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SYNTHESIS OF NEW 9-(5-PHENYL-1H-ISOXAZOLINE-3-YL)-9H- CARBAZOLE DERIVATIVES WITH POTENTIAL ANTI- INFLAMMATORY ACTIVITY

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ABSTRACT

*Carbazole on reaction with Acetic anhydride in the presence of acetic acid and Zinc dust offereded 1-(9H-carbazole-9-yl)ethanone **(1)** condensation of **(1)** with various aromatic aldehydes offerded 1-(9H-carbazol-9-yl)-3-phenylpropyl-2-en-1-one (Chalcones, **1a-1j**) . which on cyclization with Hydroxyl amine Hydrochloride yielded 9-(5-phenyl-1H-Isoxazoline-3-yl)-9H-carbazole **(2a-2j).** The structures of newly synthesized compounds were confirmed by FTIR, [1]HNMR, MASS spectral data and elemental analysis. Compounds were screened for invitro anti inflammatory activity by Inhibition of Albumin Denaturation technique and invivo anti inflammatory activity by Carageenan induced Hind paw oedema method. All the synthesized substituted carbazoles have shown moderate to good anti inflammatory activity.*

KEYWORDS : *Carbazole, Acetic anhydride, Aromatic aldehydes, Hydroxylamine Hydrochloride, Anti inflammatory activity.*

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INTRODUCTION

Carbazole alkaloids have received considerable attention since their discovery in the 1960's and the realization of their pharmacological potential[1]. This has generated enormous interest in synthetic products containing carbazole nucleous and has resulted in the development of carbazole chemistry during the last several years. Due to wide scope for synthetic investigation leading to more to more potent synthetic leads, voluminous synthetic work has been done. More recently, the area of biological interest of carbazole has been extended to various diseases such as cancer and cardiovascular disorders. In addition, carbazole also exhibit antioxidant[2], antidiabetic[3], antimitotic[4], antimicrobial[5,6], anti-vascular[7], antitumour[8,9], anticancer[10,11],antipsychotic[12],and anticonvulsant[13] activities. Isoxazoline a five-membered unsaturated heterocyclic ring containing three carbon atoms, one nitrogen and oxygen atoms (adjacent) and a

carbon-nitrogen containing double bond. Isoxazolines has exhibited an array of biological activities ranging from antifungal[14],antibacterial[15], anticonvulsant[16], anti-inflammatory[17] ,anti-viral[18], analgesic[19], antitumor [20], chemotherapeutic [21] activity. Encouraged by the diverse biological activities of compounds, in this work Carbazole derivatives were synthesized via cyclization with substituted chalcone intermediates in the presence of hydroxylamine hydrochloride, with an objective to develop potent anti inflammatory agents.

MATERIALS AND METHODS:

All the chemicals used to synthesize the title compounds were of laboratory grade and purchased from S.D. Fine Chemicals and Sigma Aldrich. All the reactions were carried out under prescribed laboratory conditions. Melting points of the synthesized compounds were determined by open capillary and are uncorrected. The purity of the compounds was checked using precoated TLC plates (MERCK, 60F). The appropriate mobile phases (Solvent system for TLC) as applicable were developed using silica Gel G as stationary phase. The developed chromatographic plates were visualized under UV at 254nm. The Infrared spectra for the synthesized compounds were recorded using SHIMADZU-FTIR 8400S spectrometer using KBr as a back ground. 1H NMR spectra of the synthesized compounds were taken using BRUKER Advance-II 400 NMR spectrometer using Tetra methyl silane as an internal standard. 1H NMR spectra were recorded with MeOD as a solvent & the chemical shift data were expressed as delta values related to TMS. Mass spectra of the synthesized compounds were taken using 2010EV LCMS SHIMADZU instrument at 70 eV. Male Wister rats (125-150g) were used for the study. Animals were procured from NIMHANS Bangalore and were maintained in the standard environmental conditions and free access to feed (Amrut feed India) and water *ad libitum.* The Institutional Animal Ethical Committee approved the protocol of the study.

Synthesis of 1-(9H-carbazole-9-yl)ethanone.

In a round bottom flask 34 gm (0.1mole) of carbazole was dissolved in 20ml (21.5 gm = 0.21 mole) of acetic anhydride, 20ml (21gm=0.35mole) of glacial acetic acid and 0.1 gm of zinc dust, refluxed for 30 min and then poured gently into a beaker containing 500ml of ice water stirred continuously. Filtered and recrystalized with water and dried in air.

Synthesis of 1-(9H-carbazol-9-yl)-3-phenylpropyl-2-en-1-one. (1a-1j).

A mixture of 1-(9H-carbazole-9-yl)ethanone (0.01 mol) 1.86 g and aromatic aldehydes (a-j) (0.01 mol) of each was stirred separately in 90 % ethanol (30 ml) and then an aqueous solution of NaOH (40%, 15 ml) added to it. The mixture was kept overnight at room temperature and then it was poured onto crushed ice, stirred and acidified with dil HCl. The solid separated was filtered and recrystalized from ethanol.

Synthesis of 9-(5-phenyl-1H-Isoxazoline-3-yl)-9H-carbazole. (2a-2j).

A solution of 2.8 g of the 1-(9H-carbazol-9-yl)-3-(4-methoxyphenyl)but-2-en-1-one (chalcone 0.01 mole) in 90 % ethanol (30 ml) was refluxed with 1 ml Hydroxyl amine Hydrochloride 80% (0.02 mole) for 4 h. The Solution was cooled and poured on the crushed ice. The precipitated crude product was filtered and recrystalized from ethanol.

ANTI INFLAMMATORY STUDIES OF THE SYNTHESIZED COMPOUNDS.

***In vitro* Anti-Inflammatory Activity by Inhibition of Albumin Denaturation technique.**

The synthesized compounds are screened for anti-inflammatory activity by using

inhibition of albumin denaturation technique, which was studied according to Sakat *et al*[22].

Diclofenac sodium was used as standard drug. The standard drug and test compounds were dissolved in minimum amount of dimethyl formamide (DMF) and diluted with phosphate buffer (0.2 M, pH 7.4). Final concentration of DMF in all solutions was less than 2.5%. Test solution (1 mL) containing different concentrations of drug was mixed with 1 mL of 1 m M albumin solution in phosphate buffer and

incubated at 27° ± 1° C in BOD incubator for 15 min. Denaturation was induced by keeping the reaction mixture at 60° ± 10° C in water bath for 10 min. After cooling, the turbidity was measured at 660 nm (UV-Visible Spectrophotometer SL-159, Elico India Ltd.). Percentage of inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and average was taken. The results are tabulated in.Table No 2. The % of inhibition was calculated as per the fomula,

% inhibition= [{Abs control- Abs sample}/Abs control] x 100,

In vivo **Anti-Inflammatory Activity by Carrageenan Induced Rat Paw Edema Method:**

Anti-inflammatory activity was assessed by the method described by Winter et al[23]. The rats were divided into three groups of six animals each. First group (negative control) received 1 ml of normal saline, second group (positive control) received 10 mg/kg p.o Diclofenac sodium and third group receive 150 mg/kg, p. o. of Test compounds respectively. After 1 h, the rats were challenged with subcutaneous injection of 0.1 ml of 1 % w/v solution of carrageenan (Sigma Chemical Co, St. Louis MO, USA) into the plantar side of the left hind paw. The paw was marked with ink at the level of lateral malleolus and immersed in mercury up to the mark. The plethysmograph apparatus used for the measurement of rat paw volume (Singh and Ghosh 1968). The paw volume was measured immediately after injection (0 h) and followed by every hour till the 3 h after injection of carrageenan to each group. The difference between the initial and subsequent reading gave the actual edema volume. The results of Percent inhibition of inflammation are shown in Table .3.

Percent inhibition of inflammation was calculated using the formula,

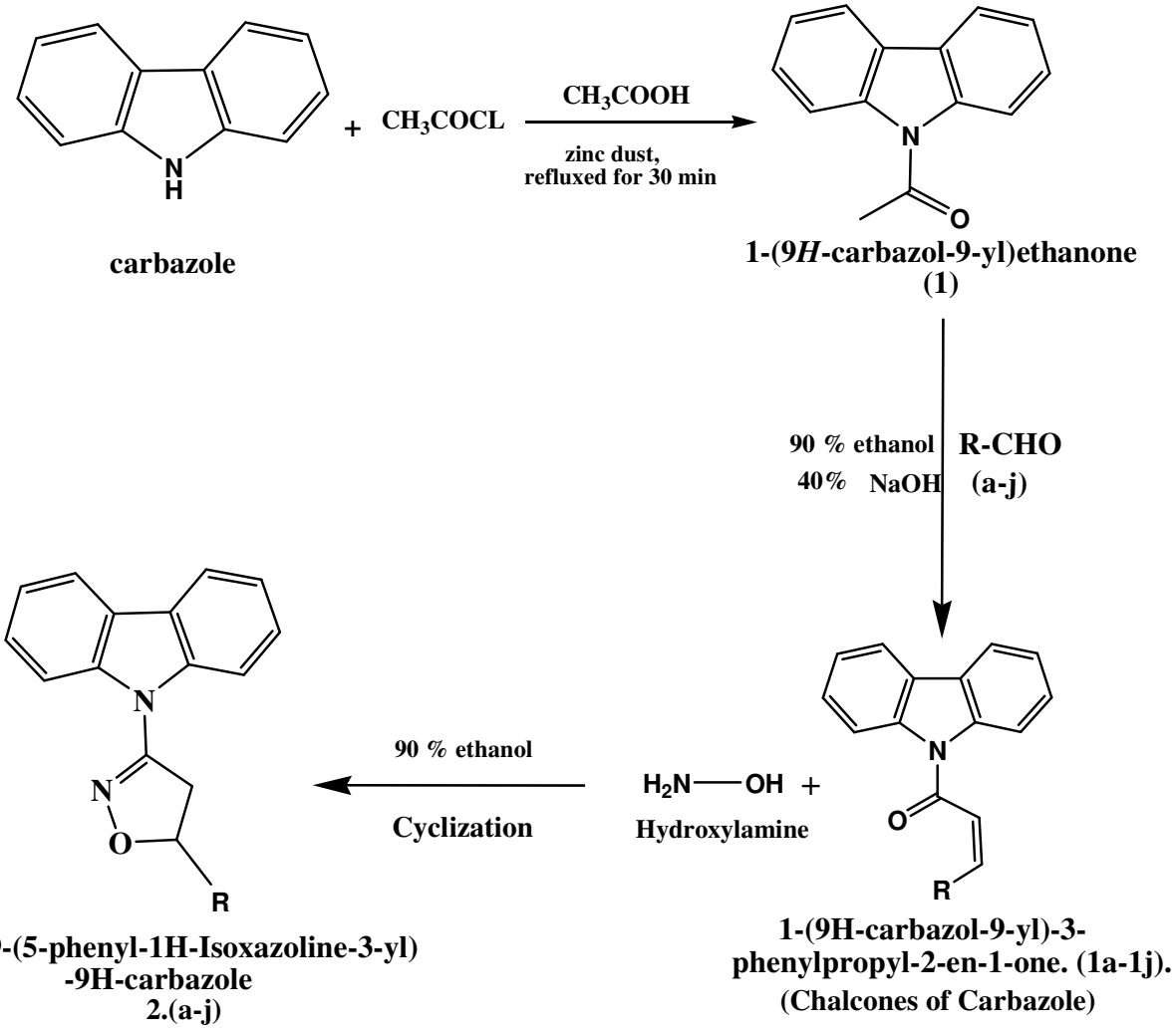
% inhibition = 100 (1-Vt/Vc),

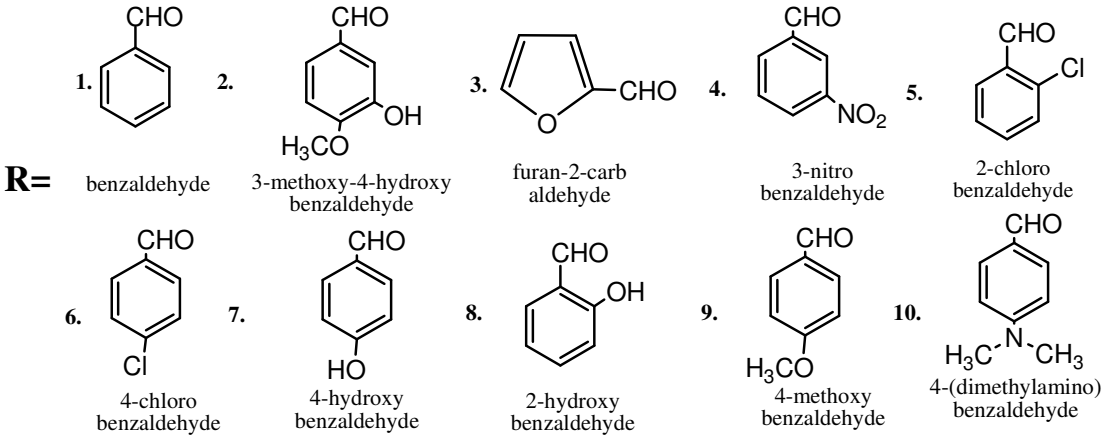
where 'Vc' represents edema volume in control and 'Vt' edema volume in group treated with test extracts.

RESULTS AND DISCUSSION.

All the synthesized compounds were purified by successive recrystallization using ethanol. The purity of the synthesized compounds was checked by performing TLC. The structures of the synthesized compounds were determined on the basis of their FTIR and 1HNMR data and mass spectrum. Table no 1, shows the charecterisation data of the synthesized compounds. The synthesized compounds were screened for *invitro* anti inflammatory activity by inhibition of albumine denaturation technique and *invivo* anti inflammatory activity by carageenan induced paw edema method on wister albino rats and compared with Diclofenac sodium as a standard drug. The SEM values are calculated by using SPSS software. The study indicated that compounds 2b, 2d, 2g and 2h exhibited potent anti inflammatory activity. Other groups have less significant anti inflammatory activity. Structural activity of the title compounds for anti inflammatory activity reveals that the compounds having OH, NO_2 and OCH_3 groups shows more activity. The above results establish the fact that carbazole linked with isoxazoline substituted with aromatic aldehydes can be studied further to search a newer anti inflammatory compound. From the data of anti inflammatory activity it is clearly concluded that the synyhesized compounds are having good anti inflammatory activity. There fore, in search of new generation of active compounds, it may be worthwhile to explore the possibility in this area by fusing and substituting different moieties which may results in better pharmacological activities. Hence in the present study, carbazole and isoxazoline substituted with different aromatic aldehydes showed highly potent and more specific anti inflammatory activity.

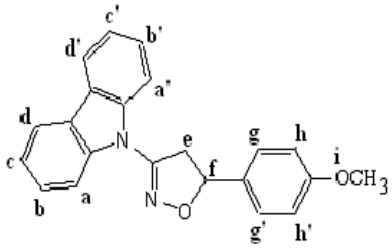
Fig no .1, (scheme 1). Synthesis of 1-(9H-carbazole-9-yl)ethanone.





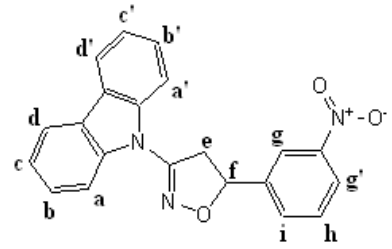
Spectral data and structure of the new synthesized compounds.

1). Compound. 2b, 9-(5-(4-methoxyphenyl)-1H-isoxazoline-3yl)-9H-carbazole.



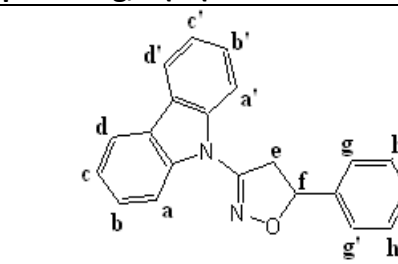
FT-IR (cm^{-1}), N-H Str, 3433.42, C-H Str (Ar-H) 3054.23, C=O Str (OCH_3), 1275.41, C-O-N *df* (isoxazoline), 1612.05, C=N Str (isoxazoline),1251.25.
1H-NMR (DMSO) δ ppm , 7.55 (d 2H Ar-H at d,d'),7.40(d 2H Ar-H at a, a'),7.08 (d 2H Ar-H at b, b'), 7.02 (d 2H Ar-H at g, g'), 7.00 (d 2H Ar-H at c, c'), 6.70 (d 2H Ar-H at h, h'), 4.5 (t 1H CH_2 of isoxazoline at f), 3.73 (s 1H OCH_3 at i), 1.8 (d 2H CH_2 of isoxazoline at f).
LC-MS m/z, 342.137[M^{+1}].

2). Compound. 2d , 9-(5-(nitrophenyl)1H-isoxazoline-3yl)-9H-carbazole



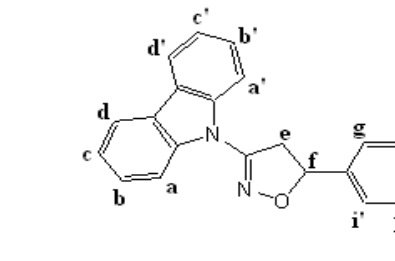
FT-IR (cm^{-1}), N-H Str, 3430.26,C-H Str (Ar-H) 3062.33, C=O Str ,1674.47, N-O Str 1572.21, C-O-N *df* (isoxazoline), 1630.20 C=N Str (isoxazoline),1263.15
1H-NMR (DMSO) δ ppm , 7.53 (d 2H Ar-H at d,d'),7.29(d 2H Ar-H at a, a'),7.04 (d 2H Ar-H at b, b'), 8.12 (d 2H Ar-H at g, g'), 7.00 (d 2H Ar-H at c, c'), 7.45 (d 2H Ar-H at h h'), 7.58 (s 1H Ar-H at i), 4.32 (t 1H CH_2 of isoxazoline at f), 1.8 (d 2H CH_2 of soxazoline at f).
LC-MS m/z, 357.111 [M^{+1}].

3).Compound.2g,4-(3-(9H-carbazol-9-yl)-4,5-dihydro-1H-isoxazoline-5-yl)phenol



FT-IR (cm^{-1}), N-H Str, 3460.40, C-H Str (Ar-H) 3164.23 C=O Str 1645.41, Ar-OH Str 3300.40, C-O-N *df* (isoxazoline), 1615.20, C=N Str (isoxazoline),1265.55.
1H-NMR (DMSO) δ ppm , 7.56 (d 2H Ar-H at d, d'),7.19(d 2H Ar-H at a, a'),7.24 (d 2H Ar-H at b, b'), 7.02 (d 2H Ar-H at g, g'), 7.07 (d 2H Ar-H at c, c'), 6.66 (d 2H Ar-H at h, h'),5.0 (t 1H OH at i),), 4.33 (t 1H CH_2 of isoxazoline at f), 1.9 (d 2H CH_2 of soxazoline at f).
LC-MS m/z, 328.121 [M^{+1}].

4). Compound. 2h, 3-(3-(9H-carbazole-9-yl)-4,5-dihydroisoxazole-5-yl)phenol



FT-IR (cm^{-1}), N-H Str, 3460.40, C-H Str (Ar-H) 3164.23 C=O Str 1645.41, Ar-OH Str 3300.40, C-O-N *df* (isoxazoline), 1615.20, C=N Str (isoxazoline),1265.55
1H-NMR (MeOD) δ ppm , 7.56 (d 2H Ar-H at d, d'),7.19(d 2H Ar-H at a, a'),7.24 (d 2H Ar-H at b, b'), 6.75 (d 2H Ar-H at g,), 7.07 (d 2H Ar-H at c, c'), 7.06 (d 2H Ar-H at h,),6.66 (d2H Ar-H at i i'), 5.01 (t 1H ,OH at j) 4.33 (t 1H CH_2 of isoxazoline at f), 1.9 (d 2H CH_2 of soxazoline at f).
LC-MS m/z, 328.363 [M^{+1}].

No .1.Characterization data of the synthesized compounds.

sample code	Mol. formula	Mol. weight	Melting point (^oC)	% yield	R_f Value	CHN composition calculated			
						C	H	N	O
2a	$C_{21}H_{16}N_2O$	312.126	126-138	76.4	0.45	81.53	4.89	13.58	5.31
2b	$C_{22}H_{18}N_2O_2$	342.137	139-140	50	0.56	77.40	5.61	12.31	4.69
2c	$C_{19}H_{14}N_2O_2$	302.122	131-135	67.1	0.50	75.73	5.02	13.94	5.31

2d	$C_{21}H_{15}N_3O_3$	357.111	124-127	54.4	0.56	70.77	4.53	15.72	8.98
2e	$C_{21}H_{15}ClN_2O$	346.815	123-124	73.8	0.43	72.93	4.66	12.15	4.92
2f	$C_{21}H_{15}ClN_2O$	346.813	112-119	81.8	0.46	72.93	4.66	12.66	4.69
2g	$C_{21}H_{16}N_2O_2$	328.121	120-125	60.3	0.43	77.04	5.23	12.84	4.89
2h	$C_{21}H_{16}N_2O_2$	328.363	125-127	65.7	0.57	77.52	4.65	12.91	4.92
2i	$C_{21}H_{18}N_2O_3$	358.389	96-100	46.1	0.53	74.35	4.82	11.82	9.00
2j	$C_{23}H_{21}N_3O$	355.162	132-138	79.6	0.59	78.38	5.72	15.90	5.31

Compound Code	**Absorbance Value Mean ± SE**	**Inhibition of Denaturation (%)**
Control	0.089 ± 0.032	- -
Diclofenac Na	0.038 ± 0.023*	57.30
2.a	0.049 ± 0.011	44.50
2.b	0.039± 0.019*	56.16
2.c	0.045 ± 0.002	49.43
2.d	0.033 ± 0.016*	62.92
2.e	0.051 ± 0.021*	42.69
2.f	0.045 ± 0.011	49.43
2.g	0.031 ± 0.033*	65.17
2.h	0.032 ± 0.041*	64.76
2.i	0.048 ± 0.031	46.08
2.j	0.056 ± 0.011	37.70

Table. No 2. *invitro* Anti-inflammatory activity by Albumine Denaturation

Technique:

Values are expressed as mean ±SE (n=6)

*Indicates significant difference at p<0.001 when compared to control

Table No. 3. *In vivo* Anti-Inflammatory Activity by Carrageenan Induced Rat Paw Edema Method:

Sl.No.	**Compound code**	**Dose Mg/kg**	**Mean difference in Paw volume ± SEM after 3 hrs.**	**% inhibition of paw volume**
1	Control	0.5ml	2.30± 0.05	-
2	Diclofenac Na	10mg/kg	0.55 ± 0.03*	76.03
3	2.a	150mg/kg	1.33± 0.05	43.20
4	2.b		1.07± 0.06*	53.39
5	2.c		1.30± 0.04*	31.81
6	2.d		1.12± 0.10*	51.14
7	2.e		1.83 ± 0.05*	24.09
8	2.f		1.63± 0.04	31.05
9	2.g		1.02± 0.04*	55.19
10	2.h		1.05± 0.07*	54.72
11	2.i		1.78± 0.06	22.09
12	2.j		1.63± 0.04	29.95

Values are expressed as mean ±SEM (n=6)

*Indicates significant difference at p<0.001 when compared to control

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REFERENCES

1) Chaudhary DN, Basak SK, Das BP. Synthesis of Pyrrole and Carbazole alkaloids. Curr.Sci, 47, 1978, 490-491.
2) P. Sauerberg, I. Pettersson, L. Jeppesen, P.S. Bury, J.P. Mogensen, K. Wassermann, C.L. Brand, J. Sturis, H.F. Woldike, J. Fleckner, S.T. Andersen, *J. Med. Chem.*, 45, 2002, 789.
3) S. Venkatesan, J. Kumar and S. M. Krishna Ganesh, *Asian Journal of Plant Science and Research,* 2(3), 2012, 263-268.
4) T.E. Barta, A.F. Barabasz, B.E. Foley, L. Geng, *J. Bioorg. Med. Chem. Lett*., 19, 2009, 3078.
5) F.F. Zhang, L.L. Gan, C.H. Zhou, *Bioorg. Med. Chem. Lett*., 20, 2010, 1881.
6) V.V. Mulwad, C.A. Patil, *Indian J. Chem*., 44B, 2005, 2355.
7) A.I. Danish, K.J.R. Prasad, *Indian J. Chem*., 43B, 2004, 1548.
8) N. Ty, G. Duperyre, G.C. Chabot, J. Seguim, L. Quentin, A. Chiarone, F. Tillequin, D. Scherman, *Eur. J. Med. Chem*.12010,129-132 .
9) E.R. Pereira, L. Belin, M. Sancelme, M. Prudhomne, M. Ollier, *J. Med. Chem*., 39, 1996, 4471.
10) T. Lemster, U. Pindur, G. Lenglet, S. Depauw, C. Dassi, M.D. Cordonnier, *Eur. J. Med. Chem*., 44, 2009, 3235.
11) M. Laronze, M. Boisburn, S. Leonce, B. Pfeiffer, P. Renard, O. Lozach, L. Meijer, A. Lansiuax, C. Bailly, *Bioorg. Med. Chem*., 13, 2005, 2263.
12) H. Kaur, S. Kumar, P. Vishwakarma, M. Sharma, K.K. Saxena, A. Kumar, *Eur. J. Med. Chem*., 45, 2010, 2777.
13) J. Bouchard, S. Wakim, M. Leclerc, *J. Org. Chem*., 69, 2004, 5705.
14) Korgaokar S. S, Patil P. H, Shab M. T and Parekh H. H, *Indian J Pharm. Soc.* 58, (1996).222
15) Nauduri D and Reddy G. B, *Chem. Pharm. Bull. Tokyo*, 46, 1998.1254
16) Uno H, Kurokawa M, Masuda Y and Nishimuura H, *J Med. Chem.*, 22, 1979, 180.
17) Shivkumar B and Nargund L. V. G, *Indian J Heterocyclic Chem.*, 8, 1998.27
18) Diana G. D, Mckinlay M. A, Otto M. J, Akullian V and Oglesby C, *J Med. Chem.*, 28, 1985.1905
19) Kano H, Adachi J, Kido R. and Hirose K.; *J Med. Chem.*, 10(3), 411 (1967).
20) Getal, *J Antibiot.*, 28(1) 1975, 91
21) Sadanandam A, Rajam M. V, Subhash K, and Rajanarendar E, *Indian Bot. Report.*, 3(1), 1984. 38
22) Sakat, S., A.R. Juvekar and M.N. Gambhire, *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. Int. J. Pharm. Pharmacol. Sci., 2(1): 2010, 146-155.
23) Winter, C.A, E.A. Risley and G.W. Nuss, Carrageenin-induced edema in hind paw of the rat as an assay for antiiflammatory drugs. Proceedin. Soc. Exp. Biol. Med. 111: 1962, 544-7.

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