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Full Paper

Synthesis, Antibacterial, Antifungal and Antiviral Activity Evaluation of Some New bis-Schiff Bases of Isatin and Their Derivatives

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Abstract: Twelve new bis-Schiff bases of isatin, benzylisatin and 5-fluoroisatin **3a-3l** were prepared by condensation of isatin, benzylisatin and 5-fluoroisatin with primary aromatic amines. The chemical structures of the products were confirmed by ^{1}H- and ^{13}C-NMR, IR and mass spectral data. The compounds were screened for antiviral activity against a panel of DNA and RNA viruses. Minimum cytotoxic and minimum virus-inhibitory concentrations of these compounds were determined. Compounds **3c** and **3i** were the most cytotoxic in HEL cells. These newly synthesized bis-Schiff bases were also tested for their antibacterial and antifungal activities. They did not display activity against *S. cerevisiae* (ATCC 28383) or *C. albicans* (CIP 1180-79).

Keywords: Isatin, bis-Schiff base, antibacterial, antiviral, antifungal.

Introduction

Isatin has been known for about 150 years and has been recently found, like oxindole and endogenous polyfunctional heterocyclic compounds, to exhibit biological activity in mammals [1]. Isatin also is a synthetically versatile substrate that can be used to prepare a large variety of heterocyclic compounds, such as indoles and quinolines, and as a raw material for drug synthesis [2]. Isatin is further known to be a color reagent for the amino acid proline, forming a blue derivative [3]. This property has been exploited for the determination of this amino acid in pollens [4] and other vegetable materials [5] using paper chromatography or for the detection of polymer-bound compounds possessing proline residues [6]. Some isatin derivatives exhibit antiplasmodial activity [7]. Schiff bases and Mannich bases of isatin are known to possess a wide range of pharmacological properties including antibacterial, [8-10] anticonvulsant, [11,12] anti-HIV, [13-16] antifungal [17-20] and antiviral activity [21]. Bis-Schiff bases are characterized by their capacity to completely co-ordinate a metal ion, forming chelate rings [22]. The Schiff bases of isatin have also been used as ligands for complexation of metals such as copper II [23]. These complexes catalyzed the oxidation of carbohydrates. Bis-Schiff bases can act as inhibitors of human α-thrombin [24]. Recently it has been reported that a bis-imine of isatin has antimicrobial properties [25] and affects cell viability [26]. Here we report the synthesis and characterization of new bis-Schiff bases of isatin, benzylisatin [27] and 5-fluoroisatin, which could be considered as potential biologically active compounds.

Results and Discussion

The desired bis-Schiff bases of isatin and its derivatives were prepared by the reactions of isatin, 5-fluoroisatin and benzylisatin with commercially available aromatic diamines in the presence of catalytic amounts of glacial acetic acid in EtOH under reflux condition (Scheme 1). The physico-chemical properties of bis-Schiff bases thus prepared are summarized in Table 1.

Scheme 1

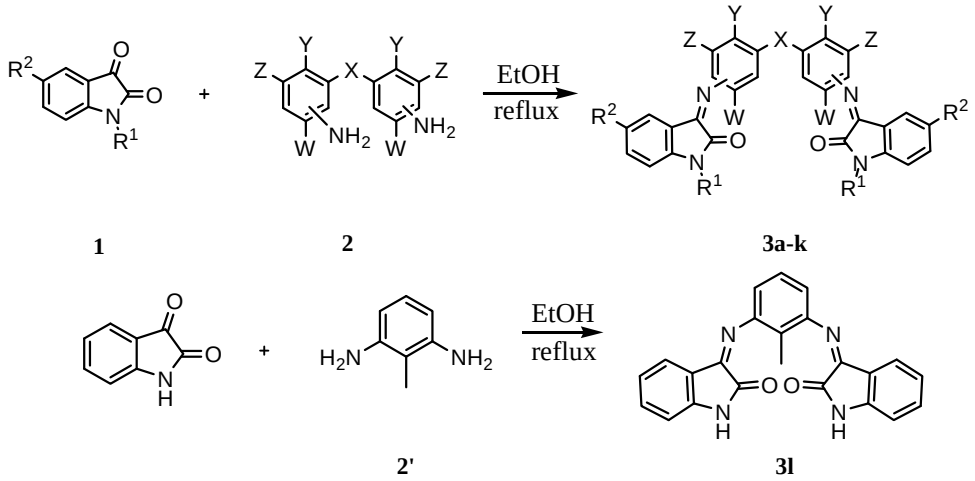

Table1. Physico-chemical properties of compounds **3a-3l**.

Compd.	X	Y	Z	W	R^1	R^2	Position of C=N relative to X	m.p. (˚C)	color	time(h)	Yield (%)
3a	CH_2	H	H	H	H	H	3,3′	>260	ochre	17	77
3b	CH_2	H	H	H	H	H	3,4′	248-250	light ochre	20	70
3c	CH_2	H	H	H	H	F	4,4′	>260	ochre	0.5	99
3d	CH_2	H	H	H	H	F	3,3′	>260	light brown	5	68
3e	CH_2	Cl	Et	Et	H	H	4,4′	>260	orange-yellow	15	74
3f	CH_2	H	H	H	Bn	H	4,4′	180-182	orange	8	98
3g	CH_2	H	H	H	Bn	H	3,3′	204-206	oval-yellow	3	82
3h	O	H	H	H	H	H	3,4′	>260	canary-yellow	28	91
3i	O	H	H	H	H	F	4,4′	>260	brown-yellow	0.5	87
3j	O	H	H	H	Bn	H	4,4′	204-206	dark orange	1	88
3k	CO	H	H	H	H	H	4,4′	242-244	yellow	0.5	70
3l	-	-	-	-	-	-	-	>260	brick-red	19	80

Derivatives **3a-3l** were evaluated for their *in vitro* biological properties against several human pathogens [28] (Table 2).

Table 2. Antimicrobial activities of derivatives **3a-3l**.

Sample CIP	Antimicrobial activity (MIC) (µg/mL)			
	***S. cerevisiae* (28383)[a]**	***S. aureus* (4.83)**	***C. albicans* (1180-79)**	***E. coli* (54127)**
3a	<50	<50	<50	<50
3b	<50	<50	<50	<50
3c	<50	<50	<50	<50
3d	<50	<50	<50	<50
3e	<50	<50	<50	<50
3f	<50	<50	<50	<50
3g	<50	<50	<50	<50
3h	<50	<50	<50	<50
3i	<50	<50	<50	<50
3j	<50	<50	<50	<50
3k	<50	<50	<50	<50
3l	<100	<100	<100	<100

[a] ATCC Number

The compounds were also evaluated for their cytotoxicity (Table 3) and antiviral activity (Table 4) in human embryonic lung (HEL) and human epithelial (HeLa) cells and African green monkey kidney (Vero) cells, according to well-established procedures [29-31]. Derivatives **3a-3l** were found to possess no antifungal activities against *S. cerevisiae* (ATCC 28383) and *C. albicans* (CIP 1180-79). Moreover, no antibacterial activities against Gram-positive and Gram-negative bacteria were noted, as is shown in Table 2. The minimum cytotoxic concentration of the compounds varied from 8 μg/mL to ≥ 400 μg/mL, compounds **3c** and **3i** being the most cytotoxic (at 8 and 16 μg/mL) in HEL cells (Table 3).

Table 3: The minimum cytotoxic concentration of compounds **3a-3l**.

compound	Minimum cytotoxic concentration(μg/mL)*		
	HEL	**Vero**	**HeLa**
3a	80	80	≥16
3b	80	80	≥16
3c	16	≥16	80
3d	80	80	80
3e	80	16	16
3f	40	40	200
3g	200	200	200
3h	400	400	≥16
3i	8	40	≥16
3j	400	≥80	80
3k	80	≥16	80
3l	400	400	≥400
Brivudin	>500	500	>500
Ribavirin	>500	>500	>500
Acyclovir	>500	-	-
Ganciclovir	>100	-	-
(S)-DHPA	-	500	>500

The results of antiviral activity of the new Schiff bases of isatin and 5-flouroisatin are shown in Table 4. None of the compounds exhibited specific antiviral activity, which means that they did not inhibit the replication (induction of viral cytopathogenicity) of any of the viruses tested at a concentration that was ≥ 5-fold lower than the minimum cytotoxic concentration.

Table 4. Antiviral activity of compounds **3a-3l**.

Compound	Minimum virus-inhibitory concentration* (μg/mL)				
	HEL				
	Herpes simplex virus-1(KOS)	Herpes simplex virus-2(G)	Vaccinia virus	Vesicular stomatitis virus	Herpes simplex virus-1 KOS ACV^r (TK$^-$)
3a	>16	>16	>16	>16	>16
3b	>16	>16	>16	>16	>16
3c	>3.2	>3.2	>3.2	>3.2	>3.2
3d	>16	>16	>16	>16	>16
3e	>16	>16	9.6	>16	>16
3f	>8	>8	>8	>8	>8
3g	>40	>40	>40	>40	>40
3h	>80	>80	>80	>80	>80
3i	>1.6	>1.6	>1.6	>1.6	>1.6
3j	>80	>80	>80	>80	>80
3k	>16	>16	>16	>16	>16
3l	>80	>80	>80	>80	16
Brivudin	0.16	>500	60	>500	500
Ribavirin	500	>500	300	>500	>500
Acyclovir	2.4	20	>500	>500	300
Ganciclovir	0.48	4	>100	>100	12
(S)-DHPA	-	-	-	-	-
Compound	Vero				
	Para-influenza-3 virus	Reovirus-1	Sindbis virus	Coxsackie virus B4	PuntaToro virus
3a	>16	>16	>16	>16	>16
3b	>16	>16	>16	>16	>16
3c	≥16	>16	>16	>16	>16
3d	>16	>16	>16	>16	>16
3e	>3.2	>3.2	>3.2	>3.2	>3.2
3f	>8	>8	>8	>8	>8
3g	>40	>40	>40	>40	>40
3h	>80	>80	>80	>80	>80
3i	>8	>8	>8	>8	>8
3j	>80	>80	>80	>80	>80
3k	>16	>16	>16	>16	>16(80)
3l	>80	>80	>80	>80	>80
Brivudin	>100	>100	>100	>100	>100
Ribavirin	300	300	300	>500	60
Acyclovir	-	-	-	-	-
Ganciclovir	-	-	-	-	-
(S)-DHPA	>100	300	>100	>100	

Table 4. Cont.

Compound	Minimum virus-inhibitory concentration* (μg/mL)		
	HeLa		
	Vesicular stomatitis virus	Coxsackie virus B4	Respiratory syncytial virus
3a	9.6	>16	>16
3b	3.2	>16	>16
3c	>16	>16	>16
3d	9.6	>16	>16
3e	>3.2	>3.2	>3.2
3f	>40	>40	>40
3g	>40	>40	>40
3h	>16	>16	>16
3i	>16	>16	>16
3j	>16	>16	>16
3k	48	>16	>16
3l	>400	>400	>400
Brivudin	>500	>500	>500
Ribavirin	60	>500	60
Acyclovir	-	-	-
Ganciclovir	-	-	-
(S)-DHPA	500	>500	>500

*Required to reduce virus- induced cytopathogenicity by 50%.

Experimental

General

Chemical materials and solvents were obtained from Merck, Fluka and Aldrich chemical companies. Melting points were determined in open capillary tubes in Buchi 530 circulating oil apparatus and are not corrected. FT-IR spectra (KBr) were run on a Shimadzu FTIR-8300 spectrophotometer. NMR spectra were recorded on a Bruker Avance DPX-250 spectrometer (^{1}H-NMR 250 MHz, ^{13}C-NMR 62.9 MHz) in $CDCl_3$ or DMSO-d_6 solvents using TMS as an internal standard. Mass spectra were obtained on a Shimadzu GCMS-QP 1000 EX instrument at 70 ev. The determination of the prepared products and reaction monitoring were carried out on silica gel 254 analytical sheets obtained from Fluka. Column chromatography was carried out by silica gel 60 Merck (230-270).

General procedure for preparation of bis-Schiff bases of isatin and its derivatives

Isatin, benzylisatin [27] or 5-fluoroisatin (13.6 mmol) and the aromatic diamines (6.8 mmol) were dissolved in warm ethanol (20 mL) containing glacial acetic acid (0.45 mL). The reaction mixture was refluxed for several hours. After standing at room temperature, the resulting solid was separated by filtration and vacuum dried.

3,3´-[Methylenebis(3,1-phenylenenitrilo))bis[1,3-dihydro)-2H-indol-2-one (**3a**): IR (cm^{-1}): 1652 (C=N), 1726 (C=O), 3168 (N-H); ^{1}H-NMR (DMSO-d_6) δ (ppm): 4.07 (2H, s, CH_2), 6.29-7.43 (16H, m, ArH), 10.96 (2H, s, N-H); ^{13}C-NMR (DMSO-d_6) δ (ppm): 44.40 (CH_2), 115.63-155.53 (aromatic carbons), 159.76 (C=N), 168.29 (C=O); MS (m/z, %): 458 (12.5), 457 (34.9), 456 (100.0), 327 (64.2), 312 (39.2), 299 (53.5), 284 (29.9), 283 (27.1), 200 (18.5), 180 (29.9), 165 (48.5), 44 (15.7).

3,3´-[Methylenebis[(3,1)-(4´,1´)phenylenenitrilo))bis[1,3-dihydro)-2H-indol-2-one (**3b**): IR (cm^{-1}): 1612 (C=N), 1732 (C=O), 3213 (N-H); ^{1}H-NMR (DMSO-d_6) δ (ppm): 4.02 (2H, s, CH_2), 6.37-7.56 (16H, m, ArH), 10.97 (2H, s, N-H); ^{13}C-NMR (DMSO-d_6) δ (ppm): 40.74 (CH_2), 111.84-151.01 (aromatic carbons), 155.31(C=N), 163.85 (C=O); MS (m/z, %): 456 (23.9), 326 (6.0), 312 (28.5), 299 (100.0), 198 (20.2), 182 (11.4), 180 (50.9), 166 (21.7), 44 (27.5).

3,3´-[Methylenebis(4,1-phenylenenitrilo))bis[1,4-dihydro)-5-fluoro-2H-indol-2-one (**3c**): IR (cm^{-1}): 1618 (C=N), 1739 (C=O), 3261 (N-H); ^{1}H NMR (DMSO-d_6) δ (ppm): 4.03 (2H, s, CH_2), 6.09-7.41 (14H, m, ArH), 11.01 (2H, s, N-H); ^{13}C-NMR (DMSO-d_6) δ (ppm): 44.30 (CH_2), 113.23-153.68 (aromatic carbons), 159.16 (C=N), 164.38 (C=O); MS (m/z, %): 492 (1.4), 467 (2.8), 439 (2.1), 368 (52.8), 339 (10.0), 313 (22.8), 236 (28.5), 83 (50.7), 57 (100.0).

3,3´-[Methylenebis(3,1-phenylenenitrilo))bis[1,3-dihydro)-5-fluoro-2H-indol-2-one (**3d**): IR (cm^{-1}): 1622 (C=N), 1733 (C=O), 3290 (N-H); ^{1}H-NMR (DMSO-d_6) δ (ppm): 4.06 (2H, s, CH_2), 6.78-7.48 (14H, m, ArH), 10.86 (2H, s, N-H); ^{13}C-NMR (DMSO-d_6) δ (ppm): 40.50 (CH_2), 112.02-150.67 (aromatic carbons), 150.67 (C=N), 163.73 (C=O); MS (m/z, %): 493 (1.4), 492 (0.7), 368 (7.1), 313 (10.7), 178 (22.1), 147 (22.1), 91 (24.2), 43 (100.0).

3,3´-[Methylenebis(2-chloro-3,5-diethyl-4,1-phenylenenitrilo))bis[1,3-dihydro)-2H-indol-2-one (**3e**): IR (cm^{-1}): 1614 (C=N), 1735 (C=O), 3247 (N-H); ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.86-1.05 (12H, t, $4CH_3$), 4.19 (CH_2), 6.66-7.70 (10H, m, ArH), 10.98 (2H, s, N-H); ^{13}C-NMR (DMSO-d_6) δ (ppm): 13.21-13.73 ($2CH_3$), 23.02-23.77 ($2CH_2$), 37.50 (CH_2), 112.10- 147.13 (aromatic carbons), 156.83 (C=N), 163.42 (C=O); MS (m/z, %): 637 (0.7), 603 (2.1), 577 (4.2), 555 (9.2), 368 (34.2), 339 (7.8), 313 (15.0), 236 (15.0), 97 (34.2), 96 (56.4), 43 (100.0).

3,3´-[Methylenebis(4,1-phenylenenitrilo))bis [1,3-dihydro)-1-phenylmethylene-2H-indol-2-one (**3f**): IR (cm^{-1}): 1604 (C=N), 1732 (C=O); ^{1}H-NMR ($CDCl_3$) δ (ppm): 4.06 (2H, s, CH_2), 5.00 (4H, s, CH_2), 6.41-7.70 (26H, m, ArH); ^{13}C-NMR (DMSO-d_6) δ (ppm): 44.40 (CH_2), 110.04-148.83 (aromatic

carbons), 154.57 (C=N), 163.78 (C=O); MS (m/z, %): 636 (3.5), 442 (2.8), 417 (100.0), 388 (12.8), 326 (27.8), 207 (20.0), 180 (17.8), 106 (37.8), 91 (35.7), 43 (13.5).

3,3´-[Methylenebis(3,1-phenylenenitrilo))bis[1,3-dihydro)-1-phenylmethylene-2H-indol-2-one (**3g**): IR (cm^{-1}): 1604 (C=N), 1732 (C=O); ^{1}H-NMR ($CDCl_3$) δ (ppm): 4.01 (2H, s, CH_2), 4.96 (4H, s, CH_2), 6.53-7.67 (26H, m, ArH); ^{13}C-NMR ($CDCl_3$) δ (ppm): 44.36 (CH_2), 110.06-150.90 (aromatic carbons), 154.58 (C=N), 163.67 (C=O); MS (m/z, %): 636 (1.4), 417 (100.0), 326 (32.1), 285 (14.2), 198 (40.0), 180 (43.5), 165 (27.1), 106 (17.1), 91 (35.0), 44 (5.0).

3,3´-[Oxybis[(3,1)-(4´,1´)phenylenenitrilo))bis[1,3-dihydro)-2H-indol-2-one (**3h**): IR (cm^{-1}): 1620 (C=N), 1733 (C=O), 3195 (N-H). ^{1}H NMR (DMSO-d_6) δ (ppm): 6.16-7.77 (16H, m, ArH), 10.94 (2H, s, N-H); ^{13}C NMR (DMSO-d_6) δ (ppm): 111.27-155.49 (aromatic carbons), 158.91 (C=N), 163.69 (C=O); MS (m/z, %): 458 (3.8), 330 (14.6), 329 (56.3), 302 (17.9), 301 (48.8), 234 (10.8), 200 (44.4), 171 (14.3), 128 (18.3), 108 (53.2), 92 (33.8), 66 (100.0), 44 (53.9).

3,3´-[Oxybis(4,1-phenylenenitrilo))bis[1,3-dihydro)-5-fluoro-2H-indol-2-one (**3i**): IR (cm^{-1}): 1618 (C=N), 1739 (C=O), 3261 (N-H); ^{1}H-NMR (DMSO-d_6) δ (ppm): 6.25-7.43 (14H, m, ArH), 11.07 (2H, s, N-H); ^{13}C-NMR (DMSO-d_6) δ (ppm): 111.27-154.00 (aromatic carbons), 155.41 (C=N), 163.66 (C=O); MS (m/z, %): 495 (2.8), 494 (2.1), 426 (22.1), 396 (9.2), 368 (14.2), 313 (31.4), 264 (22.1), 236 (28.5), 168 (30.0), 97 (43.5), 69 (65.0), 43 (100.0).

3,3´-[Oxybis(4,1-phenylenenitrilo))bis[1,4-dihydro)-1-phenylmethylene-2H-indol-2-one (**3j**): IR (cm^{-1}): 1604 (C=N), 1732 (C=O); ^{1}H-NMR ($CDCl_3$) δ (ppm): 5.01 (4H, s, CH_2), 6.72-7.71 (26H, m, ArH); ^{13}C-NMR ($CDCl_3$) δ (ppm): 44.42 (CH_2), 110.03-154.65 (aromatic carbons), 155.33 (C=N), 163.76 (C=O); MS (m/z, %): 638 (0.7), 577 (3.5), 551 (5.0), 419 (100.0), 328 (20.0), 288 (6.4), 237 (12.8), 207 (20.7), 180 (7.1), 146 (7.1), 91 (27.1), 43 (26.4).

3,3´-[Methanonebis(4,1-phenylenenitrilo))bis[1,4-dihydro)-2H-indol-2-one (**3k**): IR (cm^{-1}): 1593 (C=N), 1735 (C=O, amide), 1660 (C=O, ketone), 3222 (N-H); ^{1}H-NMR (DMSO-d_6) δ (ppm): 6.25-7.58 (16H, m, ArH), 10.99 (2H, s, N-H); ^{13}C-NMR (DMSO-d_6) δ (ppm): 119.74-134.76 (aromatic carbons), 164.20 (C=N), 197.53 (C=O); MS (m/z, %): 470 (5.3), 341 (27.7), 313 (35.4), 221 (14.6), 212 (7.8), 180 (4.3), 120 (98.5), 104 (14.6), 92 (36.1), 76 (30.8), 44 (100.0), 43 (99.0).

3,3´-[(2-Methyl-1,3-phenylene)dinitrilo)bis[1,3-dihydro)-2H-indole-2-one (**3l**): IR (cm^{-1}): 1616 (C=N), 1716 (C=O), 3209 (N-H); ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.50 (3H, s, CH_3), 6.57-7.06(11H, m, ArH), 10.42 (1H, s, N-H); ^{13}C-NMR (DMSO-d_6) δ (ppm): 18.88 (CH_3), 117.84-152.52 (aromatic carbons), 163.35 (C=N), 167.51 (C=O); MS (m/z, %):380 (0.7), 368 (13.5), 339 (2.8), 313 (8.5), 285 (5.0), 264 (5.0), 236 (11.4), 129 (9.2), 97 (28.5), 69 (42.8), 43 (100.0).

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Sample availability: Contact the authors.