

Synthesis and Antimicrobial Evaluation of Novel isatin Derivatives

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ABSTRACT

The proposed research program is continuation of the research work, which has been carried out on the synthesis and biological studies of some isatin (indole 2,3-diones). In this connection fifty-seven derivatives were prepared. The substitution was made at position such as 1 and 3. The isatin derivatives (MP V_{A-F}) were prepared by the substituted of different aldehydes by reacting the mixture of (3Z)-3-[(4-hydrazinylphenyl)imino]-1,3-dihydro-2H-indol-2-one with the presence of with glacial acetic acid. Isatin derivatives with acid anhydride were prepared and isatin derivatives were also reacted with isatin having 85% yield. All these new compounds have been screened for their antimicrobial activity against test organism.

Keyword: isatin, Antimicrobial Activity, p-chloro aniline and hydrazine hydrate.

Received 29/12/2013

Revised 02/02/2014

Accepted 18/02/2014

INTRODUCTION

Isatin chemically 1H-indole-2,3-dione, was first obtained by Erdman and Laurent in 1841 [1] as a product from the oxidation of indigo by nitric and chromic acids. In nature, isatin is found in plants of the genus *Isatis*, in *Calanthe discolor* LINDL and in *Couroupitaguianensis*. [2]. Isatin and its derivatives possess a broad range of biological and pharmacological properties and are widely used as starting materials for the synthesis of a broad range of heterocyclic compounds and as substrates for drug synthesis [3]. Many methodologies have been adopted for the synthesis of isatin derivatives and to explore its possible role in treatment of various diseases. Among these the method developed by Sandmeyer [4] is the oldest and the most frequently used for the synthesis of isatin. It consists in the reaction of aniline with chloral hydrate and hydroxylamine hydrochloride in aqueous sodium sulfate to form an isonitrosoacetanilide, which after isolation, when treated with concentrated sulfuric acid produce isatin.

Isatin nucleus having both the keto and lactam moiety has aroused tremendous curiosity due to its diverse biological and pharmacological studies. It is widely distributed in mammalian tissues and body fluids [5]. Isatin has also drawn great attention from being discovered as a component of endogenous monoamine oxidase (MAO) inhibitory activity (tribulin) and subsequently identified as a selective inhibitor of MAO B at low concentrations [6]. A large number of human and animal diseases exists in both developed and developing countries [7] and a number of synthetic isatin derivatives were found to have variety of pharmacological properties including anticonvulsant [8], antibacterial, antifungal [9], antitubercular [10], anti-inflammatory [11], analgesic [12], antiviral and anti-HIV activities [13]. Some of the isatin derivatives possess promising antimicrobial activity like a series of chloroisatin-3-semicarbazone and hydrazones which has been designed for antitubercular activity against *Mycobacterium tuberculosis* [14]. In view of the immense pharmacological activities exhibited by the molecules it was considered worthwhile to synthesize novel isatin derivatives and to explore their pharmaceutical importance in terms of antimicrobial activity for its use in various microbes generated ailments.

MATERIALS AND METHODS

Melting points were measured using Kshitij Innovations apparatus in open capillaries and are uncorrected. The purity of the compounds was checked by TLC on silica gel G plates using ethyl acetate: n-hexane (1:2) solvent system and iodine vapours as a visualizing agent. IR spectra were recorded using KBr pellets on a Shimadzu Affinity-1 FTIR spectrophotometer. ¹H-NMR spectra were recorded on a Bruker Advance 400

spectrometer at 400.13 MHz using CDCl₃ solvent and TMS as internal standard. Chemical shifts were expressed in δ ppm unit.

Synthesis of (3Z)-3-[(4-chlorophenyl)imino]-1,3-dihydro-2H-indol-2-one (I):

Equimolar mixture of isatin and p-chloro aniline (0.01 mol) was dissolved in warm ethanol and water (3:1) and was then refluxed on a steam bath for 6 hrs. The completion of the reaction was checked by Thin layer chromatography using silica as adsorbent as chloroform and ethylacetate (3:1) as solvent system. The mixture was then kept for 24hrs at room temperature. The product were separated by filtration and recrystallized from warm ethanol [15].

Synthesis of (3Z)-3-[(4-hydrazinylphenyl)imino]-1,3-dihydro-2H-indol-2-one (II):

A mixture of (3Z)-3-[(4-chlorophenyl)imino]-1,3-dihydro-2H-indol-2-one (I) (0.05 mol) and hydrazine hydrate (3ml) in dioxane (30ml) was refluxed for 2hrs. The completion of the reaction was checked by Thin layer chromatography using silica as adsorbent as n-hexane and ethylacetate (2:1) as solvent system. The separated solid was filtered off and recrystallized from dioxane to give as yellow crystals [16].

Synthesis of (3Z)-3-[2-(4-[(3Z)-2-oxo-1,2-dihydro-3H-indol-3-ylidene]amino)phenyl]hydrazinylidene]-1,3-dihydro-2H-indol-2-one (III):

Equimolar quantities (0.01 mol) of (3Z)-3-[(4-hydrazinylphenyl)imino]-1,3-dihydro-2H-indol-2-one (II) and isatin were dissolved in warm ethanol and water (3:1) and was then refluxed on a steam bath for 6 hrs. The completion of the reaction was checked by Thin layer chromatography using silica as adsorbent as chloroform and ethylacetate (3:1) as solvent system. After standing for 24hrs at room temperature, the product was separated by filtration and recrystallized from warm ethanol [16].

Synthesis of (3Z)-3-[(3-methyl-1H-imidazol-5-yl)imino]-1,3-dihydro-2H-indol-2-one (IV):

A mixture of (3Z)-3-[(4-hydrazinylphenyl)imino]-1,3-dihydro-2H-indol-2-one (II) (0.01mol) and acetic anhydride (2ml) was refluxed for 6 hr. The completion of the reaction was checked by Thin layer chromatography using silica as adsorbent as n-hexane and ethylacetate (1:1) as solvent system. After cooling the mixture was poured into ice-water and extracted with ether and the solvent was evaporated. The obtained solid was recrystallized from ethanol [17].

Synthesis of (3Z) substituted phenyl-3-[(4-hydrazinylphenyl)imino]-1,3-dihydro-2H-indol-2-one (V_{A-F}):

A mixture of (3Z)-3-[(4-hydrazinylphenyl)imino]-1,3-dihydro-2H-indol-2-one (II) (0.01mol) and substituted aromatic benzaldehyde (0.01mol) in 40 ml ethanol along with glacial acetic acid (2, 3 drops) was refluxed for 8-12 hr. The reaction mixture was cooled. The completion of the reaction was checked by Thin layer chromatography using silica as adsorbent as n-hexane and ethylacetate (1:1) as solvent system. The solid obtained was filtered off and purified by recrystallization from ethanol [39].

Chemistry

IR, ¹H-NMR and elemental analysis were consistent with the assigned structure.

(3Z)-3-[2-(4-[(3Z)-2-oxo-1,2-dihydro-3H-indol-3-ylidene] amino) phenyl] hydra-zinylidene]-1,3-dihydro-2H-indol-2-one (III): Yield: 85% ; Melting Point: 185°C; IR(KBr, cm⁻¹) – 3362, 3358, 3324 (N-H str); 3093, 3065, 3022, 2969, 2921, 2897, 2757, 2687 (C-H str); 1736 (C=O str); 1628 (C=N str); 1612 (C=C str); 1383 (C-N str); 747, 749 (C-H bend); ¹H-NMR (DMSO, δ ppm) – 7.543 (1H, s, Ar NH); 7.515- 6.442 (12H, m, Ar H); 6.418 (1H, s, Ali NH); Anal. Calcd. for C₂₂H₁₅O₂N₅: C, 69.28; H, 3.96; N, 18.36; O, 8.39% **found**; C, 68.75; H, 3.45; N, 18.8; O, 9% Mol. Wt.: 381.

Physical characteristics of (3Z)-3-[(3-methyl-1H-imidazol-5-yl)imino]-1,3-dihydro-2H-indol-2-one (IV): Yield: 54% ; Melting Point: 205°C; IR(KBr, cm⁻¹) – 3386, 3246 (N-H str); 1617 (C-N str); 1339 (C-N str), 1599 (C=C str); 1617 (C=N str); 2822, 3096, 3032, 2822 (C-H str); 1736 (C=O str), 1145 749 (C-H Bend); ¹H-NMR (DMSO, δ ppm) – 7.524 (1H, s, Ar NH); 11.021 (1H, s, Ar NH); 7.497- 6.867 (7H, m, Ar H); Anal. Calculated for C₁₆H₁₃ON₄: C, 69.55; H, 4.38; N, 20.28; O, 5.79% **found** C, 69; H, 4.05; N, 21.03; O, 5.92% ; Mol. Wt.: 254.

Physical characteristics of (3Z)-3-[(4-[(2E)-2-benzylidenehydrazinyl]phenyl)imino]-1,3-dihydro-2H-indol-2-one (V_A): Yield: 62% ; Melting Point: 165°C; IR(KBr, cm⁻¹) – 3386, 3277 (N-H str); 3092, 3062, 3023, 2890, 2961, 2819 (C-H str); 1728 (C=O str); 1585 (C=C str); 1613 (C=N str); 1342 (C-N str); ¹H-NMR (DMSO, δ ppm)– 8.107 (1H, s, Ar NH); 7.985 (1H, s, CH); 4.255 (1H, s, NH); 7.390 – 6.418 (13H, m, Ar H); Anal. Calculated for C₂₁H₁₆ON₄: C, 74.10; H, 4.974; N, 16.46; O, 4.70% **found** C, 74; H, 4.96; N, 16.12; O, 4.92%; Mol. Wt.: 340.

Physical characteristics of (3Z)-3-[(4-[(2E)-2-(2 chlorobenzylidene)hydrazinyl]phenyl) imino]-1,3-dihydro-2H-indol-2-one (V_B): Yield: 75% ; Melting Point: 170°C; IR(KBr, cm⁻¹) – 3389, 3357 (N-H str); 1731 (C=O str); 789 (C-Cl str); 1614 (C=N str); 2969, 2890, 2818, 2745, 3027, 3062, 3062 (C-H str); 1591 (C=C str), 1086 (C-Cl), 1340 (C-N str); ¹H-NMR (DMSO, δ ppm) – 8.625 (1H, s, Ar NH); 3.351 (1H, s, Ali NH);

2.670 (2H S, NH₂), 3.327 (1H, S, Ar CH-Cl); 7.998 – 6.900 (12H, m, Ar H); Anal. Calculated for C₂₁H₁₅ON₄Cl – C, 66.79; H, 5.43; Cl, 9.96; N, 14.86; O, 2.96% **found** C, 66.79; H, 5.43; Cl, 9.96; N, 14.86; O, 2.96%; Mol.Wt.: 374.

Physical characteristics of (3Z)-3-((4-[(2E)-2-(2-methoxybenzylidene)hydrazinyl] phenyl)imino)-1,3-dihydro-2H-indol-2-one (Vc):Yield: 67% ; Melting Point: 160°C; IR(KBr, cm⁻¹) – 3421, 3393 (N-H str); 1724 (C=O str); 3098, 2948, 2886, 2805, 2673 (C-H str); 1269 (C-O-C str); 1591 (C=C str); 1616 (C=N str); 747 (C-H bend), 1329 (C-N str), 747 (C-H bend); ¹H-NMR (DMSO, δppm)– 7.210 (1H, S, Ar NH); 4.079 (1H, S, Ali NH), 2.275(1H, S, NH₂); 3.462 (3H, S, OCH₃), 7.18 – 6.789 (12H, m, Ar H); Anal. Calculated for C₂₃H₁₈O₂N₄–C, 71.34; H, 4.90; N, 15.13; O, 8.64% **found** C, 71.02; H, 4.96; N, 15.45; O, 8.57% ;Mol.Wt.: 382.

Physical characteristics of (3Z)-3-((4-[(2E)-2-(2,4-dihydroxybenzylidene)hydrazinyl] phenyl)imino)-1,3-dihydro-2H-indol-2-one (Vd):Yield: 33.8% ; Melting Point: 190°C; IR(KBr, cm⁻¹) – 3357 (O-H str); 3267 (N-H str); 3028, 3089, 2984, 2814, 2867 (C-H str); 1619 (C=C str); 1222, 1293 (C-O str); 730 (C-H bend), 1348 (C-O-H str), 1736 (1736), 1597 (C=N str); ¹H-NMR (DMSO, δppm)– 5.971, 5.499 (1H, S, Ar OH); 4.356 (1H, S, Ali NH); 7.077 (1H, S, Ar NH); 7.058 (1H, S, Ali CH); 7.039 – 6.450 (11H, m, Ar CH); Anal. Calculated for C₂₁H₁₆O₃N₄–C, 67.73; H, 4.33; N, 15.05; O, 12.89% **found** C, 67.76; H, 4.69; N, 15.05; O, 12.5%; Mol.Wt.: 372.

Physical characteristics of (3Z)-3-((4-[(2E)-2-(3-methylbenzylidene)hydrazinyl] phenyl)imino)-1,3-dihydro-2H-indol-2-one (Ve):Yield: 61% ; Melting Point: 145°C; IR(KBr, cm⁻¹)– 3362, 3358 (N-H str); 1738 (C=O str); 1628 (C=N str); 1592 (C=C str); 3065, 3022, 2969, 2921, 2837, 2897, 2757, 2687 (C-H str); 1334 (C-N str); 791 (C-H bend); ¹H-NMR (DMSO, δppm)– 7.987 (1H, S Ar NH); 3.568 (1H, Ali NH); 2.735 (2H, S, NH₂); 2.275 (3H, S, Ali CH₃); 9.474 (1H, S, CH); 7.654-6.870 (12H, m, Ar H); Anal. Calculated for C₂₂H₁₈ON₄–C, 74.03; H, 5.52; N, 18.29; O, 4.17% **found** C, 74.03; H, 5.92; N, 15.79; O, 4.26%; Mol.Wt.: 354.

Physical characteristics of (3Z)-3-((4-[(2E)-2-[4-(dimethylamino)benzylidene] hydrazinyl]phenyl)imino)-1,3-dihydro-2H-indol-2-one (Vf): Yield: 72% ; Melting Point: 168°C; IR(KBr, cm⁻¹)– 3357 (N-H str)1684 (C=O str), 1654 (C=N str); 1588 (C=C str); 1250 (C-N-H str); 1364 (C-N str), 3357 (N-H str), 1684 (C=O str), 3197, 3179, 3156, 3150, 2674, 2974 (C-H str), 1269 (C-N str); ¹H-NMR (DMSO, δppm)– 4.079 (1H, S, Ali NH); 7.210 (1H, S, Ar NH); 3.462, 3.357 (6H, D, CH₃); 7.186-6.789 (12H, m, Ar H); Anal. Calculated for C₂₃H₂₁ON₄–C, 72.04; H, 5.52; N, 18.26; O, 4.17% **found** C, 72.73; H, 5.36; N, 18.42; O, 3.49%; Mol.Wt.: 383.

BIOLOGICAL SCREENING

All the synthesized compounds were subjected to antimicrobial screening at a concentration of 100µg/ml involving four bacterial strains *Bacillus subtilis* & *Staphylococcus aureus* (gram +ve) and *Escherichia Coli* & *Klebsiella Pneumoniae* (Gram -ve) using Ampicillin as standard at the same concentration. The work, in reference, was carried out by Agar disc diffusion method. The response of organisms to the synthesized compounds were measured in terms of zone of inhibition and compared with that obtained with standard.

1. Preparation of Nutrient Broth Medium:

To prepare Nutrient Broth Medium, 10gm of beef extract, 10gm of peptone and 5 mg of sodium chloride were suspended in 1000 ml of purified water. It was mixed properly and heated up to boiling to dissolve the medium completely. The pH was adjusted (8.0 - 8.4) with 5M sodium hydroxide solution and boiled for 10 minutes. The mixture was filtered & sterilized by maintaining at 115°C for 30 minutes and the pH was adjusted to 7.3 ± 0.1.

2. Preparation of Nutrient Agar Medium

To prepare Nutrient Agar Medium, 5gm of beef extract, 5gm of Peptone, 10 gm of agar and 2.5 mg of sodium Chloride were suspended in 500 ml of purified water. It was mixed properly and heated upto boiling to dissolve the ingredients completely. The mixture was filtered, sterilised by maintaining at 115° for 30 minutes and the pH was adjust to 7.3 ± 0.1. It was then cooled and poured into sterilized petridish. All the plates were kept for 4-5 hours in laminar airflow until the media got solidified. The plates were then kept in an incubator at 37°C.

3. Preparation of solutions:

A solution (100µg/ml) of standard drug (Ampicillin) was prepared by dissolving 10 mg of Ampicillin into 100 ml of sterile water.

4. Preparation of test solution:

10 mg of the synthesized compound(s) was dissolved in 100 ml of DMSO to prepare the test sample of 100 µg/ml concentration. A 24 hour old culture was used for the preparation of bacterial suspension. Likewise suspensions of all the organisms were prepared as per standard procedure.

5. Preparation of discs:

Discs of 6-7 mm in diameter were punched from No. 1 Whattmann filter paper with sterile cork borer of same size. These discs were sterilized by keeping in oven at 140°C for 60 minutes. Standard and test

solutions were added separately to these discs which were air dried later on.

6. Diffusion Test

A filter-paper disk impregnated with the compound to be tested was placed on the surface of the agar carefully by using sterilized forceps. The compound diffused from the filter paper into the agar. The larger the clear area around the filter disk, the more effective was regarded the compound.

These petridishes were kept upto one hour for diffusion at room temperature and then for incubation at 37°C for 24 hours in an incubator. The zones of inhibition after 24 hours were measured in millimeters. The size of the zone of inhibition was measured as a determination of compound's effectiveness.

RESULTS AND DISCUSSION

A mixture of (3*Z*)-3-[(4-hydrazinylphenyl)imino]-1,3-dihydro-2*H*-indol-2-one (II) (0.01mol) and substituted aromatic benzaldehyde (0.01mol) in 40 ml ethanol along with glacial acetic acid (2, 3 drops) was refluxed for 8-12 hr. Substantial proof for the formation of V_A-V_F has been provided by differences in melting points and yield value from that of parent compound. Compound V_A on reaction with various substituted aromatic benzaldehyde yielded which on 40 ml ethanol along with glacial acetic acid (2, 3 drops) was refluxed for 8-12 hr yielded novel isatin derivatives (V_A-V_F). The primary structural difference within this series involves the nature of various substituted aldehydes. Synthesized compounds were found to be crystalline in nature and easily soluble in methanol, DMF, DMSO and DMF but insoluble in chloroform, ethyl acetate, benzene hexane and toluene. With the help of analytical techniques such as melting point, IR and ¹H-NMR, synthesized derivatives were characterized. All the compounds showed IR and NMR signals for different kinds of protons at their respective positions.

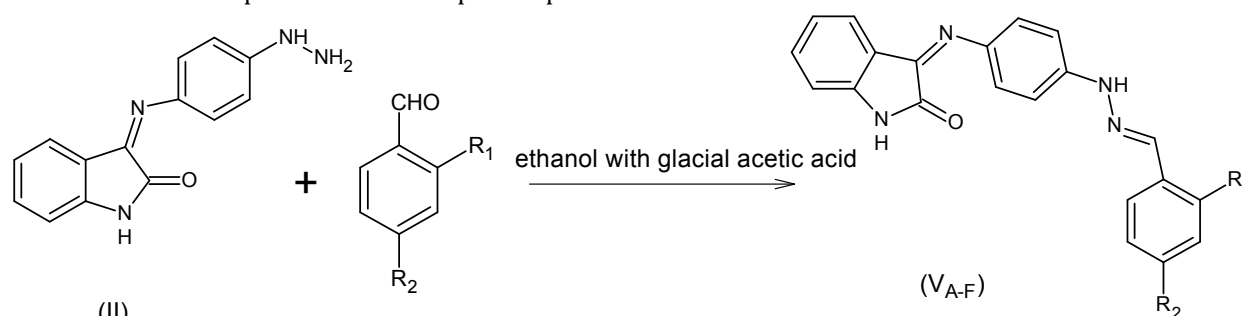


Table 1: Antibacterial Activity of synthesized isatin derivatives (V_A-V_F)

	Zone of Inhibition			
	B. subtilis	S. aureus	E.coli	K. pneumoniae
Amplicin	24	18	20	22
MPVa	13	11	12	10
MPVb	15	12	14	15
MPVc	14	10	10	11
MPVd	10	8	9	10
MPVe	16	12	12	14
MPVf	13	9	12	13

All compounds have been screened for their antimicrobial activity. From the screening results it was observed that the presence of electron withdrawing group and ester linkage made the compounds to exhibit moderate to significant activity in comparison to standard drug Ampicillin. Compound MPV_b and MPV_e exhibited promising antibacterial activity. However other compounds of the series also exhibited moderate to significant activity against the microorganisms as mentioned above. Therefore compound MPV_b, MPV_c, MPV_d, MPV_e and MPV_f can be recommended for further studies. The above results established the fact that aromatic benzaldehyde substituted with various isatin (substituted) can be studied further to explore out newer antimicrobial compounds.

CONCLUSION

The analytical and other informational data, available in literature so far, have rendered thiophene significantly important class of heterocyclic compounds and their applications in ever challenging chemotherapy of various ailments/ infections etc. since last two decades immensely hiked interests of

medicinal chemist and biochemist.

This particular research study, in reference, would extend great deal of help to researchers in reckoning and determining the best and most productive, economical, suggestive and conclusive access to various thiophenes of clinical importance superseding other compounds of their class.

Further combinatorial libraries of these compounds can be generated which can be screened for optimal pharmacological activities by optimization techniques using 2D and 3D QSAR investigation.

REFERENCES

1. Gharib A., Daneshtalab M., Scheeren J. W., Bamoharram F. F., (2009). "Novel catalytic synthesis of 6,7-dimethoxyisatin with the use of heteropolyacids (HPAs) as acid solid catalyst" Polish Journal of Chemical Technology; vol: 11(3); 2009; pp. 5-7.
2. da Silva J. F. M., Garden S. J., Pinto A. C., (2001). "The chemistry of isatins: a review from 1975 to 1999" J. Braz. Chem. Soc; vol: 12(3); pp. 273-324.
3. Clay C. M., (2009). thesis of master degree on "Synthesis of isatin Derivatives Used for the Inhibition of Pro-Apoptotic Jurkat T cells" Wright State University.
4. Rehn S., (2004). "Synthesis of indole and oxindole derivatives incorporating pyrrolidino, pyrrolo or imidazolo moieties" Karolinska Institutet, Stockholm, Sweden.
5. Zhou Y., Zhao Z. Q., Xie Z. X., (2001). "Effects of isatin on rotational behavior and DA levels in caudate putamen in Parkinsonian rats" Brain Research; vol: 917; pp. 127-132
6. Tang Y., Zhang J., Miao Y., Chen G., (2010). "1-(Morpholinomethyl)indoline-2,3-dione" Acta Cryst; vol: E66; pp. 1-6.
7. Ali M. M., Jesmin M., Salahuddin M. S., Habib M. R., (2008). "Antimicrobial activity of some Schiff bases Derived from Benzoin, Salicylaldehyde, Aminophenol and 2,4 Dinitrophenyl Hydrazine" Mycobiology; vol: 36(1); pp. 70-73
8. Bari B. S., Agrawal A. O., (2008). "Rapid and Efficient Synthesis of Some Isatin Derivatives Based on Novel Heterocycles Containing Oxadiazole Moiety" Quarterly Journal of Applied Chemistry; vol: 1; pp. 34-40
9. Babu G. S., Swathy T., Malathy P. S., Srinivas B., (2010). "Synthesis, Characterization and Evaluation of Novel substituted benzo-indolylideneacetohydrazide Derivatives as Anti-Microbial Agents" Der Pharmacia Lettre; vol: 2(4); pp. 408-415.
10. Aboul-Fadl T., Bin-Jubair F. A. S., Aboul-Wafa O., (2010). Schiff bases of indoline-2,3-dione (isatin) derivatives and nalidixic acid carbohydrazide, synthesis, antitubercular activity and pharmacophoric model building " European Journal of Medicinal Chemistry; vol: 45; pp. 4578-4586.
11. Srinivas B., Priya V. R., Babu G. S., Rao J. V., (2010). "Synthesis and Screening of New Isatin Derivatives" Der Pharma Chemica; vol: 2(6); pp. 378-384
12. Mana S., Pahari N., Sharma N. K., Priyanka., (2010). "Synthesis and Characterization of novel thiazolo-isoxazole fused isatin as analgesic and anti-inflammatory agent" The Pharma Research; vol: 3; pp. 51-59
13. Bhargava B., Pathak D., Siddiqui N., Alam M. S., (2010). "Search for Biological Active Isatins: A Short Review" International Journal of Pharmaceutical Sciences and Drug Research; vol: 2(4); pp. 229-235
14. Pandeya S. N., Raja A. S., Nath G., (2006). "Synthesis and antimicrobial evaluation of some 4- or 6-chloroisatin derivatives" Indian journal of Chemistry; vol: 45B; pp. 494-499
15. Mana S., Pahari N., Sharma N. K., Priyanka., (2010). "Synthesis and Characterization of novel thiazolo-isoxazole fused isatin as analgesic and anti-inflammatory agent" The Pharma Research., Vol: 3; pp. 51-59.
16. Bhuiyan H. M., Rahman M. K., Hossain K., (2006). "Synthesis and antimicrobial evaluation of some new thienopyrimidine derivatives" Acta Pharma; Vol: 56; 2006; pp. 441-450.
17. García-Valverde M., Torroba T., (2005). "Special Issue: Sulfur-Nitrogen Heterocycles" Molecules; vol: 10; ; pp. 318-320.

Citation of this article

Kiran Mishra. Synthesis and Antimicrobial Evaluation of Novel isatin Derivatives. Int. Arch. App. Sci. Technol; Vol 5 [1] March 2014: 28-32