ejpmr, 2017,4(12), 210-215

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME NOVEL SUBSTITUTED STILBENE CARBOXAMIDE DERIVATIVES

Chitra Rajput*

India.

***Corresponding Author: Chitra Rajput**

India.

Article Received on 04/10/2017 **Article Revised on 25/10/2017** **Article Accepted on 15/11/2017**

ABSTRACT

In this study we have synthesized 8 new derivatives of stilbene. Series A1 to A4 and B1 to B4 by modified Perkin condensation reaction. The synthesized compounds were tested for their in vitro antibacterial, antifungal and antioxidant activity. The results of the in vitro antioxidant tests showed that most of the synthesized compounds showed moderate (%RSA >50%) to mild (%RSA >40%) radical scavenging activity. The Compound 3-(4-Fluoro-phenyl)-N-methyl-2-phenyl acrylamide (A1), 3-(2-fluorophenyl)-2-phenyl-N-o-tolylacrylamide (A2), 3-(4-Fluoro-phenyl)-2-phenyl-N-m-tolyl-acrylamide (A3), showed in vitro antibacterial activity comparable to that of the standard Penicillin against *Staphylococcus aureus.* The results of the in vitro antifungal activity showed that most of the synthesized derivatives have proven their antifungal potential against *Aspergillus niger and Penicillum chrysogunum.*

KEYWORDS: Stilbene, Antibacterial activity, Antifungal activity, Antioxidant activity.

INTRODUCTION

Stilbenes are small (MW 210-270 g/mol), naturally occurring compounds found in a wide range of plant sources, aromatherapy products, and dietary supplements. Stilbenes exist as stereoisomers in *E* and *Z* forms, depending on where functional groups are attached in relation to one another on either side of the double bond. Naturally occurring stilbenes overwhelmingly exist in the *Z* form. It has been postulated and scientifically verified that the *E* and *Z* forms of stilbenes elicit different pharmacological activities. Research has revealed the *stilbene* exhibit potent activity across various cytotoxicities,[1,2,3,4,5] anti-cancer assay[6,7] and anti-oxidant assays.[8,9,10,11,12] One such study demonstrated *trans*-resveratrol to be ten times more potent in its ability to induce apoptosis in the HL60 leukaemia cell line compared to *cis*-resveratrol.[13] Additional research has shown *trans*-stilbene compounds to be significantly more potent in their ability to inhibit cyclooxygenase I (COX-I) activity compared to *cis*-stilbene compounds.[14] Stilbene based synthetic derivatives provides early lead new drug to treat atherosclerosis.[15,16] These compounds, namely flavonoids, isoflavonoids, and lignans, have also generated much scientific research in their potential clinical applications in the treatment of diseases.

There are several stilbenes that have been recognized and classified Based phytochemical knowledge, it is postulated that many more stilbene compounds have yet to be identified. Stilbenoid compounds can be both constitutive and confined to the wood pulp of the host, or induced in response to environmental stressors. Induction of stilbene synthesis and secretion occurs in the fruit and or leaves of its host. Stilbenes that have been induced are often referred to as phytoalexins, due to their protective actions upon secretion.[17] These secondary metabolites act as protective agents to defend the plant against viral and microbial attack, excessive ultraviolet exposure, and diseases.[10] Upon environmental threat, the plant host activates the phenylpropanoid pathway and stilbene structures are produced and secreted as a consequence. Which specific stilbene that is produced depends largely on its host, the region of origin, and the environmental stimuli. The most well-known and well-characterized stilbene compound is resveratrol. Primarily found in peanuts, red wine, and grapes[18], resveratrol has been shown to be a potent anti-inflammatory, anti-cancer and chemoprotective agent.[19, 20,]

Based on encouraging therapeutic evidence, resveratrol research has fuelled a great deal of interest in characterizing structurally similar stilbene compounds and in synthesizing modified stilbenes substituted with various functional groups.

Hydroxylated stilbenes have been widely investigated because of their biological role in plant defense against pathogens and for their pharmacological properties. Previously published results have shown that grape wines synthesize antimicrobial compounds in response to fungal infection. These compounds, which are referred to

as phytoalexins, belong to the family of stilbenes.[21] Considering that the stilbenes shows the activity against the bacterial and fungal infection,[13,21, 23,] we have undertaken the synthesis and screening of stilbene derivatives for their antibacterial and antifungal activity for present study.

Stilbenes are very interesting compound because of their biological applications. In present study Arylaldehyde, Phenyl acetic acid and substituted Amine use for synthesis of phenyl cinnamic acid derivatives. Literature review shows that the modified Perkin condensation method is feasible method and give *E* form of isomer.[24,25,26,27] The purpose of this study is to synthesize phenyl cinnamic acid derivatives. An attempt has been made to assess their antioxidant activity and antimicrobial activity.

MATERIALS AND METHODS

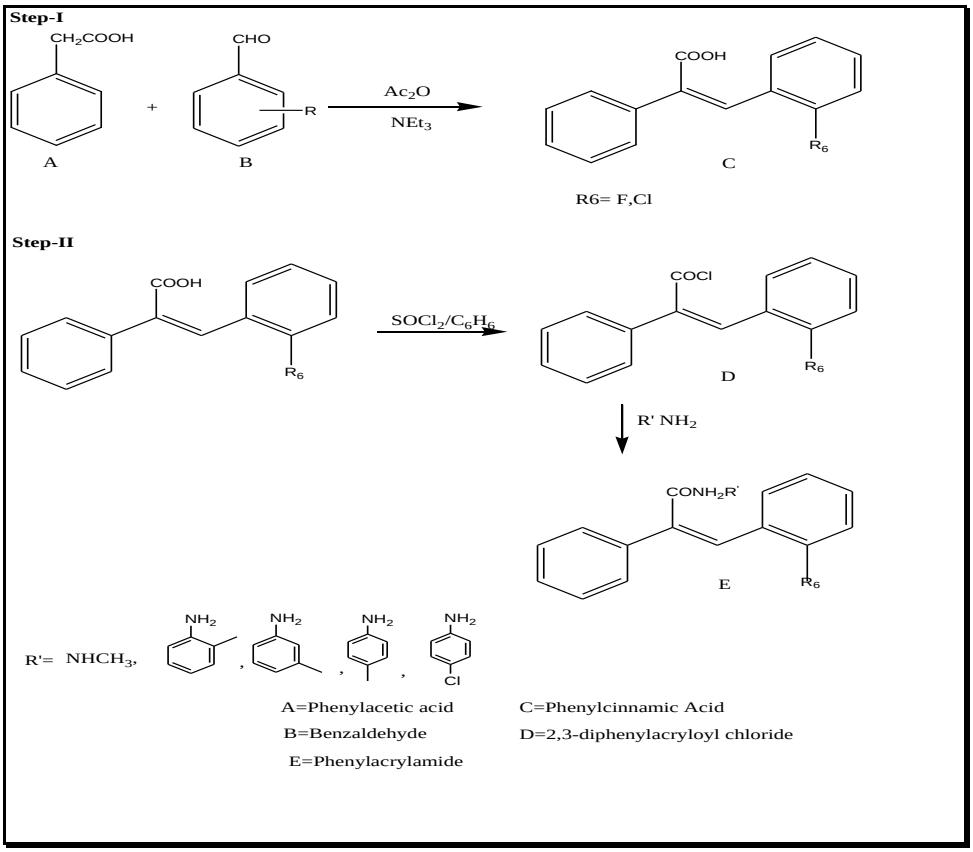
General

All the chemicals used were procured from Aldrich, Spectrochem and Rankem Ltd. and purified using standard procedure if required. Melting points were recorded on an open capillary tube on Superfit melting point apparatus and are uncorrected. The purity of all the final compounds was assessed by thin layer chromatography (TLC). The Silica gel G was used for TLC. Completion of the reaction was monitored by TLC with Hexane and ethyl acetate (in varying proportion) system. TLC plates were visualized using iodine chamber. Structures of compounds were confirmed by IR, MASS and 1H NMR spectra. IR spectra were recorded in KBr disk on Perkin Elmer FTIR instrument. MASS spectra were recorded on SHIMADZU LC-MS 2010 EV Single qudrapole and are reported in ES-MS. 1H NMR spectra were recorded on "BRUKER ADVANCE II 400 NMR Spectrophotometer" with tetramethylsilane (TMS) as the internal standard in CDCl3.

General procedure for production of E-3-phenyl-2-phenylprop-2-enoic acid (Phenyl Cinnamic Acid)

Step 1: 2m mol phenyl acetic acid and 2 m mol of benzaldehyde and trimethyl amine (0.5ml) in acetic anhydride (5ml) were refluxed for 12 hours. Poured into hot saturated NaCO3 (sodium carbonate) solution 50ml left overnight. Mixture extracted with 100ml of ether and ether extracts were discarded. The aqueous solution was acidified with dil HCl and precipitated out product was filtered. Recrystalisation from Ethylacetate and Hexane gave pure product.

Step 2: A mixture of carboxylic acid (0.5m mol) and thionyl chloride 1ml & benzene 1ml was refluxed for 6 hours. The excess of thionyl chloride & benzene were removed at reduced pressure and residue was kept under vacuum for 30min. It was subsequently mixed with methylamine solution (40% 5ml) and kept for 2 hrs at room temperature and precipitate was filtered and washed with 2% NaOH solution and with water and then dried. An analytical sample was prepared by Recrystalisation from Ethyl acetate & Hexane (1:9) solvent system.



Scheme 1: Synthesis of compounds A1 toA4 and B1 to B4.

SPECTRAL DATA OF SOME STILBENE DERIVATIVES

3-(4-Fluoro-phenyl)-N-methyl-2-phenyl acrylamide (A1): Yellow solid, Yield 50%, Rf value 0.53, m.p. 110-112, IR Vibration were seen at 1616.10 for (C=C s), 1677.35 for (C=O s), 3443.78 for (N-H s), 1252.73 for (C-F s). In ^{1}H NMR there are well-resolved Resonance peak at 7.992(s,1H,NH); 7.379-7.372(s,1H,arom); 7.282-7.262(m,3H, arom); 7.146-7.114(m,2H, arom); 6.872-6.836 (m,2H, arom); 6.653-6.625 (d,2H, arom); 2.200(m, 3H,C-H). The LC-MS of the compound (A1) shows molecular ion peak at M/Z 225(255.10).

3-(4-Fluoro-phenyl)-2-phenyl-N-o-tolyl acrylamide (A2): Buff solid, yield 60%, m.p105-107. Rf value 0.49. IR Vibration were seen at 1583.24 for (C=C s), 1630.35, for (C=O s), 3417.30 for (N-H s), 1252.73 for (C-F s). In ^{1}H NMR there are well-resolved Resonance peak at 8.009 (s,1H,NH); 7.277-7.192 (m,5H,arom); 7.149-7.118 (m,3H, arom); 6.874-6.867 (m,2H, arom); 6.656-6.628(m,3H, arom); 2.100(m, 3H,C-H). The LC-MS of the compound (A2) shows molecular ion peak at M/Z 331(331) by which have confirmed for its authenticity.

3-(4-Fluoro-phenyl)-2-phenyl-N-m-tolyl-acrylamide (A3): Yellow solid yield 52%, m.p 98-100, Rf value 0.65. IR Viration were seen at 1677.14 for (C=C s), 1584.10 for (C=O s), 3431.42 for (N-H s), 1268.33 for (C-F s).

3-(4-Fluoro-phenyl)-2-phenyl-N-p-tolyl-acrylamide (A4): Yellow Solid yield 50, Rf value 0.62, m.p 103-105. IR Vibration were seen at 1615.00 for (C=C s), 1689.13 for (C=O s), 3414.46 for (N-H s), 1252.67 for (C-F s).

3-(2-chlorophenyl)-N-methyl-2-phenylacrylamide (B1): White Solid yield 62%, m.p117-120, IR Vibration were seen at 1616.97 (C=C s), 1674.23 (C=O s), 703.89 (C-Cl s). In ^{1}H NMR there are well-resolved Resonance peak at 8.016 (s, 1H, NH); 7.385-7.282 (m, 7H, arom); 7.269-7.135 (m, 2H, arom); 6.878-6.843 (m, 1H, arom); 1.175 (m, 3H, C-H). The LC-MS of the compound shows molecular ion peak at M/Z 271(271.05).

3-(4-Chloro-phenyl)-2-phenyl-N-o-tolyl-acrylamide (B2): Buff Solid, yield 52%, m.p102-105, Rf value 0.5. IR Viration were seen at 1615.00 for (C=C s), 1689.13 for (C=O s), 3412.46 for (N-H s).). In ^{1}H NMR there are well-resolved Resonance peak at 7.854 (s, 1H, NH); 7.371-7.353 (m, 3H, arom); 7.268-7.192(m,4H, arom); 7.037-6.990(m,3H, arom); 6.866-6.809(m,3H, arom); 1.200(m, 3H, CH_3). The LC-MS of the compound shows molecular ion peak at M/Z 347(347.10) by which have confirmed for its authenticity.

3-(4-Chloro-phenyl)-2-phenyl-N-m-tolyl-acrylamide (B3): Buff solid, yield 43%, m.p112-115, Rf 0.5. IR Vibration were seen at 1614.50 for (C=C s), 1677.20 for (C=O s), 3435.46 for (N-H s). 3-(4-Chloro-phenyl)-2-phenyl-N-p-tolyl-acrylamide (B4): Buff Solid yield 46% m.p104-107, Rf 0.55. IR Vibration were seen at 1615.50 for (C=C s), 1677.20 for (C=O s), 3435.55 for (N-H s).

***In- vitro* antibacterial activity**[21,22,28]

The agar cup plate method was used for the assessment of in vitro antibacterial activity of the synthesized compounds against *Escherishia coli* and *Staphylococcus aureus*. Penicillin was used as the standard of a clinically used antibacterial agent. The concentration of the compounds used was 2% solution. Drug-free controls were included and results are presented as zone of inhibition (mm). The values of zone of inhibition were determined after 24 hour of static incubation at 37^{o}C.

Table 1: Antibacterial Activity of Phenyl cinnamic Acid Derivatives.

Dose of Compound: 2% **Cup Size:** 4 mm

Sr. No.	Compounds	*Escherishia coli*	*Staphylococcus aureus*
1	A1	-ve	15 mm
2	A2	-ve	14mm
3	A3	-ve	14 mm
4	A4	-ve	13 mm
5	B1	-ve	10 mm
6	B2	-ve	13 mm
7	B3	-ve	12 mm
8	B4	-ve	-ve
9	DMSO	-ve	-ve
10	Penicillin	17 mm	40 mm

DMSO = Dimethyl sulfoxide
-ve = No antibacterial activity observed Zone of inhibition in millimeters **(**mm),
Std = Penicillin

***In-vitro* antifungal activity**[21,23,29]

The poison plate method was used for the assessment of in vitro antifungal activity of the synthesized compounds against *Aspargillus niger* and *Penicilium chrysogenum*. Grysofulvin was used as the standard. The concentration of the compounds used was 2% solution. Drug-free controls were included and results are presented as the growth of organism observed or not. The growth of

organism was determined after 2-3 days of static incubation at 30°C.

Table 2: Antifungal Activity of Phenyl cinnamic Acid Derivatives.

Sr. No.	Compounds	*Aspergillus niger*	*Penicillum chrysogunum*
1	A1	+ve	+ve
2	A2	-ve	-ve
3	A3	-ve	-ve
4	A4	+ve	+ve
5	B1	+ve	+ve
6	B2	+ve	+ve
7	B3	+ve	+ve
8	B4	-ve	-ve
9	DMSO	+ve	+ve
10	Grysofulvin	-ve	-ve

DMSO= Dimethylsulfoxide **+ve =** No antifungal activity observe
-ve = Antifungal Activity observed Std = Griseofulvin

***In-vitro* antioxidant activity**[8,9,11,10]

Free radical scavenging ability of the test compounds were determined by using the DPPH• radical. An ethanol solution of DPPH• (33mg in 1000 ml) was mixed with different concentration of each test compound (1000-2500 µg/ml) and the absorbance of DPPH• (2, 2-diphenyl-1-picryl-hydrazyl) radical change at 517 nm was measured 30 min later. Reaction solution without DPPH• was used as blank and DPPH• solution as control. Ascorbic acid was used as standard and results are mentioned as percentage radical scavenging activity.

The radical scavenging activities were expressed as the inhibition percentage and were calculated using the formula:
Radical scavenging activity (%) = [(A0 – A1 / A0) ×100]
Where A0 was the absorbance of the control (blank, without compound) and A1 was the absorbance of the compound. The radical scavenging activity ascorbic acid was also measured and compared with that of the different synthesized compound.

Table3:% DPPH Radical scavenging Activity of Phenylcinnamic acid and Ascorbic acid.

Comp. Code	Concentration (µM)	Absorbance			Average	± Standard Deviation	DPPH Absorbance	% RSA
		1	2	3				
A1a	1500	0.131	0.135	0.127	0.131	0.0040	0.2120	38.21
A1b	1000	0.141	0.143	0.140	0.141	0.0015	0.2120	33.33
A1c	500	0.154	0.148	0.151	0.151	0.0030	0.2120	28.77
A2a	1500	0.116	0.110	0.112	0.113	0.0031	0.2120	46.86
A2b	1000	0.117	0.115	0.119	0.117	0.0020	0.2120	44.81
A2c	500	0.121	0.120	0.121	0.121	0.0006	0.2120	43.08
A3a	1500	0.105	0.104	0.106	0.105	0.0010	0.2120	50.47
A3b	1000	0.106	0.101	0.111	0.106	0.0050	0.2120	50.00
A3c	500	0.107	0.111	0.104	0.107	0.0035	0.2120	49.37
A4a	1500	0.113	0.114	0.113	0.113	0.0006	0.2120	46.54
A4b	1000	0.114	0.117	0.111	0.114	0.0030	0.2120	46.23
A4c	500	0.115	0.115	0.116	0.115	0.0006	0.2120	45.60
B1a	1500	0.445	0.440	0.450	0.445	0.0050	0.7310	39.12
B1b	1000	0.449	0.448	0.446	0.448	0.0015	0.7310	38.76
B1c	500	0.451	0.453	0.453	0.452	0.0012	0.7310	38.12
B2a	1500	0.445	0.443	0.447	0.445	0.0020	0.7310	39.12
B2b	1000	0.452	0.447	0.442	0.447	0.0050	0.7310	38.85
B2c	500	0.449	0.448	0.449	0.449	0.0006	0.7310	38.62
B3a	1500	0.425	0.421	0.428	0.425	0.0035	0.7310	41.91
B3b	1000	0.430	0.432	0.428	0.430	0.0020	0.7310	41.18
B3c	500	0.435	0.436	0.433	0.435	0.0015	0.7310	40.54
B4a	1500	0.540	0.538	0.539	0.539	0.0010	0.7310	26.27
B4b	1000	0.540	0.543	0.537	0.540	0.0030	0.7310	26.13
B4c	500	0.540	0.541	0.541	0.541	0.0006	0.7310	26.04

Comp. Code	Concentration (µM)	Absorbance			Average	± Standard Deviation	DPPH Absorbance	% RSA
		1	2	3				
AA50	50	0.024	0.018	0.018	0.020	0.0035	0.3770	94.69
AA40	40	0.029	0.033	0.028	0.030	0.0026	0.3770	92.04
AA30	30	0.052	0.051	0.047	0.050	0.0026	0.3770	86.74
AA20	20	0.069	0.073	0.074	0.072	0.0026	0.3770	80.90
AA10	10	0.121	0.120	0.113	0.118	0.0044	0.3770	68.70

RESULT AND DISCUSSION

Chemistry: Firstly we prepared *E*-3-phenyl-2-phenylprop-2-enoic acid by reacting with benzaldehyde and phenyl acetic acid with triethyl amine in acetic anhydride. According to literature after preparing *E*-3-phenyl-2-phenylprop-2-enoic acid it was reacted with methylamine to give Phenylacrylamide (stilbene).We synthesize different derivatives of stilbene by replacing chlorine by various amines. For this we adopted procedure as described in experimental section.

Biological Evaluation

Antibacterial activity: All the synthesized compounds were tested for their in vitro antibacterial activity and activities of the compounds are shown in Table 1. All experiments were performed in comparison with Penicillin, a known antibacterial agent. Compound A1 (15mm), A2, A3, was shown (14mm) and A4 , B2(13mm), B3 (12mm), B2(10mm) showed zone of inhibition,B4 showed no activity against *Staphylococcus aureus.* While for ***Escherishia coli*** compound not showed any antibacterial activity.

Antifungal activity

The antifungal activities of the synthesized compounds are shown in Table 2. All experiments were performed in comparison with Griseofulvin, a known antifungal agent. Compounds A1, A3, B4, have displayed antifungal activity against *Aspergillus niger*. Compounds A2, A3, A4 inhibited the growth of *Penicillium chrysogenum* and have proved their antifungal potential

CONCLUSION

The objective of the present work was to synthesize, purify, characterize and evaluate the Antimicrobial, Antifungal, Antioxidant activity of some novel stilbene derivatives. The yield of different synthesized compounds were found to be in the range of 40-60% and the characterization was done by melting point and TLC. Characteristic IR bands show several functional vibrational modes, which confirm the completion of reaction. Some structures were confirmed by ^{1}H NMR, NMR and Mass spectral studies.

The compounds were investigated for *in vitro* DPPH free radical scavenging potential. The percentage of radical scavenging compound seen as mild to potent scavenging potential (26-50%) RSA.

The antibacterial activity was evaluated against *E. coli* and *S. aureus* using agar cup plate method In antibacterial study Compounds A1,A2,A3, A4, B2 was shown potent and B1,B2 showed moderate antibacterial activity against *Staphylococcus aureus*

The antifungal activity was evaluated against *A. niger* and *P. chrysogenum* using poison plate method. Compounds A1, A3, B4, have displayed antifungal activity against *Aspergillus niger*. Compounds A2, A3, A4 inhibited the growth of *Penicillium chrysogenum* and have proved their antifungal potential.

REFERANCES

1. Yshihiko inamori, et al. *Chem. Pharma Bull.* 1984; 32(2): 801-804.
2. A Koji ohsumi, Ryusuke Nakagawa, Yumiko Fukuda, Toshihiro Hatanaka, Yoshihiro Morinaga, Yukio Nihei, Kazuo Ohishi, Yasuyo Suga, Yukio Akiyama, and Takashi Tsuji *j. Med. Chem.*, 1998; 41: 3022−3032.
3. Mark Cushman, Dhanapalan Nagarathnam; D. Gopal, Asit K. Chakraborti Chii M. Lin and Ernest H. *J. Med. Chem*., 1991; 34: 2579-2588.
4. Rosa Martí-Centelles, Eva Falomir, Juan Murga, Miguel Carda, J. Alberto Marco *Eur. J. Med. Chem.*, 2015; 103; 488–496.
5. Dênis Pires de Lima', Rodrigo Rotta, Adilson Beatriz, Maria Rita Marques,Raquel et al *Eur. J. Med. Chem.*, 2009*;* 44(2); 701–707.
6. Henrik C. Hansen, Fabrizio S. Chiacchia, Reena Patel, Norman C.W. Wong, *Eur. J. Med. Chem.*, 2010; 45(5): 2018–2023.
7. Virendra Kachhadia, Sridharan Rajagopal, Thanasekaran Ponpandian, Vignesh, Karnambaram Anandhan, Daivasigamani Prabhu *Eur. J. Med. Chem*., 2016; 108; 274–286.
8. Yi-Zhong Cai a, Mei Sun b, Jie Xing c, Qiong Luo d, Harold Corke a. *Life Sciences*, 2006; 78: 2872–2888.
9. Oscar M Mosquera, Yaned M Correa, Diana C Buitrago, Jaime Niño. *Mem Inst Oswaldo Cruz, Rio de Janeiro*, 2007.
10. Bavaresco L, Fregoni C, Cantu E, Trevisan M. Stilbene Compounds: From the Grapevine to Wine. *Drug Exp Clin Res.*, 1999; 25: 57-63.
11. Mingfu Wang, Yi Jin, and Chi-Tang Ho. *J. Agric. Food Chem.*, 1999; 47: 3974-3977.
12. Mitko Miliovskya, Ivan Svinyarova, Yavor Mitrevb, Yana Evstatievac, Dilyana Nikolovac, *Eur. J. Med.* Chem., 2013; 66: 185–192.
13. René Csuk', Sabrina Albert, Bianka Siewert, Stefan Schwarz *Eur. J. Med. Chem.*, 2012*;* 54: 669–678.
14. Waffo-Teguo P, Hawthorne ME, Cuendet M, *et al*. Potential cancer-chemopreventive activities of wine stilbenoids and flavans extracted from grape (Vitis vinifera) cell cultures. *NutrCancer.*, 2001; 40: 173.
15. Pace-Asciak Cr, *et al.* The red wine phenolics trans-reveratrol and quercetin block human platelet aggregation and eicosanoid synthesis: implications for protection against coronary heart disease. *Clin Chim Acta.*, 1993; 235(2): 207-19.
16. Phytoestrogens: recent developments. *Planta Med.*, 2003; 69: 589-99.
17. Jeandet P, Bessis R, Sbaghi M, Meunier P, Trollat P. Production of phytoalexin resveratrol by grapes in response to botryis attack under natural conditions. *J Phytopathol*, 1995; 143: 135-139.
18. Aggarwal BB, Bhardwaj A, Aggarwal RS, Seeram NP, Shishodia S, Takada Y. Role of resveratrol in

prevention and therapy of cancer: preclinical and clinical studies. *Anti-Cancer Res.*, 2004; 24: 2783-40.
19. Stojanovic S, Sprinz H, Brede O. Efficiency and mechanism of the anti-oxidant action of trans-resveratrol and its analogues in the radical liposome oxidation. *Arch Biochem Biophys*, 2001; 391; 79-89.
20. Elżbieta Wyrzykiewicz a, Monika Wendzonka a, Bogdan Kędzia. *Eur. J. Med. Chem.*, 2006; 41: 519–525.
21. Hyun Jung Lee,a Jai Woong Seo,a Bong Ho Lee,b Kyoo-Hyun Chunga. *Bioorg. Med. Chem. Lett.*, 2004; 14: 463–46
22. Elżbieta Wyrzykiewicz a, Monika Wendzonka a, Bogdan Kędzia. *Eur. J. Med. Chem*., 2006; 41: 519–525.
23. Shazia N. Aslama, Philip C. Stevensona,b, Tetsuo Kokubunb, David R. Halla. *Microbiol. Res.*, 2009; 164: 191-195.
24. Mark Cushman, sf Dhanapalan Nagarathnam, D. Gopal,' Hu-Ming He, Chii M. Lin: and Ernest Hamel *J. Med. Chem.*, 1992; 35: 2293-2306.
25. Arun K. Sinha' Vinod Kumar, Abhishek Sharma, Anuj Sharma and Rakesh Kumar *Tetrahedron*, 2007; 63(45)5: 11070-11077.
26. Denis Pires de Lima a, Rodrigo Rotta a, Adilson Beatriz a, Maria Rita Marques b, Raquel C. Montenegro c, Marne C. Vasconcellos c, Claudia Pessoa c, Manoel O. de Moraes c, Letı´cia V. Costa-Lotufo c, Alexandra Christine Helena Frankland Sawaya d,Marcos Nogueira Eberlin d. Eur. J. Med. Chem., 2009; 44: 701-707.
27. Gy. Tasi, I. Palinko, T. Kortvelyesi, L. Nyerges J. Mol. Struct. (Theochem), 1997; 391: 189-192.
28. British Pharmacopoeia, 2005; IV: Appendix XIV, A300.
29. Indian Pharmacopoeia.