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Synthesis and biological study of some novel schiff's bases of indazolone derivatives

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ABSTRACT

*A series of novel Schiff's bases of Indazolone derivatives were prepared by the reaction of o-hydrozinobenzoic acid with con HCl and it gives 1,2-dihydro-indazol-3-one. The 3-indazolone is react with 4 substituted aniline, it produces various derivative of schiff's bases of Indazolone. The Indazolone derivatives synthesized were Ia, Ib ,Ic, Id, Ie, If, Ig and Ih. The structures of the synthesized compounds were confirmed by their IR,HNMR. All the synthesized compounds were tested for their antimicrobial activity against Gram+, Gram – bacteria. Among the synthesized compounds , the compounds Ia and Ih shown high anti bacterial activity against Staphylococus aureus(1mg/ml). Ib,Ic,Id,If and Ih exhibited high activity against Klebsiella aerogenes(0.5mg/ml). Ia , Ic and Ih possessed potent activity against E.coli(0.5mg/ml). The analgesic activity was determined by using hot plate method. The compounds Ib,Ig,Ih,(***p<0.001),Id and Ic (**p<0.01) possess significantly. The anti inflammatory activity was determined by Carragenan induced paw edema method. The compounds Ib ad Ig were found to exhibit high (***p<0.001) anti-inflammatory. Id and Ie were found to exhibit moderate (**p<0.01) anti inflammatory.*

Key words: Indazolone derivative, Analgesic activity, Anti inflammatory activity, Anti microbial activity.

INTRODUCTION

Indazolone and its derivatives show a wide spectrum of pharmacological activities as cell apoptosis[1], rheumatoid arthritis[2], antiproliferativeeffect[3], Occular hypertension and glaucoma[4], psychoticactivity[5], hypotensiveactivity[6], obesity[7], tumorcell cytotoxic assay[8] ,anti hyperlipidemic activity[9], Trichomonacidal activity[10], Analgesic and

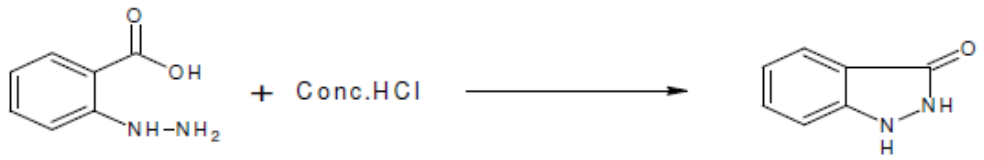
antipyretic activity[11],antiprotozoal activity[12],antidabetic activity[13],anti-inflammatory activity[14],antiarthritic effect[15],local anaesthetic activity[16],platelet anti aggregating effect[17],anti spermatogenic activity[18],antihypertensive[19]activities. Medicinal compounds of indazole derivatives are Benzydamine (anti inflammatory),Tetrdamine (analgesic)[20],Granisetron (antiemetic), lonidamine(antispermatogenic)[21],AF-2364 (anti spermatogenic).

Indazole nucleus is an effective pharmacophore in medicinal chemistry and shows diverse biological & pharmacological activities. The indazole nucleus is a seldom used, but effective pharmacophore in medicinal chemistry**,** its constitute an important class of heterocyclic compounds that display interesting biological properties and powerful pharmacological activities, such as anti-cancer, and anti-platelet activities, plus serotonin 5-HT3 receptor antagonist[22] anti-depressant , anti-inflammatory, analgesic, antipyretic, male contraceptives (anti-spermatogenetic agent), anti– hypertensive, anti-hyperlipidemic, dopamine antagonist, anti-tumor, anti-emetic and anti-HIV activities etc. However compared to indole and benzimidazole, indazole chemistry remains poorly studied due to the limited synthetic approaches to these compounds **.** However, the development of efficient and general methodologies for the synthesis of indazoles and derivatives has met with limited success and several limitations remain[23]**.**
O-hydrazino benzoic acid react with hydrochloric acid and it gives 3-indazolone. The 3-indazolone react with substituted aniline and it produced Schiff's base derivatives of Indazolone. (Scheme of work)

EXPERIMENTAL SECTION

Melting points were determined on a Veego-Vmpt (silicon bath melting point apparatus and are uncorrected. Purity of the compounds was checked using precoated TLC plates. IR spectra were recorded on a Perkin-Elmer FTIR 5000 spectrometer, using KBr discs.^{1}HNMR spectra in DMSO were recorded on a Burner 300 MHz spectrometer and the chemical shifts were reported as parts per million (δppm) field using TMS as an internal standard.

Step-1: Synthesis of 3-indazolone from o**-hydrazinobenzoic acid**
In a 1-Lit. Round-bottomed flask to which a reflux condenser is attached are placed 23.1 g. (0.125 mol) of o-hydrazinobenzoic acid hydrochloride, 70ml of water, and 7 ml of concentrated hydrochloric acid (sp. gr. 1.18). The mixture is refluxed for 30 minutes. The resulting pale yellow solution is transferred in two portions to a 23- cm. evaporating dish and concentrated on the steam bath to about one-fourth its original volume. The indazolone separates at an early stage of the evaporation but re dissolves as the concentration of acid increases. Sodium carbonate is added to the warm solution in small portions until the acid is neutralized, [The submitter reports that the described method of purification gives a better product than is obtained by solution in dilute sodium hydroxide and re precipitation with acid] and the suspension is allowed to stand for 2 hours. The nearly colorless indazolone is removed by filtration, washed with two 25-ml. portions of cold water, and air-dried. The yield of product, m.p.246–249, is (90–98%). The indazolone may be purified by re crystallization from methanol. It separates as an white needles, m.p. 250–252[24].



O-hydrazinobenzoic acid 1, 2-dihydro-indazol-3-one

Step – 2: Synthesis of Schiff's base from 3-indazolone

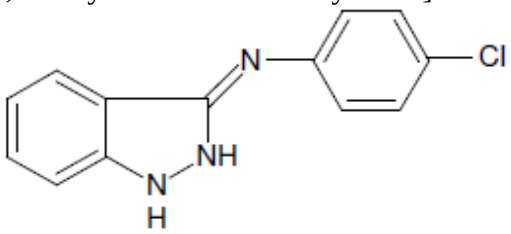
In a 100mL R.B. flask fitted with a reflux condenser; place 2.6828 gm (0.02mol) of 3-indazolone, X gm (0.022mol) of various *p* – substituted aniline and 80 ml of rectified spirit or ethanol. Heat the solution under reflex, using a water bath, for 20 - 30 minutes, add water until cloudiness persists and cool the solution under ice bath oily product is formed, the product was separated by filtration, vacuum dried and recrystallized from methanol[25,26]

3-Indazolone *P*-substituted aniline Schiff's base derivatives

Phyto chemical investigation[27,28,29]

COMPOUND-Ib

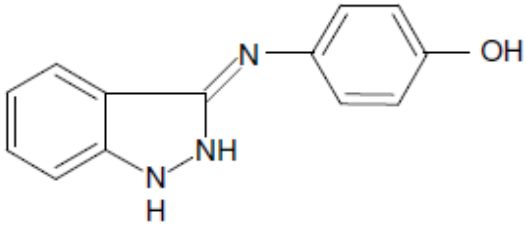
Name : 4-chloro-*N*-[(3*Z*)-1, 2-dihydro-3*H*-indazol-3-ylidene] aniline



IR spectra 3500-3300cm-^{1} N-H stretching,1687cm-^{1},C=O stretching,1488-1458cm-^{1}C=C stretching,3063-2733cm-^{1}C-H stretching, 1323-1228cm-^{1} C-N stretching, 1643-1622cm-^{1} C=N stretching1091cm-^{1}Ar-Cl, 850-550cm-^{1}C-Cl.

COMPOUND-Ic

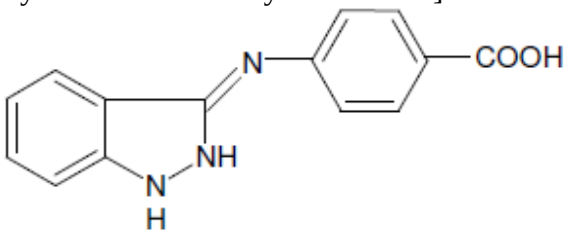
Name : 4-[(3Z)-1, 2-dihydro-3H-indazol-3-ylideneamino] phenol



IR spectra(KBr pellet) 3300-2500cm-^{1} O-H stretching,1643cm-^{1}, 1510-1461cm-^{1}C=Cstretching, 3217-2890cm-^{1}C-Hstretching, 3500-3300cm-^{1}N-H stretching, 1348cm-^{1} C-N stretching, 1641-1620cm-^{1} C=N stretching. 1101cm-^{1}Ar-OH.

COMPOUND-Ie

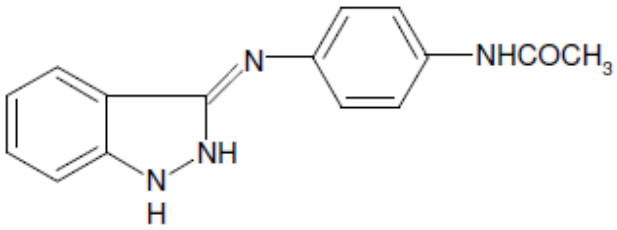
Name : 4-[(3*Z*)-1, 2-dihydro-3*H*-indazol-3-ylideneamino] benzoic acid



IR spectra 3300cm-^{1} O-H stretching,1643-1622cm-^{1},C=O stretching, 1157cm-^{1}C-O stretching1488-1458cm-^{1}C=C stretching,3060-2852cm-^{1}C-H stretching,3500-3300cm-^{1}N-H stretching,1323cm-^{1} C-N stretching, 1643-1622cm-^{1} C=N stretching. 1045cm-^{1}Ar-Cl, 813-790cm-^{1}C-Cl.

COMPOUND-Ig

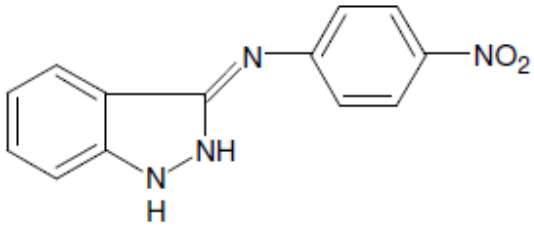
Name : *N*-{4-[(3)-1,2-dihydro-3*H*-indazol-3ylideneamino]phenyl} acetamide



IR spectra 1687cm-^{1},C=O stretching,1487cm-^{1}C=C stretching,3060-2852cm-^{1}C-H stretching,3500-3300cm-^{1}N-H stretching,1643-1622cm-^{1} C-N stretching.

COMPOUND-Ih

Name : *N*-[(3*Z*)-1, 2-dihydro-3*H*-indazol-3-ylidene]-4-nitroaniline



IR spectra 1477cm-^{1}C=C stretching,3072-2854cm-^{1}C-H stretching,3500-3300cm-^{1}N-H stretching,1328-1299cm-^{1} C-N stretching,1625cm-1 C=N stretching,1467-1328cm-^{1}N=O stretching.

Anti-bacterial activity

The in vitro antibacterial activities of the Indazolone derivatives were assessed against one Gram-positive bacteria Viz.*Staphylococcus aureus*(1mg/ml) and two Gram-negative bacteria

Viz. *E.coli and K.aerogens* (0.5mg/ml) by broth dilution method recommended by National Committee for clinical Laboratory Standards[19]. Bacteria were grown overnight in Muller Hinton agar medium broth at 37°C, harvested by centrifugation and then washed twice with sterile distilled water. Stock solutions of the series of compounds were prepared in DMSO.

Protocol[30-33]

Assay was carried out by disc diffusion method. The method followed was spread plate technique. Prepare Muller Hinton agar medium and sterilized in the autoclave and dispensed 15ml into each Petri-plate. Label with appropriate name of the organisms by sterile technique inoculate each label plate with the respective organism by spread method. Using sterile forceps, place the standard disc of ofloxacin over the agar surface in the Petri-plates. Then the filter paper discs (sterile) of 5mm were soaked in 1ml (1mg/ml) of the test solution and control DMSO solvent. After evaporating the solvent in a sterile atmosphere the drug impregnated discs were placed in Petri-plates. Gently press each disc down with a wooden end of a cotton swap (or) sterile forceps to ensure that the discs adhere to the surface of the agar. The plates were refrigerated for 1hrs to arrest the growth and for easier diffusion of test compounds. Then the plates were removing from refrigerator and incubate all plates in an incubator and inverted position for 24hrs at 37^{o}C.

The antibacterial activity of the compounds was evaluated against gram positive organism *Staphylococcus aureus* and negative organism *Klebsiella aerogenes* and *E.Coli*. The zone of inhibition was measured as parameter of activity. Ofloxacin 10µg/disc was used as standard compound. *Staphylococcus aureus*: The compounds Ia and Ih shown higher antibacterial activity than standard ofloxacin, while compounds Ib, Ic, Id, Ie, If and Ig shown least activity. *Klebsiella Aerogenes*: The compounds Ib, Ic, Id, If, and Ih exhibited high activity than standard ofloxacin, while compounds Ia, Ie and Ih exhibited least activity. *E.Coli*: The compounds Ia, Ic and Ih possessed potent activity. While compounds Ib, Id, Ie, If and Ig exhibited good activity. (Table 8)

Anti inflammatory activity[34,35,36,37]

Male albino rats weighting approximately, 150-200 gm were divided in to 18 groups and each of 6 animals. A mark was made on the hid paw just behind tibiotarsal junction so that every the Paw was dipped in the mercury column up to the fixed mark to ensure constant Paw volume. The paw volume of each animal was measured before the administration of the drug.

The dosage of the drug administered to different groups were as follows

Group –I : A control group received orally 0.2 ml of DMSO. Group – II : The standard group received orally 10mg /kg of body weight of diclofenac sodium.Group –III to VII: The III to VII groups received compound code I-b, I-d, I-e, I-g, and I-h, drugs respectively, all the above test compounds were dissolved in 0.5ml solution of DMSO and given 30 minutes before the commencement of the study. After that 0.1ml of 1% w/v Carrageenan solution in normal saline was injection into the sub plantar tissue of the left hind paw of the rat. The volume of the mercury displaced in the plethmograph was measured at 0 min, 30 min, 60min, 120min & 240min.

The compounds such Ib, and Ig possess high significant (*** $p<0.001$) anti-inflammatory activity, because these above mentioned compounds markedly reduce the paw volume than

compared to control. The compounds Id and Ie possess moderate significant (** $p<0.01$) reduction of inflammation when compare to the compounds Ib and Ig it possess least reduction of inflammation but when compared to control it possess significant (* $p<0.05$) anti-inflammatory activity. (Table 5,6)

Analgesic activity[38,39,40]

Albino mice of same sex were divided into twelve groups. Each group consists of three animals. The paw licking of jump response of the animal, when placed on the hot plate maintained at constant temperature of 55^{o}C was considered as basal reaction. The jump response or paw licking was noted. The dosage of drug administered to different groups was as follows.

Group I: A control group received orally 0.5ml of carboxy methyl cellulose (1%w/v). Group II: The standard group received orally 2.5ml of Diclofenac sodium . Group III to VII: The III to VII groups received compound code I-b, I-d, I-e, I-g and I-h, drugs respectively. The reaction time of animals on the hot plate at 30, 60, 90 & 120mts after drug administration was noted. The compound such as Ib, Ig and Ih posess highly significant analgesic activity, Id and Ie significant activity. The compounds such Ib, Ig and Ih, possess highly significant analgesic activity, Id and Ie possess significantly activity. (Table 7)

Statistical analysis[41]

All the data were presented as mean ±SEM and analyzed by Dunnett's test and unpaired Students t-test for the possible significant inter relation between the various groups. The value of $p<0.01$, $p<0.05$, $p<0.001$was considered statistically significant.

RESULTS AND DISCUSSION

The 3-indazolone nucleus was obtained starting from *o*-hydrazinobenzoic acid by cyclization reaction with help of concentrated HCl acid. The 3-indazolone was subject to condensation with different *p*-substituted aniline to afford the corresponding Schiff's base derivatives (compound-Ia - Ih).The structure of the synthesized compounds was confirmed by IR, ^{1}H, ^{13}C NMR and Mass spectra. The derivatives of physical character, solubility and TLC were determined. (Table 1,2,3,4)

Nitrogen functional groups are more basic, more nucleophilic and more reactive than their oxygen counterparts in comparable structures. The reaction involves nucleophilic addition of amine followed by elimination of water, neither acid or base catalysis is needed. For best results, the reaction was carried out in different solvents (ethanol, water and DMF) and in different cooling conditions (cool with ice bath, refrigerator in 30mins at 0^{o}C and stand for 24 hrs at room temperature). In good yield was come from using ethanol as solvent with ice bath or refrigerator.

Scheme of work

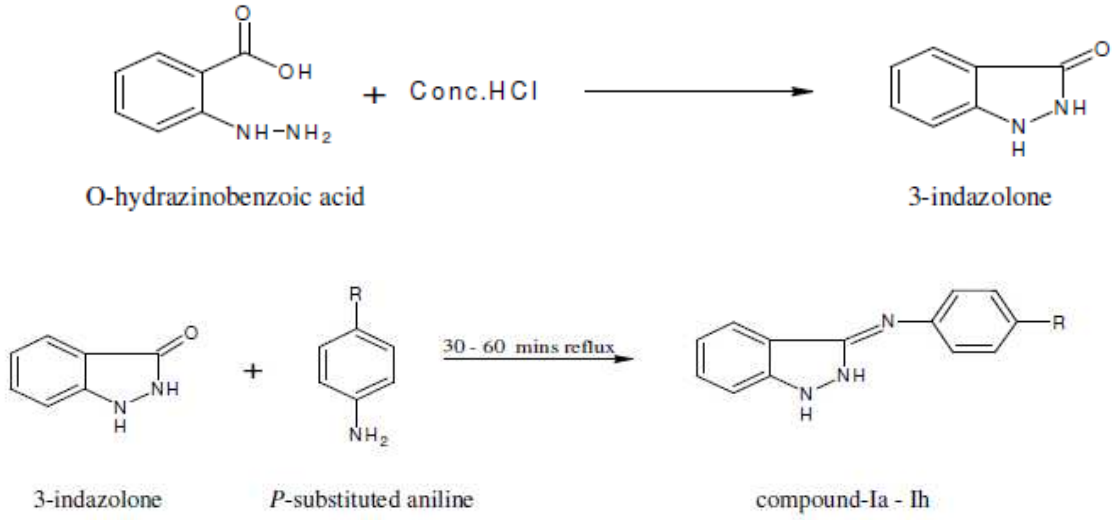


Table 1: Molecular formula and Practical yield of synthesized compounds

S.No	Compound code	R	Molecular formula	Practical Yield
1	Ia	H	$C_{13}H_{12}N_3$	1..23
2	Ib	Cl	$C_{13}H_{11}N_3Cl$	1.29
3	Ic	OH	$C_{13}H_{13}N_3O$	1.16
4	Id	OCH_3	$C_{14}H_{15}N_3O$	1.74
5	Ie	COOH	$C_{14}H_{13}N_3O_2$	1.79
6	If	$COCH_3$	$C_{15}H_{15}N_3O$	1.87
7	Ig	$NHCOCH_3$	$C_{15}H_{18}N_4O$	1.85
8	Ih	NO_2	$C_{13}H_{12}N_4O_2$	1.96

Table 2:Physical characters of Schiff's base of Indazalone derivatives

S.No	Compound code	Molecular formula	Molecular weight	Colour and nature of compounds	Percentage yield	Melting point
1	Ia	$C_{13}H_{12}N_3$	210.26	DualWhite crystalline powder	29.28%	262°C
2	Ib	$C_{13}H_{11}N_3Cl$	244.70	White crystalline powder	26.43%	284°C
3	Ic	$C_{13}H_{13}N_3O$	227.26	Dark black crystalline powder	25.50%	297°C
4	Id	$C_{14}H_{15}N_3O$	241.29	Grayish black crystalline powder	36.09%	288°C
5	Ie	$C_{14}H_{13}N_3O_2$	255.27	Pale yellow crystalline powder	35.09%	306°C
6	If	$C_{15}H_{15}N_3O$	253.30	Pale brown crystalline powder	36.95%	276°C
7	Ig	$C_{15}H_{18}N_4O$	270.33	Pale brown crystalline powder	34.19%	312°C
8	Ih	$C_{13}H_{12}N_4O_2$	256.26	Yellow crystals	38.28%	308°C

Table 3: Solubility characters of synthesized compounds

S.No	Compounds code	Soluble in	Insoluble in
1	Ia	Hot water, Alcohol, DMSO, DMF, $CHCl_3$	Cool water
2	Ib	Hot water , Alcohol, DMSO, DMF,acetone	Cool water
3	Ic	Hot water, Alcohol, DMSO, DMF,acetone	Cool water
4	Id	Hot water, Alcohol, DMSO, DMF,ether	Cool water
5	Ie	Hot water, Alcohol, DMSO, DMF,acetic acid	Cool water
6	If	Hot water, Alcohol, DMSO, DMF, acetone	Cool water
7	Ig	Hot water, Alcohol, DMSO, DMF, $CHCl_3$	Cool water
8	Ih	Hot water, Alcohol, DMSO, DMF,$CHCl_3$	Cool water

Table 4: TLC characters of synthesized compounds

S.no	Compounds code	Colour of spot	Rf Values
1	Ia	Yellow colour spot	0.50
2	Ib	yellowish brown colour spot	0.51
3	Ic	Brownish yellow colour spot	0.49
4	Id	Brownish yellow colour spot	0.52
5	Ie	Brown colour spot	0.53
6	If	Yellowish brown colour spot	0.52
7	Ig	Light brown colour spot	0.49
8	Ih	Yellow colour spot	0.50

Table 5: Anti inflammatory activity of Indazolone derivatives using Carrageenan induced paw oedema method

Group	Compound	Dose(mg/kg)	Basal paw volume	Paw volume after the drug administration (Mean ± SEM)				
				0min	30min	60min	90min	120min
I	Control	0.2ml	0.344±0.042	0.718±0.019	0.732±0.017	0.746±0.009	0.762±0.012	0.780±0.021
II	Standard	10ml	0.312±0.021	0.712±0.014	0.702±0.024	0.550±0.021	0.396±0.011	0.332±0.014
III	Ib	10ml	0.344±0.014	0.726±0.022	0.712±0.032	0.636±0.022	0.498±0.018	0.406±0.036
IV	Id	10ml	0.325±0.024	0.728±0.026	0.718±0.032	0.622±0.018	0.532±0.032	0.488±0.011
V	Ie	10ml	0.316±0.020	0.719±0.020	0.706±0.031	0.614±0.036	0.524±0.032	0.476±0.046
VI	Ig	10ml	0.301±0.016	0.699±0.016	0.686±0.040	0.562±0.012	0.418±0.033	0.340±0.032
VII	Ih	10ml	0.342±.0.022	0.732±0.002	0.720±0.040	0.640±0.012	0.574±0.032	0.484±0.038

mean±SEM

** - p<0.05 Significant ,** - p<0.01 Moderate significant ,*** - p<0.001 highly Significant from control*

Table 6: Anti inflammatory activity of Indazolone derivatives using Carrageenan induced paw odema method

Group	Compound	Dose(mg/kg)	**Paw volume after the drug administration (Mean±SEM)**				
			30min	60min	90min	120min	**Inference**
I	Control(DMSO)	0.2ml	--	--	--	--	--
II	Standard(Diclofenac sodium)	10ml	4.10%	26.27%	48.03%	57.44%	***
III	Ib	10ml	2.73%	14.75%	34.64%	47.95%	**
IV	Id	10ml	1.91%	16.62%	30.18%	37.43%	*
V	Ie	10ml	3.55%	17.69%	31.23%	38.97%	*
VI	Ig	10ml	6.28%	24.66%	45.14%	56.41%	***
VII	Ih	10ml	1.64%	14.21%	24.66%	37.95%	*

*mean±SEM; * - p<0.05 Significant ,** - p<0.01 Moderate significant ,*** - p<0.001 highly Significant from control*

Table 7: Analgesic activity of Indazolone derivatives using Hot plate

Group	Compound	Dose (mg/kg)	Basal reaction (sec)	Rectal temperature after the drug administration (mean ± SEM)				
				0min	30min	60min	90min	120min
I	Control	0.2ml	3.3±0.33	3.3±0.30	3.0±0.28	3.2±0.34	3.3±0.32	3.3±0.30
II	Standard	10ml	3.1±0.30	3.1±0.32	5.7±0.33	7.3±0.30	10.6±0.29	9.6±0.32
III	Ib	10ml	3.0±0.32	3.0±0.33	4.3±0.30	6.2±0.32	8.6±0.31	9.8±0.29
IV	Id	10ml	3.3±0.31	3.2±0.28	3.8±0.28	5.5±0.30	6.7±0.32	7.3±0.31
V	Ie	10ml	3.0±0.33	3.0±0.30	3.4±0.31	5.8±0.32	6.9±0.28	7.6±0.30
VI	Ig	10ml	2.9±0.31	2.9±0.33	5.5±0.30	6.9±0.33	9.6±0.31	10.9±0.32
VII	Ih	10ml	3.2±0.32	3.2±0.29	4.1±0.29	6.1±0.32	8.4±0.30	9.7±0.31

method *mean±SEM** - p<0.01 Significant *** - p<0.001 highly Significant from control*

Table 8 : Anti bacterial activity of Schiff's base of Indazolone derivatives

Compound Code	Organism used			Inference
	Gram positive	Gram Negative		
	Staphylococcus aureus	*Klebsiella aerogenes*	*E.Coli*	
	1mg/ml	0.5mg/ml	0.5mg/ml	
Control	0 mm	0 mm	Resistant	A
Standard	16 mm	20 mm	Resistant	A* and A
Ia	18 mm	18 mm	16 mm	A*
Ib	10 mm	22 mm	9 mm	A*
Ic	11 mm	20 mm	18 mm	A*
Id	9 mm	25 mm	8 mm	A*
Ie	7 mm	19 mm	6 mm	A*
If	9 mm	20 mm	8 mm	A*
Ig	11 mm	20 mm	10 mm	A*
Ih	18 mm	13 mm	13 mm	A*

Control - DMSO *A – Inactive*
Standard - Ofloxacin 10μg/disc *A* - Active*

CONCLUSION

In conclusion, a series of novel some Schiff's bases of Indazolone derivatives were prepared. Test compounds Ib and Ig were found to exhibit high anti-inflammatory activity. Test compounds Id and Ie were found to exhibit moderate anti-inflammatory activity.

The compounds such Ib, Ig and Ih possess highly significant activity, Id and Ie possess significantly analgesic activity.

Test compounds, Ia and Ih shown high antibacterial activity, while compounds Ib, Ic, Id, Ie, If and Ig shown least activity against *staphylococcus aureus*. Test compounds Ib, Ic, Id, If, and Ih exhibited high activity, while compounds Ia, Ie and Ih exhibited least activity against *Klebsiella aerogenes*. Test compounds Ia, Ic and Ih possessed potent activity, while compounds Ib, Id, Ie, If and Ig exhibited good activity against *E.coli*.

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