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Research Article

Synthesis of 3-[4-(2-Amino-6 (substituted phenyl)-pyrimidin-4-yl)-phenylimino]-5-chloro-1, 3-dihydro-indol-2-one derivatives of 5-chloroisatin as potential antimicrobial agents

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ABSTRACT

In the present study novel pyrimidine derivative of 5-chloroisatin were synthesized and evaluated for their in vitro antimicrobial activity. 5-chloroisatin was reacted with 4-aminoacetophenone to give 3-(4-acetylphenylimino)-5chloro-1, 3-dihydroindol-2-one, which was further reacted with different aromatic aldehyde in alkaline ethanolic medium to give chalcone (a-o). Final pyrimidine derivative were synthesized by reaction of chalcone with Guanidine. All synthesized compounds were characterized by IR and H^1NMR spectroscopy. Compounds were evaluated for their antimicrobial activity. Among the synthesized compounds, compound 5, 6, 8, 11 and 12 showed antibacterial activity while compound 4, 8 and 9 showed antifungal activity.

Keyword: Isatin; 5-chloroisatin, pyrimidine; antimicrobial; antibacterial; antifungal; chalcone

INTRODUCTION

Even after the discovery of so many good antimicrobial agents, infectious diseases are the major cause of death worldwide, because of the development of resistance by microbes. Antimicrobial resistance is becoming a serious threat to global public health. As per WHO 48000 people develop multidrug resistance tuberculosis each year. To combat this, there is a need to develop new antimicrobial agents. Indole fragment prevails a variety of pharmacologically and biological active molecules. Isatin (Indole 2, 3-Dione) is a heterocyclic moiety with indole nucleus. It is reported to possess a broad spectrum of activity like antimicrobial [1], antiviral [2], antimalarial and antitubercular [3], anti HIV[4], anticancer [5], cytotoxic [3, 6] anticonvulsant[7, 8] etc. In view of the fact mentioned above, we synthesized a series of novel pyrimidine derivatives of 5-chloroisatin and evaluated them for *in vitro* antimicrobial activity.

MATERIALS AND METHODS

General

All the chemical and solvent used were of laboratory grade. Melting points were determined by capillary method and are uncorrected. Purity of compounds was cheked by TLC on preparatory silica gel G plates. Iodine chamber and UV lamp were used for visuliasation of spot. Characterizations of compounds were done by IR and H^1 NMR spectroscopy. IR spectra were recorded in KBr pellets on FT IR spectrometer. H^1NMR were recorded on spectrometer in DMSO using

tetramethylsilane as an internal standard. The chemical shifts of the compounds were reported in ppm. Physicochemical properties of synthesized compounds are reported in table 1.

Synthesis Method

Synthesis of 3-(4-acetylphenylimino)-5-chloro-1, 3-dihydro indol-2-one (Schiff bases):

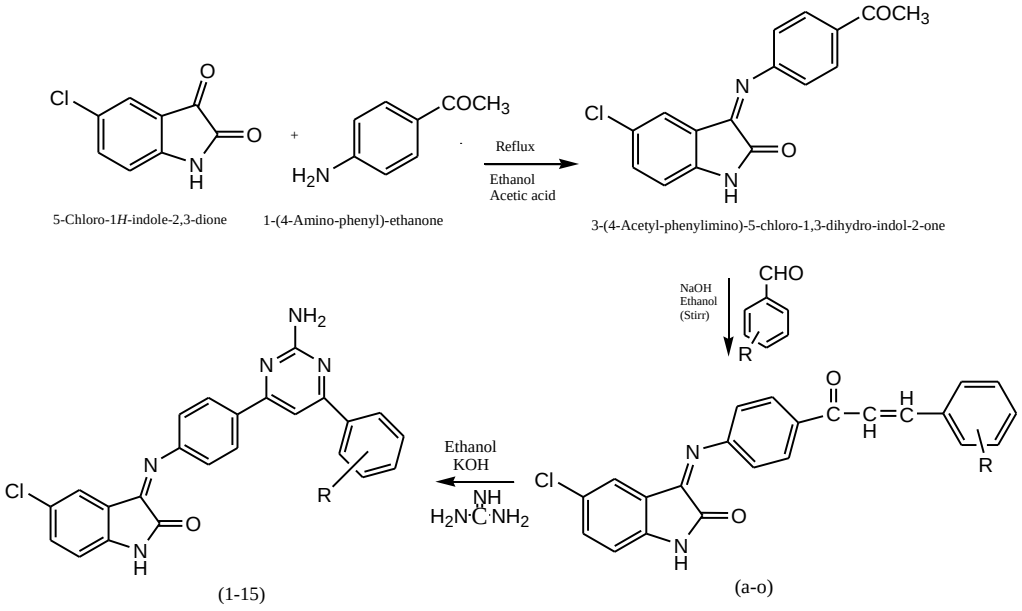
3-(4-Acetyl-phenylimino)-5-chloro-1, 3-dihydro-indol-2-one (a schiff base) was prepared by refluxing 5-chloroisatin (0.01 mol) with 4-aminoacetophenone (0.01 mol) for 1h in ethanol using catalytic amount of acetic acid. The content was put undisturbed for 24h. The Schiff base [3-(4-acetylphenylimino)-5-chloro-1, 3-dihydro indol-2-one] so obtained was filtered and recrystallised using ethanol. [9, 10, 11]

Synthesis of chalcone derivatives of 5-chloroisatin (a-o)

A mixture of 3-(4-acetylphenyl)-5-chloro-1, 3-dihydro-indol-2-one (0.01mol) and substituted aromatic aldehydes (0.01mol) was dissolved in ethanol. A solution of 10 ml of NaOH was added dropwise to the mixture. The mixture was stirred for 2-3h till it become thick. The solid was filtered and recrystallised with ethanol to obtain chalcone (a-o). The progress of reaction was monitored by TLC. [11, 12]

Synthesis of pyrimidine derivative of 5-Chloro-isatin (1-15)

To a solution of chalcone derivatives (1a-1o) (0.01mol) and guanidine hydrochloride (0.01mol) in ethanol, solution of KOH was added. The mixture was reflux for 10 hr. The content was then cooled and poured in crushed ice. The solid was filtered, washed with water and recrystallised using ethanol. [13, 14]



Scheme 1: Scheme for preparation of schiff base, chalcone (a-o) and pyrimidine derivatives (1-15) of 5-chloro isatin

Table shows the value of substituent -R in scheme:

Compounds		-R	Compounds		-R
a	1	-H	i	9	2,4-Cl
b	2	2-OCH_3	j	10	*p*-$N(CH_3)_2$
c	3	4-OCH_3	k	11	2-Cl
d	4	4-OH	l	12	2-Br
e	5	4-Cl	m	13	3-NO_2
f	6	4-Br	n	14	3,4-OCH_3
g	7	2-NO_2	o	15	4-OH,3-OCH_3
h	8	4-NO_2	-----	------	------

Characterization data of compounds of 1-15 (5-chloroisatin derivatives):

1. 3-[4-(2-Amino-6-phenyl-pyrimidin-4-yl)-phenylimino]-5-chloro-1, 3-dihydro-indol-2-one
   IR (KBr) (cm^{-1}): 3446.79 (N-H str , amine), 2929.87 (C-H str, aromatic),1690 (C=O str.), 1651 (C=N str.). ^{1}H NMR (DMSO) (δ-ppm): 7.99 (1H, s, N-H), 6.58-7.66 (13H, m, Ar-H), 4.036 (2H, s, -NH)
2. 3-[4-(2-Amino-6-(2-methoxy-phenyl)-pyrimidin-4-yl)-phenylimino]-5-chloro-1, 3-dihydro-indol-2-one
   IR (KBr) (cm^{-1}): 3415.90 (N-H str , amine), 2921.2 (C-H str, aromatic),1705 (C=O str.), 1660 (C=N str.), 1107.6 (-C-O-CH_3, ether). ^{1}H NMR (DMSO) δ; 7.82(1H, s, N-H), 6.46-7.26 (12H, m, Ar-H), 4.11 (2H, s, -NH), 3.69 (3H, s,-OCH_3).
3. 3-[4-(2-Amino-6-(4-methoxy-phenyl)-pyrimidin-4-yl)-phenylimino]-5-chloro-1, 3-dihydro-indol-2-one
   IR (KBr) (cm^{-1}): 3411.90 (N-H str , amine), 2917.2 (C-H str, aromatic),1708 (C=O str.), 1662(C=N str.), 1109.9 (-C-O-CH_3, ether).^{1}H NMR (DMSO) δ; 7.82(1H, s, N-H), 6.44-7.6 (12H, m, Ar-H), 4.13 (2H, s, -NH), 3.60 (3H, s,-OCH_3).
4. 3-[4-(2-Amino-6-(4-hydroxy-phenyl)-pyrimidin-4-yl)-phenylimino]-5-chloro-1, 3-dihydro-indol-2-one
   IR (KBr) (cm^{-1}): 3436.90 (N-H str , amine), 3241.4(O-H str), 2912.3 (C-H str, aromatic), 1710 (C=O str.), 1662(C=N str. aromatic), 1107.9 (-C-O-CH_3, ether).^{1}H NMR (DMSO) δ; 8.293(1H, s, N-H), 6.56-7.78 (12H, m, Ar-H), 5.03(1H, s, -OH), 4.051 (2H, s,-NH_2)
5. 3-[4-(2-Amino-6-(4-chloro-phenyl)-pyrimidin-4-yl)-phenylimino]-5-chloro-1, 3-dihydro-indol-2-one
   IR (KBr) (cm^{-1}): 3446.79 (N-H str , amine), 2929.87 (C-H str, aromatic),1712.5 (C=O str.), 1655 (C=N str.). ^{1}H NMR (DMSO) δ; 7.99(1H, s, N-H), 6.52-7.76 (12H, m, Ar-H), 3.98 (2H, s, -NH)
6. 3-[4-(2-Amino-6-(4-bromo-phenyl)-pyrimidin-4-yl)-phenylimino]-5-chloro-1, 3-dihydro-indol-2-one
   IR (KBr) (cm^{-1}): 3390.7 (N-H str , amine), 2891.8 (C-H str, aromatic),1722.1 (C=O str.), 1642 (C=N str.). ^{1}H NMR (DMSO) δ; 7.92(1H, s, N-H), 6.62-7.78 (12H, m, Ar-H), 4.051 (2H, s, -NH_2)
7. 3-[4-(2-Amino-6-(2-nitro-phenyl)-pyrimidin-4-yl)-phenylimino]-5-chloro-1, 3-dihydro-indol-2-one
   IR (KBr) (cm^{-1}): 3396.4 (N-H str , amine), 2887.17 (C-H str, aromatic),1715.9 (C=O str.), 1635 (C=N str.). ^{1}H NMR (DMSO) δ; 7.97(1H, s, N-H), 6.51-7.76 (12H, m, Ar-H), 4.23(2H, s, -NH_2)
8. 3-[4-(2-Amino-6-(4-nitro-phenyl)-pyrimidin-4-yl)-phenylimino]-5-chloro-1, 3-dihydro-indol-2-one IR (KBr) (cm^{-1}): 3396.7 (N-H str , amine), 2882.8 (C-H str, aromatic),1716.6 (C=O str.), 1656 (C=N str.).^{1}H NMR (DMSO) δ; 7.93(1H, s, N-H), 6.55-7.86 (12H, m, ArH), 4.23 (2H, s, -NH_2)
9. 3-[4-(2-Amino-6-(2, 4-dichloro-phenyl)-pyrimidin-4-yl)-phenylimino]-5-chloro-1, 3-dihydro-indol-2-one IR (KBr) (cm^{-1}): 3385.5(N-H str , amine), 2886.9 (C-H str, aromatic),1706.1 (C=O str.), 1648.1(C=N str.). ^{1}H NMR (DMSO) δ; 7.92(1H, s, N-H), 6.59-7.74 (11H, m, Ar-H), 4.21 (2H, s, -NH_2).
10. 3-[4-(2-Amino-6-(4- dimethylamino-phenyl)-pyrimidin-4-yl)-phenylimino]-5-chloro-1, 3-dihydro-indol-2-one. IR (KBr) (cm^{-1}): 3422.3 (N-H str , amine), 2903.7 (C-H str, aromatic),1733.6 (C=O str.), 1642.5 (C=N str.). ^{1}H NMR (DMSO) δ; 7.77(1H, s, N-H), 6.65-7.74 (12H, m, Ar-H), 2.81(2H, s, -NH_2)
11. 3-[4-(2-Amino-6-(2-chloro-phenyl)-pyrimidin-4-yl)-phenylimino]-5-chloro-1, 3-dihydro-indol-2-one. IR (KBr) (cm^{-1}): 3396.7 (N-H str , amine), 2882.17 (C-H str, aromatic), 1716.6 (C=O str.), 1647.6 (C=N str.). ^{1}H NMR (DMSO) δ; 7.99 (1H, s, N-H), 6.52-7.76 (12H, m, Ar-H), 3.98 (2H, s, -NH)
12. 3-[4-(2-Amino-6-(4-bromo-phenyl)-pyrimidin-4-yl)-phenylimino]-5-chloro-1, 3-dihydro-indol-2-one IR (KBr) (cm^{-1}): 3423.4 (N-H str , amine), 2910.8 (C-H str, aromatic),1712.5 (C=O str.), 1647.6 (C=N str.) ^{1}H NMR (DMSO) δ; 7.92(1H, s, N-H), 6.64-7.78 (12H, m, Ar-H), 4.051 (2H, s, -NH_2)
13. 3-[4-(2-Amino-6-(3-nitro-phenyl)-pyrimidin-4-yl)-phenylimino]-5-chloro-1, 3-dihydro-indol-2-one IR (KBr) (cm^{-1}): 3398.1 (N-H str , amine), 2890.6 (C-H str, aromatic),1719.1 (C=O str.), 1649.5(C=N str.). ^{1}H NMR (DMSO) δ; 7.97(1H, s, N-H), 6.51-7.76 (12H, m, Ar-H), 4.23(2H, s, -sNH_2)
14. 3-[4-(2-Amino-6-(3, 4-dimethoxy-phenyl)-pyrimidin-4-yl)-phenylimino]-5-chloro-1, 3-dihydro-indol-2-one. IR (KBr) (cm^{-1}): 3403.2

(N-H str, amine), 2881.2 (C-H str, aromatic), 1715.0 (C=O str.), 1660 (C=N str.), 1116.72(-C-O-CH_3, ether). ^{1}H NMR (DMSO) δ; 7.82(1H, s, N-H), 6.44-7.26 (11H, m, Ar-H), 4.11 (2H, s, -NH), 3.65(6H, s,-OCH_3).

15. 3-[4-(2-Amino-6-(4-hydroxy-3-methoxy-phenyl)-pyrimidin-4-yl)-phenylimino]-5-chloro-1, 3-dihydro-indol-2-one. IR (KBr) (cm^{-1}): 3403.2 (N-H str , amine), 3244.8 (O-H str), 2881.2 (C-H str, aromatic), 1715.0 (C=O str.), 1655.7 (C=N str.), 1123. 2 (-C-O-CH_3, str, ether). ^{1}H NMR (DMSO) δ; 7.92(1H, s, N-H), 6.46-7.26 (11H, m, Ar-H), 4.11 (2H, s, -NH), 3.71 (6H, s,-OCH_3).

Evaluation of antimicrobial activity

Antimicrobial activity

The antimicrobial activity of the synthesized compounds was determined by tube dilution method. The antibacterial activities of compounds were tested *in vitro* against *Staphylococcus aureus*, *Bacillus subtilis* (Gram-positive) and *Escherichia coli*, *P. aeuroginosa* (Gram-negative) in double strength nutrient broth- I.P. (bacteria) and *Candida albicans* and *Aspergillus niger* (anti fungal) in sabourand's glucose broth media- I.P. (fungi) [15, 16]. The samples were incubated at 37 °C for 24 h (bacteria), at 25 °C for 7 d (*A. Niger*) and at 37 °C for 48 h (*C. albicans*) and the results were recorded in terms of minimum inhibitory concentration (MIC). Ciprofloxacin and fluconazole were taken as standard drugs for antibacterial and antifungal activity, respectively.

Determination of MIC

MIC of compounds was determined by two fold serial dilution technique. A stock solution of the synthesized compounds/standard drugs (100µg/ml) was prepared in dimethylsulfoxide. Further dilution of test compound and standard drugs were prepared in test medium to provide concentration of 50, 25, 12.5, 6.25, 3.125 and 1.56 µg/ml. To all the test tubes 0.1ml of suspension of bacteria in saline was added and tubes were incubated at required temperatures. MIC was determined by lowest concentration of sample that prevents the development of turbidity. The observed MIC is presented in Table 2.

RESULT AND DISCUSSION

The 5-choloroisatin was refluxed with 4-aminoacetophenone in the presence of ethanol and glacial acetic acid to get Schiff base - 3-(4-acetylphenylimino)-5-chloro-1, 3-dihydro indol-2-one.This Schiff base was further treated with different aldehyde to yield chalcones (a-o)[11, 12]. These chalcones reacted in equimolar quantity with guinidine hydrocholoride to yield different pyrimidine derivatives i.e. - 3-[2-amino-6 (substituted phenyl)-pyrimidin-4-ylimino)-5-chloro-1, 3-dihydro-indol-2-one (1-15) [13, 14] (Scheme 1). Further these synthetic compounds were characterized by their physicochemical (table 1) and spectral means as discussed in experimental. These synthesized compounds were evaluated for their *in vitro* antibacterial and antifungal activity by tube dilution method as per the method of Cappucino and Sherman [15]. Ciprofloxacin was taken as standard drug for antibacterial activity while fluconazole for antifungal activity. The result of antimicrobial activity demonstrate that Compound 5(MIC_{sa} = 0.814 µM, MIC_{bs} 0.814 µM, MIC_{pa} 0.814 µM and MIC_{ec} 0.814 µM) and compound 6 (MIC_{sa} = 0.728 µM, MIC_{bs} 0.728 µM, MIC_{pa} 0.728 µM and MIC_{ec} 0.728 µM) are active against both gram positive and gram negative strain. Compound 8 (MIC_{sa} = 0.791µM, MIC_{bs} 0.791 µM) and compound 12 ((MIC_{sa} = 0.728µM, MIC_{bs} = 0.728µM) are active against *S. aureus* and *B. subtilis*. Besides compound 11 (MIC_{pa} = 0.814µM) and compound 12 (MIC_{ec} = 0.728µM) showed activity against *P. aeuroginosa* and *B. subtilis*. In case of antifungal activity compound 3(MIC_{ca} = 0.822µM), compound 8(MIC_{ca} = 0.791µM) and compound 9 (MIC_{ca} = 0.746µM) are active against *C. albican* while compound 4 (MIC_{ca} = 0.856µM) and compound 8 (MIC_{ca} = 0.791µM) are active against *A. niger*. (Table 2). The structure and activity relationship of compounds shows that most of compounds with electron withdrawing group have antimicrobial activity viz. compound 5 (4-Cl), compound 6 (4-Br), compound 8 (4-NO_2), compound 11(2-Cl), compound 12 (2-Br).

Table 1: Physicochemical properties of synthesized compounds

Compounds	Molecular Formula	Mol. Weight	Melting Point (° C)	Rf value	% Yield
1	$C_{24}H_{16}ClN_5O$	425.87	167-169	0.56	55.0
2	$C_{25}H_{18}ClN_5O_2$	455.90	198-201	0.51	59.0
3	$C_{25}H_{18}ClN_5O_2$	455.90	188-190	0.43	62.0
4	$C_{24}H_{16}ClN_5O_2$	441.87	173-176	0.47	65.0
5	$C_{24}H_{15}Cl_2N_5O$	460.31	176-178	0.49	57.0
6	$C_{24}H_{15}BrClN_5O$	504.77	179-181	0.53	51.0
7	$C_{24}H_{15}ClN_6O_3$	470.87	193-195	0.62	54.0
8	$C_{24}H_{15}ClN_6O_3$	470.87	201-204	0.58	60.0
9	$C_{24}H_{14}Cl_3N_5O$	493.03	183-185	0.40	62.0
10	$C_{26}H_{21}ClN_6O$	468.94	178-180	0.67	65.0
11	$C_{24}H_{15}Cl_2N_5O$	460.31	191-193	0.44	69.0
12	$C_{24}H_{15}ClBrN_5O$	504.77	220-223	0.50	62.0
13	$C_{24}H_{15}ClN_6O_3$	470.87	239-241	0.53	70.0
14	$C_{26}H_{20}ClN_5O_3$	485.13	227-229	0.60	71.0
15	$C_{25}H_{18}ClN_5O_3$	471.90	232-234	0.48	68.0

Solvent front: chloroform: benzene: acetic acid

Table 2: Antimicrobial activity (µM/ml) of synthesized 5-Chloroisatin derivatives

Compounds	Minimum Inhibitory Concentration (MIC) Bacterial strains				Fungal strains	
	Gram Positive		Gram Negative			
	S. aureus	***B. subtilis***	***P. aeruginosa***	***E.coli***	***C. albicans***	***A. niger***
1	1.786	1.786	1.786	1.786	1.786	1.786
2	1.645	1.645	1.645	1.645	1.645	1.645
3	1.645	1.645	1.645	1.645	**0.822**	1.645
4	1.712	1.712	1.712	1.712	1.712	**0.856**
5	**0.814**	**0.814**	**0.814**	**0.814**	1.628	1.628
6	**0.728**	**0.728**	**0.728**	**0.728**	1.460	1.460
7	1.582	1.582	1.582	1.582	1.582	1.582
8	**0.791**	**0.791**	1.495	1.495	**0.791**	**0.791**
9	0.746	0.746	1.590	1.590	**0.746**	1.590
10	1.628	1.628	1.628	1.628	1.628	1.628
11	1.457	1.457	**0.814**	1.457	1.457	1.457
12	**0.728**	**0.728**	1.458	**0.728**	1.458	1.458
13	1.582	1.582	1.582	1.582	1.582	1.582
14	1.524	1.524	1.524	1.524	1.524	1.524
15	1.578	1.578	1.578	1.578	1.578	1.578
Std.	0.471	0.471	0.471	0.471	0.510	0.510

Standard drugs: Ciprofloxacin (antimicrobial) and fluconazole (antifungal)

CONCLUSION

The result of the antimicrobial activity shows that compound 5 and compound 6 have antimicrobial action and are active against both gram positive and gram negative strain. Compound 8 and compound 12 are active against *S.* aureus and *B.* subtilis while compound 11 and 12 shows activity against *P.* aeuroginosa and *E.* coli, respectively. In case of antifungal activity most promising activity was shown by compound 9 against *C. albican* and by compound 8 against *A. niger*.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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