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Research Article

SYNTHESIS, CHARACTERIZATION, ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF ISATIN DERIVATIVES

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ABSTRACT

Heterocyclic compounds have a wide range of application in the field of chemistry. The fusions of several rings resulting in polycyclic structures from biologically active heterocyclic templates are always of interest to both organic and medicinal chemists. In view of these facts and continuation of our work on some pharmacologically active heterocyclic compounds by condensation of (3)-3-hydrazinylidine-1, 3-dihyro-2h-benzo (g) indol-2-one has been condensed with different thiadiazole derivatives. The chemical structures of synthesized compounds where confirmed by means of IR, NMR spectral data and compounds were screened for their antibacterial, antifungal activity. All the novel Isatin derivatives exhibited mild to moderate activity. Compound14 & 15 exhibited better activity than the other test compounds.

Keywords: Heterocyclic Compound, thiadiazole, antibacterial, antifungal.

INTRODUCTION

Isatin (1H-Indole 2, 3-di-one) belong to an important class of heterocyclic compounds in medicinal chemistry associated with wide range of biological activities such as antibacterial[1-3], antifungal[4-6], antiprotozoal[7-8], antiviral[9-11], anthelmintic[12-13] and CNS activities[14-15]The biological importance of the compounds inspired us to synthesize some new isatin hydrazones to get more potent compounds and screen for Antimicrobial activity. Synthesis of the title compounds was affected as shown in Scheme-1. The chemical structures of synthesized compounds were confirmed by means of ^{1}HNMR, IR, mass spectral data and elemental analysis. All the synthesized compounds were screened for antibacterial and antifungal activity by an agar diffusion method[16].

EXPERIMENTAL METHODS

All the chemicals used in the synthesis were obtained from standard commercial sources. Reactions were monitored by TLC using silica gel-G (Merck grade) as the adsorbent and the solvent systems are indicated at appropriate places. Silica gel (100-200 mesh, Merck grade) has been used for column chromatography.All the melting points were determined in open capillaries, using Boitus melting point apparatus, expressed in ^oC and are uncorrected.The ^{1}H NMR spectra of the compounds were recorded on Bruker spect, 400 MHz NMR spectrophotometer using TMS as an internal standard and the values are expressed in δ ppm

I. Synthesis of 1H-benzo[g]indole-2, 3-dione (iii)[17-18]

a. Synthesis of isonitrosoacetanilide (II) – General Procedure

In a 5 lit. R.B. flask were placed chloralhydrate (0.54 mol) and 1200 ml of water. To this solution, were then added crystallized sodium sulphate (1300 g) followed by a solution of α-naphthylamine (0.5 mol) (I) in 300 ml of water and concentrated hydrochloric acid (0.52 mol). Finally, a solution of hydroxylamine HCl (1.58 mol) in 500 ml of water was added. The content of flask was heated over a wire-guage by a mecker burner, so that vigorous boiling begins in about 45 minutes. After 1-2 minutes of vigorous boiling the reaction was complete. During the heating period itself, the crystals of isonitroacetanilide started separating out. On cooling under the current of water, the entire product was solidified. It was filtered under suction, air dried and purified by recrystallization from suitable solvent (s).

b. Synthesis of 1H-benzo[g]indole-2, 3-dione (III) – General Procedure

Sulphuric acid (600 g, d. 1.84, 326 ml) was warmed to 50^0 C in a one-litre R.B. flask fitted with an efficient mechanical stirrer and to this, finely powdered isonitrosoacetanilide (0,46 mol) (II) was added at such a rate so as to maintain the temperature between 60 and 70^0 C, but not higher. External cooling was applied at this stage so that the reaction could be carried but more rapidly. After the addition of isonitroso compound was completed, the temperature of the solution was raised to 80^0 C and maintained at that temperature for 10 minutes, to complete the reaction. Then, the reaction mixture was cooled to room temperature and poured on crushed ice (2.5 kg). After standing for about half-an-hour, the product separated was filtered, washed several times with small portions of cold water and dried. Purification of the compound was effected by recrystallization from methanol.

Ii. Synthesis of 1, 3, 4-thiadiazole (VII)

a. Synthesis of Thiosemicarbazone (VI) – General Procedure

5 gm of thiosemicarbazide HCl & 4.5 gm of anhydrous sodium acetate was added to 25 ml of water, heated gently until a clear solution was obtained. A solution of 5 ml of appropriate aromatic aldehydes in 25 ml of rectified spirit was added and warmed. This mixture was heated gently on a water bath for 15min. This thiosemicarbazone was rapidly crystallized out in the solution still being heated. It was washed thoroughly with water and dried.

b. Synthesis of 1, 3, 4-Thiadiazole (VII):– General Procedure

A solution of appropriate aromatic aldehydes, thiosemicarbazone (1gm) and anhydrous sodium acetate was prepared. 10ml of Br_2 was mixed with 40ml of acetic acid. The above solution was added drop wise into the slurry with constant stirring until a yellow colour was produced. Then the stirring was continued for about 15mins and cooled. Add crushed ice to the above solution, filter and dried.

III. Synthesis of 3-(1',3',4'-thiadiazol-2'yl-imino)-1H-benzo[e]indol-2-one (VIII)

Equimolar quantity (0.01 mol) of naphthylisatin (III), 1, 3, 4-Thiadiazole (VII) (0.01 mol) and few drops of glacial acetic acid (0.01 mol) were dissolved in 10 ml of warm methanol and refluxed for 4hrs. After standing for approximately 24 hr at room temperature, the products were separated by filtration, vacuum dried and recrystallized from warm methanol. The synthesized compounds have been characterized by the physical & spectral data.

The overall reaction involving the formation of Isatin derivatives are shown in **Scheme-I.**

ANTIMICROBIAL STUDIES

Since the isatin derivatives were reported to possess antimicrobial activity, the Isatin derivatives prepared during the course of the present work were tested for antibacterial and antifungal activity[19].

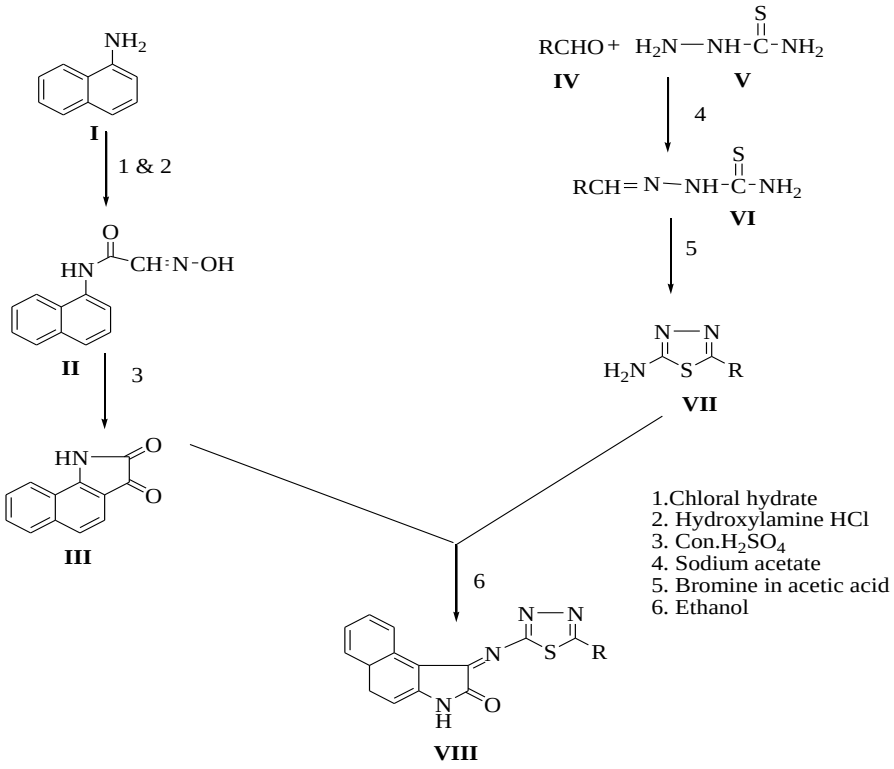
ANTIBACTERIAL ACTIVITY

Experimental procedure

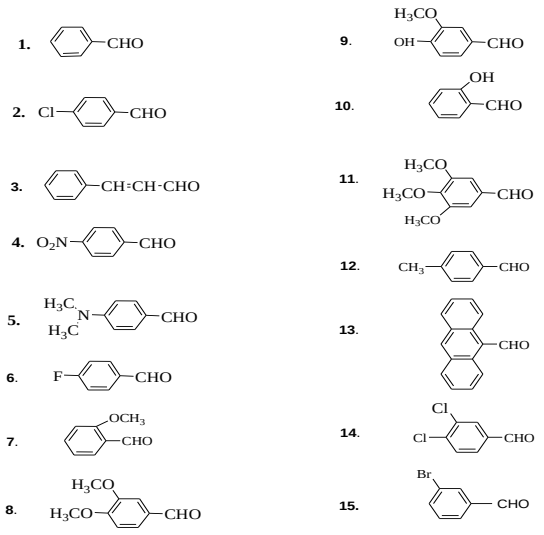
Nutrient agar (Hi-media) was dissolved and distributed in 25 mL quantities in 100 mL conical flasks and were sterilized in an autoclave at 121^0C (15 lbs/sq.in) for 20 minutes . The medium was inoculated at one percent level using 18hrs old cultures of the test organism mentioned above, aseptically into sterile petridishes and allowed to set at room temperature for about 30 min.

In a size of 4 inch petridishes, four cups of 8mm diameter at equal distance were made in each plate. In each plate, one cup was used for control i.e. Dimethyl sulfoxide (DMSO), another for standard benzylpenicillin and all the test compounds and the standard were tested at 100 µg and 150 µg dose levels.

The plates thus prepared were left for 90 min in a refrigerator for diffusion. After incubation for 24 hrs at 37^0c ± 1^0c, the plates were examined for inhibition zones. The experiments were performed in duplicate and the average diameters of the zones of inhibition measured was recorded. The results are shown in **Table- 3.**



RCHO



Scheme-I

ANTIFUNGAL ACTIVITY

The antifungal activity was tested by cup-plate method and compared with the standard Clotrimazole (10 µg/ml). Dimethylsulfoxide (DMSO) was used as a solvent and control.

Experimental procedure

Potato-dextrose-agar (Hi-media) was dissolved and distributed in 25 ml quantities in 100 ml conical flasks and were sterilized in an autoclave at 121^{0}C (15 lbs/sq.in) for 20 min. The medium was inoculated at one percent level using 48hrs old cultures of the test organism mentioned above aseptically into sterile petridishes and allowed to set at room temperature for about 30 min.

In 4 inch petridishes, four cups of 8mm diameter at equal distance were made in each plate. In each plate, one cup was used for control i.e. Dimethyl sulfoxide (DMSO), another for standard Clotrimazole. Each compound was tested at two different dose levels (100 µg and 150 µg).

The plates thus prepared were left for 90 min. in a refrigerator for diffusion. After incubation for 48 hrs at 25^{0}c, the plates were examined for inhibition zones. The experiments were performed in duplicate and the average diameter of the zones of inhibition measured were recorded. The results are shown in **Tables –4.**

RESULTS AND DISCUSSION

Among the compounds tested, NT-14 was found to be more potent against *B.subtilis* and *E.coli* at both the dose levels tested. This compound was also active against *S.aureus* and *P.vulgaris*. Compounds NT-05, NT-11 and NT-12 also showed antimicrobial activity at the dose levels tested.

Among the compounds tested for antifungal activity, compounds NO-14 & NO-15were found to be more potent than the other compounds at both the dose levels tested, but less potent than the standard drug fluconazole.

The above results indicate the necessity of halogen at the *para* position of the phenyl ring. Since the halogen substitution has contributed favorably to the inhibitory activity, a Isatin with two or more such substituents on the aromatic ring at different positions can be synthesized with a hope to get promising antibacterial & antifungal compounds.

The results also indicate, in general, a simple aryl group in the place of substituted aryl group contributes favorably to the inhibitory activity. Compounds with more number of electron releasing or electron with drawing substituents on the aromatic ring at different positions can be synthesized to draw meaningful conclusions with respect to the influence of electronic effects on the antimicrobial activity.

Table 1: Physical characterization data of the compounds (NT1-NT15)

S. No.	Compd	Substituents R	Mol. Formula	Mol. Weight	M.P.(^{0}C)	Yield (%)
1	NT1	Phenyl	$C_{20}H_{12}N_4OS$	356	297±2^{0}C	32.17
2	NT2	4''-chlorophenyl	$C_{20}H_{11}N_4OSCl$	390	305±2^{0}C	24.35
3	NT3	Styryl	$C_{22}H_{14}N_4OS$	382	234±2^{0}C	28.55
4	NT4	4''-nitrophenyl	$C_{20}H_{11}N_4O_3S$	401	230±2^{0}C	45.28
5	NT5	4''-(dimethylamino)phenyl	$C_{22}H_{17}N_4OS$	399	248±2^{0}C	37.01
6	NT6	4''-fluorophenyl	$C_{20}H_{11}N_4OSF$	373	225±2^{0}C	31.42
7	NT7	3''-methoxyphenyl	$C_{21}H_{14}N_4O_2S$	386	215±2^{0}C	11.86
8	NT8	3'',4''-dimethoxyphenyl	$C_{22}H_{17}N_4O_3S$	416	276±2^{0}C	17.38
9	NT9	4''-Hydroxy, 3''-methoxy phenyl	$C_{21}H_{14}N_4O_3S$	402	243±2^{0}C	20.62
10	NT10	2''-Hydroxyphenyl	$C_{20}H_{12}N_4O_2S$	372	296±2^{0}C	22.81
11	NT11	3'',4'',5''-trimethoxyphenyl	$C_{23}H_{23}O_4N_4S$	451	296±2^{0}C	22.81
12	NT12	5'-p-tolyl-1',3',4'-oxadiazol-2'-ylimino	$C_{23}H_{23}O_4N_4S$	373	296±2^{0}C	22.81
13	NT13	anthracene-9''-yl	$C_{28}H_{16}O_4N_4S$	456	296±2^{0}C	22.81
14	NT14	2'',4''-dichlorophenyl	$C_{20}H_9ON_4SCl_2$	424	296±2^{0}C	22.81
15	NT15	3''-bromophenyl	$C_{20}H_{11}N_4SBr$	425	296±2^{0}C	32.41

Table 2: Spectral data of the compounds (NT1 – NT15)

compound	IR (KBr, cm^{-1})	^{1}H-NMR (δ ppm)
Phenyl	**3400 (NH), 1632 (C=N), 1579 (C=C), 1358 (C-N), 689 (C-S), 1359 (C= O)**	**7.89 (1H, s, NH), 7.01-7.76 (6H, m, C- 4, 5, 6, 7, 8, 9), 7.65 (2H, s, C-2'' and 6''), 7.31 (3H, m, C-3'', 4''and 5'')**
4''-chlorophenyl	**3342 (NH), 1630 (C=N), 1582 (C=C), 1358 (C-N), 695 (C-S), 1740 (C=O), 1110 (C-Cl).**	**7.92 (1H, s, NH), 7.12-7.67 (6H, m, C- 4, 5, 6, 7, 8, 9), 7.49 (2H, s, C-2'' and 6''), 7.28 (3H, m, C-3'' and 5'')**

Styryl	**3400 (NH), 1641 (C=N), 15794(C=C), 1348 (C-N), 655 (C-S), 1628 (C= O)**	**7.85 (1H, s, NH), 7.05-7.72 (6H, m, C- 4, 5, 6, 7, 8,9), 7.29 (2H, s, C-2''' and 6'''), 7.20 (2H, m, C-3''',5'''), 7.16 (1H, m, C-4''), 5.62 (1H, s, C-1''), 6.61 (1H, s, C-2'').**
4''-nitrophenyl	**3350 (NH), 1592 (C=N), 1358 (C-N), 689 (C-S), 1720 (C=O), 1564 (Ar- C=C), 1545 (Asy-C-NO_2), 1325 (Sym-C- NO_2)**	**7.92 (1H, s, NH), 7.09-7.74 (6H, m, C- 4, 5, 6, 7, 8, 9), 7.83 (2H, s, C-2'' and 6''), 8.12 (2H, m, C-3'',5'')**
4''-(dimethylamino)phenyl	**3200 (NH), 1632 (C=N), 1598(C=C), 1258(C-N), 695 (C-S), 1720 (C=O), 1564 (Ar- C=C).**	**8.12 (1H, s, NH), 6.98-7.64 (6H, m, C- 4, 5, 6, 7, 8,9), 7.38 (2H, s, C-2'' and 6''), 6.72 (2H, m, C-3'', 5''), 2.91 (6H, s, 2-CH_3)**
4''-fluorophenyl	**3165 (NH), 1635 (C=N), 1570 (C=C), 1358 (C-N), 684 (C-S), 1740 (C=O), 1578 (Ar- C=C), 1058 (C-F).**	**7.9 (1H, s, NH), 6.84-7.53 (6H, m, C- 4, 5, 6, 7, 8,9), 7.58 (2H, s, C-2'' and 6''), 7.65 (2H, s, C-3'', 5'')**
3''-methoxyphenyl	**3320 (NH), 1635 (C=N), 1582 (C=C), 1358 (C-N), 675 (C-S), 1689 (C= O), 1240 (-OCH_3).**	**8.12 (1H, s, NH), 6.86-7.86 (6H, m, C- 4, 5, 6, 7, 8,9), 6.85 (2H, s, C-2'' and 6''), 7.52 (2H, m, C-3'', 5'')**
3'',4''-dimethoxyphenyl	**3255 (NH), 1603 (C=N), 1579 (C=C), 1457 (C-N), 798 (C-S), 1709 (C= O), 1564 (Ar- C=C), 3020 (=C-H), 1230 & 1171(-OCH_3).**	**7.92 (1H, s, NH), 6.96-7.69 (6H, m, C- 4, 5, 6, 7, 8,9), 7.00 (1H, s, C-6''),7.03(1H, s, C-2''),7.35 (1H, m, C-5''), 3.69 (6H, s, 2OCH_3)**
4''-Hydroxy, 3''-methoxy phenyl	**3390 (NH), 1635 (C=N), 1579 (C=C), 1358 (C-N), 685 (C-S), 1359 (C= O), 1564 (Ar- C=C), 3157 (O-H), 1185 (O-CH_3).**	**8.02 (1H, s, NH), 6.86-7.96 (6H, m, C- 4, 5, 6, 7, 8,9), 6.65 (1H, s, C-6''), 6.93 (1H, s, C-5''), 7.05 (1H, m, C-2''), 3.95 (1H, s, OCH_3), 5.05 (1H, s, OH)**
2''-Hydroxyphenyl	**3310 (NH), 1632 (C=N), 1667 (C=C), 1384 (C-N), 689 (C-S), 1701 (C= O), 1530 (Ar- C=C), 3180 (O-H)**	**7.95 (1H, s, NH), 6.68-7.65 (6H, m, C- 4, 5, 6, 7, 8,9), 6.75 (1H, s, C-6''), 6.83 (1H, s, C-5''), 7.12 (1H, s, C-4''), 7.45 (1H, m, C-3''), 4.95(1H, s, OH)**
3'',4'',5''-trimethoxyphenyl	**3410 (NH), 1630 (C=N), 1579 (C=C), 1358 (C-N), 689 (C-S), 1629 (C= O), 1564 (Ar- C=C), 1220 (O-CH_3)**	**7.9 (1H, s, NH), 6.84-7.53 (6H, m, C- 4, 5, 6, 7, 8,9), 6.64 (2H, s, C-2'' & C-6''), 3.82 (9H, s, -3OCH)**
5'-p-tolyl-1',3',4'-oxadiazol-2'-ylimino	**3210 (NH), 1635 (C=N), 1560 (C=C), 1358 (C-N), 689 (C-S), 1720 (C=O), 1564 (Ar- C=C), 3085 (O-H)**	**7.91 (1H, s, NH), 7.14-7.72 (6H, m, C- 4, 5, 6, 7, 8,9), 7.49 (2H, s, C-2'' and C-6''), 7.10 (2H, s, C-3'' and C-5''), 2.42 (3H, s, CH)**
anthracene-9''-yl	**3400 (NH), 1632 (C=N), 1579 (C=C), 1358 (C-N), 670 (C-S), 1359 (C= O)** **7.92 (1H, s, NH), 6.92-7.21 (6H, m, C- 4, 5, 6, 7, 8, 9)**	**7.35 (4H, s, C-2'', 5'', 7'', 10''), 7.67 (4H, s, C-3'', 4'', 8'' and 9''), 7.74 (1H, s, C-6'').**
2'',4''-dichlorophenyl	**3150 (NH), 1632 (C=N), 1579 (C=C), 1358 (C-N), 685 (C-S), 1659 (C= O), 1564 (Ar- C=C), 824 (C-Cl)**	**8.2 (1H, s, NH), 6.84-7.53 (6H, m, C- 4, 5, 6, 7, 8, 9), 7.31 (1H, s, C-3''), 7.51 (1H, s, C-6''), 7.19 (1H, s, C-5'')**
3''-bromophenyl	**3400 (NH), 1632 (C=N), 1579 (C=C), 1358 (C-N), 689 (C-S), 1659 (C= O), 1564 (Ar- C=C), 578 (C-Br)**	**8.1 (1H, s, NH), 6.84-7.53 (6H, m, C- 4, 5, 6, 7, 8, 9), 7.81 (1H, s, C-2''), 7.49 (1H, s, C-4''), 7.19 (1H, s, C-5''), 7.59 (1H, s, C-6'')**

Table 3: Antibacterial activity of Isatin derivatives (compounds NT-1 to NT-15)

Compound	Zone of inhibition (in mm)							
	B.subtilis		*S.aureus*		*E.coli*		*P.vulgaris*	
	100 µg	150 µg	100 µg	150 µg	100µg	150 µg	100 µg	150 µg
Standard	20	22	19	22	21	23	16	19
Control	-	-	-	-	-	-	-	-
NO-1	09	11	08	09	06	08	07	08
NO-2	12	14	13	14	10	11	12	13
NO-3	08	10	08	10	09	11	08	11
NO-4	12	13	10	12	12	14	09	11
NO-5	11	12	09	11	09	10	08	10
NO-6	14	17	12	14	14	11	12	12
NO-7	11	13	10	11	09	11	09	10
NO-8	12	14	09	11	10	12	08	10
NO-9	10	12	09	10	08	11	10	11
NO-10	10	11	08	10	08	11	08	10
NO-11	13	16	12	14	14	16	10	12
NO-12	11	13	09	11	08	10	08	10
NO-13	08	11	07	09	09	10	08	09
NO-14	16	18	14	16	12	12	13	12
NO-15	12	15	13	15	12	12	13	14

Note: "–"No zone of inhibition

Table 4: Antifungal activity of Isatin derivatives (compounds NT-1 to NT-15)

Compound	Zone of inhibition (in mm)			
	A.niger		*P.crysogenum*	
	100 µg	150 µg	100 µg	150 µg
Standard	23	25	22	24
Control	-	-	-	-
NO-1	14	15	16	17
NO-2	20	21	18	21
NO-3	15	17	16	18
NO-4	19	21	19	20
NO-5	19	21	20	22
NO-6	20	22	19	22
NO-7	18	19	17	18
NO-8	18	20	18	19
NO-9	18	19	15	16
NO-10	17	18	16	17
NO-11	18	19	20	21
NO-12	16	18	16	17
NO-13	15	16	16	17
NO-14	22	24	20	22
NO-15	19	20	17	18

REFERENCES

1. Pandeya SN and Sriram D. Acta pharm Turc. 1998;40:33-38.
2. Sarangapani M and Reddy VM. Indian J Pharm Sci. 1994;6:174-177.
3. Varma RS and Nobles WL. J Pharm Sci. 1975;64: 881-882.
4. Khan SA, Siddiqui N, Imran M and Haque SW. Indian J Pharm Sci. 2004;66(6): 830.
5. Pandeya SN, Sriram D, Nath G and De Clercq E. Sci Pharm. 1999;67:103-111.
6. Pandeya SN, Sriram D, Nath G and De Clercq E. Pharm Acta Helv. 1999;74:11-17.
7. Imams A and Varma RS. Experientia. 1975;31:1287-1288.
8. Varma RS and Khan IA. Polish J Pharm. 1977;29:549-594.
9. Debrac Quenelle, Kathy A. Keith and Earl R. Kern. Antiviral Research. 2006;71:24-30.
10. Selvan P, Chandramohan M, Delereq E, Myrian Witvrow and Christophe Pannecouque. Eur J Pharm Sci. 2000;114:313.
11. Sriram D. Tanusree and Balasubramani. Biorg & Medchem Lett. 2005;15:4452.
12. Sarciron SE, Audin P, Delebre I, Gabrion C, Pentavy AF and Paris J. J Pharm Sci. 1993;82:605-609.
13. Et-Sawi EA, Mostafa TB and Mostafa BB. J Egypt Soc Parasitol. 1998;28:481-486.
14. Pandeya SN, Senthil Raja A and Stables JP. J Pharm Sci. 2002;5:266-270.
15. Varma M, Pandeya SN, Singh K and Stables JP. Acta pharm. 2004;54:49-56.
16. Analytical Microbiology. Ed. E. Kavanagh, Academic press, Newyork. 1963:249.
17. Alam M, Younas M, Zafar MA and Naeem. Pak J Sci Ind Res. 1989;32: 246.
18. Atmakuru Ramesh, Seshaiah Krishnan Sridhar and Muniyandy Sarvanan. Eur J Med Chem. 2001;36:615-625.
19. Andrews JM. Journal of Antimicrobial Chemotherapy. 2001;48:5.