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# Synthesis and Screening of Some New Isatin Containing Thiazole Derivatives for Antimicrobial Activity

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**Abstract**

In the present work, a series of new N'-[{5-(4-aryl) 1,3-thiazol-2yl}carbohydrazide-methyl]-3(4-arylimino) indol-2-one analogs (**5a-g**) had heen synthesized from 3-(4-arylimino)-2-oxo-1-indole-acetylthiosemicarbazide (**4a-g**) in ethanol, in the presence of phenacyl bromide or substituted phenacyl bromides. The compound **5e** was characterized by its elemental analysis, IR, $^1$HNMR and Mass Spectroscopy. The synthesized compounds (**5a-g**) were evaluated for *in vitro* antibacterial activity and antifungal activity against various strains of bacteria and fungi.

***Keywords**: Isatin, 1,3 Thiazole, Antibacterial, Antifungal.*

## Introduction

Over the past several years, the emergence of microorganisms resistant to nearly all the class of antimicrobial agents has become a serious public health concern. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. The health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses not only because many of them produce toxic reactions but also due to emergence of drug resistant bacteria.Literature survey revealed that isatin possess diverse biological activities such as antibacterial [1], antifungal [2], antiviral [3], anti-mycobacterial [4], anti cancer [5], anti-inflammatory [6] and anticonvulsant [7]. Thiazole moiety also display diverse pharmacological activities like antimicrobial [8], anti-inflammatory [9], anti-viral[10], anti-psychotic[11], antiarrhythmic and anticoagulant[12] activities. The study

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of above pharmacophores reveals that the combination of these two entities may result in increased antimicrobial activity.

In the view of biological importance of these two moieties, it was planned to synthesize a new series of Isatin containing 1,3-thiazole derivatives and to evaluate the new compounds for their anti-microbial activity.

## Experimental

All solvents, reagents and catalysts were of analytical grade and used without further purification. The melting points were determined by open capillary method and were uncorrected. The purity of compounds was confirmed by thin layer chromatography using Silica Gel glass plates as the stationary phase and with suitable mobile phase. IR spectra were recorded using KBr on FTIR-8400S Shimadzu. $^1$H NMR spectra were recorded on Joel-FT-NMR-300MHz using DMSO-$d_6$ as solvents and TMS as internal standard. Mass spectra were recorded on Varian500 LC-MS.

Synthetic route of N'-[{5-(4-aryl)1,3-thiazol-2yl}carbohydrazide-methyl]-3 phenylimino indol-2-one analogs is given in Figure 1.

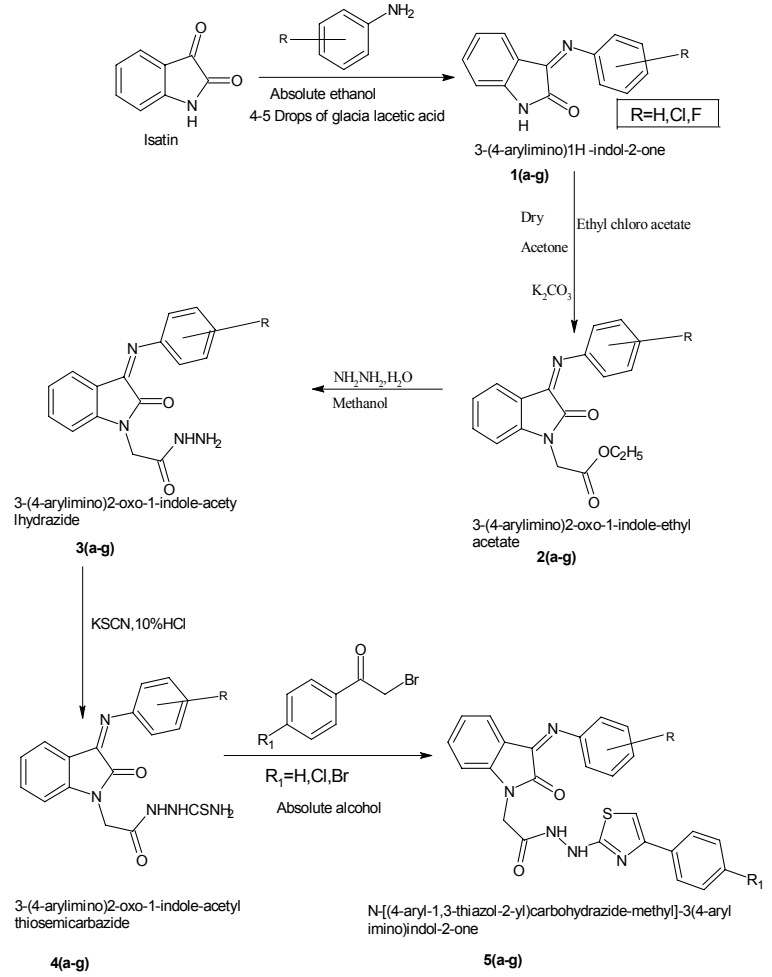



**Figure 1.** Synthesis of N'-[{5-(4-aryl)1,3-thiazol-2yl)carbohydrazide-methyl]-3phenylimino indol-2-ones.

*Synthesis of 3-phenylimino-1H-indol-2-one (1) [14]*

A mixture of indole-2,3-dione (1.47g, 0.01mol) and aniline (0.91ml, 0.01mol) in absolute ethanol (20ml) was refluxed for half an hour in the presence of 2-3 drops of glacial acetic acid. On cooling, crystals which was separated out, filtered and recrystallised from ethanol to give 1.05 g. Yield: 71.6 %; m. p.: 208-212 o C (210 o C) ; IR (KBr): 3416, 1614, 1728 $cm^{-1}$.

*Synthesis of 3-phenylimino-2-oxo-1-indole-ethylacetate (2) [15]*

A mixture of 3-phenylimino-1*H*-indol-2,3-dione **(1)** (2.21 g, 0.01mol), ethyl chloroacetate (1.22 ml, 0.01mol) and potassium carbonate (2.2g, 0.015mol) in dry acetone was refluxed for 20 h. The reaction mixture was poured onto crushed ice. The solid separated was filtered, washed with water and recrystallised from methanol to give 1.53 g. Yield: 64%; m. p.: 105-106$^{o}$ C; IR (KBr): 1330, 1614, 1724, 1716 $cm^{-1}$.

*Synthesis of 3-phenylimino-2-oxo-1-indole-acetylhydrazide (3) [16]*

A mixture of 3-phenylimino-2-oxo-1-indole-ethylacetate **(2)**( 3.07g 0.01mol) and hydrazine hydrate (99%, 0.5ml, 0.01mol) in methanol (20mL) were refluxed for about 5 h on steam bath. After completion of reaction (monitored by TLC), the mixture was cooled and the resulting solid was filtered, dried and recrystallised from ethanol to give 2.02 g. Yield: 62%; m. p.: 220-221$^{o}$ C; IR (KBr): 1330 , 3398 , 1614, 1743, 1606 $cm^{-1}$.

*Synthesis of 3-phenylimino-2-oxo-1-indole-acetylthiosemicarbazide(4) [10]*

A mixture of 3-phenylimino-2-oxo-1-indole-acetylhydrazide (2.93g,0.01 mol) (3)was refluxed with 10ml of 10% HCl and potassium thiocyanate (0.015 mol) for 4 h. The reaction mixture was allowed to cool to room temperature. The solid formed was collected by filtration, washed with water, dried and then recrystallized from ethanol. Yield: 67%; m. p.: 225-2260 C; IR (KBr): v ($cm^{-1}$) 1330 (>N-$CH_2$), 3414(-$NH_2$), 1614(C=N), 1739(>C=O), 1693 (>C=O),1238(>C=S).$^1$H NMR (δ-ppm): 4.6 (s, 2H, N-$CH_2$), 7.10-7.65 (m, 9H, Ar-H),8 (s,2H,>CS-$NH_2$),8.8(s,H, CS-NH), 9.2 (s, H, CO-NH).

Mass (m/z): 354 (M+1), 264(M- $C_6H_5N^+$ ) 100%, 133 ($C_3H_6N_3OS^+$), 131 ($C_8H_6NO^+$), 91 ($C_6H_5N$) ), 77 ($C_6H_5^+$)

Mass fragmentation patern of 3-phenylimino-2-oxo-1-indole-acetylthiosemicarbazide was given in Figure 2.

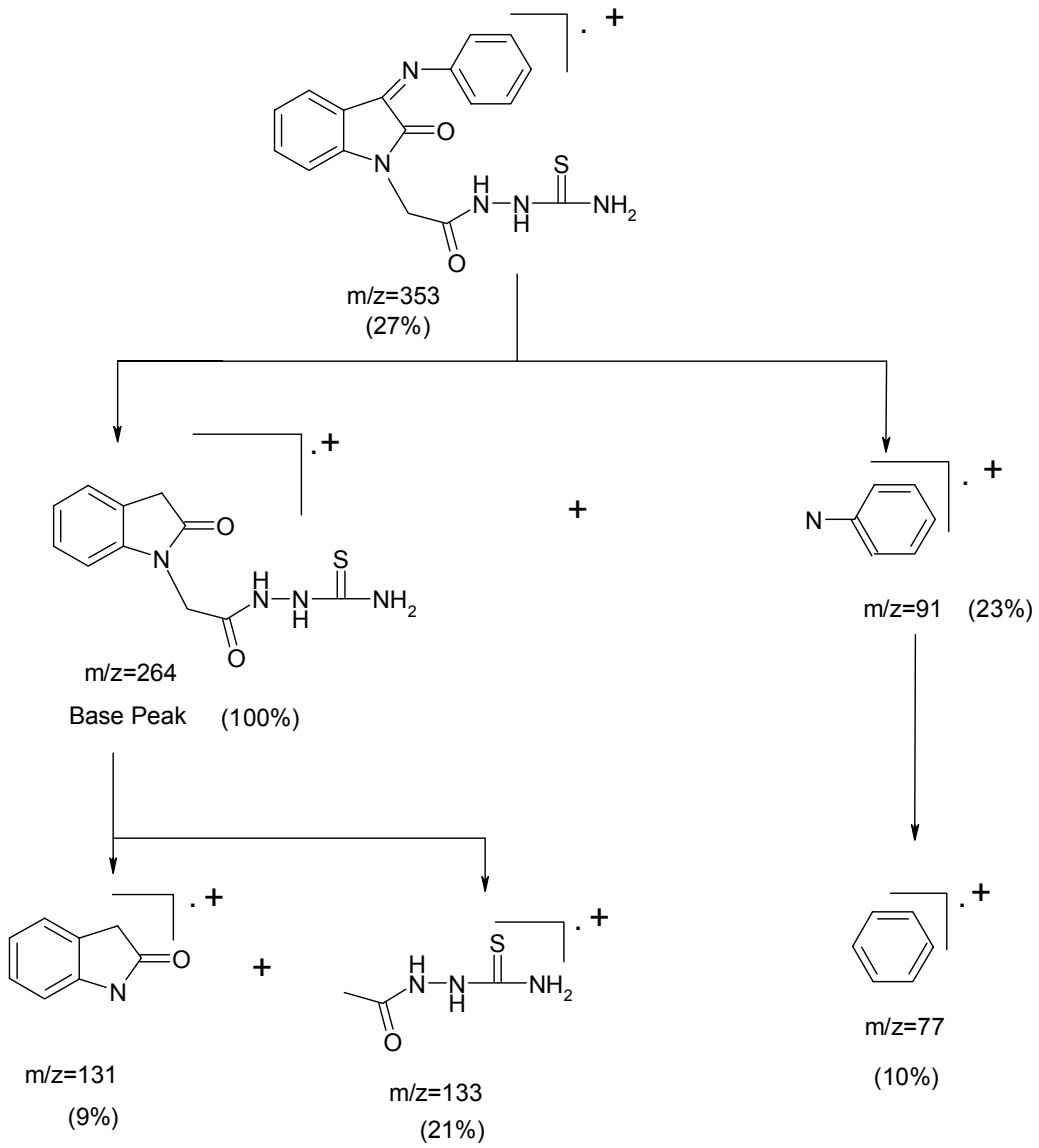



**Figure 2**: Mass fragmentation pattern of 3-phenylimino-2-oxo-1-indole acetylthiosemicarbazide(4)

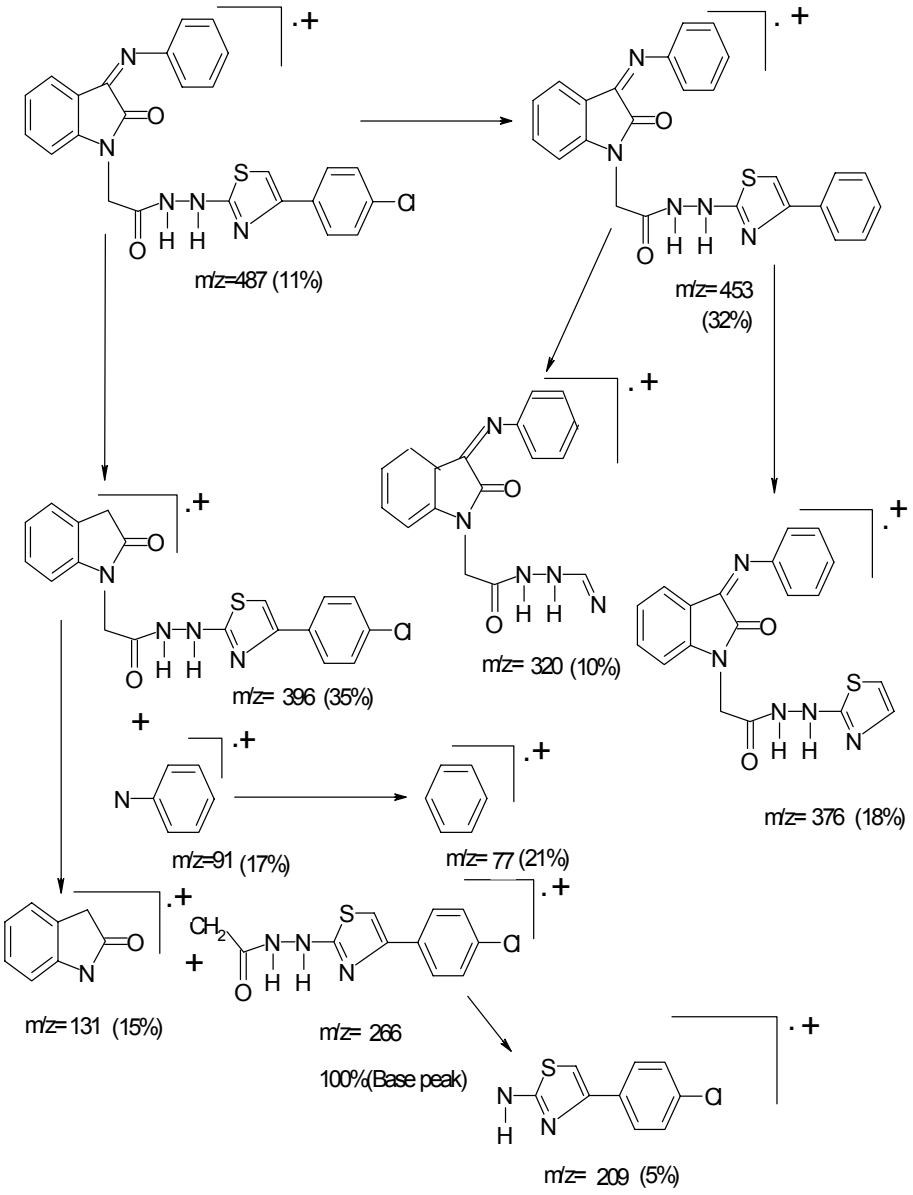



**Figure 3.** Fragmentation pattern of synthesized compound (5e).

*Synthesis of N'-[{5-(4-chlorophenyl)1,3-thiazol-2yl}carbohydrazide-methyl]-3 phenylimino indol-2-one [10]*

A mixture of 3-phenylimino-2-oxo-1-indole-acetylthiosemicarbazide (3.53 g,0.01 mol) (4) and chloro phenacyl bromide (0.01 mol)was refluxed in ethanol (50 mL) for 8–10 h. The solid that separated out in reaction mixture, was filtered off and crystallized from DMF or directly purified with column chromatography. Yield: 82%; m. p.:230-234 (231) $^{O}$C; IR (KBr): 3171.08,1744.67,1670.41,1612.54,1341.19 cm$^{-1}$.

*Spectral data of representative compound*

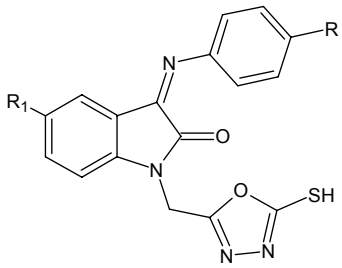
N'-[{5-(4-chlorophenyl)1,3-thiazol-2yl}

carbohydrazide-methyl]-3 phenylimino indol-2-one (**5e**):

IR (KBr): 1060, 1342, 1614, 1662, 2582 $cm^{-1}$ ; $^1$HNMR (DMSO – $d_6$) δ (ppm): 4.6 (s, 2H, N-$CH_2$), 7.1-8.00 (m, 13H, Ar-H),6.8 (thiazole CH) ,9.62(s,H, CS-NH), 10.43 (s, H, CO-NH).); MS (m/z): 488.9 [$M^+$+1],487,453[M-Cl], 397.9 ($C_{19}H_{14}ClN_4O_2S+$), 376 [M-$C_6H5Cl$], 266.7($C_{11}H_9ClN_3OS^+$) (100%), 209($C_9H_6ClN_2S^+$), 131($C_8H_6NO^+$), 91($C_6H_5N^+$), 77($C_6H_5^+$).

Other Isatin derivatives were prepared similarly. The physical constants are recorded in Table 1.

**Table 1**. Physical data of 5-substituted-3-(4-arylimino)-1-[5-mercapto(1, 3, 4 – oxadiazolyl)]-methyl-indol-2-one analogs.




| Sample code | R | $R_1$ | Molecular formula | Mol. Wt. (gm) | Melting point ($^O$C) | $R_f$* |
|---|---|---|---|---|---|---|
| 5a | H | H | $C_{25}H_{19}N_5O_2S$ | 453.5 | 250 $^O$C | 0.78 |
| 5b | H | Br | $C_{25}H_{18}N_5O_2SBr$ | 532.4 | 258 $^O$C | 0.80 |
| 5c | Cl | Br | $C_{25}H_{17}N_5O_2SClBr$ | 566.8 | 221 $^O$C | 0.87 |
| 5d | Cl | H | $C_{25}H_{18}N_5O_2S$ Cl | 487.9 | 263 $^O$C | 0.78 |
| 5e | H | Cl | $C_{25}H_{18}N_5O_2S$ Cl | 487.9 | 231 $^O$C | 0.86 |
| 5f | F | H | $C_{25}H_{18}N_5O_2SF$ | 471.6 | 226 $^O$C | 0.81 |
| 5g | F | Cl | $C_{25}H_{17}N_5O_2S$ ClF | 505.9 | 241 $^O$C | 0.83 |

*Mobile phase – Toluene:Acetone:Glacial acetic acid(5:0.5:3drops)v/v/v

*Biological Evaluation [17]*

*In vitro antibacterial & antifungal activity*

All the compounds were evaluated for their in-vitro anti-bacterial activity against *S. aureus NCIM 2079, B. subtilis ATCC 6633, E. coli ATCC M 200, P. vulgaris NCIM 2813* and anti-fungal against *C. albicans NCIM 3471, A. niger NCIM 545* standard strains using disc diffusion method [21] which were procured from the Department of Microbiology, R.C. Patel College of Arts, Science and Commerce, Shirpur, Dist Dhule, India. Each disc contains 200 µg/ml of the tested compounds. Paper Disc Diffusion method was performed using Mueller-Hinton (Hi-Media) Agar (anti-bacterial) and Potato Dextrose (Hi-Media)

Agar (anti-fungal). Suspensions of each microorganism were prepared to contain approximately 106 colony forming units (cfu)/ ml and applied to plates. The surface of the medium was allowed to dry. The 200 µg/ml (in DMSO) compound impregnated discs were applied to the surface of inoculated plates. The Petri plates were incubated at 37 °C for antibacterial activity, and at 26 °C overnight approx. 48–72 hr for anti-fungal activity. The Petri plates were examined for anti-bacterial activity after 18–24 h of incubation (Table 2).

**Table 2.** Biological activities of the compounds 5(a-g) at 200 µg/ml.

| Com. No. | R | $R_1$ | Zone of inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Antimicrobial activity (200 µg/ml) | | | | Antifungal activity (200 µg/ml) | |
| | | | *S. aureus* | *B. subtilis* | *E. coli* | *P. vulgaris* | *C. albicans* | *A. niger* |
| 5a | H | H | NS | 7.1 | NS | NS | NS | NS |
| 5b | H | Br | NS | 7.45 | 8.75 | NS | 7.14 | 7.11 |
| 5c | Cl | Br | 6.82 | 9.64 | 9.78 | 8.95 | 8.24 | 8.93 |
| 5d | Cl | H | 6.46 | 7.82 | 10.43 | 8.87 | 8.21 | 7.84 |
| 5e | H | Cl | 6.79 | 7.56 | 7.9 | 7.34 | 7.69 | 8.87 |
| 5f | F | H | 6.92 | 10.89 | 11.32 | 10.13 | 8.1 | 7.49 |
| 5g | F | Cl | 7.41 | 9.23 | 12.71 | 9.75 | 7.14 | 7.23 |
| A | Ampicilin | | 14.76 | 8.53 | 16.34 | 17.23 | - | - |
| B | Norfloxacin | | 8.45 | 12.25 | 11.76 | 12.18 | - | - |
| C | Fluconazole | | - | - | - | - | 10.62 | 12.47 |

NS: Not Significant

*Minimum Inhibitory Concentration Determination*

The solution of the newly synthesized compounds and standard drugs were prepared at 500, 250, 125, 62.5, 31.25, 15.63, 7.8, 3.9, 1.95, 0.98, 0.48, 0.24, 0.12 mg/ml concentrations in the wells of microplates by diluting in the liquid double stranded Nutrient Broth. The bacterial suspensions used for inoculation were prepared of $10^5$ cfu/ml by diluting fresh cultures at MacFarland 0.5 density ($10^7$ cfu/ ml). Suspensions of the bacteria at $10^5$ cfu/ ml concentration were inoculated to the two-fold diluted solution of the compounds. There were 104 cfu/ml bacteria in the wells after inoculations. Nutrient Broth was used for diluting the bacterial suspension and for two-fold dilution of the compound. DMSO, pure microorganisms and pure media were used as control wells. A 10 µl bacteria inoculum was added to each well of the micro dilution trays. The trays were incubated at 37 $^oC$ in a humid chamber and MIC endpoints were read after 24 h of incubation. For antifungal activity, same procedure was used. The lowest concentration of the compound that completely inhibits

macroscopic growth was determined and minimum inhibitory concentrations (MICs) were reported (Table 3).

**Table 3.** MIC value of compounds **5a – 5g**.

| Com. No. | R | $R_1$ | MIC (µg/ml) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | *S. aureus* | *B. subtilis* | *E. coli* | *P. vulgaris* | *C. albicans* | *A. niger* |
| 5a | H | H | - | 250 | - | - | 250 | - |
| 5b | H | Br | - | 250 | 125 | - | 125 | 250 |
| 5c | Cl | Br | 125 | **62.5** | **62.5** | 125 | 125 | 125 |
| 5d | Cl | H | 125 | 125 | **62.5** | 125 | 125 | 125 |
| 5e | H | Cl | 125 | 125 | 125 | **62.5** | 250 | 250 |
| 5f | F | H | 125 | **62.5** | **31.25** | **62.5** | 125 | 250 |
| 5g | F | Cl | **62.5** | **62.5** | **31.25** | **62.5** | 125 | 125 |
| A | Ampicilin | | 0.48 | 0.48 | 3.9 | 3.9 | - | - |
| B | Norfloxacin | | 0.12 | 0.12 | 0.12 | 0.12 | - | - |
| C | Fluconazole | | - | - | - | - | 0.98 | 1.95 |

## Result and discussion

In this present work, nucleophillic addition of 4-substituted aniline with indole-2,3-dione yields 3-(4-arylimino)-indol-2-one (**1a-g**) which on further treatment with ethylchloro acetate undergo alkylation reaction in presence of anhydrous $K_2CO_3$ in dry acetone yields 3-(4-arylimino)-2-oxo-1-indole-ethylacetate (**2a-g**), which was further converted in to 3-(4-arylimino)-2-oxo-1-indole-acetylthyrazide (**3a-g**) through the reaction using hydrazine hydrate as a reagent. Then further thiosemicarbazone formation from compound (**3a-g**) in presence of KSCN & 10%HCl, yields 3-phenylimino-2-oxo-1-indole-acetylthiosemicarbazide (**4a-g**). Compound (**4a-g**) was refluxed in ethanol, in the presence of phenacyl bromide or substituted phenacyl bromide yielded N'-[{5-(4-aryl) 1,3-thiazol-2yl}carbohydrazide-methyl]-3(4-arylimino) indol-2-one analogs(5a-g) . Synthesis of title compounds showed in scheme was performed by conventional and microwave method.

IR spectrum of final compound (5e) exhibited isatin carbonyl at 1744 (C=O str.),3171.08 ($2^0$ NH str.),1670.41(amide C=O str.)1612 (C=N str.), 1341(S-C str.), 786 (C-Cl str.), which confirms the formation of final compound.

The 1H-NMR (DMSO-$d_6$) spectrum of (**5e**) as a representative compound of series , all protons were seen according to the expected chemical shift and integral values at (δ ppm) 4.6 (s, 2H, N-$CH_2$), 7.1-8.00 (m, 13H, Ar-H),6.8 (thiazole CH) ,9.62 (s,H, CS-NH), 10.43 (s, H, CO-NH).

Positive ESI (Electron spray Ionization) mass spectrum of compound (**5e**) was according to expected molecular weight presented. The m/z ratio 266.7 found at 100% abundance i.e. base peak. In the mass spectra of (**5e**), the molecular ion peak at 488.9 [M++1] confirms the formation of final structure. The fragment ion peak were observed at 487, 453 [$M^+$-Cl], 397.9 ($C_{19}H_{14}ClN_4O_2S^+$), 376 [$M^+$-$C_6H_4Cl$], 266.7 ($C_{11}H_9ClN_3OS^+$) (100%), 209 ($C_9H_6ClN_2S^+$), 131 ($C8H6NO^+$), 91 ($C_6H_5N^+$) , 77 ($C_6H_5^+$). Structure of the compound (**5e**) was confirmed by the physical data(TLC, melting point) & spectral data (IR, $^1$H NMR, and MS).

From the spectral data (IR, NMR, Mass) of compound (**5e**) it was confirmed that the synthesized compound is N'-[{5-(4-chloro phenyl) 1,3-thiazol-2yl}carbohydrazide-methyl]-3-phenylimino indol-2-one.

The compound **5f, 5g** with the flouro substitution at the R position was found to be highly active against S. *aureus & B*. subtilis. Compounds **5d** with Cl substitution at R & **5e** with Cl substitution at R1- position were found to be moderately active against S. aureus and B. subtilis.

In case of gram-negative bacteria E. Coli, P. vulgaris, compounds **5f** with the flouro substituent at R- position & compounds **5g** with the flouro & chloro substituents at R & R1 position respectively were found to be highly active. **5d** was found to be moderate activite against E.coli and the compound 5e was moderately active against P. vulgaris.

All the compound moderate activity in case of *C. albicans* and for *A. niger*. All the compounds screened for antimicrobial activity showed significant activit.

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