Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Der Pharmacia Sinica, 2011, 2 (1): 194-200

Der Pharmacia Sinica

ISSN: 0976-8688
CODEN (USA): PSHIBD

Design, synthesis and pharmacological screening of 1-acetyl-3-aryl-5-(4-methoxyphenyl)pyrazoles as a potential anti-inflammatory agents

[*]Kapupara Pankaj P.[1], [2]Suhagia Bhanubhai N., [1]Bhandari Anil and [3]Jivani N P

[1]Department of Pharmacy, Jodhpur National University, Jodhpur, Rajasthan, India
[2]Department of Pharmaceutical Chemistry, L. M. College of Pharmacy, Ahmedabad, Gujarat, India
[3]R. B. Patel Mahila Pharmacy College, Atkot, Gujarat, India

---

ABSTRACT

4-methoxybenzaldehyde was react with various aromatic ketones by using alkali as catalyst to afford (E)-3-(4-methoxyphenyl)-1-aryl-prop-2-en-1-ones (2a-j). Compounds (2a-j) on reaction with hydrazine hydrate in the presence of glacial acetic acid to gives 1-Acetyl-3-aryl-5-(4-methoxyphenyl)pyrazoles (3a-j). The obtained compounds were evaluated for their anti-inflammatory activites as well as gastric ulcerogenic effects. Results showed that 3i and 3j showed potent anti-inflammatory activity in carageenan-induced rat paw edema test with low gastric ulcerogenicity compared with etoricoxib.

Key words: pyrazoles, antiinflammatory activity, chalcones, COX-2.

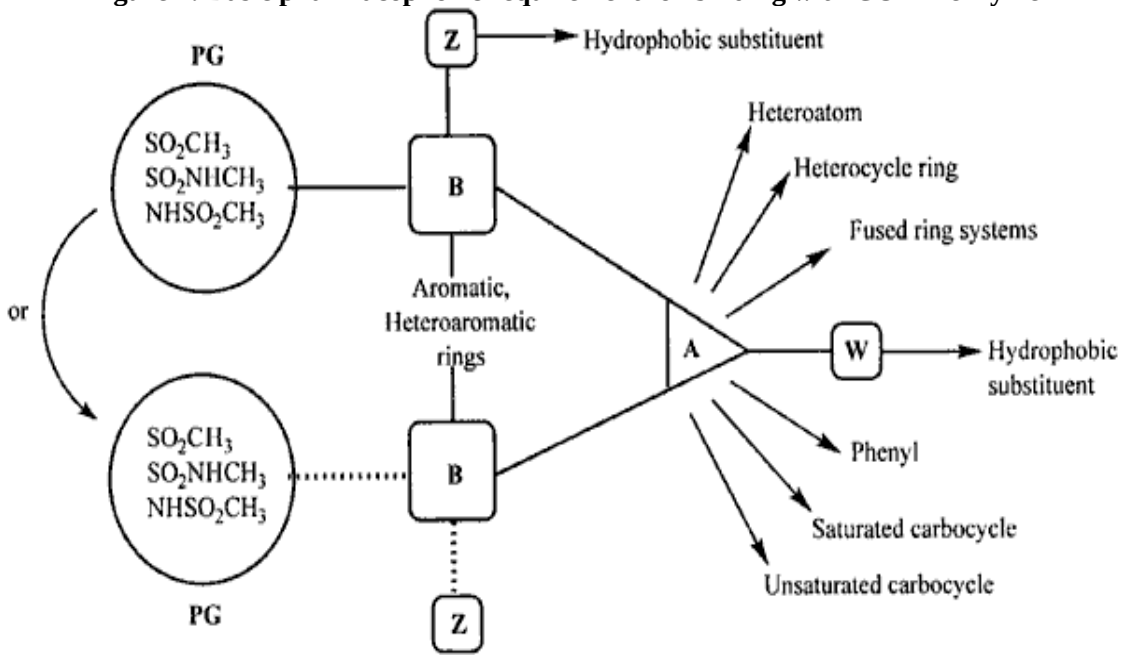
INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are an unhomogeneous family of pharmacologically active compounds used in the treatment of acute and chronic inflammation, pain, and fever. However, nevertheless NSAIDs are the most widely used drugs, their long-term clinical employment is associated with significant side effects and the steady use determines the onset of gastrointestinal lesions, bleeding, and nephrotoxicity.[1,2] Therefore the discovery of new safer anti-inflammatory drugs represents a challenging goal for such a research area. Although several mediators support the inflammatory processes, the main target of NSAIDs is cyclooxygenase (COX),[3] the enzyme involved in the first step of the conversion of arachidonic acid to prostaglandins (PGs).These latter regulate important functions in the gastric,renal, and ematic systems and are known to mediate all inflammatory responses. Classical NSAIDs, such as indomethacin, inhibit both isoforms of COX:[4] COX-1, which is constitutively expressed in most tissues and organs and catalyzes the synthesis of PGs involved in the regulation of

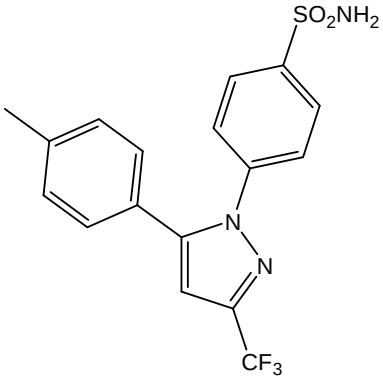
physiological cellular activities; COX-2, which is mainly induced by several stimuli such as cytokines, mitogens, and endotoxins in inflammatory sites.[5] Thus, their therapeutical effects are mainly due to the decrease of proinflammatory PGs produced by COX-2, whereas their unwanted side effects result from the inhibition of constitutive COX-1 isoform. Recently highly selective COX-2 inhibitors belonging to the classes of diarylheterocycles and methanesulfonanilides have been developed and marketed.

Furthermore, it had been reported that many compounds having a diaryl pyrazoles skeleton possessed significant anti-inflammatory activity.[6-8]

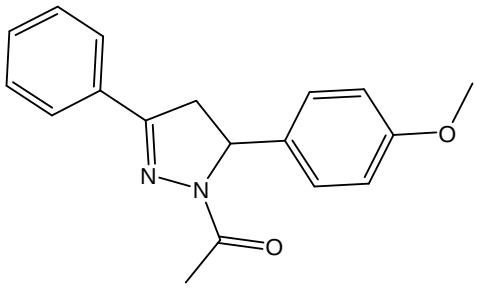
Figure 1: Basic pharmacophoric requirement for binding with COX-2 enzyme



Basic pharmacophoric requirement for binding with COX-2 enzyme are (figure 1) two suitably substituted aryl rings on the adjacent atoms of the five membered or six membered ring system.[9] Celecoxib is COX-2 inhibitor which has five membered heterocycle (imidazole) as centered ring.



Celecoxib



Targeted compounds

Based on pharmacophoric requirement, it was thought of interest to study the effect of 2,4-diaryl substituent on central pyrazole ring for antiinflammatory activity. Based on this we have

designed series of 1-acetyl-3-aryl-5-(4-methoxy phenyl)pyrazoles as a potential anti-inflammatory agents.

MATERIALS AND METHODS

Melting points were determined on electro thermal apparatus using open capillaries and are uncorrected. Thin-layer chromatography was accomplished on 0.2-mm precoated plates of silica gel G60 F254. Visualization was made with UV light (254 and 365nm) or with an iodine vapor. IR spectra were recorded on a FTIR-8400 spectrophotometer. 1H NMR spectra were recorded on a Bruker AVANCE II (300 MHz) spectrometer in CDCl3. Chemical shifts are expressed in δ ppm downfield from TMS as an internal standard. Mass spectra were determined using direct inlet probe on a GCMS-QP 2010 mass spectrometer. All reagents were purchased from Fluka (New Delhi, India), Sigma Aldrich (New Delhi, India) and Rankem (New Delhi, India) and used without further purification.

General procedure for synthesis of (E)-3-(4-methoxyphenyl)-1-aryl-prop-2-en-1-ones (2a-j)
A solution of substituted acetophenone (0.01 mole) in minimum quantity of ethanol (10 ml) was added to a 4-methoxybenzaldehyde (1.78 gm, 0.01 mole) in ethanol (10 ml). In this mixture 40 % NaOH (1 ml) was added to make it alkaline. The reaction mixture was stirred for 24 hr. at room temperature. The product was isolated by filtration and crystallized from suitable solvent to give pure product.

General procedure for synthesis of 1-Acetyl-3-aryl-5-(4-methoxyphenyl)pyrazoles (3a-j)
A mixture of (E)-3-(4-methoxyphenyl)-1-aryl-prop-2-en-1-ones (0.01 mole) and hydrazine hydrate (0.04 mole) in acetic acid (20 ml) was refluxed on an oil-bath for 10-11 hrs. The resulting solution was poured on crushed ice. The solid product was isolated and crystallized from suitable solvent to give analytically pure product.

1-Acetyl-5-(4-methoxyphenyl)-3-phenyl-pyrazole (3a); Yield 64%, m.p. 156-158 °C; IR (KBr, cm^{-1}): 2959 (C=C-H), 1656 (C=O), 1567 (C=N), 1491 (C=C), 1134 (C-N); ^{1}H NMR (300 MHz $CDCl_3$) δ, ppm: 2.46 (s, 3H, $COCH_3$), 3.60 (s, 3H, OCH_3), 3.76-3.80 (dd, 1H, CH_2), 5.59-5.63 (t, 1H, H_C), 7.87-7.91 (m, 9H, Ar); EI/MS m/z: 294 (M+1).

1-Acetyl-5-(4-methoxyphenyl)-3-(4-methoxyphenyl)-pyrazole (3b); Yield 59%, m.p. 144-146 °C; IR (KBr, cm^{-1}): 2945 (C=C-H), 1661 (C=O), 1556 (C=N), 1153 (C-N); ^{1}H NMR (300 MHz $CDCl_3$) δ, ppm: 2.45 (s, 3H, $COCH_3$), 3.74 (s, 3H, OCH_3), 3.75-3.79 (d, 1H, CH_2), 3.98 (s, 3H, OCH_3), 5.53-5.63 (t, 1H, H_C), 7.28-8.30 (m 8H, Ar); MS m/z: 324 (M+1).

1-Acetyl-5-(4-methoxyphenyl)-3-(3-nitrophenyl)-pyrazole (3c); Yield 68%, m.p. 189-190 °C; IR (KBr, cm^{-1}): 2978 (C=C-H), 1674 (C=O), 1588 (C=N), 1491 (C=C), 1146 (C-N), 1246 (N=O); ^{1}H NMR (300 MHz $CDCl_3$) δ, ppm: 2.46 (s, 3H, $COCH_3$), 3.60 (s, 3H, OCH_3), 3.76-3.80 (dd, 1H, CH_2), 5.59-5.63 (t, 1H, H_C), 7.47-7.98 (m, 8H, Ar); MS m/z: 339 (M+1).

1-Acetyl-3-(4-chlorophenyl)-5-(4-methoxyphenyl)-pyrazole (3d); Yield 71%, m.p. 132-134 °C; IR (KBr, cm^{-1}): 2954 (C=C-H), 1677 (C=O), 1589 (C=N), 1495 (C=C), 1166 (C-N); ^{1}H

NMR (300 MHz $CDCl_3$) δ, ppm: 2.40 (s, 3H, $COCH_3$), 3.56 (s, 3H, OCH_3), 3.76-3.80 (d, 2H, CH_2), 5.43-5.56 (t, 1H, H_C), 7.65-8.12 (m, 8H, Ar); MS m/z: 328 (M+1).

1-Acetyl-3-(3,4-dimethoxyphenyl)-5-(4-methoxyphenyl)-pyrazole (3e); Yield 62%, m.p. 151-153 °C; IR (KBr, cm^{-1}): 2967 (C=C-H), 1684 (C=O), 1575 (C=N), 1495 (C=C), 1156 (C-N); ^1H NMR (300 MHz $CDCl_3$) δ, ppm: 2.55 (s, 3H, $COCH_3$), 3.63 (s, 3H, OCH_3), 3.76-3.80 (d, 2H, CH_2), 3.92 (s, 6H, Ar'-OCH_3), 5.51-5.60 (t, 1H, H_C), 7.30-7.96 (m, 9H, Ar); MS m/z: 354 (M+1).

1-Acetyl-3-(2,5-dimethoxyphenyl)-5-(4-methoxyphenyl)-pyrazole (3f); Yield 69%, m.p. 116-118 °C; IR (KBr, cm^{-1}): 2955 (C=C-H), 1664 (C=O), 1568 (C=N), 1498 (C=C), 1202 (C-N); ^1H NMR (300 MHz $CDCl_3$) δ, ppm: 2.43 (s, 3H, $COCH_3$), 3.64 (s, 3H, OCH_3), 3.76-3.82 (d, 2H, CH_2), 3.85 (s, 6H, Ar'-OCH_3), 5.50-5.69 (t, 1H, H_C), 7.80-8.30 (m, 8H, Ar); MS m/z: 339 (M+1).

1-Acetyl-5-(4-methoxyphenyl)-3-(2-methoxyphenyl)-pyrazole (3g); Yield 56%, m.p. 113-114 °C; IR (KBr, cm^{-1}): 2977 (C=C-H), 1664 (C=O), 1584 (C=N), 1488 (C=C), 1135 (C-N); ^1H NMR (300 MHz $CDCl_3$) δ, ppm: 2.46 (s, 3H, $COCH_3$), 3.60 (s, 3H, OCH_3), 3.76-3.80 (d, 2H, CH_2), 3.95 (s, 3H, OCH_3), 5.49-5.69 (t, 1H, H_C), 7.45-8.23 (m, 8H, Ar); MS m/z: 324 (M+1)..

1-Acetyl-3-(2-hydroxyphenyl)-5-(4-methoxyphenyl)-pyrazole (3h); Yield 56%, m.p. 128-130 °C; IR (KBr, cm^{-1}): 3350 (O-H), 2957 (C=C-H), 1674 (C=O), 1588 (C=N), 1491 (C=C), 1146 (C-N); ^1H NMR (300 MHz $CDCl_3$) δ, ppm: 2.40 (s, 3H, $COCH_3$), 3.20 (s, 3H, OCH_3), 3.45-3.65 (d, 2H, CH_2), 5.46-5.57 (t, 1H, H_C), 7.27-7.80 (m, 8H, Ar), 10.50-10.65 (s, 1H, OH); MS m/z: 310 (M+1).

1-Acetyl-5-(4-methoxyphenyl)-3-(4-nitrophenyl)-pyrazole (3i); Yield 74%, m.p. 162-164 °C; IR (KBr, cm^{-1}): 2950 (C=C-H), 1646 (C=O), 1567 (C=N), 1534 (C=C), 1145 (C-N), 1269 (N=O); ^1H NMR (300 MHz $CDCl_3$) δ, ppm: 2.35 (s, 3H, $COCH_3$), 3.45 (s, 3H, OCH_3), 3.65-3.84 (d, 2H, CH_2), 5.60-5.65 (t, 1H, H_C), 7.47-8.11 (m, 8H, Ar); MS m/z: 339 (M+1).

1-Acetyl-3-(3-bromophenyl)-5-(4-methoxyphenyl)-pyrazole (3j); Yield 66%, m.p. 131-133 °C; IR (KBr, cm^{-1}): 2962 (C=C), 1664 (C=O), 1567 (C=N), 1495 (C=C), 1167 (C-N); ^1H NMR (300 MHz $CDCl_3$) δ, ppm: 2.46 (s, 3H, $COCH_3$), 3.60 (s, 3H, OCH_3), 3.76-3.80 (d, 2H, CH_2), 5.59-5.63 (t, 1H, H_C), 7.17-7.93 (m, 8H, Ar); MS m/z: 339 (M+1).

Anti-inflammatory activity

Animals

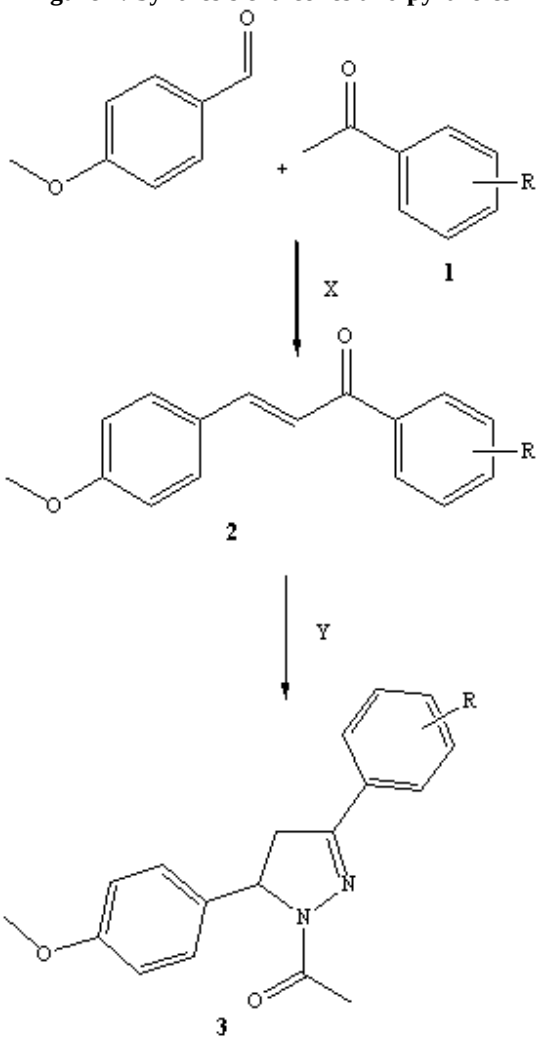
Albino wistar rats (300–350 g;) were housed in a controlled environment and provided with standard rodent chow and water. Animal care was in compliance with the CPCSEA regulations (RKCP/MED/RP/10/05).

Carrageenan-induced paw edema

Rats received a subplantar injection of 0.2 mL saline containing 2% γ-carrageenan in the right hind paw. A suspension of tested compounds (50 mg/kg) or an equivalent volume of vehicle, were administered intraperitoneally 30 min before carrageenan. Control animals received the

same volume of vehicle. The volume of the paw was measured by plethysmometry immediately after the injection. Subsequent readings of the volume of the same paw were carried out after 1h and 3h and compared to the initial readings. The sigma statistical software was used for statistical evaluation of activity data.

Figure 2: Synthesis chalcones and pyrazoles



X = 40% NaOH, Y = $NH_2NH_2.H_2O$, glacial acetic acid

RESULTS AND DISCUSSION

Chemistry

The synthesis of pyrazoles (3a-j) were performed following the steps shown in reaction scheme-I. The compounds (2a-j) were synthesized by reacting 4-methoxybenzaldehyde with various aromatic ketones by using alkali as catalyst.[10] Compounds (2a-j) on reaction with hydrazine hydrate in the presence of glacial acetic acid to gave compounds (3a-j).[11] The purity of all the new synthesized compounds was checked by TLC. The structures of the synthesized compounds were assigned on the basis of spectral data like IR, ^{1}HNMR, mass spectral analysis and elemental

analysis. All the newly synthesized compounds were in full agreement with the proposed structures.

Anti-inflammatory activity

The preliminary anti-inflammatory activity of the synthesized 1-Acetyl-3-aryl-5-(4-methoxyphenyl)pyrazoles (3a-j) derivatives were evaluated against carrageenan-induced rat paw edema using the method by Kasahara et al.[12]

Table 1: Anti-inflammatory activity of 1-Acetyl-3-aryl-5-(4-methoxyphenyl)pyrazoles (3a-j) derivatives at 50 mg/kg dose level

Comp no.	R	% inhibition		
		1h	2h	3h
3a	H	18.22 ± 2.12*	30.38 ± 1.76*	31.69 ± 1.54*
3b	4-methoxy	24.77 ± 4.48**	34.22 ± 3.65**	41.04 ± 4.16**
3c	3-nitro	33.88 ±5.34*	50.88 ± 3.22**	66.26 ± 5.13*
3d	4-Chloro	28.45 ± 1.33*	40.00 ± 1.91*	49.22 ± 5.45**
3e	3,4-dimethoxy	19.61 ± 3.71*	29.61 ± 1.66*	40.96 ± 6.69**
3f	2,5-dimethoxy	22.76 ± 4.23**	23.34 ± 1.71*	34.96 ± 4.34**
3g	2-methoxy	17.08 ± 2.33**	30.00 ± 2.93**	3.61 ± 6.06*
3h	2-hydroxy	21.74 ± 1.45*	29.34 ± 2.33**	45.07 ± 5.61**
3i	4-nitro	38.00 ± 4.12**	55.00 ± 4.50**	77.14 ± 1.60*
3j	2-bromo	34.32 ± 3.23**	51.22 ± 2.33*	73.07 ± 5.61**
Etoricoxib		**41.23 ± 2.13***	**58.33 ± 3.67****	**85.53 ± 1.63***

***Significant difference at P < 0.05; *Significant difference at P < 0.01*

Results of the anti-inflammatory activity of the tested compounds as well as etoricoxib are shown in Table 1. Results showed that most of the tested compounds exhibited significant ($P < 0.05$) inhibition against carrageenan induced rat paw edema and comparable anti-inflammatory activity relative to etriocoxib. Among these derivatives, compounds 3i and 3j were found to be equally potent like etoricoxib.

Gastric ulcerogenic effects were determined in rats[13] for representative examples of the synthesized compounds, 3i and 3j. Results indicated that compounds 3i and 3j did not induce any ulcerogenic effect at 50 mg/kg dose. At higher doses, the tested compounds exhibited low gastric ulcerogenicity compared with indomethacin, which caused severe ulceration at all doses,

Table 2: Ulcerogenic effects of compounds 3i and 3j in comparison with etoricoxib

Compounds	Dose mg/kg	Ratio of ulcered animals	Ulcer index (mean ± SEM)
Etoricoxib	50	1/6	0.45 ± 0.12*
	100	1/6	0.67 ± 0.15**
3i	50	0/6	0.00
	100	1/6	0.39 ± 0.23**
3j	50	0/6	0.00
	100	1/6	0.71 ± 0.20**

***Significant difference at P < 0.05; *Significant difference at P < 0.01*

CONCLUSION

We reported here the synthesis of different 1-Acetyl-3-aryl-5-(4-methoxyphenyl)pyrazoles. The synthesized compounds were tested for their anti-inflammatory activity. Results showed that the most tested compounds exhibited significant anti-inflammatory activity in the carageenan-induced rat paw edema test with low gastric ulcerogenicity compared with indomethacin. So it was indicated that diaaryl pyrazoles may binding with COX-2 receptor and produced anti-inflammatory activity. In this series order of anti-inflammatory activity was 4-nitro > 3-bromo > 3-nitro > 4-chloro > 4-methoxy.

Acknowledgements
Authors are thankful to R. K. College of Pharmacy for providing laboratory facilities. Authors are also thankful to RSIC Chandigarh for providing ^{1}H NMR spectral analysis of the compounds.

REFERENCES

[1] C. Bombardier, L. Laine, A. Reicin, D. Shapiro, M.C. Hochberg, T.K. Kvien, T.J. Schnitzer, *N. Eng. J. Med*., **2000**, 343, 1520.
[2] F.E. Silverstein. G. Faich, J.L. Goldstein, L.S. Simon, T. Pincus, A. Whelton, *JAMA.*, **2000**, 284, 1247.
[3] J. Vane, R. Botting, *FASEB J*., **1987**, 1, 89–96.
[4] W.L. Smith, D.L. Dewitt, *Adv. Immunol*., **1996**, 62, 167–215.
[5] H.R. Herschman, *Biochim. Biophys. Acta.*, **1996**, 1299, 125–140.
[6] S.K. Agarwal, R. Tadiparthi, P. Aggarwal, S. Shivkumar, PCT Int. Appl. WO 2004 9,560 through *Chem. Abstr.*, **2004**, *140*, 146156m.
[7] R.H. Green, C.D. Hartely, A. Naylor, PCT Int. Appl. WO 2003 0296427 through *Chem. Abstr.*, **2003**, *138*, 14066f.
[8] T.D. Venu, K.M. Khanum, B.K. Manuprasad, S. Shashikanth, R. Mohamed, B.S. Vishwanth, *Bioorg. & Med. Chem. Letters*, **2008**, 18, 4409–4412.
[9] A. Palomer, F. Cabre, J. Pascual, J. Campos, M.A. Trujillio, A. Entrena, M.A. Gallo, L. Garcia, D. Mauleon, A. Espinosa, *J. Med. Chem.*, **2002**, 45, 1402-1411.
[10] Y. R. Prasad, A.L. Rao, R, Rambabu, *Eur. J. Chem*., **2008**; 5: 461-466
[11] B. A. Bhat, K. A. Dhar, S. C. Puri, A. K. Saxena, M. Shanmugavel, G. N. Qazi, *Bioorg. & Med. Chem. Letters*, **2005,** 15, 3177-3180.
[12] Y.H. Kasahara, S. Hikino, M. Tsurufuji, K. Watanabe, P. Ohuhi, *Planta Med.*, **1985**, 51, 325-331.
[13] H. Ikuta, H. Shirota, S. Kobayashi, Y. Yamagishi, K. Yamada, I. Yamatsu, K. Katayama, *J. Med. Chem.*, **1987**, 30, 1995-1998.