antioxidant and antimicrobial activity. ^{1}H-NMR, elemental analysis and IR spectra confirmed the structure of the synthesized compounds. The results of antimicrobial screening indicate that all compounds exhibited moderate activity against tested microorganisms. The antioxidant activity also displayed considerable results, all the tested compounds presented significant Fe^{3+} reducing power, similar to ascorbic acid. All the compounds of the present study are in agreement with the *Lipinski's Rule of Five* and have desirable molecular properties for drug likeness.

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