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Original Article

Design, Synthesis and Antimicrobial, Antifungal and Anti-Inflammatory Evaluation of Some (4-Substituted Phenyl)[5-(4- Substituted Phenyl)-3-Phenyl-4,5-Dihydro-1*H*-Pyrazol-1-yl]-Methanone Derivatives

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ABSTRACT

It is evident from reported articles that bacterial infections often produce pain & inflammation. In normal practice, two groups of agents (chemotherapeutics & NSAID's) are prescribed simultaneously. Unfortunately, none of the drugs available to possesses these activities in a single molecule. Therefore, our aim is to find a compound having dual effect of anti-inflammatory & antimicrobial activitiy (twin drug concept). Pyrazole and its derivatives have been reported for these dual activities. Here an attempt has been made to synthesize eight (4-substituted phenyl)[5-(4-substituted phenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl]methanone derivatives. The synthesized derivatives were assessed for their *in-vitro* anti-microbial and anti-inflammatory activity. Chalcone derivative was synthesized by Claisen Schmidt condensation between acetophenone and substituted benzaldehyde. It was refluxed with hydrazine hydrate to form pyrazole ring which on reaction with substituted benzoyl chloride in acetone and triethylamine to form to get the parent compound from which the final derivatives has been synthesized. The structures of newly synthesized compounds so obtained were confirmed by using IR, H-NMR, MS spectral data.

Keywords: Synthesis, Pyrazole ring, Claisen Schmidt Condensation, Anti-bacterial, Anti-fungal, Anti-Inflammatory screening.

INTRODUCTION

The advancement and changes in the culture and lifestyle the new disease are being existed among the human population. The treatment of many infectious diseases are challenging due to resistance to antimicrobial agents And the major drawback of current treatment with anti-inflammatory agents are their GI side effects like GI irritation, ulceration etc. Also on long term usage they cause severe CVS & thrombotic side effect. So it is necessary to continue the search for new drugs. Pyrazole and its derivatives was found to be having diverse activity like anti-inflammatory, antimicrobial, antifungal, ant diabetic and anticancer etc. In market different pyrazoleanalogous having anti-inflammatory activity are available such as. Celecoxib, Deracoxib, Kebutazone, Remifenazone, Pyrazofurin. Therefore it was planed to synthesize the novel series of pyrazole derivatives to screen their activity like anti-inflammatory and antimicrobial and antifungal activity.[1-3]

MATERIALS AND METHODS

The entire chemicals were supplied by S. D. Fine Chem. (Mumbai), Finar Chem. Ltd (Ahmedabad) and Loba Chemie. Pvt. Ltd. (Mumbai). Melting points were determined by open tube capillary method. Purity of compounds was checked by thin layer chromatography (TLC) on silica gel-G in solvent system hexane-ethyl acetate (1:1) and the spots were located under iodine vapours and UV light.[4] IR spectra of all compounds were recorded on FT-IR 8400S Shimadzu spectrophotometer using KBr. Mass spectra were obtained using 2010EV LCMS Shimadzu instrument.

GENERAL PROCEDURE

General Procedure for synthesis of Chalcone derivative. (3a-b) (Claisen Schmidt Condensation)

A solution of sodium hydroxide (2.2 g) in water (20 ml) and rectified spirit (15 ml) was cooled in a conical flask kept in an ice bath. To the cooled solution, acetophenone (5 ml, 0.043 mole) was added followed by the addition of benzaldehyde (4.4 ml, 0.043 mole). The reaction mixture was stirred all the time with a mechanical stirrer and the temperature of the reaction mixture kept at above 25^0C. The mixture was stirred for 1 hr. Separated product was filtered under suction and washed well with cold water till filterate become neutral to pH paper. It was recrystallized from methanol.[5]

General Procedure for synthesis of pyrazole ring. (4a-b)

Chalcone derivative (10 g) was dissolved in glacial acetic acid (30 ml). Hydrazine hydrate (8-10 ml) was added to it and refluxed for 9 hr. The solution was cooled in ice–bath. The separated product is filtered at the pump and washed with water till filterate become neutral to pH paper. It was recrystallized from methanol.[6]

General Procedure for synthesis of substituted benzoyl chloride (6)

This preparation should be done in a fume cupboard. Substituted benzoic acid (1 mole) and redistilled thionyl chloride (15-20 ml) were taken dry round bottom flask and refluxed for 4-5 hr till no more hydrogen chloride was evolved. First the reaction mixture was dissolved after 20min it was start to convert in pale yellow liquid. Evaporate excess $SOCl_2$ in water bath. Cool the solution.[7]

General Procedure for synthesis of pyrazole ring. (4a-b)

Pyrazole ring derivative (1 mole) was dissolved in acetone (25-30 ml). To a solution 4-5 ml triethylamine and substituted benzoyl chloride (1 mole) was added and refluxed for 28-30 hr. The solution is cooled in ice–bath. The separated product is filtered at the pump. It was recrystallised from rectified spirit.

Or

Pyrazole ring derivative (1 mole) was dissolved in pyridine (25-30 ml). To a solution substituted benzoyl chloride (1 mole) was added and refluxed for 24-25 hr. The solution was cooled in ice–bath & poured into dilute HCl and solid thus obtained was filtered, washed with water. It was recrystallized from rectified spirit.[8]

PHARMACOLOGICAL SCREENING

Antibacterial Activity

The microbiological assay was based upon a comparison of inhibition of growth of microorganisms by measured concentrations of test compounds with that produced by known concentration of a standard antibiotic. Two methods generally employed were turbidometric (tube dilution) method and filter paper disc method. In the turbidometric method inhibition of growth of microbial culture in a uniform dilution of antibiotic in a fluid medium is measured. It was compared with the synthesized compounds. Here the presence or absence of growth was measured. The cylinder plate method depends upon diffusion of antibiotic from a vertical cylinder through a solidified agar layer in a petridish or plate to an extent such that growth of added micro-organisms is prevented entirely in a zone around the cylinder containing solution of the antibiotics. The cup-plate method is simple and measurement of inhibition of microorganisms was also easy. Here we have used this method for antibacterial screening of the test compounds.[9,10]

Name of Microorganisms

Gram +ve microorganisms
Staphylococcus aureus (MTCC No. 96)
Bacillus subtillis (MTCC No. 121).
Gram -ve microorganism
Escherichia coli (MTCC No. 521)

The cultures of microorganisms have been procured from Microbiology section, Department of Pharmaceutics, S.K.Patel College of Pharmaceutical Sciences, Kherva, Gujarat.

Preparation of medium

Nutrient agar 2%
Peptone 1%
Beef extract 1%
Sodium chloride 0.5%
Distilled water up to 100ml

All the ingredients were weighed and added to water. This solution was heated on water bath for about one and half-hour till it became clear. This nutrient media was sterilized by autoclave at 121°C at 15psi.

Apparatus

All the apparatus like petridishes, pipettes, glass rods, test-tubes etc. were properly wrapped with papers and sterilized in hot air oven.

Antibacterial screening method

Disc Diffusion Method

- All the Petri dishes were sterilized in oven at 160°C for 1 hour.
- Agar media, borer and test solutions were sterilized in autoclave at 121°C at 15psi.
- Molten sterile agar was poured in sterile petridishes asceptically.
- The agar was allowed to cool and the bacterial suspension was poured into the petridishes asceptically.

- Placing the sterile filter paper discs in the agar plate and solution of the compounds was added by using pipette (0.1ml) in appropriate four quadrants of petridishes aseptically.
- Petridishes were incubated at 37°C for antimicrobial and 24ºC for antifungal for 24 hrs and observed the zone of inhibition.

MIC

- Minimum inhibitory concentration is the lowest concentration of antimicrobial compound found to inhibit the growth of particular test organism. MIC of different antimicrobial compounds is determined by liquid dilution method.
- MIC of the synthesized compounds was determined by tube dilution techniques.
- Serial dilution of the substance under examination was placed into culture tubes containing suitable medium and inoculated with the test organism. After incubation, the minimum concentration of test compound that inhibited the growth of the organism was observed.

Antifungal Screening

Culture

The synthesized compounds were screened for their antifungal activity against fungi *Candida albicans.*

Apparatus

All the apparatus like Petri dishes, pipettes, glass rods, test-tubes etc. were properly wrapped with papers and sterilized in hot air oven.

Preparation of Sabouraud Dextrose Broth

Enzymatic digest of Casein	5g
Enzymatic digest of Animal Tissue	5g
Dextrose	20g
Final pH	5.6
±0.2 at 25 °C	
Purified water	1000ml

All the ingredients were weighed and added to water. This solution was heated on water bath for about one and half-hour till it became clear. This nutrient media was sterilized by autoclaving at 121°C (15lbs psig) for 15 minutes.[8,9]

Preparation of standard solution

The standard drug Clotrimazole was dissolved in appropriate quantity of DMF to obtain the concentration range of 100, 250 and 500µg/ml and the zone of inhibition was checked.

Preparation of test solution

Specified quantity (100mg) of the compound was accurately weighed and dissolved in 100ml of DMF to get the 1000µg/ml stock solution. Further dilution was made to obtain the concentration in the range 750µg/ml, 500µg/ml and 250µg/ml.

Procedure

30g of the medium was suspended in 1000ml of purified water. The mixture was allowed to boil till it forms a homogeneous solution. The medium was autoclaved at 121°C for 15minutes at 15psi. Media was cooled to the temperature of approximately 40°C temperature and microorganisms were inoculated to the media. 150ml was transfer to a petriplates aseptically. Two such plates were prepared for each organism. Plates were allowed to cool for 20 minutes. Here both high and low strength disks were applied for each compound to be tested. The Petridishes were then incubated at 24°C for 24 hours after which zone of inhibition was measured.

Discussion of anti microbial activity

- All the synthesized compounds were screened for their anti-microbial activity against Gm +ve organisms *S.aureus* & *B.subtilis* and Gm –ve organism *E.coli* and anti-fungal activity against *C.albicans.*

- Compounds $7a_4$, $7b_4$, $7a_2$, $7b_2$ have shown good anti-bacterial activity but less potent as compared to standard reference drug ciprofloxacin. Among them $7a_4$, $7b_4$ have shown highest anti bacterial activity.
- Compounds $7a_1$, $7b_1$ have shown moderate antibacterial activity.
- Compounds $7a_3$, $7b_3$ have shown least antibacterial activity
- Compounds $7a_4$, $7b_4$, $7a_2$, $7b_2$ have shown good anti-fungal activity but less potent as compared to standard reference drug clotrimazole.
- Compounds $7a_1$, $7b_1$, $7a_3$, $7b_3$ have shown less antifungal activity.
- Compounds $7b_1$, $7a_3$, $7b_3$ were found to be very least potent towards antifungal activity.

Anti-inflammatory activity

Carrageenan induced rat paw edema method.

- Mice were assigned into 6 groups of 3 animals each.
- They were marked with picric acid for individual animal identification.
- The animals were deprived of food overnight (allowed free access to water *ad libitium*)
- First 0.1ml of 1%w/v of carrageenan in normal saline was injected in to the subplanter region of the left hind paw of rat.
- Synthetic compounds & std. compounds were administered after 1hr of the injection of carrageenan. Dose volume not exceeding 0.5ml/100gm orally was administered.
- Group I: The solvent control received normal saline.
- Group II: Positive control received phenylbutazone (100mg/kg).
- Group III-VI: Received pyrazole derivatives $7a_2$, $7b_2$, $7a_4$, $7b_4$ at a dose of 100mg/kg suspended in 1%w/v Tween-80.
- Immediately after administered the test compounds & Std. compounds, the volume of its displacement was measured using plethysmometer. The reading was recorded at 0, 1, 2, 3 hrs.
- % inhibition of oedema was calculated at the end of 3 hrs by using the formula.[12-14]

% inhibition = 100× (1-Vt/Vc)

Vt/Vc= oedema volume in the mice treated with the test drug and control respectively.

No. of animals used in each Group (n) = 3, Values are expressed as Mean±SEM

Dose of test compound = 100mg/kg, Dose of Phenylbutazone = 100mg/kg

Discussion of Anti-inflammatory activity

- The pharmacological screening of the synthesized compounds showed anti-inflammatory activity ranging from 37.8 to 44.5% inhibition of rat paw edema volume after 3hr, where as the standard drug phenylbutazone showed 66.6% inhibition of rat paw edema volume after 3hr.
- The compound $7a_4$ & $7b_4$ have shown to be a moderately potent than phenylbutazone which is used as standard drug. Compounds $7a_2$ & $7b_2$ have shown less potency than compound $7a_4$ & $7b_4$ and phenylbutazone.

CONCLUSION

All the synthesized compounds were characterized by UV, IR, Mass and some of by 1H-NMR spectroscopy & report of them support the structures of compounds. All the synthesized compounds were screened for antimicrobial activity against *S.aureus*, *B.subtilis* and *E.coli* and antifungal activity against *C.albicans* by disc diffusion method

and *in-vitro* anti-inflammatory activity by carrageenan induced rat paw oedema method. Compounds 7a4, 7b4, 7a2, 7b2 have shown good anti-bacterial and anti-fungal activity Compounds 7a1 & 7b1 have shown moderate antibacterial activity. Compounds 7a3 & 7b3 have shown least antibacterial activity compared to standard ciprofloxacin and clotrimazole. Compounds 7a4, 7b4, 7a2, 7b2 shown better antimicrobial activity have been evaluated for anti-inflammatory activity. The compounds 7a4 & 7b4 have shown to be a moderately potent than phenylbutazone which is used as standard drug. Compounds 7a2 & 7b2 have shown less potency than compound 7a4 & 7b4 and phenylbutazone. Compounds having chlorine and bromine at para position of benzene ring have shown better activity while electron withdrawing group such as nitro have shown less activity.

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REFERENCES

1. Morrow D J, Goodman and Gilman's.The Pharmacological Basis Of therapeutics; 10 Edn; Mc Graw-Hill pub, New York, **2001**, pp 669-87.
2. Rang H P., Dale M M., Ritter J M., Moore P K. Pharmacology. 5 Edn; Churchill Livingstone's pub, New York, **2003**, pp 217-243.
3. Mohan H. In Textbook of Pathology.Inflammation and Healing; 3 Edn; Jaypee brother's medical publishers Ltd., **1998**, pp 133-90.
4. Gupta D, Bhadauria R, Soan V, "Synthesis and antimicrobial activity of N- substituted Pyrazole derivatives" *Inter. J. of pharm. & applied sci.* **2010**, *2*, 0976-6936.
5. Ahluwalia V, Aggarwal R, Comprehensive Practical Organic Chemistry; Universities Press, **2000**, pp 44.
6. Manna F, Secci D, Chimenti F, Chimenti P , Fioravanti R,Ferlini C, Bolasco A, Scambia G, "Synthesis of some pyrazole derivatives and preliminary investigation of their affinity binding to P-glycoprotein." *Bioorg. & Med. Chem.* **2005**, *15*, 4632-4635.
7. Furniss BS., Vogel's Textbook of Practical Organic Chemistry; 5th Edition; Addison Wesley Longman, **1998**, pp 1273-1274.
8. Minu M, Thangadurai A, Wakode SR, Agrawal SS, Narasimhan B, " Synthesis, antimicrobial activity and QSAR studies of novel 2,3-disubstituted-3,3a,4,5,6,7-hexahydro-2H-indazoles." *Bioorg. & Med. Chem. Lett.* **2009**,*19*, 2960-2964.
9. Pelczar MJ., Chan ECS., and Krieg NR.In Microbiology; 5th Edition; Tata McGraw Hill Publishing Company Limited, New Delhi, **1986**, pp 73-98, 687-688, 504-508. Microbiological Assay. Indian Pharmacopeia. **1996**, vol-II, pp 100-103.
10. Kokare CR., Pharmaceuutical Experiments and Techniques, **2007**, 2, pp 153-162.
11. Shailesh Prafulbhai Zala, Badmanaban R. and Dhrubo Jyoti Sen; Chapter *Synthesis, Bioevaluation of 2,4,5-Triphenyl-1H-Imidazole-1yl Derivative*: Lambert Academic Publishing GmbH & Co. KG, Germany: **2012,** 1-89.
12. OECD **2001**- Guideline on Acute oral toxicity, Environmental health and safety monograph on testing and adjustment, no.425.
13. Winter C A, Risely E A, Nuss G W. "Carrageenan induced edema in hindpaw of the rats as an assay for anti-inflammatory drugs". *Proc. Soc. Exptl. Bio.Med.* **1962**, *1*, 544-547.
14. Winter C A, Risely E A, Nuss G W. "Carrageenan induced edema in hindpaw of

the rats as an assay for anti-inflammatory drugs", *J. Pharmacol. Exp. Ther.* **1963**, *3*, 141-569.

Table 1. Physicochemical Properties of synthesized compound (1st Section)

Sr No.	Compound Code	R_1	Molecular Formula	Molecular Wt.	Melting point (^{0}C)	% Yield (%)	R_f	Log P
1	3a	H	$C_{15}H_{12}O$	208.25	53-55	85.00	0.7	4.01
2	3b	OCH_3	$C_{16}H_{14}O_2$	238.28	70-75	85.00	0.7	3.96
3	4a	H	$C_{15}H_{14}N_2$	222.28	142-145	80.0	0.5	2.28
4	4b	OCH_3	$C_{16}H_{16}N_2O$	252.31	120	82.00	0.4	2.20

Table 2. Physicochemical Properties of synthesized compound (2nd Section)

Sr No.	Compound Code	R_2	Molecular Formula	Molecular Wt.	Melting point(^{0}C)	% Yield (%)	R_f	Log P
1	$7a_1$	H	$C_{22}H_{18}N_2O$	326.39	160-162	74.0	0.5	3.41
2	$7b_1$	H	$C_{23}H_{20}N_2O_2$	356.41	180-183	74.0	0.4	3.33
3	$7a_2$	Cl	$C_{22}H_{17}ClN_2O$	360	195-197	70.0	0.48	4.18
4	$7b_2$	Cl	$C_{23}H_{19}ClN_2O_2$	390.86	215-217	70.0	0.4	4.09
5	$7a_3$	NO_2	$C_{22}H_{17}N_3O_3$	371.38	150-153	65.0	0.47	3.37
6	$7b_3$	NO_2	$C_{23}H_{19}N_3O_4$	401.41	165-170	65.0	0.37	3.29
7	$7a_4$	Br	$C_{22}H_{17}BrN_2O$	405.28	205-210	70.0	0.45	4.35
8	$7b_4$	Br	$C_{23}H_{19}BrN_2O_2$	435.31	220-225	70.0	0.32	4.27

Table 3. Spectral characteristics of synthesized compound

Compound	UV (λ_{max})	IR (υ, cm^{-1})	Mass (m/e)	^{1}H-NMR (δ, ppm)
$7a_1$	304 nm	1658(C=O), 2356,2337(C=N)	326.9 (M)	-
$7b_1$	305 nm	1658(C=O),2339,2358(C=N),1031(Ar-OCH_3)	356.7 (M)	2.0 (d, 2H, C**H**$_2$ Pyrazole),3.7 (s, 3H, Ar-O**CH**$_3$),4.9 (t, 1H, C**H** Pyrazole),6.9 (d, 2H, C**H** Ar-OCH_3),7.2 (d, 2H, C**H** Ar-OCH_3),7.4-7.5 (m, 8**H**, ArH),7.9(d, 2**H**, ArH)
$7a_2$	281 nm	1668(C=O),2349(C=N), 1174(C-Cl)	361.1(M+) 363(M+2)	2.0 (d, 2H, C**H**$_2$ Pyrazole),4.9 (t, 1H, C**H** Pyrazole),7.0-7.1 (m, 10H, Ar**H**),7.6 (d, 2H, Ar**H**),7.9 (d, 2H, Ar**H**)
$7b_2$	267nm	1695(C=O),2337, 2358(C=N), 1174(C-Cl), 1089(Ar-OCH_3)	391.8(M+) 393 (M+2)	-
$7a_3$	299 nm	1658(C=O), 2339,2358(C=N)	371.9(M)	-
$7b_3$	269 nm	1708(C=O), 2387(C=N), 1047(Ar-OCH_3)	402.1 (M+1)	-
$7a_4$	290 nm	1668(C=O), 2308(C=N), 1080(P-Br)	406.9(M+1) 408 (M+2)	-
$7b_4$	272nm	1641(C=O), 2337,2358(C=N), 1076(P-Br), 1029(Ar-OCH_3)	436.8 (M+) 438 (M+2)	-

Table 4. Screening of Antibacterial activity by zone of inhibition[11]

Compound code	Concentration (µg/ml)	Zone of inhibition(mm)		
		Gram+ve		Gram-ve
		S.aureus	*B.subtilis*	*E.coli*
$7a_1$	5	08	08	07
	7	09	09	08
	1	10	10	10
$7b_1$	5	06	06	05
	7	07	08	07
	1	09	10	08
$7a_2$	5	12	12	10
	7	13	13	11
	1	15	14	13
$7b_2$	5	11	11	10
	7	12	12	11
	1	14	13	12
$7a_3$	5	05	05	04
	7	06	07	06
	1	07	08	07
$7b_3$	5	04	04	03
	7	05	06	04
	1	07	07	06
$7a_4$	5	14	12	11
	7	16	13	12
	1	17	15	14
$7b_4$	5	14	12	11
	7	15	12	12
	1	16	14	13
Ciprofloxacin	5	27	24	25
	7	28	25	27
	1	30	27	28

Table 5. MIC of Antibacterial Screening

Compound code	Zone of inhibition(mm)		
	Gram+ve		Gram-ve
	S.aureus	*B.subtilis*	*E.coli*
$7a_1$	400	400	400
$7b_1$	500	600	500
$7a_2$	250	300	300
$7b_2$	250	300	300
$7a_3$	400	400	400
$7b_3$	500	500	500
$7a_4$	200	200	200
$7b_4$	250	250	250
Ciprofloxacin	50	50	50

Table 6. Antifungal screening by zone of inhibition[11]

Compound Code	Concentration (µg/ml)	*Zone of inhibition(mm)*
		C.albicans
$7a_1$	500	07
	750	08
	1000	10
$7b_1$	500	06
	750	08
	1000	09
$7a_2$	500	10
	750	12
	1000	14
$7b_2$	500	10
	750	11
	1000	13
$7a_3$	500	05
	750	07
	1000	08
$7b_3$	500	04
	750	05
	1000	06
$7a_4$	500	12
	750	13
	1000	15
$7b_4$	500	11
	750	13
	1000	14
Ciprofloxacin	500	19
	750	20
	1000	22

Table 7. MIC of Antifungal Screening

Minimum Inhibitory Concentrations (µg/ml)	
Compound Code	***C.albicans***
$7a_1$	400
$7b_1$	500
$7a_2$	250
$7b_2$	300
$7a_3$	500
$7b_3$	500
$7a_4$	200
$7b_4$	250
Clotrimazole	50

Table 8. Screening of Anti-inflammatory activity

Time	Paw volume (ml)±SEM				% Inhibition		
	0 hr	1 hr	2 hr	3 hr	1 hr	2 hr	3 hr
Control	0.75±0.03	0.74±0.03	0.74±0.04	0.74±0.03	-	-	-
Phenylbutazone	0.74±0.02	0.57±0.02	0.4±0.02	0.25±0.02	24.0	46.6	66.6
$7a_2$	0.71±0.02	0.67±0.02	0.59±0.02	0.48±0.02	9.4	20.2	35.13
$7b_2$	0.73±0.03	0.63±0.02	0.53±0.02	0.46±0.03	14.8	28.3	37.8
$7a_4$	0.75±0.03	0.6±0.03	0.51±0.03	0.42±0.03	17	30.1	42.4
$7b_4$	0.75±0.03	0.59±0.02	0.49±0.01	0.41±0.03	20	33.7	44.5

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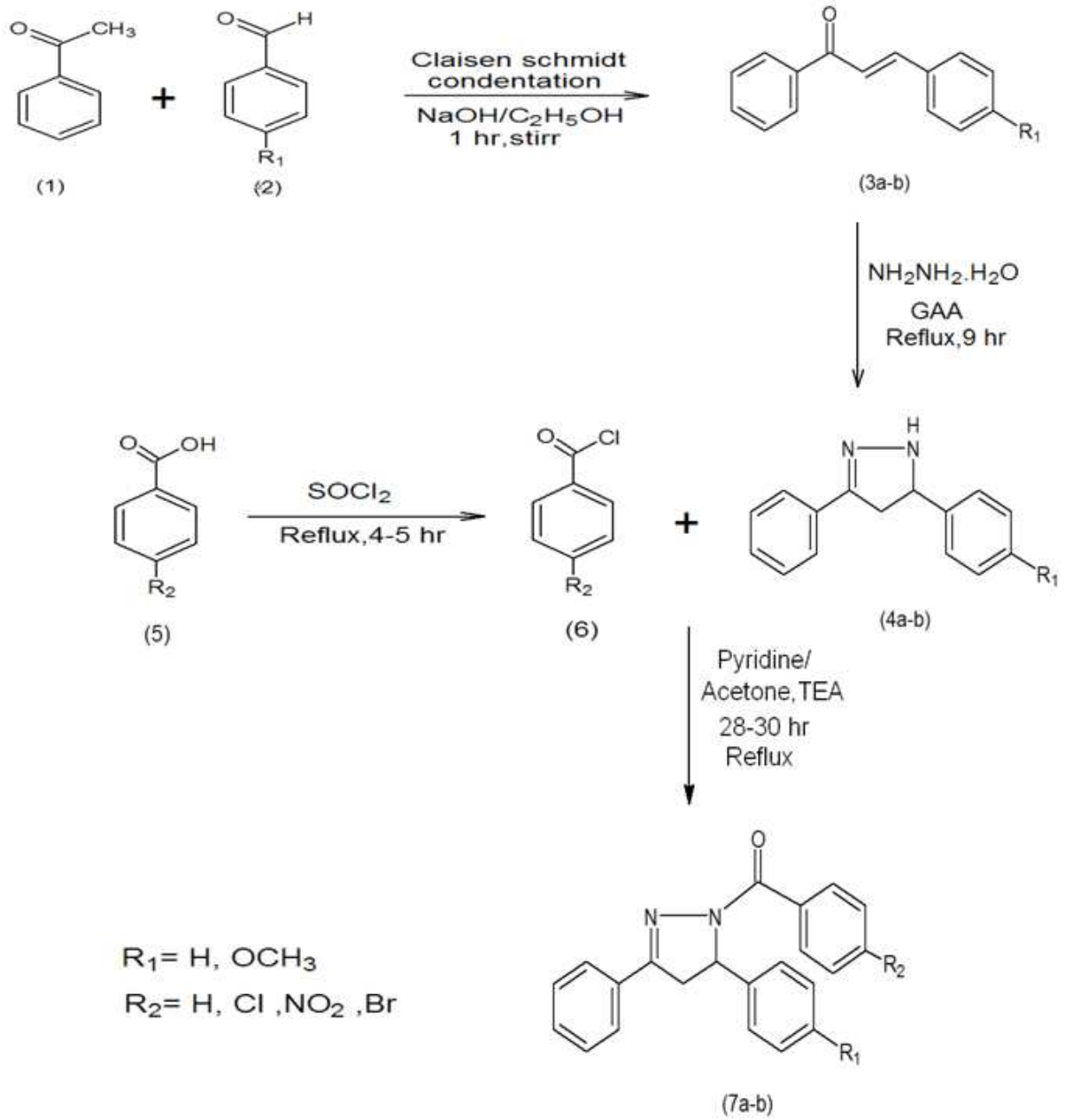

Scheme of Synthesis

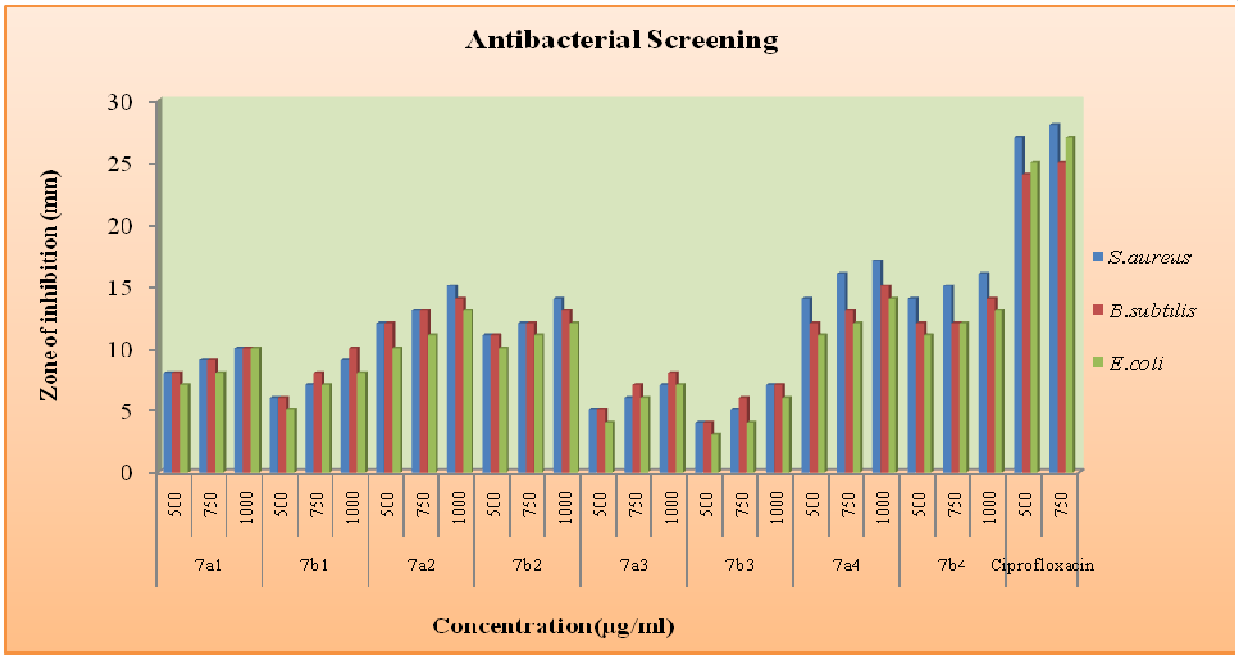

Figure 1: Histogram of Zone of Inhibition (Antibacterial Screening)

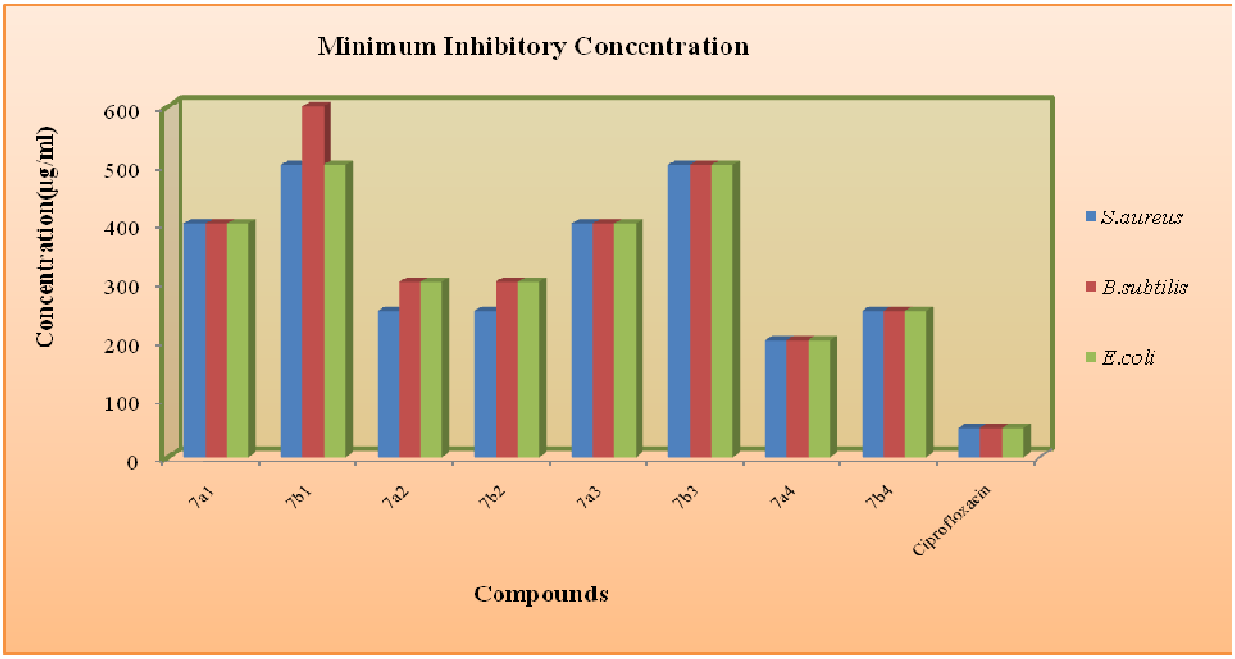

Figure 2: Histogram of MIC (Antibacterial activity)

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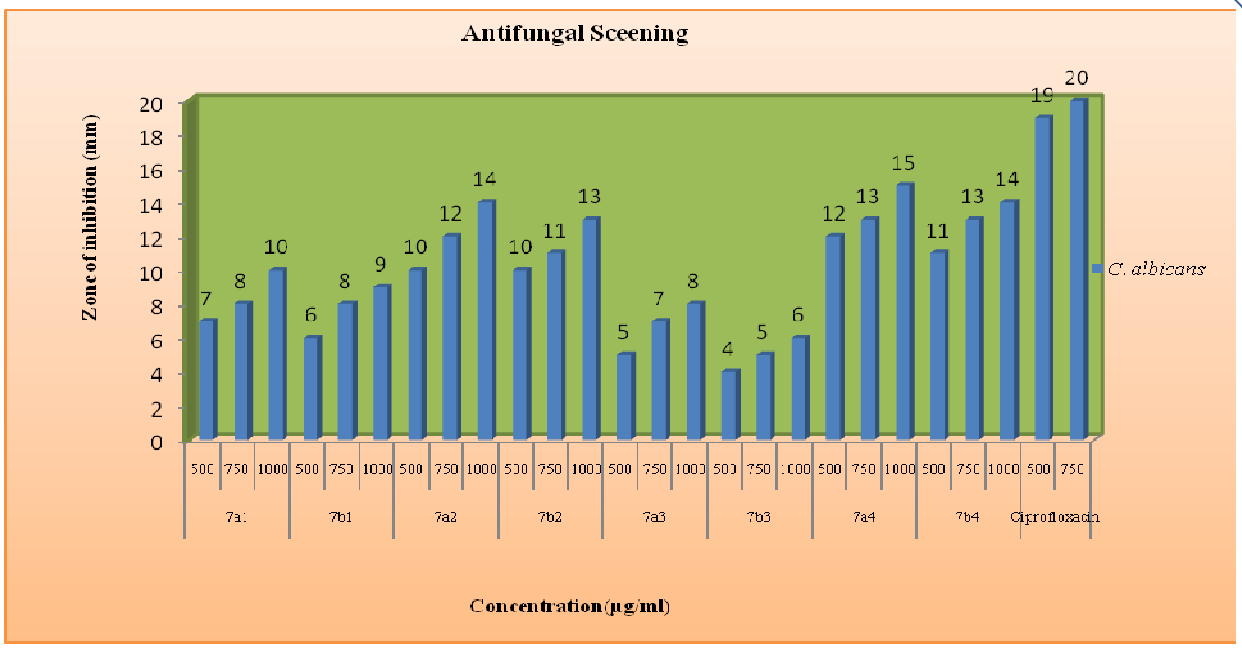

Figure 3: Histogram of Zone of Inhibition (Antifungal Screening)

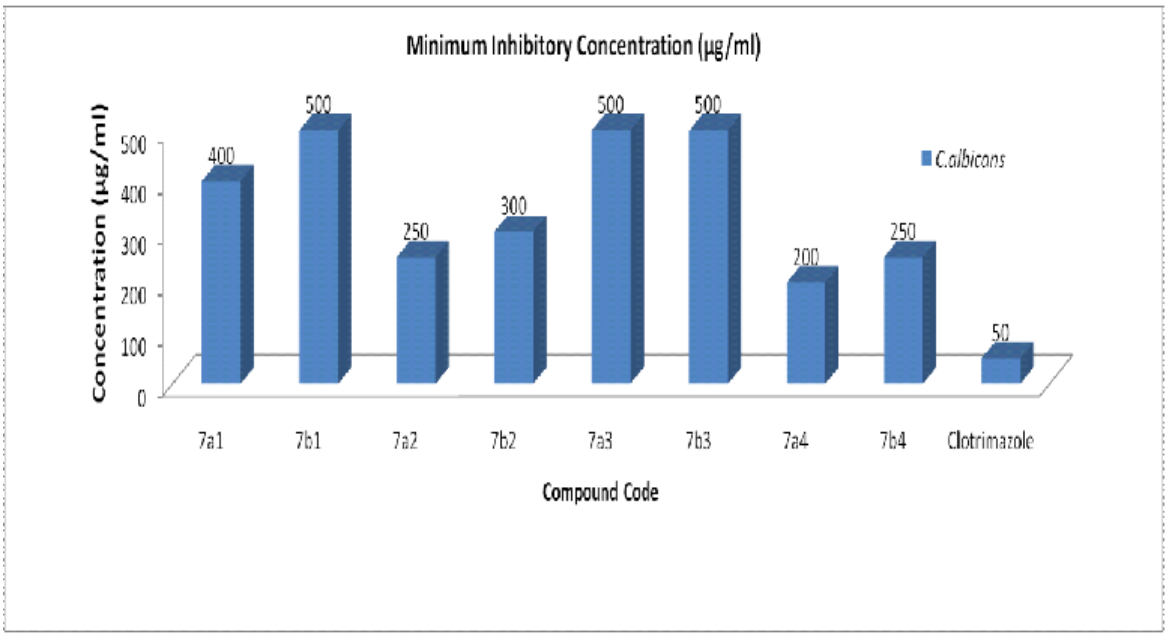

Figure 4: Histogram of MIC (Antifungal Screening)

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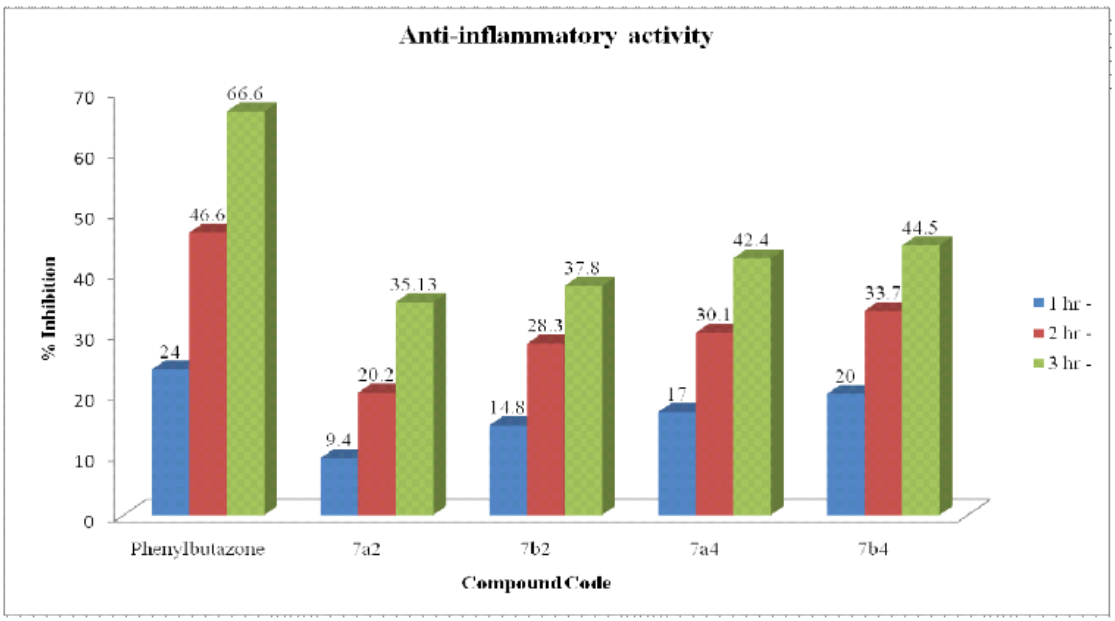

Figure 5: Histogram of Anti-inflammatory screening