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Synthesis and pharmacological screening of some novel chloro chalconesemicarbazone derivatives

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Abstract

A series of chalconyl derivatives of chloro phenyl semicarbazide (CA1-CA5) was synthesized. All the synthesized compounds were screened for their pharmacological action in protection of seizures, behavioral study, anti-inflammatory and analgesic activity. After intraperitoneal injection to mice or rats, the synthesized compounds were examined in the maximal electroshock seizure (MES), subcutaneous pentylenetetrazole (scPTZ), and neurotoxicity test models. Compound CA6 emerged as the most active molecule for anticonvulsant and anti-inflammatory activity. Some of the title compounds exhibited lesser CNS depression compared to phenytoin as was evident from the CNS studies.

Key words: chalcones, anticonvulsant, semicarbazide, anti-inflammatory activity.

INTRODUCTION

The search for new anticonvulsant drugs remains an active area of investigation since available antiepileptic drugs are effective in only 60%-80% of patients [1]. While absence (petit mal) seizures are well treated in most instances, significant therapeutic improvement is still needed for the treatment of partial-complex (focal) and generalized tonic-clonic (grand mal) seizures. In addition, most marketed anticonvulsants suffer from a broad range of undesirable side effects such as sedation, teratogenicity, cognitive dulling, blood dyscrasia, and hepatotoxicity. Failure to achieve control of seizures is frequently due to use-limiting side effects seen with increasing doses of the drugs before a satisfactory therapeutic dose is reached [2,3].

Two major pharmacological screening tests used to evaluate compounds for anticonvulsant activities are the maximal electroshock method (MES) and subcutaneous pentylenetetrazole (scPTZ) method. These tests are claimed to detect compounds possessing activity against generalized tonic-clonic (grand mal) and generalized absence (petit mal) seizures, respectively. The structural requirements for activity in the MES screen have been stated to be the presence of a large hydrophobic group which is in close proximity to at least two electron-donor atoms. For activity in the scPTZ screen, a smaller, less hydrophobic group than is required for activity in the MES screen should be present near to a minimum of two electron donor atoms. Based on these considerations, a number of aryl semicarbazones have been prepared in past time, which contained a hydrophobic moiety, namely an aryl ring, as well as four electron-donor atoms in the semicarbazono group [4-10].

Furthermore, there are several reports about the synthesis and pharmacological evaluation of new bioactive N-aroylarylhydrazones acting at the AA cascade enzyme level [11–13] and chalcones are also having anti-inflammatory activity. As a part of our ongoing research program [14–20] to find novel anti-inflammatory and analgesic compounds, herein, we have fused these both active moiety and design a scheme for synthesizing these. The analgesic and anti-inflammatory activity of synthesized compounds were performed.

On the bases of these conclusions we have applied indirect drug design approach. In present study, the aldehydic moiety is replaced with chalconyl moiety in aryl semicarbazones and synthesized some novel chalconyl derivatives of semicarbazide and screened for their pharmacological action.

MATERIALS AND METHODS

Chemistry

Melting points were measured in open capillary tubes on a Buchi 530 melting point apparatus and were uncorrected. Infrared (IR) and proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded for the compounds on Jasco IR Report 100 (KBr) and Brucker Advance (300 MHz) instruments, respectively. Chemical shifts are reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard. All exchangeable protons were confirmed by addition of D_2O. Mass spectra were measured with a Shimadzu GC-MS-QP5000 spectrophotometer. Only molecular ions (M+) and base peaks are given. Elemental analyses (C, H, and N) were undertaken with a Perkin-Elmer model 240C analyzer, and all analyses were consistent with theoretical values (±0.4%) unless indicated. The homogeneity of the compounds was monitored by ascending thin-layer chromatography (TLC) on silica gel G (Merck) coated aluminum plates, visualized by iodine vapor.

Synthesis of substituted chalcone derivatives

Substituted benzaldehydes (0.012mol) were added to a mixture of substituted acetophenones (0.01mol) in 25 ml of ethanol in a 200 ml beaker. The content of the beaker was mixed well and to that 10 ml of 10% potassium hydroxide solution was added and stirred vigorously at 25 °C until the mixture was so thick that stirring was no longer effective (3—4 h). After the completion of the stirring, the reaction mixture was kept in a refrigerator overnight. The reaction mixture was then diluted with ice-cold water (50 ml), acidified with 10% aqueous hydrochloric acid to

precipitate the chalcones. The product was filtered with suction on a Buchner funnel, washed with cold water until the washings were neutral to litmus and then washed with 10 ml of ice-cold rectified spirit. The dried product was recrystallized from chloroform.

STEP 1: Synthesis of chloro phenylurea
The substituted aniline (0.1mole) was dissolved in 20 ml of glacial acetic acid and 10 ml of water. To this, equimolar amount of sodium cynate in 80 ml of warm water was added with stirring. The reaction mixture was allowed to stand for 30 minutes, then cooled in ice and filtered with suction and dried. The product was then recrystallized from boiling water to yield the phenyl urea.
Yield 61 %, IR (KBr/cm^{-1}): 3448, 1675, 1593, 1619, 1560, 751, 692.

STEP 2: Synthesis of substituted phenyl semicarbazide
The phenylsemicarbazide were prepared by heating equimolar quantities of the phenyl urea and hydrazine hydrate i.e. 0.05 mole, in ethanol under reflux for 48 hours with stirring. The two third volume of alcohol was distilled by vaccum distillation unit and then poured into ice. The resultant precipitate was filtered, washed with water and dried. The solid phenylsemicarbazide obtained was recrystallized from 50 ml of 90% v/v ethanol.
Yield 58 %, IR (KBr/cm^{-1}): 3448, 1674, 1593, 1619, 1560, 751, 692.

STEP 3: Synthesis of substituted phenyl semicarbazones
To a solution of phenylsemicarbazide (0.01 mol) in methanol was added an equimolar quantities of appropriate chalcone (0.01 mol). The pH of the reaction mixture was adjusted to 5-6 by adding con hydrochloric acid, to facilitate the nucleophilic substitution. The reaction mixture was stirred at 60-70^{o}C for 5-6 hours. The reaction mixture was poured into a beaker containing crushed ice and allowed to stand for two hours. The precipitate so formed was filtered and washed with ice cold sodium acetate followed by ice cold methanol. The crude product was dried and recrystallized from chloroform.
Solvent system: Benzene: methanol (8:2)

Spectral and elemental analyses data of the 2-fluoro phenylsemicarbazones

4-[4-chlorophenyl-1-(1,3-diphenylallylidene)]semicarbazide **(CA1)**
1H-NMR (δ/ppm in $CDCl_3$): 7.11-7.64 (m, 14H, Ar-H), 7.71 (s, 1H, –CH=CH–), 7.93 (s, 1H, –CH=CH–), 8.35 (s, 1H, ArNH, D_2O exchangeable), 9.42 (s, 1H, CONH, D_2O exchangeable); IR (KBr/cm^{-1}): 3450 (NH), 3300–3240 (CONH), 1670 (–CH=CH–),1590 (C-N), 1616, 1558 (aromatic), 878 (Cl), 754, 697 (monosubstituted benzene); MS, m/z 374; Elemental analysis calculated/found (%) C (70.30/70.26), H (4.83/4.78), N (11.18/11.12).

4-[4-chlorophenyl-1-(1,5-diphenylpenta-2,4-dienylidene)]semicarbazide **(CA2)**
1H-NMR (δ/ppm in $CDCl_3$): 7.11-7.64 (m, J= 8.32 Hz, 12H, Ar-H) 7.69 (s, 1H, –CH=CH–), 7.78 (s, 1H, –CH=CH–), 7.87-8.12 (dd, 2H, –CH=CH–), 8.34 (s, 1H, ArNH, D_2O exchangeable), 9.44 (s, 1H, CONH, D_2O exchangeable); IR (KBr/cm^{-1}): 3448 (NH), 3310–3250 (CONH), 1675 (–CH=CH–),1593 (C-N), 1619, 1560 (aromatic), 877 (Cl), 751, 692 (monosubstituted benzene); MS, m/z 400; Elemental analysis calculated/found (%) C (71.73/71.66), H (5.02/4.98), N (10.46/10.12).

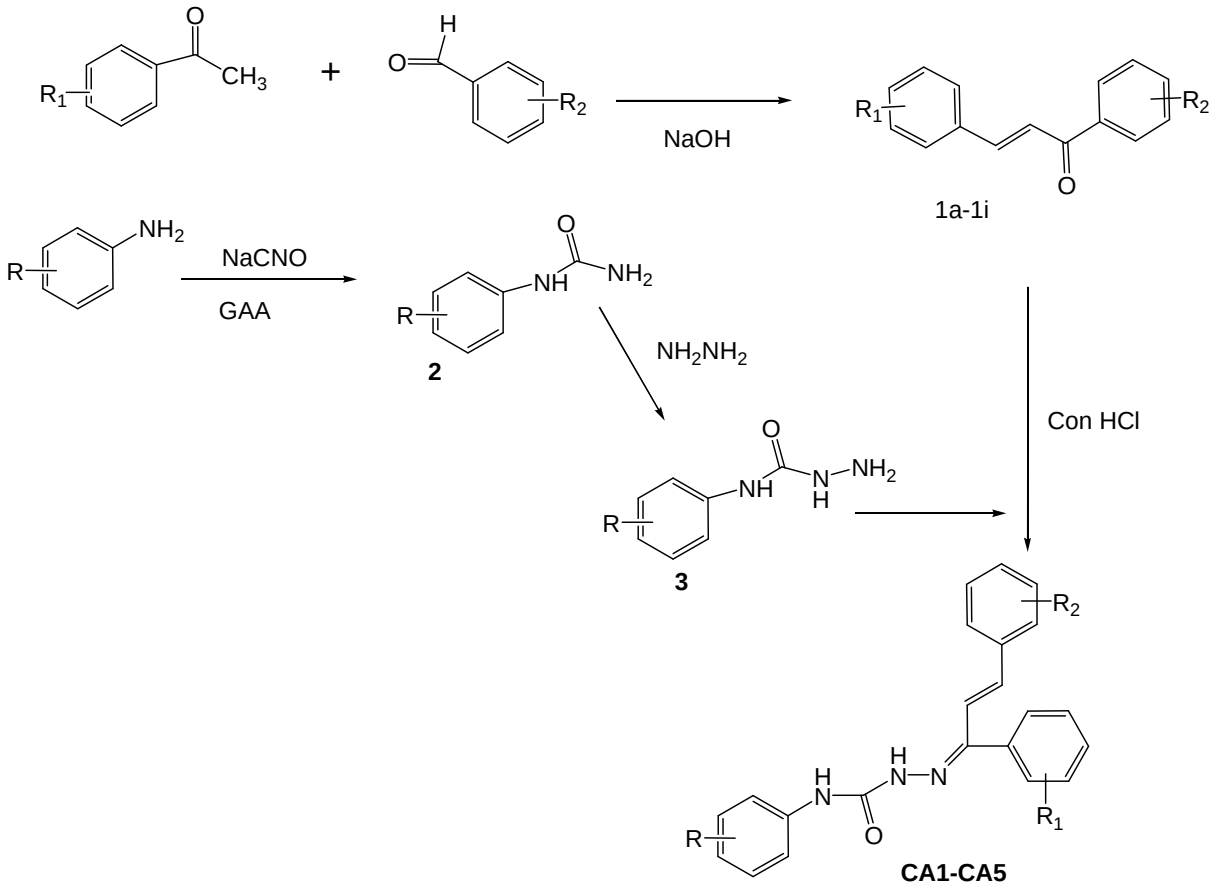

Table 1: Structure and physicochemical properties of substituted phenyl semicarbazones

Com	R	R_1	R_2	M Formula	yield (%)	mp (ºC)	Rf
CA1	p-Cl	H	H	$C_{22}H_{18}ClN_3O$	70	197	0.55
CA2	p-Cl	H	Cinnameldehyde	$C_{24}H_{20}ClN_3O$	72	91	0.63
CA3	o-Cl	H	p-Cl	$C_{22}H_{17}Cl_2N_3O$	72	93	0.69
CA4	o-Cl	p-NH_2	p-Cl	$C_{22}H_{18}Cl_2N_4O$	60	152	0.51
CA5	o-Cl	H	Cinnameldehyde	$C_{24}H_{20}ClN_3O$	64	80	0.53
CA6	o-NO_2	H	Cinnameldehyde	$C_{24}H_{20}N_4O_3$	64	110	0.63

(Mobile phase: chloroform: methanol 9:1)

4-(2-chlorophenyl)-1-[3-(4-chlorophenyl)-1-phenylallylidene] semicarbazide **(CA3)**
1H-NMR (δ/ppm in $CDCl_3$): 7.11-7.64 (m, 13H, Ar-H), 7.74 (s, 1H, –CH=CH–), 7.9 (s, 1H, –CH=CH–), 8.35 (s, 1H, ArNH, D_2O exchangeable), 9.43 (s, 1H, CONH, D_2O exchangeable); IR (KBr/cm^{-1}): 3451 (NH), 3300–3247 (CONH), 1678 (–CH=CH–),1597 (C-N), 1613, 1548 (aromatic), 877 (Cl), 756, 696 (monosubstituted benzene); MS, m/z 409; Elemental analysis calculated/found (%) C (64.40/64.26), H (4.18/4.08), N (10.24/10.12).

4-[2-chlorophenyl-1-(1,5-diphenylpenta-2,4-dienylidene)]semicarbazide **(CA5)**
1H-NMR (δ/ppm in $CDCl_3$): 7.12-7.63 (m, 14H, Ar-H), 7.74 (s, 1H, –CH=CH–), 7.93 (s, 1H, –CH=CH–), 7.97-8.22 (dd, 2H, –CH=CH–), 8.38 (s, 1H, ArNH, D_2O exchangeable), 9.46 (s, 1H, CONH, D_2O exchangeable); IR (KBr/cm^{-1}): 3453 (NH), 3310–3245 (CONH), 1673 (–CH=CH–),1592 (C-N), 1615, 1556 (aromatic), 872 (Cl), 752, 696 (monosubstituted benzene); MS, m/z 400; Elemental analysis calculated/found (%) C (71.73/71.66), H (5.02/4.92), N (10.46/10.33).

Pharmacology

Anticonvulsant activity

All the synthesized compounds were screened for their anticonvulsant activity by MES method. The electroshock assay in mice is used primarily as an indication for compounds which are effective in grand mal epilepsy. Tonic hind limb extensions are evoked by electric stimuli which are suppressed by anti-epileptics. The behavioral and electrographic seizures generated in this model are consistent with the human disorder. These data are presented in Table 2.

Table 2: anticonvulsant screening of the substituted phenyl semicarbazones by MES method

Comp No	Dose[a] (mg/kg)	Mean ± SEM[b]	Protection (%)	Potency
Normal control	--	8.98±0.0600	-	-
Phenytoin	25	1.8±0.1183***	79.95	100
CA1	30	6.95±0.0494	22.60	28.26
CA2	30	6.8±0.0494*	24.27	30.35
CA3	30	4.6±0.0199***	51.44	64.34
CA4	30	6.2±0.1844***	30.95	38.71
CA5	30	2.88 ±0.0122***	67.92	84.95
CA6	30	2.43±0.1493***	72.93	91.21

[a] The compounds were tested at a dose of 30 mg/kg (i.p.).; [b] Each score represents the mean ± SEM of six mice, significantly different from the control score,; * $P < 0.05$, ** $P < 0.01$ (Dunnett's post hoc test)
[c]Tested at 5 mg/kg p.o.

CNS depressant evaluation

The synthesized compounds have been tested for the CNS depression effects and the results are summarized in table 3 and table 4.

Anti-inflammatory activity

The anti-inflammatory activity was determined *in vivo* using the carrageenan-induced rat paw edema test and the results are summarized in table 5.

Analgesic activity

The peripheral analgesic activity was evaluated using acetic acid-induced writhing test in mice and the results are summarized in table 6.

Table 3: Behavioral study of substituted phenyl semicarbazones by actophotometer

Compound[a]	Activity score[b] Control (24 h prior)	Post-treatment	
		0.5 h after	1 h after
CA1	320± 27.73	231 ± 17.22	303 ± 21.11
CA2	180 ± 30.22	161 ± 9.69 *	164 ± 11.02*
CA3	303 ± 22.66	241 ± 2.21**	247 ± 1.02*
CA4	519 ± 13.38	421 ± 26.78 *	459 ± 29.87 *
CA5	373 ± 32.75	20 ± 6.12*	22 ± 2.35*
CA6	320± 27.73	231 ± 17.22	243 ± 21.11
Phenytoin[c]	267 ± 31.12	44 ± 4.56**	46 ± 2.44**

[a] The compounds were tested at a dose of 30 mg/kg (i.p.).; [b] Each score represents the mean ± SEM of six mice, significantly different from the control score,; * $P < 0.05$, ** $P < 0.01$ (Dunnett's post hoc test); [c]Tested at 5 mg/kg p.o.

Table 4: CNS study of substituted phenyl semicarbazones in forced swim pool test

Comp[a]	Immobility time[b]	
	Control (24 hrs prior)	Post- treatment (60 min after)
DMSO	158.67 ± 11.68	168.53 ± 12.32
CA1	128.67 ± 11.56	180.30 ± 12.45*
CA2	119 ± 12.35	130.3 ± 11.25*
CA3	142 ± 11.70	168.00 ± 11.73
CA4	125.67 ± 11.90	155.30 ± 12.35*
CA5	57 ± 12.16	130.3 ± 11.26**
CA6	124 ± 12.70	183.00 ± 11.64
carbamazepine	138.4 ± 17.3	240.60 ± 14.10*

[a] The compounds were tested at a dose of 30 mg/kg (i.p.).; [b] Each value represents the mean ± SEM of six rats significantly different from the control; * $P < 0.05$ and ** $P < 0.01$ (Dunnett's post hoc test).

Table 5: Anti-inflammatory activity of substituted phenyl semicarbazones

Com code	Dose (mg/kg)	Time (hrs)	Thickness variation	% inhibition
Control		1	0.2563±0.0119	-
		2	0.35±0.01768	-

		3	0.4125±0.0125	-
Diclofenac sodium	25	1	0.1563±0.0257*	39.01
		2	0.1375±0.05154**	60.71
		3	0.06875±0.03287**	83.33
CA1	30	1	0.1988±0.03590	22.43
		2	0.1788±0.02577	31.74
		3	0.08125±0.01573**	79.68
CA2	30	1	0.1563±0.01197*	39.01
		2	0.1313±0.02954**	62.48
		3	0.0250±0.0**	78.93
CA3	30	1	0.1313±0.006250**	48.77
		2	0.08125±0.01875**	76.78
		3	0.03125±0.01197**	92.42
CA4	30	1	0.1575±0.04732**	38.54
		2	0.1263±0.02772**	69.62
		3	0.0375±0.0125**	90.9
CA5	30	1	0.1763±0.04002	31.21
		2	0.1375±0.01197**	73.21
		3	0.0375±0.0125**	90.9
CA6	30	1	0.1563±0.01197*	39.01
		2	0.1313±0.02954**	62.48
		3	0.0250±0.0**	93.93

a) Number of animals in each group n = 6.; b) Thickness variation is the difference between the thickness of the carrageenan-treated paw and the saline-treated paw.; c) Percentage of inhibition obtained by comparison with the standard drug. * and ** differed from control group $P < 0.05$ and $P < 0.01$, respectively.

Table 6: Analgesic activity of substituted phenyl semicarbazones

Compound	**Dose(mg/kg)**	**Number of writhings (mean ± SEM)**	**Activity (%)**
Control	--	83 ± 6.72	----
Aspirin	50	18 ± 2.66**	78.31
CA1	30	23.8 ± 7.59*	71.32
CA2	30	25.8 ± 7.59*	68.91
CA3	30	42.5 ± 5.12*	48.79
CA4	30	16.1±3.41**	80.60
CA5	30	24.2±2.86*	70.84
CA6	30	29.62±4.11**	64.31

a) Number of animals in each group n = 6.; b) Percentage if inhibition obtained by comparison with vehicle control group.; c) Analgesic activity relative to aspirin. * and ** differed from control group $P < 0.05$ and $P < 0.01$, respectively.

RESULTS AND DISCUSSION

The compounds (CA1-CA6), were injected intraperitoneally into mice and evaluated as anticonvulsant in the maximal electroshock (MES), using doses of 30 mg/kg. These observed data are presented in Table 2. All the compounds showed anti-MES activity indicative of their ability to prevent seizure spread. All the compounds showed protection against MES model at 30 mg/kg more or less depending upon the substitutions. The substitution with different substituents on the phenyl of the aldehydic and acetophenic group of chalcone moiety plays an important role in protection of seizures. When the phenyl group of aldehydic and acetophenic moiety of chalcone is substituted with electron withdrawing group the compounds exhibited better activity in comparison to the previous study. The order of activity regarding substitution on chalconyl group is NO_2>Cl>cinnameldehyde>H. compound CA6 was the most potential compound, shown 91.21 percent protection in comparison to the standard drug phenytoin. As the carbon chain increases in length (compound CA2 and CA5) the activity is decreased. The chlorine substitution favors the activity. As the result observed from compound CA4, the substitution of chlorine with amino group shown more favor for the anticonvulsant activity. By comparing the results it is observed Cl substitution at position two is more favorable than at position 4 in the derivatives. The compounds with no substitution or less substitution (CA1) were showed very less protection in comparison to the substituted compounds.

In the behavioral despair test, all the compounds showed less to more decrease in motor activity as indicated by the actophotometer scores, in which the standard drug phenytoin also showed behavioral despair side effect. The compound CA5 had shown the significant decrease in motor activity.

The synthesized derivatives were also studied for CNS depressant effect by porsolts's forced swim pool test and compared with carbamazepine. In this study most of the compounds except CA5, showed not more significant increase in the immobility time with respect to control indicative of their CNS depressant effect, as compared to the conventional anti epileptic drugs.

The anti-inflammatory and analgesic activity of the synthesized compounds is summarized in Table 5 and Table 6 respectively. As from the tables it could be seen that most of the compounds showing anti-inflammatory and analgesic activity more or comparable to the reference drugs. Comparison of the anti-inflammatory activity of all tested compounds revealed that compound 15 was the most active compound in the synthesized compounds. The order of activity regarding aniline substitution is NO_2>o-Cl>unsubstituted aniline>p-Cl. As can be seen from Table 34, the halo substituted aniline compounds were more potent anti-inflammatory agents than the other synthesized compounds, may be due to the increase of lipophilicity, which may lead to increases in bioavailability. But in the case of p-Cl substituted aniline, the substitution disfavors the activity. Among the synthesized compounds, compound CA3, CA4, CA5 and CA6 showed the better activity in comparison to diclofenac sodium as the reference drug. In reference to the substitution on the acetophenic phenyl in chalcone moiety, the unsubstitution (CA3 and CA5) is comparatively favorable than the substitution (CA4) for activity. The lengthening of the carbon chain i.e. cinnameldehyde (CA2, CA5 and CA6) is more favorable than simple aldehydic carbon chain. The substitution on phenyl group in aldehydic group is favorable for the activity (CA3 and

CA4) than the unsubstitution (CA1). These observations clarify the role of type of substitutions, helps to increase the activity.

But in case of analgesic activity only some compounds (CA3, CA4, CA5 and CA6 showed the observation comparable to the anti-inflammatory activity. Compound CA3, CA4 and CA5 are more potent than the standard drug aspirin.

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REFRENCES

[1] McCormick D. A., Contreras D, *Annu. Rev. Physiol*, 63, **2001**, 815- 846.
[2] McNamara O. J, Pharmacological Basis of Therapeutics, McGraw-Hill, New York, **2001**, 521-548.
[3] Meador K. J, *J. Clin. Psychiatry*, 64(8), **2003**, 30-34.
[4] Lin Z., Kadaba P. K, *Med. Res. Rev*,17, **1997**, 537-572.
[5] Yogeeswari P, Raghavendran J. V, Thirumurugan R, Saxena A, Sriram D, *Curr. Drug Targets*, 5, **2004**, 553-568.
[6] Dimmock J. R., Elias D. W., Beazely M. A., Kandepu M. N. , *Curr. Med. Chem*, 6, **1999,** 1125.
[7] Ni L. , Meng C. Q., Sikorski J. A., *Expert Opin. Ther.*, 14, **2004**, 1669.
[8] Mokle S S, Sayeed M A, Kothawar, Chopde, *Int. J. Chem. Sci*., 2(1), **2004**, 96.
[9] Hsieh H K, Tsao L T, Wang J P, *J. Pharm. Pharmacol*., 52, **2000**, 163.
[10] Viana G S, Bandeira M A., Matos F, *J. Phytomedicine*, 10, **2003**, 189.
[11] 8. Mokle S S, Sayeed M A, Kothawar, Chopde, *Int. J. Chem. Sci*. **2004**, 2, 1, 96.
[12] 9. Hsieh H K, Tsao L T, Wang J P, *J. Pharm. Pharmacol*. **2000**, 52, 163.
[13] 10. Viana G S, Bandeira M A., Matos F, *J. Phytomedicine*, **2003**, 10, 189.
[14] 11. Zhao L M, Jin H S, Sun L P, Piao H R , Quan Z S, *Bioorg. Med. Chem. Lett*, **2005**, 15, 5027.
[15] 12. Dimmock J. R., Sidhu K. K., Thayer R. S., Mack P, Dutty M. J., Reid R. S., Quail J. W., *J. Med. Chem.***1993**, 36, 2243–2252.
[16] 13. Pandeya S. N., Ponnilavarasan I., Pandey A., Lakhan R., Stables J.P., *Pharmazie*, **1999,** 54, 923–925.
[17] Prabhat Das, Suman Mekap, Saumya Pani, Ranjan Sethi, Praveen Nayak, *Der Pharmacia Lettre*, **2010**, 2, 2, 151-154
[18] S. I. Shivakumar, A. A. Shahapurkar, K. V. Kalmath, B. Shivakumar, *Der Pharmacia Lettre*, **2010**, 2, 1, 22-24
[19] Hemant Kumar, Dharmendra Singh, Swatantra K.S. Kushwaha, Ajay K. Gupta, *Der Pharmacia Lettre*, **2010**, 1, 2, 193-198.
[20] Sadaf Jamal Gilani, Ozair Alam, Suroor Ahmad Khan, Nadeem Siddiqui, Harish Kumar, *Der Pharmacia Lettre*, **2009**, 1, 2, 1-8.