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SYNTHESIS, ANTIINFLAMMATORY AND ANTIBACTERIAL ACTIVITIES OF SOME SUBSTITUTED ISATIN AND ISATIN FUSED WITH 3-SUBSTITUTED 4-AMINO-5-MERCAPTO-1, 2, 4-TRIAZOLES

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ABSTRACT

The synthesis of 1, 2, 4-triazoles have attracted popularity and interest in recent years. The literature studies have shown that more work is not carried out on the fused triazole and isatin derivatives. In this context, we explored the modification in the structures of some synthesized isatin derivatives and prepared some substituted triazole fused with isatin. The present study focuses on new antiinflammatory and antimicrobial properties of 1, 2, 4-triazoles. The aryloxy acetic acid and hydrazine hydrate is useful for the preparation of substituted aryloxy acid hydrazides and extend the preparation of various isatin semicarbazones by reacting with prepared basic isatin.

The structures of compounds were confirmed on the basis of their elemental analysis, IR, ^{1}H NMR and Mass spectral analysis, were screened for antiinflammatory and antibacterial activities. Among all the synthesized compounds, **SI-5** and **1C** have shown potent antibacterial activity where as other compounds exhibited moderate antibacterial activity. The antiinflammatory and antimicrobial activities of title compounds were comparable to that of standard drug ibuprofen and norfloxacin, respectively. The pharmacological studies demonstrate that compounds SI-5, IB, IC and ID can be chosen as lead for additional modification.

Key words: Isatin, Isatin-3-hydrazones, 1, 2, 4-Triazoles, Antiinflammatory, Antibacterial activity.

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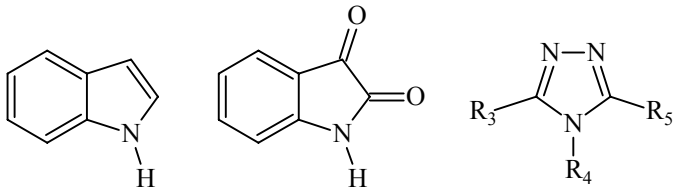
INTRODUCTION

The exploitation of a simple lead with diverse functionalities for the manufacture of heterocycles is a valuable input in the chemistry of heterocycles. Schiff base was used as substrates in the synthesis of various biologically active compounds via cycloaddition, ring closure and replacement reactions[1]. Likewise, Schiff bases derived from various heterocycles have been reported to possess: anticancer[2], anticonvulsant[3], antifungal[4] properties etc. Isatin or 1H-indole-2, 3-Dione, an indole derivative and an oxidation product of indigo dye by nitric acid and chromic acids, was first synthesized by Erdmann[5] and Laurent[6] in 1841 and is available in many plants.

In recent years, indole derivatives have acquired prominent importance due to their broad spectrum of biological activities[7]. Isatin ring consists of pyrrole ring combined with benzene ring. Pyrrole ring is a five member ring containing one nitrogen in the ring system. 1,2,4-triazole ring system consist of 5 membered cyclo-penten ring in which 1,2,4 position of the ring carbon is substituted by nitrogen (Fig. 1).

The synthetic versatility of isatin and triazole derivatives has led to the wide utilize of these compounds in organic synthesis.

Various substituted isatin derivatives were reported as antibacterial[8], antipox virus agents[9], antifungal & antiviral[10], antioxidant & cytotoxic agents[11]. On the other side 1,2,4-triazoles derivatives are also proven by being a utmost lead for different pharmacological activity[12]. In view of the above facts and in continuation with search for better biologically active molecules[13] has encouraged us to produce a few molecules of isatin with phenoxyacetohydrazide and substituted triazoles fused with isatin and carry out their preliminary antiinflammatory and antibacterial activity. In this paper, we report the synthesis and spectral studies of a series of novel isatin derivatives, substituted 1,2,4-triazoles fused with isatin and their antiinflammatory and antibacterial activity.



Indole Isatin 1,2,4-Triazole

Fig. 1: Basic structures of indole, isatin and 1,2,4-triazole

EXPERIMENTAL

Chemistry

All the chemicals used in this study were of synthetic grade and commercially procured from SD Fine Chemicals, Mumbai. Melting points (mp) were determined in open capillary method and were uncorrected. The purity of the compound was checked on silica Gel TLC plates. IR spectra were recorded on FT-IR8400S, Fourier Transform (Shimadzu) infrared spectrophotometer using the KBr disc method. The proton magnetic resonance spectra (^{1}H NMR) were recorded on Perkin-Elmer spectrophotometer-300 MHz. in DMSO-*d6,* chemical shifts are reported as parts per million (ppm) using TMS as an internal standard.

The isatin (1H-indole-2, 3-dione) and various substituted phenoxy acetic acid and their hydrazide were prepared by following the procedure reported earlier[14].

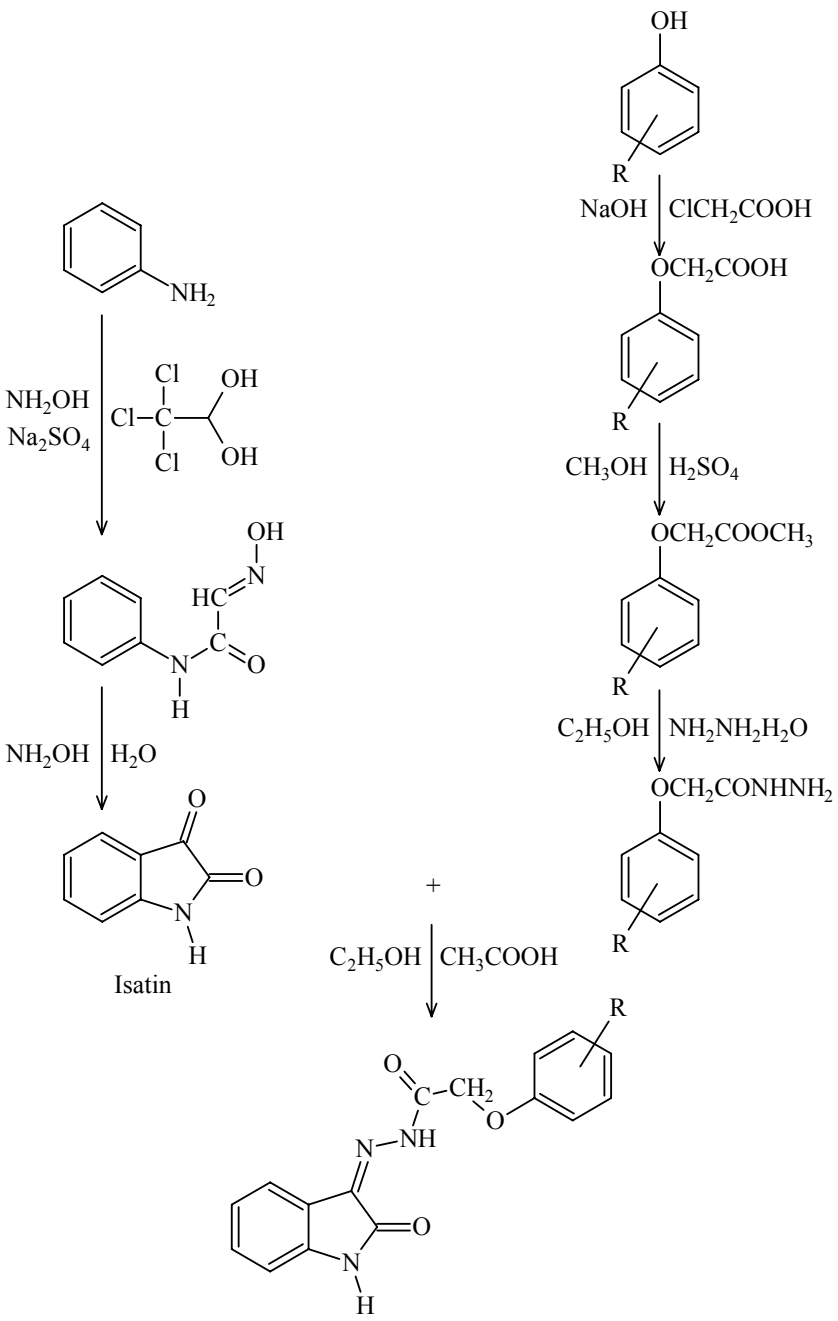
Synthesis of isatin-3-hydrazones (1-5)[15] (Scheme 1)

An equimolar quantity of isatin (0.003 M) and substituted phenoxy acetyl hydrazide (0.003 M) was dissolved in 10 mL of warm ethanol (95%) containing a few drops of glacial acetic acid. The mixture was refluxed for 30 min and then cooled in ice. The resultant solid was filtered, dried and recrystallized from ethanol (95%).

(Z)-2-(p-tolyloxy)-N'-(2-oxoindolin-3-ylidene) acetohydrazide (**SI-1**): Yield 86%, m.p. 203-205ºC; TLC solvent chloroform: ethanol (9:1), R_f value: 0.44 IR υ (cm^{-1}): 3290.40 (NH), 1702.00 (C=O), 2912.00 (aliphatic-H), 3106.70 (aromatic-H). ^{1}H-NMR δ (ppm): 7.64 (1H,- NH, 2º amine), 7.59 (1H, -NH, hydrazide), 6.79-7.40 (8H,-Ar-H), 4.90 (2H-CH_2), 2.35 (3H-CH_3).

(Z)-2-(m-tolyloxy)-N'-(2-oxoindolin-3-ylidene) acetohydrazide (**SI-2**): Yield 75%, mp. 221-223ºC; TLC solvent chloroform: ethanol (9:1), R_f value: 0.78 IR υ (cm^{-1}): 3378.12 (NH), 1666.26 (C=O), 2917.77 (aliphatic-H), 3078.80 (aromatic-H). ^{1}H-NMR δ (ppm): 7.63 (1H,- NH, 2º amine), 7.58 (1H, -NH, hydrazide), 6.10-7.32 (8H, -Ar-H), 4.58 (2H-CH_2), 2.29 (3H-CH_3).

(Z)-2-(o-tolyloxy)-N'-(2-oxoindolin-3-ylidene) acetohydrazide (**SI-3**): Yield 80%, mp. 170-172ºC; TLC solvent chloroform: ethanol (9:1), R_f value: 0.31 IR υ (cm^{-1}): 3396.02 (NH), 1675.56 (C=O), 2867.50 (aliphatic-H), 3050.20 (aromatic-H). ^{1}H-NMR δ (ppm): 7.62 (1H,- NH, 2º amine), 7.57 (1H, - NH, hydrazide), 6.81-7.17 (8H, -Ar-H), 4.54 (2H-CH_2), 2.23 (3H-CH_3).



R = 2-CH_3, 3-CH_3, 4-CH_3, C_6H_5, 4-Cl

Isatin-*3*-hydrazones (SI 1-5)

Scheme 1: Synthesis of various isatin-*3*-hydrazones

(Z)-2-(naphthalen-1-yloxy)-N'-(2-oxoindolin-3-ylidene) acetohydrazide (**SI-4**): Yield 84%, mp. 254-257ºC; TLC solvent chloroform: ethanol (9:1), R_f value: 0.42 IR υ (cm^{-1}): 3320.40 (NH), 1695.36 (C=O), 2935.23 (aliphatic-H), 3060.80 (aromatic-H). ^{1}H-NMR δ (ppm): 8.31 (1H,- NH, 2º amine), 7.31 (1H, - NH, hydrazide), 7.42-7.91 (11H, -Ar-H), 3.35 (2H-CH_2).

(Z)-2-(4-chlorophenoxy)-N'-(2-oxoindolin-3-ylidene) acetohydrazide (**SI-5**): Yield 72%, mp. 185-187ºC; TLC solvent chloroform: ethanol (9:1), R_f value: 0.59 IR υ (cm^{-1}): 3380.40 (NH), 1666.43 (C=O), 2837.26 (aliphatic-H), 3120.63 (aromatic-H). ^{1}H-NMR δ (ppm): 8.31 (1H, -NH, 2º amine), 7.08 (1H, -NH, hydrazide), 7.32-7.64 (8H, -Ar-H), 3.35 (2H-CH_2). Mass m/z 329.06

Synthesis of 4-amino-3-substituted-5-mercapto 1,2,4-triazoles[16]: (SS 2A-2D) (Scheme 2)

Equimolar concentrations of potassium dithiocarbazenates and hydrazine hydrate in 5 mL of water were refluxed for 6-7 hr. After the heating for stipulated time, the resultant mixture was heated up to solvent evaporation. The crude solid obtained, triazole, was recrystallized from ethanol. The different substituted product obtained was confirmed by specific chemical test, TLC value, m.p. with reference values.

Synthesis of 1,2,4-triazoles derivatives fused with isatin[17]: (1A-1D)

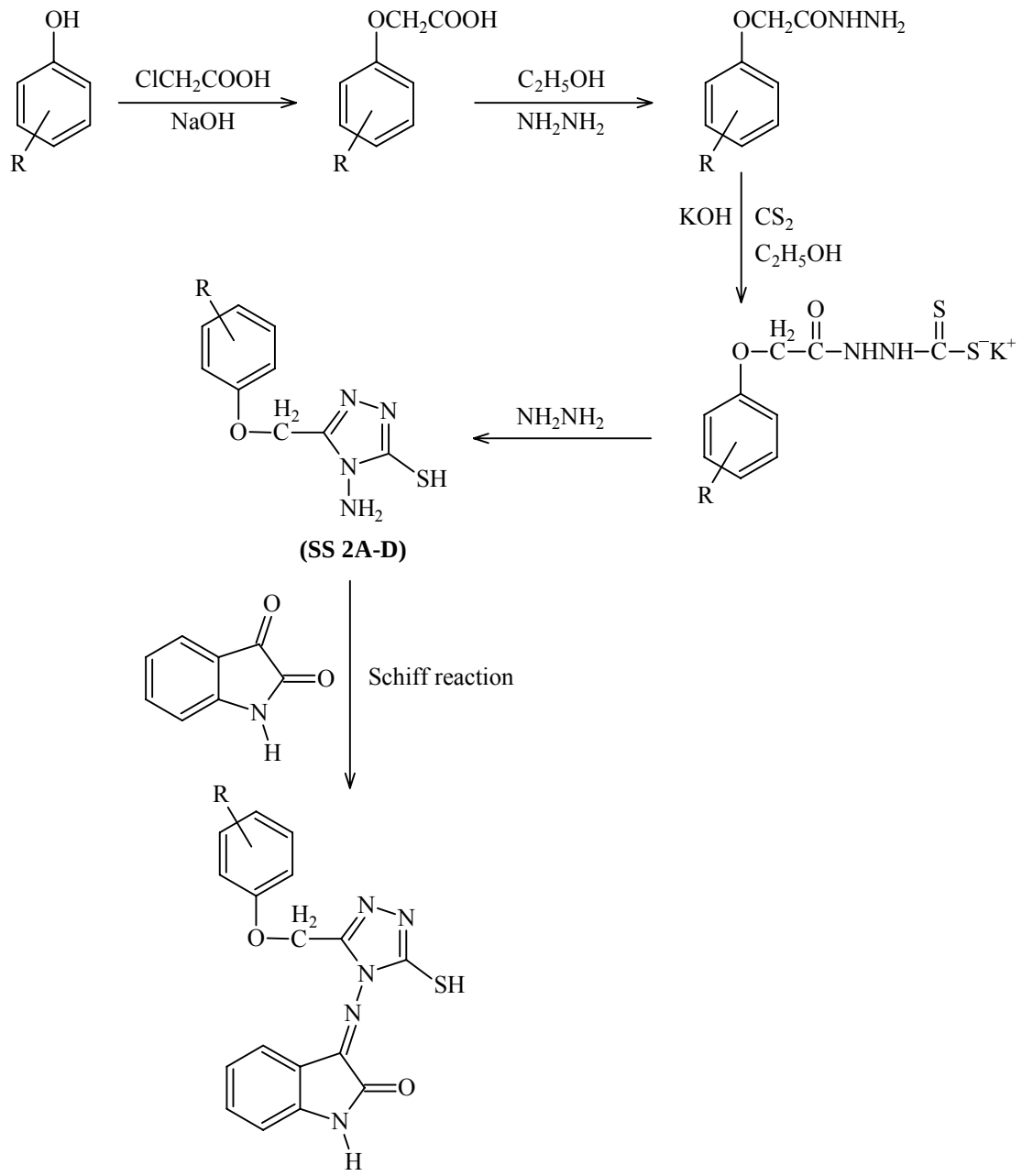
Equimolar quantities of (0.1 M) of isatin and the corresponding triazoles were dissolved in 50 mL of warm ethanol containing 0.5 mL glacial acetic acid and the reaction mixture was refluxed for 4 hr and kept at room temperature for overnight. The solid was washed with dilute ethanol, dried and recrystallized from ethanol: water (1:2).

3-(3-((m-tolyloxy) methyl)-5-mercapto-4H-1, 2, 4-triazol-4-ylimino) indolin-2-one (1A)

Yield 80%, mp. 220-221ºC; TLC solvent chloroform: ethanol (9:1), R_f value: 0.58 IR υ (cm^{-1}): 3443.62 (NH), 1729.59 (C=O), 2920.20 (Aliphatic-H), 3030.10 (Aromatic-H). ^{1}H-NMR δ (ppm): 8.372 (1H, - NH, 2º amine), 4.084 (1H, - SH), 2.309 (3H - CH_3), 6.831-8.131 (8H,-Ar-H), 5.380 (2H-CH_2). Mass m/z 365.1

3-(3-(phenoxy methyl)-5-mercapto-4H-1, 2, 4-triazol-4-ylimino) indolin-2-one (1B)

Yield 68%, mp. 218-220ºC; TLC solvent chloroform: ethanol (9:1), R_f value: 0.56 IR υ (cm^{-1}): 3446.20 (NH), 1728.85 (C=O), 2837.26 (Aliphatic-H), 3195.30 (Aromatic-H). ^{1}H-NMR δ (ppm): 7.966 (1H, - NH, 2º amine), 3.709 (1H, - SH), 6.905-7.638 (9H,-Ar-H), 5.380 (2H-CH_2). Mass m/z 351.1.



Schiff bases (1A-1D)

Scheme 2: Synthesis of various substituted triazoles and triazoles fused with isatin

3-(3-((4-chlorophenoxy) methyl)-5-mercapto-4H-1,2,4-triazol-4-ylimino)indolin-2-one (1C)

Yield 65%, mp. 279-280ºC; TLC solvent chloroform: ethanol (9:1), R_f value: 0.68 IR υ (cm^{-1}): 3267.18 (NH), 1699.51 (C=O), 2904.84 (aliphatic-H), 3098.67 (aromatic-H). ^{1}H-NMR δ (ppm): 7.847 (1H, -NH, 2º amine), 3.501 (1H, -SH), 6.885-7.581 (8H, -Ar-H), 4.751 (2H-CH_2). Mass m/z 385.03

3-(3-((o-tolyloxy) methyl)-5-mercapto-4H-1,2,4-triazol-4-ylimino)indolin-2-one (1D)

Yield 66%, mp. 240-241ºC; TLC solvent chloroform: ethanol (9:1), R_f value: 0.59 IR υ (cm^{-1}): 3262.87 (NH), 1725.88 (C=O), 2919.30 (Aliphatic-H), 3020.10 (Aromatic-H). ^{1}H-NMR δ (ppm): 8.414 (1H, -NH, 2º amine), 4.184 (1H, -SH), 2.586 (3H -CH_3), 6.890-7.619 (8H,-Ar-H), 5.280 (2H-CH_2).

Pharmacology

Animals and instruments

Adult Wister albino rats of either sex weighing between 200 to 250 g were used for the study. The selected animals were maintained by pelleted diet, water ad libitum and kept in 12/12 hr light/dark cycle. All experimental procedures were carried out in strict accordance with the guidelines prescribed by the committee for the purpose of control and supervisions on experimentation on animals (CPCSEA). Edema was produced using IV lambada carrageenan, foot volumes were measured in a plethysmograph by the water displacement method.

Antiinflammatory activity (Carrageenan-induced rat hind paw edema)

The antiinflammatory activity study was carried out by the method of Winter et al.[18], modified by Srimal and Dhawan[19]. The animals were divided into eight groups of six animals each. One group served as a standard (Ibuprofen 200 mg/Kg), another group served as control (1% w/v CMC) and the rest of the groups were used for the test drugs. The rats were dosed with test drugs orally at 200 mg/Kg body weight including the reference standard. Test compounds and standard drug were suspended in 0.5% w/v of sodium carboxyl methyl cellulose, which was used as a vehicle for the control group. A solution of 1% w/v of carrageenan was used as an inflammatory agent. Food was withdrawn overnight with adequate water before the experiment. The drugs are dosed orally with the help of oral catheter. After 30 min drug administration, the carrageenan solution (1% w/v) was injected in the sub plenter region of the left hind paw of control as well as standard and test groups. After the administration of carrageenan solution the paw volume of control, standard and

test groups were measured with Plethysmograph by the water displacement method at 1 hr, 2 hr, 3 hr and 4 hr time interval. The percentage of inhibition was calculated by using the formula: % inhibition = 200 x (1-Vt/Vc) where, Vc = Edema volume of control and Vt = Edema volume of tests. The results are presented in Table 1.

Table 1: Antiinflammatory activity of isatin-3-hydrazones & 3-(substituted-triazolo) iminoisatin

Treatmen 200 mg/Kg	Paw volume in mm, Mean ± SEM (% inhibition of paw edema)							
	1 hr	% Red	2 hr	% Red	3 hr	% Red	4 hr	% Red
Crgn	1.85 ± 0.009	NA	1.97 ± 0.043	NA	2.25 ± 0.054	NA	2.26 ± 0.053	NA
SI-1	0.805 ± 0.002**	56.48	0.802 ± 0.003**	59.28	0.782 ± 0.006**	65.24	0.745 ± 0.009**	67.03
SI-2	0.852 ± 0.005**	53.94	0.905 ± 0.002**	54.06	0.889 ± 0.025**	60.48	0.860 ± 0.005**	61.94
SI-3	0.795 ± 0.004**	57.02	0.842 ± 0.074**	57.25	0.853 ± 0.005**	62.08	0.846 ± 0.006**	62.56
SI-4	0.758 ± 0.013**	59.02	0.806 ± 0.013**	59.08	0.832 ± 0.009**	63.02	0.823 ± 0.003**	63.58
SI-5	0.694 ± 0.015**	62.48	0.742 ± 0.014**	62.33	0.746 ± 0.014**	66.84	0.702 ± 0.006**	68.93
1A	0.751 ± 0.013**	59.40	0.795 ± 0.021**	59.64	0.815 ± 0.006**	63.77	0.806 ± 0.012**	64.33
1B	0.693 ± 0.008**	62.54	0.735 ± 0.009**	62.69	0.733 ± 0.009**	67.42	0.701 ± 0.007**	68.98
1C	0.698 ± 0.009**	62.27	0.737 ± 0.008**	62.58	0.735 ± 0.012**	67.33	0.705 ± 0.009**	68.80
1D	0.697 ± 0.009**	62.32	0.741 ± 0.009**	62.38	0.737 ± 0.008**	67.24	0.702 ± 0.009**	68.93
Ibuprofen	0.692 ± 0.010**	62.59	0.736 ± 0.010**	62.63	0.690 ± 0.012**	69.33	0.575 ± 0.012**	74.55

Crgn- Carrageenan, n = 6 animals in each group; ** $p < 0.01$., * $p < 0.05$ when compared with control
SEM-standard Error of Mean

Antibacterial activity[20]

Standard nutrient agar medium

Meat extract (Bacteriological)	1.0%
Peptone	…..1.0%
Sodium chloride	…..0.5%
Agar	…..2.0%
Water	100 mL

Meat extract was taken and made up the volume to 100 mL with water and to this were added weighed quantities of peptone, salt and agar. The contents were dissolved by heating and the mixture was filtered and the pH was adjusted to 7.5. The medium was sterilized by autoclaving at 121ºC for 15 mins, cooled to 45ºC and then poured in 20 mL quantities to petri dishes. A loopful of an overnight broth culture was spread evenly over the whole part with a sterile cotton-wool swab.

The culture plates were dried in an incubator with the lid until its surface was free from visible moisture without further delay. The known concentration of the drug applied with adequate spacing to the surface of the culture plates with sterile fine pointed forceps and pressed gently to ensure full contact with the medium.

It was then transferred to the incubator for 24 hr at 37ºC. At the end of 24 hr, the diameters of the zone of inhibition produced were measured. The results are presented in Table 2.

RESULTS AND DISCUSSION

Chemistry

In the present study **(Scheme 1)**, the various substituted aryloxy acetic acid was prepared by reacting substituted phenol with sodium hydroxide followed by addition of chloroacetic acid. Further, the prepared substituted aryloxy acetic acid was treated with methanol and few drops of H_2SO_4 to get the corresponding ester, without separating the ester, the reaction mixture was further reacted with hydrazine hydrate to get the various substituted aryloxy acid hydrazides. On the other side, aniline reacted with chloral hydrate, in the presence of sodium sulphate to give the isonitrosoacetanilide. Later, isonitrosoacetanilide was treated with concentrated sulphuric acid to get isatin. Finally, the various substituted hydrazides were treated with isatin to get the substitute isatin 3-hydrazones.

Table 2: Antibacterial activity of isatin-3-hydrazones & 3-(substituted-triazolo) iminoisatin

Code No.	Dose (µg/mL)	Zone of inhibition (mm)		
		P. Vulgaris	*S. aureus*	*B. subtilus*
SI-1	100	13.5	12.5	14.5
SI-2		14.5	13.0	12.7
SI-3		12.0	11.0	13.0
SI-4		14.2	11.7	13.2
SI-5		20.0	21.5	22.0
1A		18.3	17.4	17.6
1B		13.9	12.5	11.0
1C		20.6	21.0	20.3
1D		16.3	15.9	16.8
Standard (Norfloxacin)	100	24.0	25.3	25.0

In **Scheme 2**, various 4-amino-3-substituted-5-mercapto 1,2,4-triazoles have been prepared from potassium salt dithiocarbazinates. The presence of free amino group at 4th position is a key functional group for further modification. Finally, the 4-amino-3-substituted-5-mercapto 1,2,4-triazoles were reacted with prepared isatin to get desired fused product.

Antiinflammatory activity

In vivo antiinflammatory evaluation of synthesized compounds is summarized in Table 1. Compounds **SI-1**, **SI-5**, **IB**, **IC** and **ID** were active antiinflammatory agents (67-68% inhibition) after 4 hr in comparison with control and their activity was comparable to ibuprofen. As shown in Table 1, there were no any significant difference between the activity of the compounds **SI-1**, **SI-5**, **IB**, **IC** and **ID**. The most active derivatives were found to be **SI-5**, **IB**, **IC** and **ID**. In contrast to antiinflammatory activity, the compounds having a halogen substitution on the phenyl ring showed better antiinflammatory activity.

Antibacterial activity

Antibacterial evaluation of synthesized compounds is summarized in Table 2. The antibacterial activity was screened by the Agar diffusion method by taking various bacteria (*Bacillus subtilus*, *Staphylococcus aureus*, and *Protease vulgaris*) and norfloxacin as

standard. Compounds **SI-5** and **IC** showed potent antibacterial activity when compared to standard Norfloxacin.

All the newly synthesized compounds were confirmed by their IR, ^{1}H NMR spectral data, and were screened for antiinflammatory and antimicrobial activity. The IR spectroscopic data showed that the various functional groups were present in final compounds and, ^{1}H NMR data further confirmed the presence of the number of hydrogen atoms in final compounds. The antibacterial activity was screened by the Agar diffusion method by taking various bacteria (*Bacillus subtilus*, *Staphylococcus aureus*, and *Protease vulgaris*) and norfloxacin used as standard. All the synthesized compounds showed moderate activity but compounds **SI-5** and **1C** showed very good antibacterial activity.

The antiinflammatory activity carried out by paw edema method showed that compounds **SI-1**, **SI-5**, **IB**, **IC** and **ID** comparatively had better antiinflammatory activity than the other synthesized compounds. The pharmacological studies showed that compounds **SI-5**, **IB**, **IC** and **ID** can be selected as lead for additional modification.

Statistical analysis

Values are expressed as mean ± SEM and data were analyzed by one way ANOVA followed by Dunnet's test using Graph pad instant software. P < value was considered as significant.

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