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Synthesis and Biological Evaluation of 2-(1*h*-Benzotriazol-1-Yl) Acetohydrazide Containing Isatin Derivatives

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ABSTRACT

Benzotriazole was condensed stoichiometrically with ethyl chloro acetate in presence of potassium carbonate. The resulting ethyl-1H-benzotriazole-1yl-acetate was reacted with hydrazine hydrate in ethanolic solution. The resulting 2-(1H-benzotriazole-1-yl) aceto hydrazide was characterized by physical data and spectral studies and further it was condensed with 1H-indole-2,3-dione derivatives to form 2-(1H-benzotriazole-1-yl)-N'-[(3Z)-2-oxo-1,2-dihydro-3H-indol-3-ylidene] aceto hydrazide derivatives. These derivatives were characterized by melting point, TLC, IR, ^{1}H-NMR and Mass spectrum. From the biological activity profile it was revealed that they have considerable anti-inflammatory activity & antimicrobial activity against *S. aureus, B. subtilis, Klebsiella and E. Coli*.

Keywords: Indole-2, 3-diones, Benzotriazole, Antimicrobial and invitro anti-inflammatory activities;

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INTRODUCTION:

In the last few decades, the chemistry of 1, 2, 4-triazoles and their fused heterocyclic derivatives have received considerable attention owing to their synthetic and effective biological importance. For example, a large number of 1,2,4-triazole-containing ring system have been incorporated into a wide variety of therapeutically interesting drug candidates including anti-inflammatory, CNS stimulants sedatives, antianxiety, antimicrobial agents and antimycotic activity such as fluconazole, intraconazole, voriconazole. Also, there are known drugs containing the 1, 2, 4-triazole group e.g. Triazolam, Alprazolam, Etizolam, and Furacylin [1]

Isatin is an endogenous compound identified in humans that possesses wide range of biological activities. Isatin has anxiogenic, sedative, anticonvulsant activity and acts as a potent antagonist on atrial natriuretic peptide receptors in-vitro. A series of p-substituted Isatin semicarbazones have shown anticonvulsant activity. Various isatin N-Mannich bases of isatin-3-thiosemicarbazones have shown antiviral and tuberculostatic activity. Methisazone is an effective compound against variola and vaccinia viruses. Various substituted indolinones showed antitubercular activity against M. tuberculosis. Isatin derivatives of Mannich bases had fibrinolytic, muscle relaxant, antiallergic, immunosuppressant, and antithrombotic activity[2, 3].

MATERIALS AND METHOD:

The chemicals and reagents used in the present project were of AR and LR grade, procured from Aldrich, Hi-Media, Merck, Sigma, Ranbaxy and S.D- Fine Chem. Ltd. The melting points of the synthesized compounds were determined by Thiel's melting point apparatus (open capillary tube method).^{1}HNMR reports were from Indian Institute of Science (IISC), Bangalore, mass spectrum reports and elemental analysis reports were from Uwin Global Services, Bangalore.

STEP I: Synthesis of ethyl 1*H*-benzotriazol-1-ylacetate (1) [4]:

In a 250 ml iodine flask, 60 ml of acetone, 0.01M of 1H-benzotriazole, 1 ml of ethyl chloroacetate and 3 gm of anhydrous potassium carbonate was added and stirred for 6 hours at room temperature. The solution was filtered to remove potassium carbonate. The solvent was removed under reduced pressure. The product so obtained was extracted with ether. The ether was evaporated to get needle shaped brown crystals. The yield obtained was 90.24% and M.P. was 58 ^{o}C.

STEP II: Synthesis of 2-(1*H*-benzotriazol-1-yl)acetohydrazide(2) [4] :

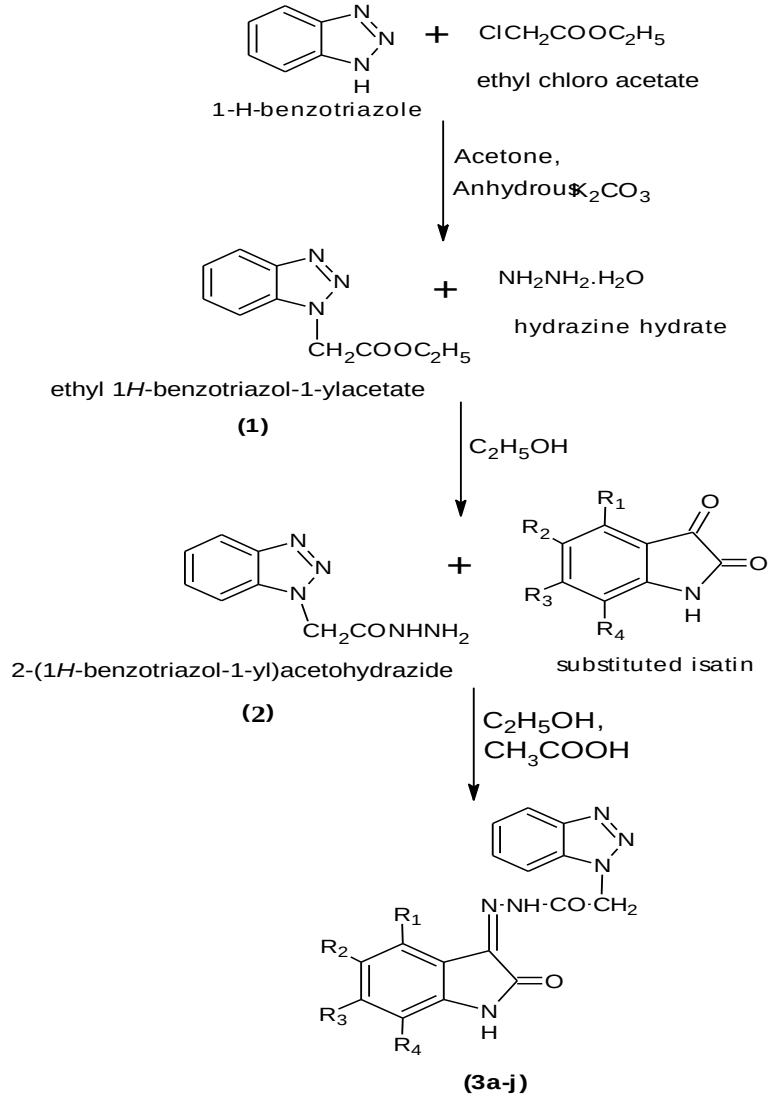
In a 250 ml iodine flask, an ethanolic solution of ethyl 1-H benzatriazol-1-yl acetate (1) (0.01M) and hydrazine hydrate (20 ml) was stirred for 4 hours at room temperature and then refluxed on

water bath for 3 hours in a 250 ml round bottom flask, the solution was kept in a freezer for overnight and then excess solvent was removed under reduced pressure. The crystals so obtained were washed with cold water and recrystallised from ethanol. The yield obtained was 8.53% and M.P. was 130 ^{0}C

STEP III: Synthesis ofSubstituted2-(1*H*-benzotriazole-1-yl)-*N*'-[(3*Z*)-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]acetohydrazide(3a-j):

A mixture of compound (2) (0.01mol) and Substituted Isatin[10-12] (1*H*-indole-2,3-dione) (0.01Ml) in ethanol (50ml) containing 3-4 drops of glacial acetic acid in a 250 ml round bottom flask was first stirred for 4 hours and then refluxed for 3 hours & kept over night at room temperature. The solid product so obtained was filtered & dried. The dried crude product was recrystallised with N, N-Dimethyl formamide: water. (7:3).

Scheme for synthesis:



Where

Compound	R_1	R_2	R_3	R_4	Compound	R_1	R_2	R_3	R_4
3a	H	H	H	H	3f	H	I	H	H
3b	H	Cl	H	H	3g	H	CH_3	H	H
3c	H	Br	H	H	3h	CH_3	H	H	H
3d	H	NO_2	H	H	3i	H	CH_3	CH_3	CH_3
3e	H	F	H	H	3ej	Cl	F	Cl	H

The purity of the synthesized compound (3a-3j) was established by TLC using silica gel G as stationary phase and Chloroform: Ethyl acetate: 8:2 as mobile phase.Other compounds (3b-3j) were synthesized in the similar manner.

BIOLOGICAL ACTIVITY:

Antibacterial activity [5,9]:

Antibacterial screening was determined based on the cup plate agar diffusion method by measuring a clear zone of inhibition in millimeters. All the samples were tested at a concentration of 1000µg/ml in dimethyl formamide. The bacterial strains employed were *Staphylococcus aureus, Bacillus subtilis, Escherichia coli*and *Klebsiella*. The antibacterial activity was carried out, by using standard reference drugs Amoxicillin (10µg/ml) and Ciprofloxacin (30µg/ml)

Antifungal activity [6,9]

The fungal strains used were *Candida albicans*and *Aspergillusniger*. Clotrimazole (10µg/ml) and Ketoconazole (10µg/ml) were used as standard reference drugs for antifungal activity.

***In-vitro* Anti inflammatory activity [7]:**

The synthesized compounds were screened for anti-inflammatory activity by in-vitro inhibition of albumin denaturation. The test compounds were dissolved in minimum amount of dimethyl formamide (DMF) and diluted with phosphate buffer (0.2M, pH 7.4).

The final concentration of DMF in all solutions was less than 2.5%. Test solution (1ml) containing different concentration of drug was mixed with 1ml of 1mg/ml albumin solution in phosphate buffer and incubated at 27º±1ºC for 15 min. Denaturation was induced by keeping the reaction mixture at 60º±1ºC in water bath for 10-20 min. After cooling, the transmittance of the turbid suspensions was measured at 660nm in UV Spectrophotometer. The percentage inhibition of denaturation was calculated with reference to control where no drug was added and compared against standard (Ibuprofen).

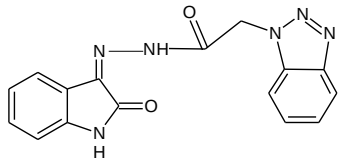
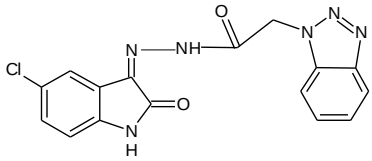
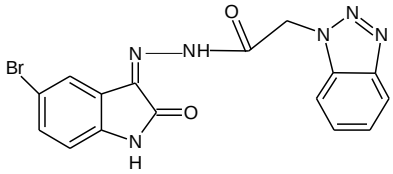
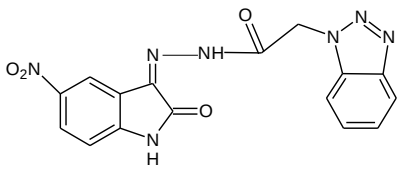
RESULT AND DISCUSSION:

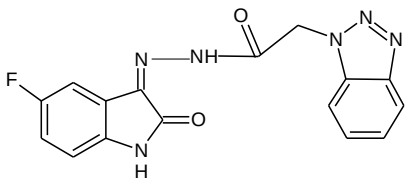
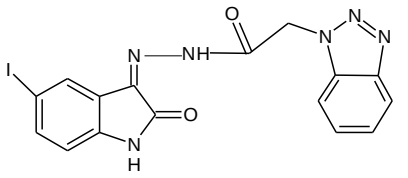
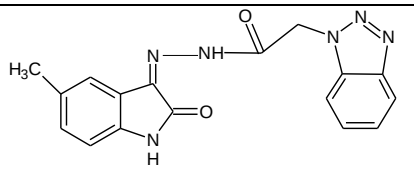
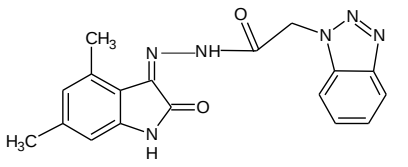
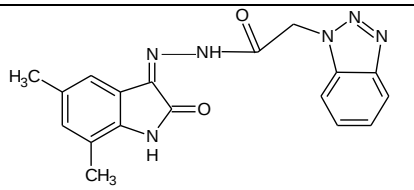
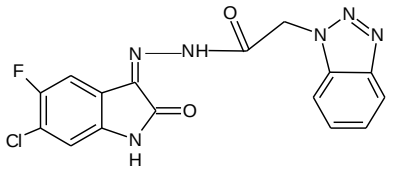
Physical characterization data (3a-3j) are presented in Table 1 and spectral data (3a-3b) are presented in Table 2.

Table 1: Physical data for Synthesized compounds (3a-3j):

Compounds	Molecular formula.	mol.wt gm/mol	M.P.	% yield	R_f*	Elemental analysis % found(calculated)		
						C	H	N
3a	$C_{16}H_{12}N_6O_2$	320.30	180°C	79.5%	0.42	62.86 (60)	3.72(3.78)	27.34 (26.24)
3b	$C_{16}H_{11}ClN_6O_2$	354.75	187°C	79.48%	0.72	55.13(54.17)	2.87(3.13)	24.33 (23.69)
3c	$C_{16}H_{11}BrN_6O_2$	399.20	140°C	85.71%	0.50	50.30(48.14)	2.62(2.78)	22.09 (21.05)
3d	$C_{16}H_{11}N_7O_4$	365.30	175°C	87.5%	0.54	52.04(52.61)	3.29(3.04)	28.03 (26.84)
3e	$C_{16}H_{11}FN_6O_2$	338.29	176°C	83.33%	0.72	57.66(56.81)	3.16(3.28)	25.93 (24.84)
3f	$C_{16}H_{11}IN_6O_2$	446.20	165°C	91.40%	0.72	46.25(43.07)	3.11(2.48)	19.53 (18.83)
3g	$C_{17}H_{14}N_6O_2$	334.33	145°C	76.92%	0.73	63.03(61.07)	4.30(4.22)	28.02 (25.14)
3h	$C_{18}H_{16}N_6O_2$	348.35	152°C	82.95%	0.53	53.05(51.56)	3.05(2.70)	24.35(22.55)
3i	$C_{18}H_{16}N_6O_2$	348.35	169°C	87.97%	0.68	63.65(62.06)	4.87(4.63)	26.45(24.12)
3j	$C_{16}H_{10}ClFN_6O_2$	372.74	160°C	86.90%	0.72	52.45(51.56)	3.10(2.70)	23.34(22.55)

Table 2: Spectral data for Synthesized compounds (3a-3j):

Comp.	Structure	IR Spectral Data (cm^{-1})	^{1}HNMR Spectral Data δ (ppm)	Mass Spectral Data
3a		3201.61 (20amine, N-H str), 1751.24 (isatin, >C=O str), 1697.24 (amide, >C=O str), 1445(N-H bend),3074.32 (Ar, C-H str), 2993.32 (alkanes, C-H str),	7.3-8.2 (8H, Ar), 8.2(1H, Ar N-H), 11.8 (1H, aliphatic amide N-H), 4.3-4.9 (2H, CH_2)	M/e =319; The other important peaks observed are: 146 and 176
3b		3336.62 (2^0amine, N-H str), 1735.36(isatin, >C=O str), 1697.24 (amide, >C=O str), 1515.94(N-H bend),3074.32 (Ar, C-H str), 2983.80(alkanes, C-H str),744.47 (C-Cl str)	7.1-8.2 (7H, Ar), 8.2(1H, Ar N-H), 11.3 (1H, aliphatic amide N-H), 4.3 (2H, CH_2)	M+1=355; The other important peaks observed are: 176 and 180
3c		3305.76 (2^0amine, N-H str), 1650.95(isatin, >C=O str), 1604.95 (amide, >C=O str), 1542(N-H bend),3053.81(Ar, C-H str), 2904.89(alkanes, C-H str), 557.03(C-Br str)	7.2-8.2 (7H, Ar), 8.2(1H, Ar N-H), 11.4 (1H, aliphatic amide N-H), 4.4 (2H, CH_2)	M/e=;398 The other important peaks observed are: 176 and 225
3d		3193.90 (2^0amine, N-H str), 1724.24(isatin, >C=O str), 1697.24 (amide, >C=O str), 3095.51Ar, C-H str), 2974.03(alkanes, C-H str), 1434.94 and 1388.23 (N=O str)	7.4-8.1 (7H, Ar), 8.1(1H, Ar N-H), 11.4 (1H, aliphatic amide N-H), 4.4 (2H, CH_2)	M/e-366; The other important peaks observed are: 176 and 191

3e		3201.31 (2^0amine, N-H str), 1751.24(isatin, >C=O str), 1703.31 (amide, >C=O str), 1485.80(N-H bend),3074.32(Ar, C-H str), 2993.32(alkanes, C-H str),1053.64(C-F str)	7.4-8.4 (7H, Ar), 8.4(1H, Ar N-H), 11.3 (1H, aliphatic amide N-H), 4.2 (2H, CH_2)	M+1=339; The other important peaks observed are: 164 and 176
3f		3193.90(2^0amine, N-H str), 1731.95(isatin, >C=O str), 1631.81 (amide, >C=O str), 1451(N-H bend),3053.28(Ar, C-H str), 3035.75(alkanes, C-H str),636.47(C-I str)	7-8.4 (7H, Ar), 8.4(1H, Ar N-H), 11.9 (1H, aliphatic amide N-H), 4.1 (2H, CH_2)	M+1=445; The other important peaks observed are: 176 and 272
3g		3377.34(2^0amine, N-H str), 1674.10(isatin, >C=O str), 1616.24 (amide, >C=O str), 1456.64(N-H bend),3093.61(Ar, C-H str), 2923.83(alkanes, C-H str),	7-8.1 (7H, Ar), 8.1(1H, Ar N-H), 11.9 (1H, aliphatic amide N-H), 4.1 (2H, CH_2)	M+1=335; The other important peaks observed are: 160 and 176
3h		3344.05(2^0amine, N-H str), 1735.35(isatin, >C=O str), 1697.24 (amide, >C=O str), 1451.64(N-H bend),3074.32(Ar, C-H str), 2923.83 and 2959.76(alkanes, C-H str),	7.3-8 (6H, Ar), 8(1H, Ar N-H), 11.2 (1H, aliphatic amide N-H), 3.7-4.3(2H, CH_2)	M/e-347; The other important peaks observed are: 170and 176
3i		3282.62(2^0amine, N-H str), 1747.39(isatin, >C=O str), 1631.67 (amide, >C=O str), 1435(N-H bend),3035.75(Ar, C-H str), 2977.89 and 2923.83(alkanes, C-H str)	7.3-8.1 (6H, Ar), 8.1(1H, Ar N-H), 11.4 (1H, aliphatic amide N-H), 3.8-4.3 (2H, CH_2)	M/e-347; The other important peaks observed are: 170 and 176
3j		3244.05(2^0amine, N-H str), 1735.76(isatin, >C=O str), 1797.35 (amide, >C=O str), 1451(N-H bend),3074.32(Ar, C-H str), 1005.77(C-F str),744.47(C-Cl str)	7.2-8.1 (6H, Ar), 8.1(1H, Ar N-H), 11.2 (1H, aliphatic amide N-H), 4 (2H, CH_2)	M/e-371; The other important peaks observed are: 176 and 198

The results of the antimicrobial screening are shown in Table 3 and figure 1,2.

The results of the *invitro* anti inflammatory screening are shown in Table 4 and figure 3

Table 3: Antimicrobial screening studies of compounds (3a-3j)

S. No.	Compound	Antibacterial activity zone of inhibition in (mm)				Antifungal activity zone of inhibition in(mm)	
		S.aureus (Gram +ve)	*B. subtilis (Gram +ve)*	*Klebsiella (Gram ve)*	*E.Coli (Gram -ve)*	*A.niger*	*C.albicans*
1	3a	10	9	7	9	9	8
2	3b	12	9	9	7	10	6
3	3c	9	8	10	9	8	7
4	3d	16	12	11	13	9	6
5	3e	13	11	7	8	13	10
6	3f	12	10	8	10	10	7
7	3g	13	11	7	8	9	8
8	3h	13	8	10	7	10	8
9	3i	10	7	8	8	8	6
10	3j	8	10	9	9	8	7
9	Amoxicillin	45	41	25	32	-	-
10	Ciprofloxacin	41	38	27	33	-	-
11	Clotrimazole	-	-	-	-	36	25
12	Ketoconazole	-	-	-	-	37	27
13	Control(DMF)	NI	NI	NI	NI	NI	NI

Note: All the values are mean of triplicates, NI: no inhibition, "- " : not tested

Table 4: *Invitro* Anti-inflammatory activity of synthesized compounds (3a-3j)

Sl. No	Compound		Blank	Concentration (mg/ml)				
				0.2	**0.4**	**0.6**	**0.8**	**1.0**
1.	3a	Inhibition of denaturation	0	21.1	29.2	41.7	48.3	53.9
2.	3b		0	21.0	27.7	33.7	47.1	56.8
3.	3c		0	20.2	18.3	24.9	39.2	54.3
4.	3d		0	22.3	33.2	44.0	57.0	73.9
5.	3e		0	20.2	28.6	41.0	41.2	50.4
6.	3f		0	12.8	23.8	29.8	39.6	44.1
7.	3g		0	20.0	20.3	33.8	41.2	51.6
8.	3h		0	20.9	25.5	40.7	51.1	53.2
9.	3i		0	20.1	23.3	33.3	38.6	51.6
10.	3j		0	19.4	22.5	42.8	46.7	53.8
11.	**Ibuprofen(std)**		0	29.0	40.0	55.5	66.1	83.4

The synthesized compounds were confirmed by the spectral and analytical data. The screening for antimicrobial activity for **(3a-3j)** compounds shows a mild to moderate inhibition. The zone of inhibition exhibited by 1000µg/ml of the compound **(3d)** against *Staphylococcus aureus* and *Bacillus subtilis* is 16mm and 12mm respectively, which is significant but less than the inhibition shown by 30µg of standard Ciprofloxacin (41mm and 38mm respectively) and 10µg of Amoxicillin (45mm and 41mm respectively).

The zone of inhibition exhibited by 1000µg/ml of the compound **(3d)** against *E-Coli* and *Klebsiella* is 13mm and 11mm respectively, which is significant but less than the inhibition

shown by 30µg of standard Ciprofloxacin (33mm and 27mm respectively) and 10µg of Amoxicillin (32mm and 25mm respectively).

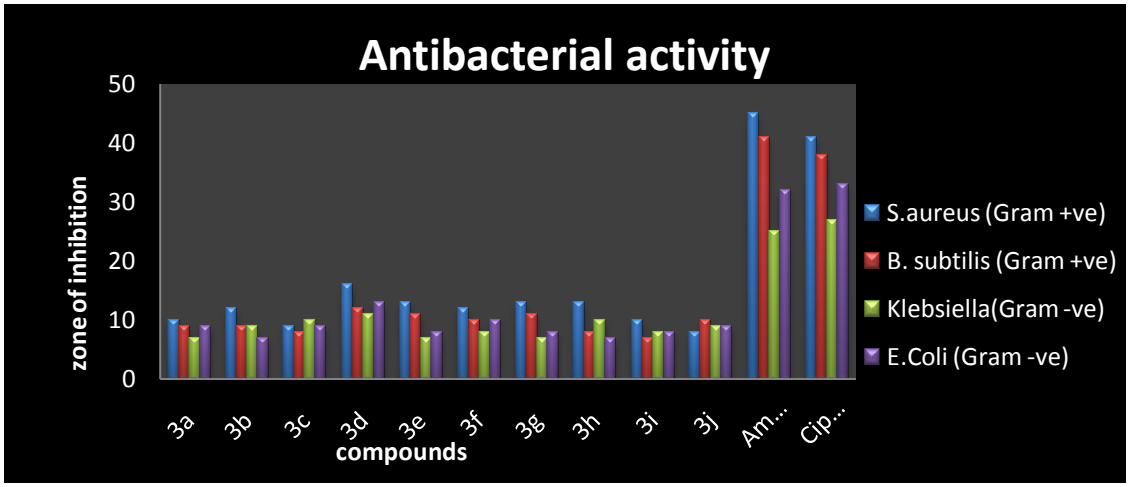


Figure 1: Antibacterial activity

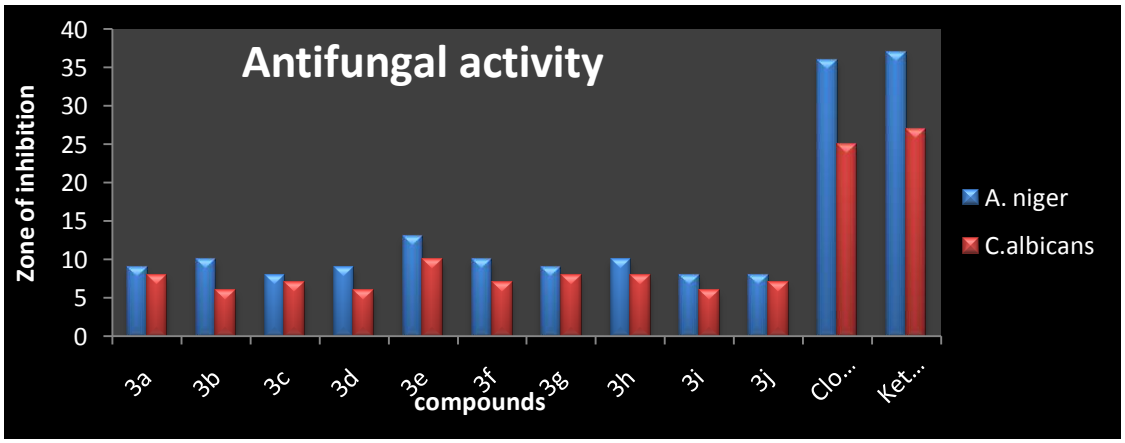


Figure 2: Antifungal activity

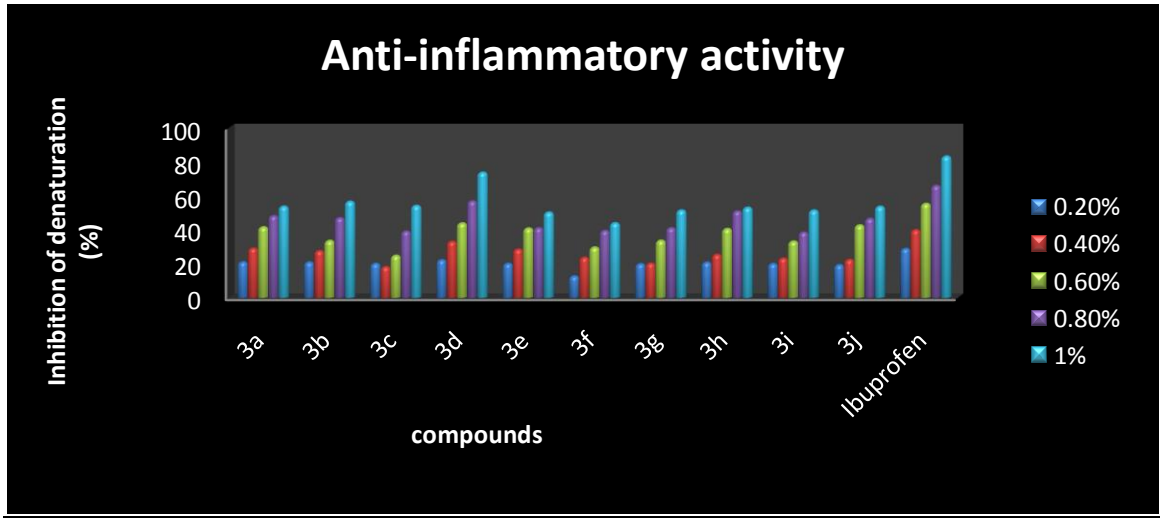


Figure 3: *In vivo* Anti inflammatory activity

The zone of inhibition exhibited by 1000µg/ml of the compound **(3e)** against *Aspergillu sniger* and *Candida albicans* is 13mm and 10mm respectively, which is significant but less than the inhibition shown by 10µg of standard Clotrimazole (36mm and 25mm respectively) and 10µg of Ketoconazole (37mm and 27mm respectively).

The synthesized compounds were screened for anti-inflammatory activity by in-vitro inhibition of albumin denaturation method. All the compounds inhibited the denaturation of serum albumin. The compound **(3d)** exhibited significant percentage inhibition of bovine serum albumin denaturation at conc. 0.2mg/ml, 0.4mg/ml, 0.6mg/ml, 0.8mg/ml and 1.0mg/ml are 22.3%,32%, 44%, 57%, 73.9% respectively, which is comparable with standard Ibuprofen.

CONCLUSIONS:

The yield of the products ranged from 67-77%. The structures of the newly synthesized compounds **(3a-3j)** are confirmed by spectral data through, IR, ^{1}H NMR, Mass spectra and Elemental analysis. All the synthesized final compounds were screened for their Antimicrobial activity and *In vitro a*nti inflammatory activity.

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