Turk J. Pharm. Sci. 8 (2), 169-178, 2011

Original article

SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME 3-SUBSTITUTED-2-OXINDOLE DERIVATIVES

Subhas S. KARKI,* Rahul HAZARE, Sujeeth KUMAR, Amit SAXENA, Arpit KATIYAR

KLE University's College of Pharmacy, Department of Pharmaceutical Chemistry, Rajajinagar, Bangalore, INDIA

Abstract

A series of 1,3,5-trisubstituted-2-oxindole derivatives have been synthesized by reaction of 2,3-dihydro-2,3-dioxindol , 1-benzyl-2,3-dihydro-2,3-dioxindole and an appropriate benzoyl hydrazide. Structures of these compounds were established by IR, ^{1}H NMR, ^{13}C NMR, Mass spectroscopy. All the compounds were evaluated for their preliminary in vitro anti-tuberculosis activity against Mycobacterium tuberculosis $H_{37}Rv$ strain using broth dilution assay method. The results show that compounds 3a, 3c, 4a, 4b and 4h exhibited anti-tubercular activity at 25 µg/mL. Some of the selected compounds were evaluated for their preliminary in vitro antibacterial activity against Bacillus subtilis and Staphylococcus aureus and displayed mild antibacterial activity on comparison to standard streptomycin.

Key words: *Isatin, Antitubercular, Antibacterial.*

Bazı 3-sübstitüe-2-oksindol Türevlerinin Sentezi ve Antimikrobiyal Aktiviteleri

2,3-dihidro-2,3-dioksindol, 1-benzil-2,3-dihidro-2,3-dioksindol ve uygun bir benzoil hidrazid reaksiyona sokularak bir seri 1,3,5-trisübstitüe-2-oksindol türevi sentezlenmiştir. Bu bileşiklerin yapısı IR, ^{1}H NMR, ^{13}C NMR ve kütle spektroskopisi ile belirlenmiştir. Tüm bu bileşiklerin in vitro anti-tüberküloz aktiviteleri broth dilüsyon yöntemi ile Mycobecterium tuberculosis $H_{37}Rv$ suşlarına karşı değerlendirilmiştir. Sonuçlar; 3a, 3c, 4a, 4b ve 4h bileşiklerinin 25 µg/mL konsantrasyonda anti-tüberküler aktiviteye sahip olduğunu göstermektedir. Seçilen bazı bileşiklerin in vitro olarak Bacillus subtilis ve Staphylococcus aureus'a karşı antibakteriyel aktiviteleri değerlendirilmiş ve streptomisin ile karşılaştırılacak olursa daha az bir antibakteriyel aktiviteye sahip olduğu gözlenmiştir.

Anahtar kelimeler: *Isatin, Antitüberküler, Antibakteriyel.*

***Correspondence:** E-mail: subhasskarki@gmail.com; Tel: +91 80 23325611; Fax: 91 80 23425373.

INTRODUCTION

Tuberculosis (TB) is a chronic necrotizing bacterial infection with wide variety of manifestations caused by *Mycobacterium tuberculosis*, which has been a scourge of humanity for thousands of years and remains one of the prevalent health tribulations in the world (1). Today, TB is among the top five causes of global mortality. World Health Organization (WHO) declared TB a global health emergency in 1993, for the first time an infectious disease achieved that dubious distinction (2,3). The active TB is currently treated with a four-drug regimen comprising isoniazid, rifampicin, pyrazinamide and ethambutol for a period of at least six months (4-6). The long treatment regimen can be difficult to fully complete, fueling the development of more infectious and virulent multidrug resistant (MDR) strains of TB, which shows very high mortality (7). Such MDR-TB is difficult and expensive to treat and is not always curable, alternative combination treatment of five-drug regimen is recommended initially, including both ethambutol and rifampicin. MDR-TB often requires prolonged treatment sometimes up to 24 months (8) and thus poses a major challenge to the control of the disease worldwide. There is now recognition that innovative drugs to combat TB are urgently required. With the completion of the genome of *M. tuberculosis* comes the promise of a new generation of potent drugs to combat the emerging epidemic of TB. In this regard, however, there have been few additions of some promising new anti-tuberculosis agents, such as the long acting rifamycins (9,10), fluoroquinolones (11-13), oxazolidinones (14,15), and nitroimidazopyrans (16).

Isatin (1H-indole-2,3-dione) is synthetically versatile substrates, where it can be used for the synthesis of a large variety of heterocyclic compounds, such as indoles and quinolines, and as raw material for drug synthesis. The synthetic versatility of isatin has led to the extensive use of this compound in organic synthesis. The synthetic versatility of isatin has stemmed from the interest in the biological and pharmacological properties of its derivatives (17).

Derivatives of 2,3-dihydro-2,3-dioxindole were reported as antibacterial (18-24), antipox virus agents (25), antifungal & antiviral (26), antioxidant & cytotoxic agents (27). In view of the above facts and in continuation of our search for various biologically active molecules (28, 29) has prompted us to synthesize some molecules of isatin with benzoyl hydrazide and carry out their preliminary anti-tubercular and antibacterial activity. In this paper we report the synthesis and spectral studies of a series of 2-oxindole derivatives and preliminary evaluation of in vitro anti-tuberculous and antibacterial activity.

EXPERIMENTAL

Melting points were determined in open capillaries and were uncorrected. R_f values were obtained using silica gel thin layer chromatography plates and a solvent system of chloroform/methanol (9:1). 2,3-dihydro-2,3-dioxindoles/5-substituted 2,3-dihydro-2,3-dioxindoles and 1-benzyl-2,3-dihydro-2,3-dioxyindoles were prepared according to literature (30,31). The infrared spectra of all compounds were determined by a diffuse reflectance technique using potassium bromide powder on a Jasco 460 FTIR machine (Jasco, Japan). ^{13}C NMR for and ^{1}H NMR spectra (400 MHz) of all compounds were generated in dimethylsulfoxide-*d*6/$CDCl_3$ on a Bruker Ultraspec spectrophotometer (Germany). LCMS for all compounds were taken on LCMS-2010A (Shimadzu, Japan).

Method for preparation of 5-substituted-indole-2,3-dione-3-benzoyl hydrazide ***(3a-c)***

To a mixture of 2,3-dihydro-2,3-dioxindole (0.005 M) equimolar quantity of benzoyl hydrazide and 4-5 drops of glacial acetic acid was refluxed in 50 mL of ethanol for 2-4 hours on

water bath. The initial colored solution slowly change in to some fluffy solid crystals in the end of the reaction, which was verified by TLC on silica plates. The solid that precipitated was collected, washed with cold ethanol and purified by ethanol and chloroform.

Method for preparation of 5-substituted-1-(4'-substitutedbenzyl)-1-H-indole-2,3-dione-3-benzoyl hydrazide ***(4a-i)***

To 0.00168 M of 1-benzyl-2,3-dihydro-2,3-dioxindole, equimolar quantity of benzoyl hydrazide and 4-5 drops of glacial acetic acid was added and refluxed in 50 mL of ethanol for 2-4 hours on water bath. The initial colored solution slowly changes in to some fluffy solid crystals at the end of the reaction, which was verified by TLC on silica plates. The compounds were purified by suitable solvents.

Biological activity

Antitubercular activity

The procedure we have followed for anti-Tb activity mainly involves the use of Middlebrook 7H-9 broth and standard strain of *Mycobacterium tuberculosis* $H_{37}Rv$. The basal medium was prepared according to manufacture's instructions (Hi-Media) and sterilized by autoclaving. 4.5 mL of broth was poured into each one of the sterile bottles; 0.5 mL of ADC supplement was added. This supplement contains catalase, dextrose and bovine serum albumin fraction v. Then a stock solution of the compound was prepared (10 mg/mL). From this appropriate amount of solution was transferred to media bottles to achieve final concentrations of 25, 50, 75 µg/mL, finally 10 µL suspension of Mtb strain (100000 organisms/mL, adjusted by McFarland's turbidity standard) was transferred to each of the tubes and incubated at 37 °C. Along with this one growth control without compound and drug controls were also set up. The bottles were inspected for growth twice a week for a period of three weeks. The appearance of turbidity was considered as growth and indicated resistance to the compound. The growth was confirmed by making a smear from each bottle and performing a ZN stain.

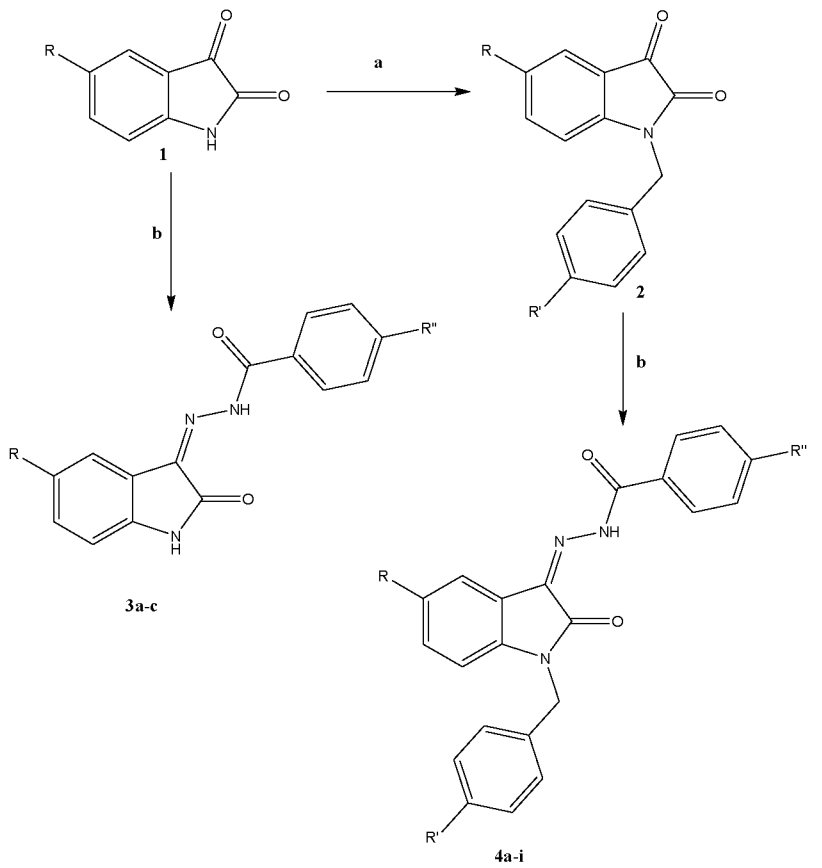
Antibacterial activity

A stock solution of compounds were 200 µg/mL was made in sterile water containing 5% DMF under aseptic conditions and further dilutions were made with the same solvent in a similar manner. All the dilutions and stock solutions were sterilized by membrane filtration. Solid agar and liquid broth culture media No. 1 were used for all the test organisms and the pH was adjusted to 7.2. Antimicrobial activity (32) of the selected compounds against different strains of bacteria was determined by cup-plate method, and activity was expressed in terms of diameters of zone of inhibition. Inoculum was prepared by washing a fresh 5 mL medium slant of test organisms with 5 mL sterile water and further diluting the 1 mL washing to 10 mL. This suspension (0.15 mL) was added to 15 mL melted medium at a temperature 45-50 °C and plates were prepared. Holes of diameter 6 mm were dug into the agar plates with a sterile borer and filled with the drug. The plates were incubated for at 35 °C for 24 h. The results were compared with that of standard streptomycin.

RESULTS AND DISCUSSION

Chemistry

Substituted 2,3-dihydro-2,3-dioxindole was a versatile starting material for the synthesis of number of proposed compounds (Scheme, Table 1). The different 5-substituted-1-benzyl-2,3-dihydro-2,3-dioxindole (substituted benzyl isatins) were prepared by reacting the 4-substituted benzyl chloride with 5-substituted-2,3-dihydro-2,3-dioxindoles in DMF in presence of Na_2CO_3 (Scheme). These synthesized compounds (3a-c, 4a-i) showed absorption bands ranging from 3372-3190 cm^{-1} for N-H stretching, 3064-2848 cm^{-1} for C-H aromatic and aliphatic respectively, 1702-1683 cm^{-1} for C=O stretching; there were also some bands for C=C and C=N at 1599-1465 cm^{-1} respectively (Table 2). In ^1H NMR spectra, the presence of a singlet between δ 4.85-4.96 ppm was observed for the methylene group and multiplets were observed between δ 8.04-6.58 ppm for aromatic protons, while for NH, the singlet were observed between δ 13.84-14.08 ppm, and for the NH in case of compounds 3a-c, it was observed between δ 10.37-11.28 ppm and these protons were exchangeable with D_2O. In the ^{13}C NMR spectra, signals from δ 164.49 ppm to 111.33 ppm were observed for aromatic carbons and δ 43.51 & δ 30.19 ppm for alkyl carbons. Liquid chromatography mass spectra (LCMS) showed accurate molecular ion peaks (Table 2).



Scheme. Synthetic route of the title compounds 3a-c and 4a-i. [Reagents: a) Benzyl chloride, DMF, reflux, 2 h b) Benzoyl hydrazide, alcohol, CH_3COOH, reflux, 4 h]

Table 1. Physicochemical data of synthesized compounds

Compd.	R	R'	R''	Yield (%)	Mp [°C]	Mol. Formul (M_r)	R_f value
3a	Cl	-	H	81	280-282	$C_{15}H_{10}N_3O_2Cl$	0.61
3b	CH_3	-	H	83	260-262	$C_{16}H_{13}N_3O_2$	0.84
3c	H	-	H	82	270-272	$C_{15}H_{11}N_3O_2$	0.78
4a	Cl	H	H	77	170-172	$C_{22}H_{16}N_3O_2Cl$	0.57
4b	CH_3	H	H	79	230-233	$C_{23}H_{19}N_3O_2$	0.60
4c	H	H	H	80	160-162	$C_{22}H_{17}N_3O_2$	0.58
4d	Cl	Cl	H	76	188-190	$C_{23}H_{15}N_3O_2Cl$	0.60
4e	CH_3	Cl	H	77	220-223	$C_{23}H_{18}N_3O_2Cl$	0.61
4f	H	Cl	H	79	214-216	$C_{22}H_{16}N_3O_2Cl$	0.65
4g	Cl	CH_3	H	69	200-202	$C_{23}H_{18}N_3O_2Cl$	0.66
4h	CH_3	CH_3	H	65	210-212	$C_{24}H_{21}N_3O_2$	0.61
4i	H	CH_3	H	68	190-193	$C_{23}H_{19}N_3O_2$	0.59

Table 2. Spectral data of synthesized compounds

Compd.	IR (ν, cm^{-1})	^1H / ^{13}C NMR (δ, ppm) (DMSO-d_6)	MS
3a	3230, 2918, 2848, 1702, 1599, 1528, 1464	6.87-6.84 (m, 1H, Ar-H), 7.25-7.23 (m, 1H, Ar-H), 7.59-7.42 (m, 4H, Ar-H), 7.88-7.84 (m, 2H, Ar-H), 11.28 (s, 1H, NH, exchangeable with D_2O), 14.03 (s, 1H, NH, exchangeable with D_2O)	299
3b	3254, 3050, 2918, 2855, 1709, 1600, 1484	2.24 (s, 3H, CH_3), 6.73 (d, 1H, J=8, Ar-H), 7.05 (d, 1H, J=12, Ar-H), 7.43 (t, 2H, J=16, Ar-H), 7.55-7.49 (m, 2H, Ar-H), 7.91 (d, 1H, J=8, Ar-H) 10.37 (s, 1H, NH, exchangeable with D_2O), 14.06 (s, 1H, NH, exchangeable with D_2O)	279
3c	3237, 3041, 2917, 2857, 1691, 1536, 1465	6.86 (d, 1H, J=8, Ar-H), 7.25 (d, 1H, J=8, Ar-H), 7.59-7.47 (m, 4H, Ar-H), 7.88 (dm, 3H, J=8, Ar-H), 11.28 (s, 1H, NH, exchangeable with D_2O), 14.03 (d, 1H, J=8, Ar-H)	265
4a	3363, 3196, 3029, 2923, 1683, 1489, 1344		389
4b	3248, 2921, 2851, 1702, 1600, 1535, 1469, 1265,	2.34 (s, 3H, CH_3), 4.96 (s, 2H, CH_2), 6.99 (d, 1H, J=8 Ar-H), 7.10 (d, 1H, J=8, Ar-H), 7.37-7.29 (m, 5H, Ar-H), 7.53 (t, 2H, J=16, Ar-H), 7.60 (t, 2H, J=16, Ar-H), 7.72 (s, 1H, Ar-H), 8.04 (d, 2H, J=8, Ar-	369

		H), 14.08 (s, 1H, NH, exchangeable with D_2O)	
4c	3372, 3195, 3059, 1703, 1678, 1612, 1538, 1488, 1255	4.92 (s, 2H, CH_2), 6.75 (d, 1H, J=8, Ar-H), 7.07 (t, 1H, J=16, Ar-H), 7.28-7.18 (m, 6H, Ar-H), 7.54-7.46 (m, 3H, Ar-H), 7.83 (s, 1H, Ar-H), 7.97 (d, 2H, J=8, Ar-H), 14.03 (s, 1H, NH, exchangeable with D_2O)	355
4d	3370, 3245, 3064, 1692, 1594, 1531, 1489, 1341, 1252	4.86 (s, 2H, CH_2), 6.58 (d, 1H, J=8, Ar-H), 7.21 (dm, 4H, J=8, Ar-H), 7.40 (t, 2H, J=16, Ar-H), 7.49 (t, 2H, J=16, Ar-H), 7.72 (s, 1H, Ar-H), 7.79 (d, 2H, J=8, Ar-H), 13.84 (s, 1H, NH, exchangeable with D_2O) 30.19 (s), 43.51 (s), 111.33 (s), 121.67 (s), 122.71 (s), 128.41 (s), 129.14 (s), 129.53 (s), 129.81 (s), 130.15 (s), 131.54 (s), 132.16 (s), 133.37 (s), 133.61 (s), 134.71 (s), 136.18 (s), 141.03 (s), 161.96 (s), 164.49 (s)	423
4e	3365, 3242, 3031, 2922, 1695, 1627, 1595, 1534, 1488,	2.27 (s, 3H, CH_3), 4.85 (s, 2H, CH_2), 6.59 (d, 1H, J=8, Ar-H), 7.05 (d, 1H, J=8, Ar-H), 7.25-7.17 (m, 4H, Ar-H), 7.46 (t, 1H, J=16, Ar-H), 7.54 (t, 1H, J=16, Ar-H), 7.66 (s, 1H, Ar-H), 7.96 (d, 2H, J=8, Ar-H), 13.95 (s, 1H, NH, exchangeable with D_2O)	403
4f	3190, 3060, 1695, 1610, 1537, 1488, 1362, 1255	6.74 (d, 1H, J=8, Ar-H), 7.11 (t, 1H, J=16, Ar-H), 7.29-7.21 (m, 5H, Ar-H), 7.48 (t, 1H, J=16, Ar-H), 7.57 (t, 1H, J=16, Ar-H), 7.86 (s, 1H), 7.99 (d, 2H, J=8, Ar-H), 14.00 (s, 1H, NH, exchangeable with D_2O)	389
4g	3369, 3192, 3030, 1699, 1612, 1543, 1474, 1443, 1343,	2.25 (s, 3H, CH_3), 4.87 (s, 2H, CH_2), 6.76 (d, 1H, J=8, Ar-H), 7.25-7.04 (m, 5H, Ar-H), 7.46 (t, 2H, J=16, Ar-H), 7.54 (t, 1H, J=16, Ar-H), 7.82 (s, 1H, Ar-H), 7.97 (d, 2H, J=8, Ar-H), 14.04 (s, 1H, NH, exchangeable with D_2O)	403
4h	3365, 3242, 3030, 2923, 1695, 1595, 1535, 1488, 1253	2.26 (s, 6H, $2CH_3$), 4.85 (s, 2H, CH_2), 6.63 (d, 1H, J=8, Ar-H), 7.25-7.01 (m, 5H, Ar-H), 7.45 (t, 2H, J=16, Ar-H), 7.53	383

		(t, 1H, J=16, Ar-H), 7.64 (s, 1H, Ar-H), 7.97 (d, 2H, J=8, Ar-H), 14.02 (s, 1H, NH, exchangeable with D_2O)	
4i	3368, 3185, 3059, 2920, 1698, 1612, 1539, 1487, 1466	2.25 (s, 3H, CH_3), 4.87 (s, 2H, CH_2), 6.76 (d, 1H, J=8, Ar-H), 7.25-7.04 (m, 5H, Ar-H), 7.46 (t, 2H, J=16, Ar-H), 7.82 (s, 1H, Ar-H), 7.97 (d, 2H, J=8, Ar-H), 14.04 (s, 1H, NH, exchangeable with D_2O)	369

Biological activity

The antitubercular activity for all compounds at concentration of 25, 50 and 100 µg/mL was tested against $H_{37}Rv$. The results of antitubercular activity have been presented in Table 3. Compounds namely 3a, 3c, 4a, 4b and 4h were capable of showing antitubercular activity at 25 µg/mL. While for the compound 3b exhibited antitubercular activity at 50 µg/mL and 4i at 75 µg/mL. Where as for remaining compounds (4c-4f) were failed to show activity in the concentrations tested.

Antibacterial activity has been done for some of the selected compounds namely 3a, 3b and 4b at lowest and highest concentrations against *Bacillus subtilis* and *Staphylococcus aureus* have been presented in Table 4. These compounds (3a, 3b and 4b) displayed mild antibacterial activity against both the tested organisms on comparison to streptomycin.

Table 3. Anti-tubercular activity of synthesized compounds (3a-c and 4a-i) against $H_{37}Rv$ strain

Sl No.	Code No.	Concentration (µg/mL)
1	3a	25
2	3b	50
3	3c	25
4	4a	25
5	4b	25
6	4c	NA
7	4d	NA
8	4e	NA
9	4f	NA
10	4g	75
11	4h	25
12	4i	75
Std	Streptomycin	7.5
Std	Ciprofloxacin	10

NA - Not active

Table 4. Antibacterial activity

Compound code	Zone of inhibition, (cm), *B. substilis*	Zone of inhibition, (cm), *S. aureus*
3a (lowest)	0.4	0.6
3a (highest)	0.9	1.2
3b (lowest)	0.6	0.7
3b (highest)	0.9	1.1
4b (lowest)	0.7	0.7
4b (highest)	1.0	1.3
Streptomycin (10 µg/mL)	3.2	3.4

CONCLUSION

We have prepared twelve compounds by reacting substituted/ unsubstituted 2,3-dihydro-2,3-dioxindole and various benzoyl hydrazide in alcohol in presence of glacial acetic acid. All synthesized compounds were characterized by TLC, MP, and spectral analysis. All compounds were screened for antitubercular, and 3a, 3b and 4b compounds for antibacterial activity. Compounds 3a, 3c, 4a, 4b and 4h were capable of exhibiting antitubercular activity against *Mycobacterium tuberculosis* $H_{37}Rv$ at 25 µg/mL. Compounds 3a, 3b and 4b displayed mild antibacterial activity against both the tested organisms on comparison to streptomycin.

REFERENCES

1. Dutt, A. K., Stead, W. W., In Tuberculosis and Nontuberculosis Mycobacterial Infections, 4th ed.; David, S., Ed.; W.B. Saunders, 1999; p 3.
2. Martien, W. B., Annual risk of tuberculous infection —time for an update? *Bull. World Health Org.*, 80, 501, 2002.
3. Snider, D. E. J., Raviglione, M., and Kochi, A., In Tuberculosis: Pathogenesis Protection and Control; Bloom, B. R., Ed.; American Society of Microbiology:Washington, DC, 1994; p 2.
4. Bloom, B. R., Murray, C. J. L., Tuberculosis: Commentary on a Reemergent Killer, *Science*, 257, 1055-1064, 1992.
5. Snider, D. E. J., Roper, W. L., The New Tuberculosis, *New Engl. J. Med.*, 326, 703-705, 1992.

6. Bass, J. B., Farer, L. S., Hopewell, P. C., O'Brien, R., Jacobs, R. F., Ruben, F., Dixie, E., Snider, J., Thornton, G., Treatment of tuberculosis and tuberculosis infection in adults and children. American Thoracic Society and The Centers for Disease Control and Prevention, *Am. J. Respir. Crit. Care Med.,* 149, 1359-1374, 1994.
7. Rattan, A., Kalia, A., Ahmed, N., Multidrug-Resistant Mycobacterium tuberculosis: Molecular Perspectives, *Emerg. Infect. Dis.,* 4, 195-209, 1998.
8. Piero, S., Giuliona, G. G., In Burgers Medicinal Chemistry and Drug Discovery, 4th ed.; Wolff, M. E., Ed.; John Wiley and Sons, Inc.: New York, 1996; p 575.
9. Ían, O., Search for New Drugs for Treatment of Tuberculosis, *Antimicrob. Agents Chemother.,* 45, 1943-1946, 2001.
10. Temple, M. E., Nahata, M. C., Rifapentine: its role in the treatment of tuberculosis, *Ann. Pharmacother.,* 33, 1203-1210, 1999.
11. Miyazaki, E., Miyazaki, M., Chen, J. M., Chaisson, R. E., Bishai, W. R., Moxifloxacin (BAY12-8039), a New 8-Methoxyquinolone, Is Active in a Mouse Model of Tuberculosis, *Antimicrob. Agents Chemother.,* 43, 85-89, 1999.
12. Tomika, H., Sato, K., Akaki, T., Kajitani, H., Kawahara, S., Sakatani, M., Comparative In Vitro Antimicrobial Activities of the Newly Synthesized Quinolone HSR-903, Sitafloxacin (DU-6859a), Gatifloxacin (AM-1155), and Levofloxacin against *Mycobacterium tuberculosis* and *Mycobacterium avium* Complex, *Antimicrob. Agents Chemother.,* 43, 3001-3004, 1999.
13. Ruiz Serrano, M. J., Alcala, L., Martinez, L., Diaz, M., Marin, M., Gonzalez-Abad, M. J., Bouza, E., In Vitro Activities of Six Fluoroquinolones against 250 Clinical Isolates of *Mycobacterium tuberculosis* Susceptible or Resistant to First-Line Antituberculosis Drugs, *Antimicrob. Agents Chemother.,* 44, 2567-2568, 2000.
14. Zurenko, G. E., Yagi, B. H., Schaadt, R. D., Allison, J. W., Kilburn, J. O., Glickman, S. E., Hutchinson, D. K., Barbachyn, M. R., Brickner, S. J., In vitro activities of U-100592 and U-100766, novel oxazolidinone antibacterial agents, *Antimicrob. Agents Chemother.,* 40, 839-845, 1996.
15. Barbachyn, M., Huchinson, D. K., Brickner, S. J., Cynamon, M. H., Kilburn, J. O., Klemens, S. P., Glickman, S. E., Grega, K. C., Hendges, S. K., Toops, D. S., Ford, C. W.,Zurenko, G. E., Identification of a Novel Oxazolidinone (U-100480) with Potent Antimycobacterial Activity, *J. Med. Chem.,* 39, 680-685, 1996.
16. Stover, K. C., Warrener, P., Vandevanter, D. R., Sherman, D. R., Arain, T. M., Langhorne, M. H., Anderson, S. W., Towell, J. A., Yuan, Y., McMurray, D. N., Kreiswirth, B. N., Barry, C. E., Baker, W. R., A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis, *Nature,* 405, 962-966, 2000.
17. Joaquim, D. S., Garden, S. J., Pinto, A. C., The Chemistry of isatins: A Review, *J. Braz. Chem. Soc.,* 12, 273-324, 2001.
18. Varma, R. S., Nobles, W. L., Substituted N-amino methyl isatins. Substituted N-Aminomethylisatins, *J. Med. Chem.*, 10, 510-513, 1967.
19. Kupini, M., Medi, M., Morvin, M., Maysinger, D., Antibacterial and antifungal activities of isatin N-Mannich bases, *J. Pharm. Sci.,* 68, 459-462, 1979.
20. Dilber, S., Saban, M., Gelinco, A., Bogavac, M., Investigation of antimicrobial activity of some isatin derivatives, *Pharmazie,* 45, 800-805, 1990.
21. Maysinger, D., Ban, J., Morvin, M., Effects of N- Mannich bases of isatins on various bacteria and fungi, *Arzneim. Forsch.,* 30, 932-935, 1980.
22. Pandeya, S. N. Gnana, S., Saravanan, M., Sriram, D., Senthil, K., Synthesis and antibacterial activity of Mannich bases of ciprofloxacin and lomefloxacin with isatin and its derivatives, *Indian J. Pharm. Sci.,* 60, 280-282, 1998.

23. Mogilaiah, K., Rao, R. B., Synthesis and antibacterial activity of some novel spiro [indole-pyrazolines], spiro[indole- pyrimidines] and spiro[indole-1,5-benzodiazepine] containing 1,8- napthyridine moiety, *Indian J. Chem.*, 37, 139-144, 1998.
24. Ravichandran, V., Mohan, S., Suresh, K., Synthesis and antimicrobial activity of Mannich bases of isatin and its derivatives with 2-[(2,6-dichloro phenyl)amino] phenylacetic acid, *ARKIVOC*, 15, 51-57, 2007.
25. Pirrung, C., Sunil, V., Koushik, D., Kathy, A., Combinatorial optimization of isatin *beta* thiosemicarbazones as antipox virus agents, *J. Med. Chem.*, 48, 3045-3050, 2005.
26. Aliasghar, J., Dariush, K., De Clercq, Chanaz, S., Synthesis, antibacterial, antifungal and antiviral activity, Evaluation of some new bis- Schiff bases of isatin and their derivatives, *Molecules*, 12, 1720-1730, 2007.
27. Venkateshwar, J., Sarangapani, M., Synthesis and evaluation of some new isatin hydrazones for their antibacterial, antioxidant and cytotoxic activities, *Asian J. Chem.*, 20, 5331-5335, 2008.
28. Karki, S.S., Hazare, R., Kumar, S., Bhadauria, V. S., Balzarini, J., De Clercq., Synthesis, anticancer and cytostatic activity of some 6H-indolo[2,3-b]quinoxalines, *Acta Pharm.*, 59, 431–440, 2009.
29. Karki, S.S., Bhadauria, V.S., Rana, V., Kumar, S., Subbarao, G.S., Das, U., Balzarini, J., De Clercq., Dimmock, J.R., 1-Arylmethyl-2,3-dioxo-2,3-dihydroindole thiosemicarbazones as leads for developing cytotoxins and anticonvulsants, *Journal of Enzyme Inhibition and Medicinal Chemistry*, 24(2), 537–544, 2009.
30. Marvel, C.S., Heirs, G.S., In: Blatt AH, editor. Isatin. Organic syntheses collection. 2nd ed., Vol 1, New York: John Wiley and Sons, Inc; 1941. p 327–330.
31. Azizian, J., Fallah-Bagher-Shaidaei, Kefayati, H., A facile one pot method for the preparation of N-alkyl isatins under microwave irradiation, *Synth. Commun.*, 33, 789 –793, 2003.
32. National Committee for Clinical Laboratory Standards (NCCLS). Performance standards for antimicrobial disk susceptibility tests, 6th ed.: Approved standard M2-A6. National Committee for Clinical Laboratory Standards, Wayne, PA, 1999.

Received: 23.08.2010
Accepted: 30.09.2010