A Simple and Green Procedure for the Synthesis of N-Benzylthioureas

Lúcia C. de Sequeira Aguiar*, Gil M. Viana, Marcus V. dos Santos Romualdo, Márcio V. Costa and Bruno S. Bonato

Instituto de Química (UFRJ), Av. Athos da Silveira Ramos 149, Centro de Tecnologia Bl. A, Lab. 617, Cidade Universitária, Rio de Janeiro, RJ 21941-909, Brasil

Received July 15, 2010: Revised May 26, 2011: Accepted May 26, 2011

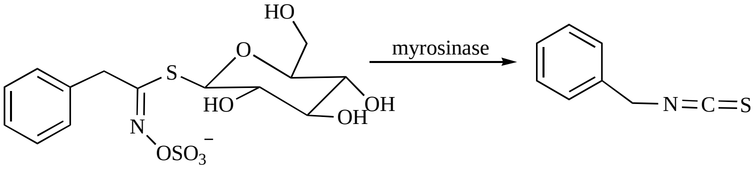
Abstract: A simple and efficient reaction protocol for the synthesis of N-benzylthioureas is described from benzylisothiocyanate (BITC: 94% pure/ GC-MS), isolated from crushed papaya seeds.

Keywords: Benzylisothiocyanate, *Carica papaya*, Glucosinolates, Green chemistry, Isothiocyanates, natural products, Thioureas.

INTRODUCTION

Worldwide demand for environmentally friendly chemical processes and products requires the development of novel and cost-effective approaches to pollution prevention. The synthetic methods that we now still use to transform our starting materials should be the focus of basic research, as they employ reagents which may be harmful for humans and for the environment.

Isothiocyanates are useful compounds as precursors for the synthesis of different classes of acyclic and heterocyclic compounds [1], and are also highly biologically active [2, 3]. It is well known in the literature that isothiocyanates can be produced by enzymatic hydrolysis of glucosinolates [3]. Plants produce many secondary metabolites used as a defense against herbivores, pests and pathogens. Among them are the glucosinolates (GSLs) that are produced by plants, primarily within Brassicaceae, Capparaceae and Caricaceae [3b]. Through enzymatic hydrolysis, GSLs are transformed primarily into isothiocyanates (ITCs) and nitriles [3]. Benzylisothiocyanate **1** (**BITC**; Scheme **1**) is derived from the action of myrosinase on benzylglucosinolate (glucotropaeolin), when papaya seeds are crushed [3]. **BITC** has been found to have effective cancer chemopreventive capatibilities [2c] and has showed biocidal activity to some extent against microorganisms [2b-c] and helminthis [3c].



glucotropaeolin BITC (**1**)

Scheme 1. BITC from papaya seeds.

We report, for the first time, the use of natural **BITC** as raw material for the synthesis of thioureas. In the literature, we find several papers describing the biological properties [2, 3] of plant extracts containing isothiocyanates, however they underestimate their use in synthesis. Symmetrical and unsymmetrical thioureas are an important class of compounds in medicinal and agricultural chemistry [4]. Thioureas are also important building blocks for the synthesis of both five and six membered heterocycles [5] and are widely used in organocatalysis [6].

The aim of this study is to evaluate a friendly methodology to synthesize N-benzylthioureas from papaya seeds. Although several reports describing the synthesis of isothiocyanates are available [7], we were keen to develop a protocol to synthesize N-benzylthioureas which would work cleanly without intermediate work-up, such as extraction or column chromatography, and which would require no use of synthetic **BITC**. We should remember that *Carica papaya* is a tropical and subtropical plant and Brazil is a great producer of papaya, the seeds of which are considered totally worthless.

MATERIALS AND METHODS

Plant Materials

The seeds of *Carica papaya* used in these studies were obtained from ripe papaya fruits of the golden variety, supplied by Agrapapaya (Linhares / BR) or bought in the markets of Rio de Janeiro. The non dried seeds were sometimes stored in containers in a freezer (-18 ºC) for several months

---

*Address correspondence to this author at the Instituto de Química (UFRJ), Av. Athos da Silveira Ramos 149, Centro de Tecnologia Bl. A, Lab. 617, Cidade Universitária, Rio de Janeiro, RJ 21941-909, Brasil; Tel: +55-021-2562-7140; Fax: +55-021-2562-7256; E-mail: luciasequeira@yahoo.com.br

1570-1786/11 $58.00+.00

and subsequently thawed in running water. A manual peeling process led to the removal of the seed sarcotestas. The seeds were then crushed with a mortar and pestle to perform the extraction.

Extraction

Approximately 250 g (8-12 fruits) of freshly ground non dried seeds of *Carica papaya* (without their sarcotestas) were added to a 2000 mL round-bottom flask containing 800 mL of distilled water (simple distillation apparatus). The distillate was collected in a vessel containing 80 mL of hexane. After more or less 2 ½ hours of distillation, 300-350 mL of the distillate (**BITC**/water) were collected. The dispersion in the collected vessel was transferred to a separatory funnel and the aqueous phase was extracted with further 20 mL of hexane. The organic phase (100 mL) was dried with anhydrous Na_2SO_4.

General Experimental Procedures for the Synthesis of N-Benzylthioureas

Method A: In this method the hexane solution of **BITC** (100 mL) was divided into two parts of 50 mL, each of which led to a different thiourea. Approximately 1 mmol of the amine, or an excess of it (5-10 mmols) when it is volatile, was added to the **BITC** hexane solution (50 mL). The reaction occurred at room temperature, under agitation, or under reflux (see Table **1**). The thiourea was obtained as a solid insoluble in the reaction medium and was recovered by filtration under vacuum. In the cases in which the product was impurified with the amine (TLC), it was treated with 5 mL of a HCl solution (10%) and after 10 minutes of stirring, the suspension was filtered and a pure thiourea was obtained.

Method B: In this method, the **BITC** hexane solution (50mL - see above) was concentrated in a rotary evaporator and the colorless oil obtained (120-190 mg) was resolubilized in *tert*-butanol (10 mL). From that point on, the reaction occured as in method A, differing only in the maintenance of the reaction under reflux. Then the *tert*-butanol, used as solvent, was removed in a rotary evaporator. The reaction mixture was resuspended in hexane, which was latter filtered to isolate the thiourea. If the amine was still present in the thiourea, the product was also treated with a HCl 10% aqueous solution, as in method A.

All amines were commercially available. Thin layer chromatography (TLC) was performed using Merck silica gel 60 F 254 on Aluminium sheets and ethyl acetate-hexane 1:1 as eluent. GC-MS experiments were run on a GCMS-QP2010 Plus Gas Chromatograph / Mass Spectrometer from Shimadzu, column: ZB-5MS; injector-temperature: 270°C; detector temperature: 230ºC; temperature program: 60°C to 290°C (5-20°C/min). ^{1}H NMR spectra were recorded at 200 MHz and 400 MHz on a Varian Gemini 200 and Bruker 400 spectrometers, respectively, and chemical shifts were recorded in parts per million relative to internal standard TMS. ^{13}C NMR spectra were recorded at 50 MHz at Varian Gemini 200 spectrometer and chemical shift were recorded in parts per million relative to TMS. Infrared spectra were recorded with Nicolet 550FTIR and Excalibur 3100 Varian spectrometers using KBr pellets. Melting points (uncorrected) were recorded in open capillary on MelTemp II (Laboratory Devices).

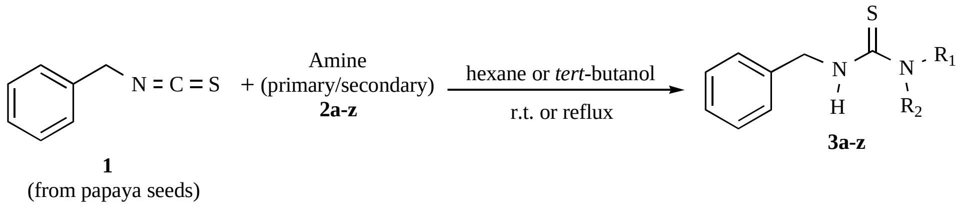
RESULTS AND DISCUSSION

Several N-benzylthioureas **3a-z** (Scheme **2**) were prepared, in a mmol scale by the reaction of ~1mmol of alkyl/aryl amines **2** (primary and secondary) with **BITC** (**1**) in a hexane (or *tert*-butanol) solution (r.t./reflux). **BITC** was isolated by the steam distillation of ~125 g of crushed non dried papaya seeds, corresponding to 4-6 fruits. The seeds of *Carica papaya* used were obtained from ripe papaya fruits of the golden variety, supplied by Agrapapaya (Brazil) or purchased in the local markets. Initially, to perform the extraction, the seed sarcotestas were withdrawn manually, and then were crushed with a mortar and pestle. The distillate was collected in a vessel containing hexane until **BITC**, co-distillated with water, was not observed anymore. From this same amount of seeds (fresh or stored in a freezer at -18^{0}C), 120-190 mg of **BITC** were obtained, after evaporation of the hexane. The purity of **BITC**, in the hexane extracts, was determined as ~94% by GC-MS.

A variety of amines **2** were used in the preparation of their respective N-benzylthioureas **3**. These results are summarized in Table **1** (method A – use of hexane as solvent and method B – use of *tert*-butanol as solvent).

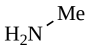
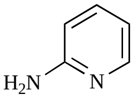
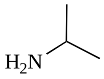
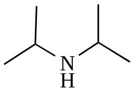
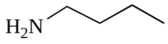
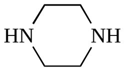
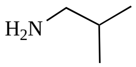
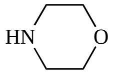
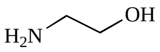
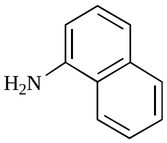
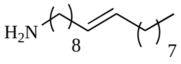
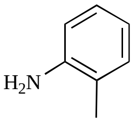
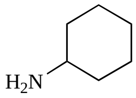
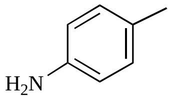
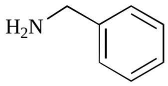
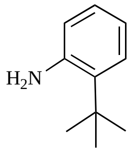
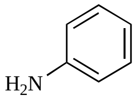
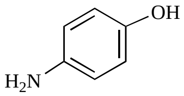
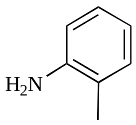
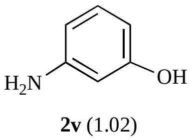
The ideal amine **2** / **BITC** ratio had to be established empirically, considering each individual process. In most cases, a slight excess of amine was added (~1mmol) to an hexane solution of **BITC** (**1**). The exceptions were the volatile amines **2a-d** (Table **1**) of which ~ 5-10mmol were added to compensate their volatilities. Based upon the average concentration of **BITC** (94%) in the hexane solutions (extraction), we estimate that the yields ranged from 57% (**3t**) to 100% (**3h**, **3i**, **3j** and **3n**).

Most of the N-benzylthioureas prepared by method A were insoluble in an apolar solvent (hexane). Therefore, they were easily isolated by a simple vacuum filtration. Although several thioureas could be prepared at room temperature/reflux, using hexane as the solvent (method A), we

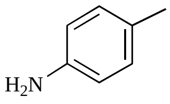
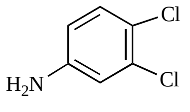
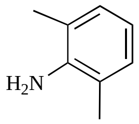
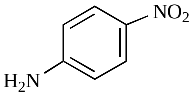
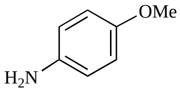
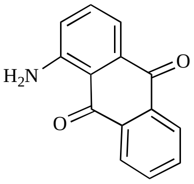
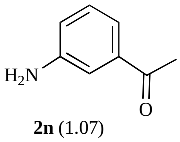


Scheme 2. Synthesis of N-benzylthioureas **3** from papaya seeds (~125g, corresponding to 4-6 fruits).

Table 1. Preparation of N-Benzylthioureas 3

Amine (mmol)	N-benzylthiourea[a] (mmol)[b]	Time/h (temperature)	Method	Amine (mmol)	N-benzylthiourea[a] (mmol)[b]	Time/h (temperature)	Method
 **2a** (5.35)c	**3a** (1.17)	6.0 (r.t.)	A	 **2o** (1.03)	**3o** (0.61)	16.0 (reflux)	A
 **2b** (9.34)	**3b** (0.78)	0.5 (r.t.)	A	 **2p** (1.07)	**3p** (0.86)	14.0 (r.t.)	A
 **2c** (7.08)	**3c** (1.11)	0.5 (r.t.)	A	 **2q** (1.16)	**3q** (1.03)	10.0 (r.t.)	A
 **2d** (7.04)	**3d** (0.96)	0.5 (r.t.)	A	 **2r** (1.20)	**3r** (1.17)	16.0 (r.t.)	A
 **2e** (1.02)	**3e** (0.77)	3.0 (r.t.)	A	 **2s** (1.00)	**3s** (0.96)	4.0 (reflux)	B
 **2f** (1.01)	**3f** (0.79)	2.0 (r.t.)	A	 **2j** (1.09)	**3j** (0.94)	3.0 (reflux)	B
 **2g** (1.05)	**3g** (1.00)	0.5 (r.t.)	A	 **2k** (1.01)	**3k** (0.91)	2.0 (reflux)	B
 **2h** (1.11)	**3h** (1.11)	2.0 (r.t.)	A	 **2t** (1.07)	**3t** (0.61)	4.0 (reflux)	B
 **2i** (1.10)	**3i** (1.10)	14.0 (reflux)	A	 **2u** (1.01)	**3u** (0.89)	2.0 (reflux)	B
 **2j** (1.09)	**3j** (1.09)	16.0 (reflux)	A	 **2v** (1.02)	**3v** (1.01)	2.0 (reflux)	B

(Table 1). Contd…..

Amine (mmol)	N-benzylthiourea[a] (mmol)[b]	Time/h (temperature)	Method	Amine (mmol)	N-benzylthiourea[a] (mmol)[b]	Time/h (temperature)	Method
 **2k** (1.01)	**3k** (0.74)	14.0 (reflux)	A	 **2w** (1.01)	**3w** (0.79)	1.5 (reflux)	B
 **2l** (1.10)	**3l** (1.08)	16.0 (reflux)	A	 **2x** (1.00)	**3x** (0.78)	16.0 (reflux)	B
 **2m** (1.01)	**3m** (0.86)	14.0 (reflux)	A	 **2y** (1.00)	**3y** (0.63)	16.0 (reflux)	B
 **2n** (1.07)	**3n** (1.07)	10.0 (reflux)	A	$NH_4OH_{(aq)}$ **2z**	**3z** (0.78)	0.5 (r.t.)	Ref.[8]

[a]Products were characterized by physical and spectroscopic methods (IR, ^{1}H NMR); [b]Amount of pure isolated products; [c]Methylammonium chloride (2a) was used.

observed that the less reactive amines reacted much more sluggishly in this condition. Therefore, the hexane was replaced by a more polar solvent. In choosing this new solvent, preservation of the environment was also taken into account. Thus, alcohols were the best choice as polar solvents for these reactions because they are biodegradable and less harmful to human health. However, due to its nucleophilicity, it had to be carefully chosen since it could compete with the amine in addition to the isothiocyanate. In fact, the attempts to use ethanol and isopropanol as solvents led to the formation of mixtures of thiourea and thiocarbamates[9] (minor products). Thus, it was found that *tert*-butanol was the ideal solvent for reactions of less reactive amines **2s-y** (method B), because this is a non-nucleophilic alcohol (steric hindrance). We have also verified that the long time reaction of amines (such as *ortho*/*para*-toluidine **2j**/**2k**) could be drastically lowered with the substitution of hexane for *tert*-butanol (16h/14h - method A; 3h/2h - method B). This modification of the usual procedure may prove useful in the synthesis of thioureas with relevant bioactivity [4a-c].

CONCLUSION

In conclusion, we have developed a simple and green procedure for the synthesis of a variety of N-benzylthioureas from papaya seeds. The desired products are obtained in good to excellent yields (~ 1 mmol/seeds of 4-6 fruits) and most of them do not require any further purification, after filtration from the crude reaction mixture.

ACKNOWLEDGEMENT

We are grateful to CNPq, CAPES and FAPERJ for financial support and Agrapapaya for providing papaya fruits.

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publishers Web site along with the published article.

REFERENCES AND NOTES

[1] (a) Daele, I.V.; Munier-Lehmann, H.; Froeyen, M.; Balzarini, J.; Calenbergh, S.V. Rational design of 5'-thiourea-substituted α-thymidine analogues as thymidine monophosphate kinase inhibitors capable of inhibiting mycobacterial growth. *J. Med. Chem.*, **2007**, *50*, 5281-5292. (b) Spallarossa, A.; Cesarini, S.; Ranise, A.; Bruno, O.; Schenone, S.; Colla, P.L.; Collu, G.; Sanna, G.; Secci, B.; Loddo, R. Novel modifications in the series of O-(2-phthalimidoethyl)-N-substituted thiocarbamates and their ring-opened congeners as non-nucleoside HIV-1 reverse transcriptase inhibitors. *Eur. J. Med. Chem.*, **2009**, *44*, 1650-1663. (c) Poitout, L.; Brault, V.; Sackur, C.; Bernetière, S.; Camara, J.; Plas, P.; Roubert, P. Identification of a novel series of benzimidazoles as potent and selective antagonists of the human melanocortin-4 receptor. *Bioorg. Med. Chem. Lett.*, **2007**, *17*, 4464-4470. (d) Mukerjee, A.K.; Ashare, R. Isothiocyanates in the chemistry of heterocycles. *Chem. Rev.*, **1991**, *91*, 1-24.

[2] (a) Valgimigli, L.; Iori, R. Antioxidant and pro-oxidant capacities of ITCs. *Environ. Mol. Mutagen.*, **2009**, *50*, 222-237. (b) Aires, A.; Mota, V.R.; Saavedra, M.J.; Monteiro, A.A.; Simões, M.; Rosa, E.A.S.; Bennett, R.N. Initial *in vitro* evaluations of the antibacterial activities of glucosinolate enzymatic hydrolysis products against plant pathogenic bacteria *J. Appl. Microbiol.*, **2009**, *106*, 2096-

2105. (c) Yuan, H.; Yao, S.; Shen, L.; Mao, J. Preparation and characterization of inclusion complexes of β-cyclodextrin-BITC and β-cyclodextrin-PEITC *Ind. Eng. Chem. Res.* **2009,** *48*, 5070-5078.

[3] (a) Traka, M.; Mithen, R. Glucosinolates, isothiocyanates and human health. *Phytochem. Rev.*, **2009**, *8*, 269-282. (b) Fahey, J.W.; Zalcmann, A.T.; Talalay, P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry*, **2001**, *56*, 5-51. (c) Kermanshai, R.; McCarry, B.E.; Rosenfeld, J.; Summers, P.S.; Weretilnyk, E.A.; Sorger, G.J. Benzyl isothiocyanate is the chief or sole anthelmintic in papaya seed extracts. *Phytochemistry*, **2001**, *57*, 427-435. (d) Rosseto, M.R.M.; do Nascimento, J.R.O.; Purgatto, E.; Fabi, J.P.; Lajolo, F.M.; Cordenunsi, B.R. Benzylglucosinolate, benzylisothiocyanate, and myrosinase activity in papaya fruit during development and ripening. *J. Agric. Food Chem.*, **2008**, *56*, 9592-9599.

[4] (a) Li, H.Q.; Yan, T.; Yang, Y.; Shi, L.; Zhou, C.F.; Zhu, H.L. Synthesis and structure–activity relationships of N-benzyl-N-(X-2-hydroxybenzyl)-N'-phenylureas and thioureas as antitumor agents. *Bioorg. Med. Chem.*, **2010**, *18*, 305-313. (b) Esteves-Souza, A.; Pissinate, K.; Nascimento, M.G.; Grynberg, N. F.; Echevarria, A. Synthesis, cytotoxicity, and DNA-topoisomerase inhibitory activity of new asymmetric ureas and thioureas. *Bioorg. Med. Chem.*, **2006**, *14*, 492-499. (c) Chung, J.; Kim, S.Y.; Lim, J.; Choi, H.; Kang, S.; Yoon, H.; Ryu, H.; Kang, D.W.; Lee, J.; Kang, B.; Choi, S.; Toth, A.; Pearce, L.V.; Pavlyukovets, V.A.; Lundberg, D.J.; Blumberg, P.M. α-Substituted N-(4-tert-butylbenzyl)-N'-[4-(methylsulfonylamino)- benzyl]thiourea analogues as potent and stereospecific TRPV1 antagonists. *Bioorg. Med. Chem.*, **2007**, *15*, 6043-6053. (d) Cunha, S.; Macedo Jr., F.C.; Costa, G.A.N.; Rodrigues Jr., M.T.; Verde, R.B.V.; Neta, L.C.S.; Vencato, I.; Lariucci, C.; Sá, F.P. Antimicrobial activity and structural study of disubstituted thiourea derivatives. *Monatsh. Chem.*, **2007**,*138*, 511-516. (e) Mahajan, A.; Yeh, S.; Nell, M.; van Rensburg, C.E.J.; Chibale, K. Synthesis of new 7-chloroquinolinyl thioureas and their biological investigation as potential antimalarial and anticancer agents. *Bioorg. Med. Chem. Lett.*, **2007**, *17*, 5683-5685. (f) Küçükgüzel, İ.; Tatar, E.; Küçükgüzel, Ş.G.; Rollas, S.; De Clercq, E. Synthesis of some novel thiourea derivatives obtained from 5-[(4-aminophenoxy)methyl]-4-alkyl/aryl-2,4-dihydro-3H-1,2,4-triazole-3-thiones and evaluation as antiviral/anti-HIV and anti-tuberculosis agents. *Eur. J. Med. Chem.*, **2008**, *43*, 381-392. (g) Yonova, P.A.; Stoilkova, G.M. Synthesis and biological activity of urea and thiourea derivatives from 2-aminoheterocyclic compounds. *J. Plant. Growth Regul.*, **2005**, *23*, 280-291. (h) Schroeder, D.C. Thioureas. *Chem. Rev.*, **1955**, *55*, 181-228.

[5] (a) Koketsu, M.; Ishihara, H. Thiourea and selenourea and their applications. *Curr. Org. Synth.*, **2006**, *3*, 439-455. (b) Potewar, T.M.; Ingale, S.A.; Srinivasan, K.V. Catalyst-free efficient synthesis of 2-aminothiazoles in water at ambient temperature. *Tetrahedron*, **2008**, *64*, 5019-5022. (c) Yella, R.; Ghosh, H.; Patel, B.K. It is "2-imino-4-thiazolidinones" and not thiohydantoins as the reaction product of 1,3-disubstituted thioureas and chloroacetylchloride. *Green Chem.*, **2008**, *10*, 1307-1312. (d) Matloobi, M.; Kappe, C.O. Microwave-Assisted Solution- and Solid-Phase Synthesis of 2-Amino-4-arylpyrimidine Derivatives. *J. Comb. Chem.*, **2007,** *9*, 275-284.

[6] (a) Zhang, Z.; Schreiner, P.R. (Thio)urea organocatalysis—What can be learnt from anion recognition? *Chem. Soc. Rev.*, **2009**, *38*, 1187-1198. (b) Connon, S.J. The design of novel, synthetically useful (thio)urea-based organocatalysts. *Synlett*, **2009**, 354-376. (c) Zhang, X.; Liu, S.; Lao, J.; Du, G.; Yan, M.; Chan, A.S.C. Asymmetric conjugate addition of carbonyl compounds to nitroalkenes catalyzed by chiral bifunctional thioureas. *Tetrahedron: Asymm.*, **2009**, *20*, 1451-1458.

[7] (a) Nath, J.; Ghosh, H.; Yella, R.; Patel, B.K. Molecular iodine mediated preparation of isothiocyanates from dithiocarbamic acid salts. *Eur. J. Org. Chem.*, **2009**, 1849-1851 and references cited therein. (b) Wong, R.; Dolman, S.J. Isothiocyanates from tosyl chloride mediated decomposition of in situ generated dithiocarbamic acid salts. *J. Org. Chem.*, **2007**, *72*, 3969-3971. (c) Munch, H.; Hansen, J.S.; Pittelkow, M.; Christensen, J.B.; Boas, U. A new efficient synthesis of isothiocyanates from amines using di-tert-butyl dicarbonate. *Tetrahedron Lett.*, **2008**, *49*, 3117-3119.

[8] **N-benzylthiourea (3z)** [621-83-0] was prepared reacting natural **BITC** (neat) with conc. $NH_4OH_{(aq)}$ (0.5 h; 0.78 mmol).

[9] Breme, K.; Fernandez, X.; Meierhenrich, U.J.; Brevard, H.; Joulain, D. Identification of new, odor-active thiocarbamates in cress extracts and structure-activity studies on synthesized homologues. *J. Agric. Food Chem.*, **2007,** *55*, 1932-1938.