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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and in vitro antibacterial activity of schiff bases of *N*-substituted isatins as effective scaffolds

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Abstract Novel Schiff bases of *N*-substituted isatin, **1–13**, were synthesized starting from isatin and *N*-(4-amino-2-methylphenyl)-4-chlorophthalimide and their structures were confirmed by spectral and elemental analyses. All new compounds were tested for their in vitro antibacterial activity against a range of Gram +ve bacterial strains, like *Bacillus subtilis* (NCIM-2156), *Staphylococcus aureus* (NCIM-2079) and *Staphylococcus epidermis* (NCIM-2493) and Gram −ve bacterial strains, like *Pseudomonas aeruginosa* (NCIM-2036), *Escherichia coli* (NCIM-2065) and *Proteus vulgaris* (NCIM-2027) following broth dilution method as recommended by the National Committee for clinical laboratory standards using ciprofloxacin as reference. Determination of minimum inhibitory concentration (MIC) and zone of inhibition showed that the molecules were more active against Gram −ve bacteria than Gram +ve bacteria. The compounds showed promising antibacterial properties with MIC ranging between 10 and 30 μg/mL.

Keywords Schiff base · Isatin · Antibacterials · MIC · Zone of inhibition

Introduction

Infectious microbial diseases, despite being treated successfully in the most of cases, remain a global health problem because of development of resistance to a number of antimicrobial agents, like β-lactam antibiotics, macrolides, quinolines and vancomycin and this pattern is shown by a variety of clinically significant species of microorganisms. Hence, there is always a vital need to discover new chemotherapeutic agents to avert the emergence of resistance and ideally shorten the duration of therapy.

Isatin (indole-2,3-dione) is an important pharmacophore and a privileged structure in medicinal chemistry. Isatin and its various derivatives are reported to show a variety of biological activities. The isatin moiety is also present in a range of compounds, which can act as inhibitors of apoptosis (Chapman *et al.,* 2002; Lee *et al.,* 2001), anticonvulsants (Verma *et al.,* 2004), antiviral (Sriram *et al.,* 2004; Pirrung *et al.,* 2005), antibacterial and antifungal (Chohan *et al.,* 2004) agents. Besides these, it also has antitumor, antiangiogenic, antitubercular and anxyolytic effects. Isoindolinediones are also reported to have a wide range of biological activities (Al-Soud and Al-Masoudi, 2001; Hashimoto, 2002; Bailleux *et al.,* 1995; Usifoh *et al.,* 2001; Sou *et al.,* 2000; Takahashi *et al.,* 2000; Shimazawa *et al.,* 1999; Hashimoto, 1998; Chapman *et al.,* 1979; Ando *et al.,* 1989; Spallarossa *et al.,* 2009; Ranise *et al.,* 2005), especially the antibacterial activity (Bansode *et al.,* 2009). In view of these facts, different Schiff bases of isatin, viz. 5-chloro-2-[2-methyl-4-(5-nitro-2-oxo-1,2-dihydro-indol-3-ylideneamino)-phenyl]-isoindole-1,3-dione (**1**); 5-chloro-2-[2-methyl-4-(2-oxo-5-trifluoromethoxy-1,2-dihydro-indol-3-ylideneamino)-phenyl]-isoindole-1,3-dione (**2**); 5-chloro-2-[2-methyl-4-(5-methyl-2-oxo-1,2-dihydro-indol-3-ylideneamino)-phenyl]-isoindole-1,3-dione (**3**) and their derivatives **4–13** have been synthesized and subjected to their antimicrobial screening.

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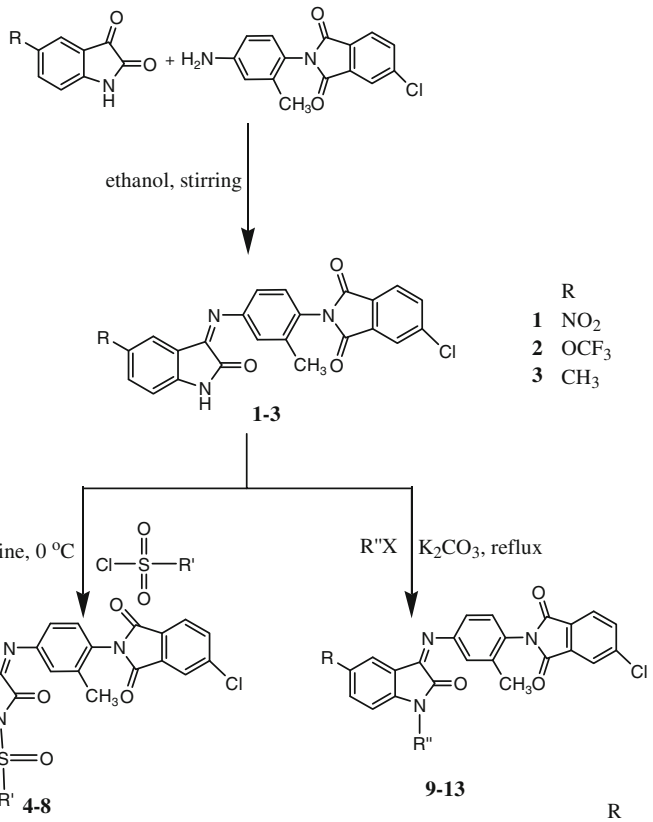
Results and discussion

Chemistry

For the synthesis of Schiff bases, **1–3**, substituted isatins (nitro, trifluoromethoxy or methyl) were treated with a stirred solution of *N*-(4-amino-2-methylphenyl)-4-chlorophthalimide in ethanol. The completion of the reaction was assessed by the appearance of a yellow colored precipitate. The precipitates were filtered, air-dried and recrystallized with ethanol–chloroform mixture to get compounds **1–3**. Similarly, compound **1** or **2** on treatment with different sulfonyl chlorides in dry pyridine gave compounds **4–8.** Compound **1** or **2** on treatment with different alkyl/aryl halides in the presence of anhydrous potassium carbonate (K_2CO_3) gave compounds **9–13** (Scheme 1). All compounds have been characterized by different spectral techniques and elemental analyses. The IR data of compound **1** showed the presence of NH stretching peak at 3,417 cm^{-1} for NH of isatin. The IR data of compound **4** and **9** showed the absence of NH stretching peaks for NH of isatin thus confirming the formation of products. In ^{13}C NMR spectra, several singlets were obtained and have been summarized. Elemental analysis also confirmed formation of the desired products.

Microbiology

The compounds were tested against a range of Gram +ve and Gram −ve bacteria using broth dilution method for determining the minimum inhibitory concentration (MIC) and disc diffusion method for zone of inhibition. Different concentrations (80, 40, 20, 10, 5, 2.5, 1.25 and 0.65 μg/mL) of all compounds were prepared using dimethylsulphoxide (DMSO) as solvent. Muller Hinton broth (M391) was used to prepare nutrient broth. The tubes were incubated at 37 °C for 48 h and checked for appearance of any turbidity, which showed the effect of the test molecules against the pathogens studied. Similarly, Whatman No. 1 sterilized discs were used for studying zone of inhibition using nutrient agar medium seeded with fresh bacteria



Scheme 1 Synthetic route for compounds **1–13.** Reagents and conditions: (i) ethanol, pyridine, rt, 6 h; (ii) pyridine, 0 °C; (iii) alkyl halide, K_2CO_3, reflux

	R
1	NO_2
2	OCF_3
3	CH_3

	R	R'
4	NO_2	p-C_6H_4 NO_2
5	NO_2	CH_3
6	OCF_3	CF_3
7	OCF_3	CH_3
8	OCF_3	p-$C_6H_4NO_2$

	R	R"
9	NO_2	C_6H_4-CH_2-
10	NO_2	HC≡C−CH_2-
11	NO_2	H_3C−C(H)(=H_2)−CH_2-
12	OCF_3	HC≡C−CH_2-
13	OCF_3	C_6H_4-CH_2-

Table 1 Antibacterial activity and log P values of compounds **1–13**

Compound	Log P	Antibacterial activity					
		MIC in μg/mL (zone of inhibition in mm)					
		Gram +ve bacterial strains			Gram −ve bacterial strains		
		S. aureus	*B. subtilis*	*P. aeruginosa*	*E. coli*	*S. epidermis*	*P. vulgaris*
1	2.75	10 (20)	15 (16)	10 (18)	10 (22)	10 (20)	15 (11)
2	5.65	10 (18)	15 (16)	10 (20)	10 (24)	10 (18)	15 (14)
3	4.61	10 (20)	25 (12)	15 (16)	20 (12)	15 (10)	20 (10)
4	2.36	10 (18)	15 (16)	10 (18)	10 (22)	10 (20)	15 (14)
5	0.47	15 (16)	15 (16)	10 (18)	10 (24)	10 (18)	15 (14)
6	7.09	15 (18)	10 (20)	15 (12)	10 (23)	15 (10)	10 (20)
7	4.91	15 (16)	15 (18)	15 (14)	10 (22)	10 (18)	15 (16)
8	3.22	10 (20)	15 (14)	10 (18)	10 (20)	10 (18)	10 (22)
9	3.87	30 (12)	25 (10)	15 (14)	15 (16)	15 (10)	15 (14)
10	2.70	25 (13)	30 (8)	15 (14)	15 (18)	20 (8)	20 (10)
11	3.31	30 (10)	30 (10)	20 (10)	20 (12)	15 (12)	20 (12)
12	6.10	30 (10)	25 (11)	15 (12)	20 (10)	15 (10)	15 (14)
13	7.62	25 (14)	25 (12)	20 (10)	15 (17)	20 (8)	20 (12)
Ciprofloxacin **(14)**	1.32	10 (26)	10 (22)	10 (24)	10 (30)	10 (28)	10 (28)

MIC minimum inhibitory concentration

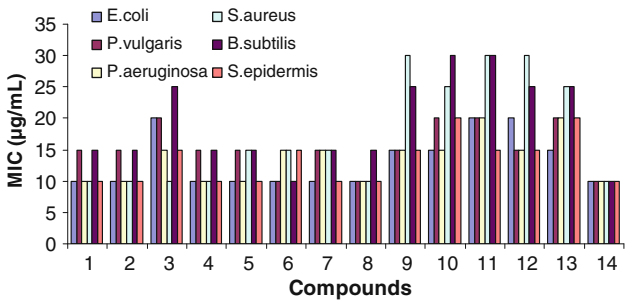


Fig. 1 Graphical presentation of antibacterial activity of compounds **1–13**

separately. The incubation was carried out at 37 °C for 24 h. Ciprofloxacin was used as a standard drug for determining the MIC and zone of inhibition. Zone of inhibition and MICs have been shown in Table 1.

The antibacterial studies showed that all compounds had moderate to good antibacterial activity against Gram +ve as well as Gram −ve bacteria. Almost all compounds were active against different bacterial strains at 10 μg/mL concentration. However, better activity was found in the case of Gram −ve bacteria than Gram +ve bacteria as this trend was substantially supported by their respective MIC values. This may be because of structural differences in the cell wall of Gram +ve and Gram −ve bacteria. Out of 13 compounds studied, seven compounds showed MIC in the range of 10–15 μg/mL (Fig. 1).

Compounds **9–13** bearing alkyl or aryl substituents at nitrogen on isatin moiety, were not as potent as derivatives **1–8** bearing H or sulfonyl group at the same position on isatin. Thus, the compounds bearing a sulfonyl group (hydrogen acceptor) attached to nitrogen or free NH group (hydrogen donor) on isatin, were more potent than other molecules studied. The plausible cause for variation in antibacterial profile of substituted derivatives may be that these atoms (electron withdrawing groups) are very useful to modulate the steric effect on phenyl ring of drug and which, in turn, alters the ease of penetration of molecule in bacterial cell wall. These groups are further expected to alter the hydrophobic–hydrophilic balance of the target molecule and thus, affect their cell permeation, which remains the topic of interest of SAR studies.

It is also believed that the lipophilic nature of compounds plays a key role for generation of antimicrobial effect by increasing the membrane permeation and hence, a strong correlation exists between lipophilicity and activity. In this context, lipophilicity of compound expressed as logP (octanol/water partition coefficient) value, the main predictor of activity, was calculated using the methodology developed by Molinspiration as a sum of fragment-based contributions and correction factors as shown in Table 1. It is evident that compounds having low logP values have shown better biological activity as is evident from their zone of inhibition than those having higher logP values. We have noticed that a preferred logP for optimum activity

always advocated for an optimum amphiphilicity of possible drug molecules.

Conclusion

In conclusion, we have developed a simple and efficient method for synthesis of Schiff bases of *N*-substituted isatins and screened them for antibacterial activity. All compounds showed moderate to good antibacterial activity. The molecules were found to be more active against Gram −ve bacteria than Gram +ve bacteria on the basis of their MIC values and zone of inhibition. The presence of electron withdrawing groups and hydrogen donor–acceptor sites on the test molecules produced better results. Most of these compounds showed bacterial inhibition comparable to the standard drug—ciprofloxacin.

Materials and methods

Chemistry

Silica gel G for TLC was obtained from E. Merck India Ltd. Substituted isatins, sulfonyl chlorides and alkyl halides were purchased from Aldrich Chemical Co., USA. *N*-(4-amino-2-methylphenyl)-4-chlorophthalimide was prepared from 4-chlorophthalimide and 4-chloro-3-methylaniline. All reactions were performed in oven-dried flasks and solvents used were anhydrous. Melting points were determined on an electrothermal apparatus and are uncorrected. Mass spectra were recorded on Micromass Quattro II. UV spectra were recorded on a Perkin-Elmer λ25 spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker AVANCE 400 MHz Fourier transform spectrometer and using an internal deuterium lock. Chemical shifts are quoted in parts per million downfield from tetramethylsilane. ^{13}C-NMR spectra were recorded at 100 MHz on Bruker AMX 400 MHz spectrometer in $CDCl_3$. IR spectra were recorded in AVATAR-330 FT-IR spectrophotometer and only noteworthy absorption levels are listed. Mass spectra were recorded at PESCIEX API 3000 series mass spectrometer. Elemental analysis was carried out using Heraeus Vario EL III Carlo Erba 1108.

General procedure for synthesis of compounds **1–3**

To a stirred solution of *N*-(4-amino-2-methylphenyl)-4-chlorophthalimide (0.5 mmol) in ethanol (10 mL) was added isatin (0.5 mmol). The reaction mixture was stirred overnight. Completion of the reaction was assessed by the appearance of a yellow coloured precipitate. The precipitate was filtered off, washed first with aqueous ethanol followed by water (H_2O), dried, and recrystallized with ethanol–chloroform mixture to get the products **1**, **2** or **3**.

*5-Chloro-2-[2-methyl-4-(5-nitro-2-oxo-1,2-dihydro-indol-3-ylideneamino)-phenyl]-isoindole-1,3-dione (**1**)*

Dark yellow solid Yield 79 % R_f: 0.46 (DCM:MeOH 9.8:0.2) mp: 234 °C. UV (MeOH) λ_{max} 224 nm. IR (KBr, cm^{-1}): 3417 (NH of Isatin), 1779, 1729 (C=O imide), 1703 (C=O of isatin), 1603 (C=N), 1470 (C=C), 1310 (N=O), 806, 740 (Ar–H), 630 (C–Cl). ^{1}H NMR δ_H (DMSO-d_6): 2.34 (s, 3H, CH_3), 7.1–7.5 (m, 3H, ArH), 7.70–8.14 (m, 3H, ArH), 8.04 (s, 1H, =NH), 7.90–8.53 (m, 3H, ArH). ^{13}C NMR δ (ppm): 177.2 (–C=N), 176.6 (C=O), 176.2 (C=O), 175.4 (NH–C=O), 151.7 (C–N), 148.9 (C–NH), 146.3 (C–NO_2), 139.1 (ArC–N imide), 138.4 (ArC–Cl), 134.8, 133.6, 131.9, 131.6, 129.8, 128.9, 128.4, 126.3, 125.4, 124.8, 123.4, 123, 121.2 (Aryl carbons), 12.9 (–CH_3). MS (EI^+) *m*/z 460.06 (100.0 %), 461.03 (26.0 %), 462.04 (32.0 %). Anal. Calcd for $C_{23}H_{13}ClN_4O_5$: C 59.95, H 2.84, N 12.16. Found: C 59.98, H 2.81, N 12.12.

*5-Chloro-2-[2-methyl-4-(2-oxo-5-trifluoromethoxy-1,2-dihydro-indol-3-ylideneamino)-phenyl]-isoindole-1,3-dione (**2**)*

Yellow solid Yield 82 % R_f: 0.49 (DCM:MeOH 9.8:0.2) mp: 245 °C. UV (MeOH) λ_{max} 225 nm. ^{1}H NMR δ_H (DMSO-d_6): 2.34 (s, 3H, CH_3), 7.1–7.5 (m, 3H, ArH), 6.78–7.56 (m, 3H, ArH), 7.70–8.14 (m, 3H, ArH), 8.04 (s, 1H, NH). MS (EI^+) *m/z* 499.05 (100 %), 500.08 (26.0 %), 501.05 (32.0 %). Anal. Calcd for $C_{24}H_{13}ClF_3N_3O_4$: C 57.67, H 2.62, N 8.41. Found: C 57.62, H 2.64, N 8.38.

*5-Chloro-2-[2-methyl-4-(5-methyl-2-oxo-1,2-dihydro-indol-3-ylideneamino)-phenyl]-isoindole-1,3-dione (**3**)*

Orange solid Yield 81 % R_f: 0.47 (DCM:MeOH 9.8:0.2) mp: 143 °C. UV (MeOH) λ_{max} 225 nm. ^{1}H NMR δ_H (DMSO-d_6): 2.28 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 7.0–7.5 (m, 3H, ArH), 7.07–7.55 (m, 3H, ArH), 7.70–8.14 (m, 3H, ArH), 8.02 (s, 1H, NH). MS (EI^+) *m/z* 429.09 (100.0 %), 430.05 (28.0 %), 431.05 (36.2 %). Anal. Calcd for $C_{24}H_{16}ClN_3O_3$: C 67.06, H 3.75, N 9.78. Found: C 67.10, H 3.71, N 9.72.

General procedure for synthesis of compounds **4–8**

To a solution of **1** or **2** (0.1 mmol) in dry pyridine (10 mL) at 0 °C sulfonyl chloride (0.1 mmol) was added drop wise. The reaction mixture was stirred overnight and monitored on TLC. Completion of the reaction was assessed by consumption of the starting material. The reaction mixture

was partitioned between dichloromethane (DCM) and H_2O. The organic layer was collected, dried over anhydrous sodium sulphate (Na_2SO_4), filtered, concentrated *in vacuo* and the product was crystallized with hexane.

*5-Chloro-2-{2-methyl-4-[5-nitro-1-(4-nitro-benzenesulfonyl)-2-oxo-1,2-dihydro-indol-3-ylideneamino]-phenyl}-isoindole-1,3-dione (**4**)*

Light brown solid Yield 72 % R_f: 0.48 (DCM:MeOH 9.8:0.2) mp: 84 °C. UV (MeOH) λ_{max} 398 nm. IR (KBr, cm^{-1}): 1781, 1727 (C=O imide), 1705 (C=O of isatin), 1604 (C=N), 1468 (C=C), 1314 (N=O), 1180 (SO_2N), 809, 744 (Ar–H), 634 (C–Cl). ^{1}H NMR δ_H (DMSO-d_6): 2.34 (s, 3H, CH_3), 7.1–7.5 (m, 3H, ArH), 7.70–8.14 (m, 3H, ArH), 8.19–8.47 (m, 4H, ArH), 7.90–8.53 (m, 3H, ArH). MS (EI^+) *m/z* 645.04 (100.0 %), 646.02 (34.0 %), 647.06 (36.0 %). ^{13}C NMR δ (ppm): 176.2 (–C=N), 175.6 (C=O), 175.2 (C=O), 174.4 (NH–C=O), 152.6 (C–NO2), 150.7 (C–N), 148.5 (C–NH), 146.3 (C–SO_2N), 145.3 (C–NO2), 138.8 (ArC–N imide), 137.4 (ArC–Cl), 134.8, 133.4, 131.8, 131.6, 129.4, 129.1, 128.9, 128.4, 127.9, 125.9, 125.4, 124.8, 124.1, 123.9, 123.2, 123, 122.2 (Aryl carbons), 12.6 (–CH_3). Anal. Calcd for $C_{29}H_{16}ClN_5O_9S$: C 53.92, H 2.50, N 10.84, S 4.96. Found: C 53.92, H 2.50, N 10.84, S 4.96.

*5-Chloro-2-[4-(1-methanesulfonyl-5-nitro-2-oxo-1,2-dihydro-indol-3-ylideneamino)-2-methyl-phenyl]-isoindole-1,3-dione (**5**)*

Dark brown solid Yield 75 % R_f: 0.58 (DCM:MeOH 9.6:0.4) mp: 94 °C. UV (MeOH) λ_{max} 392 nm. ^{1}H NMR δ_H (DMSO-d_6): 2.34 (s, 3H, CH_3), 2.92 (s, 3H, CH_3), 7.1–7.5 (m, 3H, ArH), 7.70–8.14 (m, 3H, ArH), 7.90–8.53 (m, 3H, ArH). MS (EI^+) *m/z* 538.03 (100.0 %), 539.06 (27.0 %), 540.01 (36.0 %). Anal. Calcd for $C_{24}H_{15}ClN_4O_7S$: C 53.49, H 2.81, N 10.40, S 5.95. Found: C 53.41, H 2.814, N 10.43, S 5.92.

*5-Chloro-2-[2-methyl-4-(2-oxo-1-trifluoromethanesulfonyl-5-trifluoromethoxy-1,2-dihydro-indol-3-ylideneamino)-phenyl]-isoindole-1,3-dione (**6**)*

Brown solid Yield 71 % R_f: 0.7 (DCM:MeOH 9.6:0.4) mp: 160 °C. UV (MeOH) λ_{max} 407 nm. ^{1}H NMR δ_H (DMSO-d_6): 2.34 (s, 3H, CH_3), 7.1–7.5 (m, 3H, ArH), 6.78–7.56 (m, 3H, ArH), 7.70–8.14 (m, 3H, ArH). MS (EI^+) *m/z* 631.00 (100.0 %), 632.01 (28.0 %), 633.05 (37.0 %). Anal. Calcd for $C_{25}H_{12}ClF_6N_3O_6S$: C 47.52, H 1.91, N 6.65, S 5.07. Found: C 47.56, H 1.89, N 6.63, S 5.02.

*5-Chloro-2-[4-(1-methanesulfonyl-2-oxo-5-trifluoromethoxy-1,2-dihydro-indol-3-ylideneamino)-2-methyl-phenyl]-isoindole-1,3-dione (**7**)*

Light brown solid Yield 73 % R_f: 0.6 (DCM:MeOH 9.6:0.4) mp: 130 °C. UV (MeOH) λ_{max} 399 nm. ^{1}H NMR δ_H (DMSO-d_6): 2.34 (s, 3H, CH_3), 2.82 (s, 3H, CH_3), 7.1–7.5 (m, 3H, ArH), 6.78–7.56 (m, 3H, ArH), 7.70–8.14 (m, 3H, ArH). MS (EI^+) *m/z* 577.03 (100.0 %), 578.07 (28.0 %), 579.06 (37.0 %). Anal. Calcd for $C_{25}H_{15}ClF_3N_3O_6S$: C 51.96, H 2.62, N 7.27, S 5.55. Found: C 51.96, H 2.68, N 7.22, S 5.57.

*5-Chloro-2-{2-methyl-4-[1-(4-nitro-benzenesulfonyl)-2-oxo-5-trifluoromethoxy-1,2-dihydro-indol-3-ylideneamino]-phenyl}-isoindole-1,3-dione (**8**)*

Brown solid Yield 72 % R_f: 0.43 (DCM) mp: 195°C. UV (MeOH) λ_{max} 402 nm. ^{1}H NMR δ_H (DMSO-d_6): 2.34 (s, 3H, CH_3), 7.1–7.5 (m, 3H, ArH), 6.78–7.56 (m, 3H, ArH), 7.70–8.14 (m, 3H, ArH), 8.19–8.47 (m, 4H, ArH). MS (EI^+) *m/z* 684.03 (100.0 %), 685.03 (34.0 %), 686.07 (37.0 %). Anal. Calcd for $C_{30}H_{16}ClF_3N_4O_8S$: C 52.60, H 2.35, N 8.18, S 4.68. Found: C 52.65, H 2.33, N 8.13, S 4.69.

General procedure for synthesis of compounds **9–13**

1 or **2** (0.1 mmol) and anhydrous K_2CO_3 (0.55 mmol) were taken together and crushed with a glass rod. The respective alkyl/aryl halide, R″X, was added in excess and the reaction mixture was refluxed for 4–5 h and monitored on TLC. Completion of the reaction was assessed by consumption of the starting materials. The reaction mixture was partitioned between DCM and H_2O. The organic layer was collected, dried over anhydrous Na_2SO_4, filtered, concentrated *in vacuo* and the product was crystallized with hexane.

*2-[4-(1-Benzyl-5-nitro-2-oxo-1,2-dihydro-indol-3-ylideneamino)-2-methyl-phenyl]-5-chloro-isoindole-1,3-dione (**9**)*

Dark brown solid Yield 77 % R_f: 0.8 (DCM:MeOH 9.9:0.1) mp: 65 °C. UV (MeOH) λ_{max} 407 nm. IR (KBr, cm^{-1}): 2926 (N–CH_2), 1789, 1724 (C=O imide), 1709 (C=O of isatin), 1600 (C=N), 1472 (C=C), 1317 (N=O), 805,740 (Ar–H), 637(C–Cl). ^{1}H NMR δ_H (DMSO-d_6): 2.34 (s, 3H, CH_3), 4.22 (s, 2H, CH_2), 7.06–7.14 (m, 5H, ArH), 7.1–7.5 (m, 3H, ArH), 7.70–8.14 (m, 3H, ArH), 7.90–8.53 (m, 3H, ArH). ^{13}C NMR δ (ppm): 177.2 (–C=N), 176.6 (C=O), 176.2 (C=O), 175.4 (NH–C=O), 151.7 (C–N), 148.9 (C–NH), 146.3 (C–NO_2), 139.1 (ArC–N imide), 138.4 (ArC–Cl), 137.9, 134.8, 133.6, 131.9, 131.6, 129.8,

128.9, 128.4, 127.9, 127.8, 127.7, 127.1, 126.7, 126.3, 125.4, 124.8, 123.4, 123, 121.2 (Aryl carbons), 54.1 (N–CH_2–Ph), 12.9 (–CH_3). MS (EI^+) *m/z* 550.10 (100.0 %), 551.7 (34.0 %), 552.18 (33.0 %). Anal. Calcd for $C_{30}H_{19}ClN_4O_5$: C 65.40, H 3.48, N 10.17. Found: C 65.46, H 3.43, N 10.14.

*5-Chloro-2-[2-methyl-4-(5-nitro-2-oxo-1-prop-2-ynyl-1, 2-dihydro-indol-3-ylideneamino)-phenyl]-isoindole-1,3-dione (**10**)*

Reddish brown solid Yield 75 % R_f: 0.7 (DCM) mp: 162 °C. UV (MeOH) λ_{max} 392 nm. ^1H NMR δ_H (DMSO-d_6): 2.25 (s, 1H, CH), 2.34 (s, 3H, CH_3), 3.72 (s, 2H, CH_2), 7.1–7.5 (m, 3H, ArH), 7.70–8.14 (m, 3H, ArH), 7.90–8.53 (m, 3H, ArH). MS (EI^+) *m/z* 498.07 (100.0 %), 499.05 (30.0 %), 500.05 (33.0 %). Anal. Calcd for $C_{26}H_{15}ClN_4O_5$:C 62.60, H 3.03, N 11.23. Found: C 62.64, H 3.06, N 11.18.

*5-Chloro-2-[2-methyl-4-(5-nitro-2-oxo-1-propyl-1,2-dihydro-indol-3-ylideneamino)-phenyl]-isoindole-1,3-dione (**11**)*

Brown solid Yield 72 % R_f: 0.8 (DCM:MeOH 9.8:0.2). mp: 104 °C. UV (MeOH) λ_{max} 405 nm. ^1H NMR δ_H (DMSO-d_6): 0.96 (t, 3H, CH_3), 1.59 (m, 2H, CH_2), 2.34 (s, 3H, CH_3), 2.96 (t, 2H, CH_2), 7.1–7.5 (m, 3H, ArH), 7.70–8.14 (m, 3H, ArH), 7.90–8.53 (m, 3H, ArH). MS (EI^+) *m/z* 502.10 (100.0 %), 503.2 (30.0 %), 504.5 (33.0 %). Anal. Calcd for $C_{26}H_{19}ClN_4O_5$: C 62.09, H 3.81, N 11.14. Found: C 62.02, H 3.78, N 11.18.

*5-Chloro-2-[2-methyl-4-(2-oxo-1-prop-2-ynyl-5-trifluoromethoxy-1,2-dihydro-indol-3-ylideneamino)-phenyl]-isoindole-1,3-dione (**12**)*

Orange solid Yield 76 % R_f: 0.42 (DCM) mp: 136 °C. UV (MeOH) λ_{max} 413 nm. ^1H NMR δ_H (DMSO-d_6): 2.25 (s, 1H, CH), 2.34 (s, 3H, CH_3), 3.72 (s, 2H, CH_2), 7.1–7.5 (m, 3H, ArH), 6.78–7.56 (m, 3H, ArH), 7.70–8.14 (m, 3H, ArH). MS (EI^+) *m/z* 537.07 (100.0 %), 538.04 (32.0 %), 539.05 (33.0 %). Anal. Calcd for $C_{27}H_{15}ClF_3N_3O_4$: C 60.29, H 2.81, N 7.81. Found: C 60.22, H 2.80, N 7.78.

*2-[4-(1-Benzyl-2-oxo-5-trifluoromethoxy-1,2-dihydro-indol-3-ylideneamino)-2-methyl-phenyl]-5-chloro-isoindole-1,3-dione (**13**)*

Light brown solid Yield 75 % R_f: 0.8 (DCM:MeOH 9.6:0.4) mp: 101 °C. UV (MeOH) λ_{max} 415 nm. ^1H NMR δ_H (DMSO-d_6): 2.34 (s, 3H, CH_3), 4.22 (s, 2H, CH_2), 7.14–7.16 (m, 5H, ArH), 7.1–7.5 (m, 3H, ArH), 6.78–7.56 (m, 3H, ArH), 7.70–8.14 (m, 3H, ArH). MS (EI^+) *m/z* 589.10 (100.0 %), 59.3 (36.0 %), 591.5 (33.0 %). Anal. Calcd for $C_{31}H_{19}ClF_3N_3O_4$: C 63.11, H 3.25, N 7.12. Found: C 63.15, H 3.23, N 7.15.

Antibacterial studies

All compounds were screened for their antibacterial activity against a range of Gram +ve and Gram –ve bacteria, viz. *Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcus aureus, Bacillus subtilis* and *Staphylococcus epidermis*. The MIC was determined by broth dilution method and zone of inhibition by disc diffusion method (Cruickshank *et al.,* 1975; Collins, 1976; Singh *et al.,* 2010; Srivastava *et al.,* 2007).

Determination of MIC by the broth dilution method

Different concentrations (80, 40, 20, 10, 5, 2.5, 1.25 and 0.65 μg/mL) of all the compounds were prepared using dimethylsulphoxide (DMSO) as solvent in sterile dry test tubes to determine MIC *by* the broth dilution method. Nutrient broth was prepared using Muller Hinton broth (M391) and 4.9 mL of it was taken in each test tube and was sterilized after plugging. After cooling, 0.1 mL of each dilution was added to the test tube and the final volume was made up to 5.0 mL. The test tubes were shaken to uniformly mix the inocula with the broth. The tubes were incubated at 37 °C for 48 h. Appearance of any turbidity showed that the compound was not able to inhibit the growth of bacteria, while no turbidity indicated the inhibition of microorganism by the sample.

Determination of zone of inhibition by the disc diffusion method

Discs measuring 6.25 mm in diameter were punched from Whatman No. 1 filter paper. Batches of 100 discs were dispensed to each screw capped bottles and sterilized by dry heat at 140 °C for an hour. The test compounds were prepared with different concentrations using DMSO. One milliliter containing 100 times the amount of chemical in each disc was added to each bottle, which contained 100 discs. The discs of each concentration were placed in triplicate in nutrient agar medium seeded with fresh bacteria separately. The incubation was carried out at 37 °C for 24 h. Ciprofloxacin was used as a standard drug.

Determination of logP

logP was calculated using Molinspiration and ChemDraw softwares.

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