Bioorganic & Medicinal Chemistry Letters 22 (2012) 5550–5554

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Hybrid molecules of carvacrol and benzoyl urea/thiourea with potential applications in agriculture and medicine

Umesh D. Pete [a], Chetan M. Zade [a], Jitendra D. Bhosale [a], Santosh G. Tupe [b,*], Preeti M. Chaudhary [b], Amol G. Dikundwar [c], Ratnamala S. Bendre [a,*]

[a] Department of Pesticides and Agrochemicals, School of Chemical Sciences, North Maharashtra University, Jalgaon 425001, Maharashtra, India
[b] Biochemical Sciences Division, National Chemical Laboratory, Dr. Homi Bhabha Road, Pune 411008, Maharashtra, India
[c] Solid State and Structural Chemistry Unit (SSCU), Indian Institute of Science, Bangalore 560012, India

ARTICLE INFO

Article history:
Received 9 April 2012
Revised 4 July 2012
Accepted 6 July 2012
Available online 14 July 2012

Keywords:
Benzoyl phenyl urea
Benzoyl carvacryl urea
Insect growth regulators
Crop protection agents
Antifungal agents

ABSTRACT

Benzoyl phenyl urea, a class of insect growth regulator's acts by inhibiting chitin synthesis. Carvacrol, a naturally occurring monoterpenoid is an effective antifungal agent. We have structurally modified carvacrol (2-methyl-5-[1-methylethyl] phenol) by introducing benzoylphenyl urea linkage. Two series of benzoylcarvacryl thiourea (BCTU, **4a–f**) and benzoylcarvacryl urea (BCU, **5a–f**) derivatives were prepared and characterized by elemental analysis, IR, ^{1}H and ^{13}C NMR and Mass spectroscopy. Derivatives **4b**, **4d**, **4e**, 4f and **5d**, 5f showed comparable insecticidal activity with the standard BPU lufenuron against *Dysdercus koenigii*. BCTU derivatives **4c**, 4e and BCU **5c** showed good antifungal activity against phytopathogenic fungi viz. *Magnaporthe grisae, Fusarium oxysporum, Dreschlera oryzae*; food spoilage yeasts viz. *Debaromyces hansenii, Pichia membranifaciens*; and human pathogens viz. *Candida albicans and Cryptococcus neoformans*. Compounds **5d**, **5e** and **5f** showed potent activity against human pathogens. Moderate and selective activity was observed for other compounds. All the synthesized compounds were non-haemolytic. These compounds have potential application in agriculture and medicine.

Hybrid molecules with dual mode of action are an emerging novel strategy being employed for the generation of new drug candidates for various human diseases.[1] In present study, the strategy has been used for the generation of new and more effective agrochemicals with insecticidal and antifungal properties.

Accordingly, a known antifungal monoterpenoid carvacrol was coupled with benzoyl urea or thiourea moiety present in commercially used benzoylphenyl urea (BPU) class insecticide for the synthesis of hybrid molecules and their synthesis, insecticidal and antifungal activity is discussed.

Insect pests and plant pathogenic fungi are major causes for crop losses. Many synthetic organic compounds are in use for their control; however, these traditional pesticides have drawbacks such as resistance development, unwanted side-effects, persistence in the environment, toxicity, etc. Insect Growth Regulators (IGRs) are receiving more practical attention to provide for safer foods and cleaner environment.[2] IGRs inhibit different developmental stages in the lifecycle of an insect by specific action such as inhibition of chitin biosynthesis, metamorphosis or breeding.

Benzoylphenyl urea (BPU) is one such class of IGR compounds which mainly inhibits chitin synthesis and thus interferes with the formation of insect cuticle.[3] Lufenuron, diflubenzuron, penfluzuron, novaluron, flufenoxuron, teflubenzuron, chlorfuazuron, hexaflumuron are few examples of BPU compounds currently in use for the control of a wide range of leaf-eating insects and their larvae in vegetables, fruits and mushrooms (Fig. 1).[4] BPU compounds bind to the sulphonylurea receptors (SUR), a group of ABC-transporters and inhibit the exocytotic movement of the

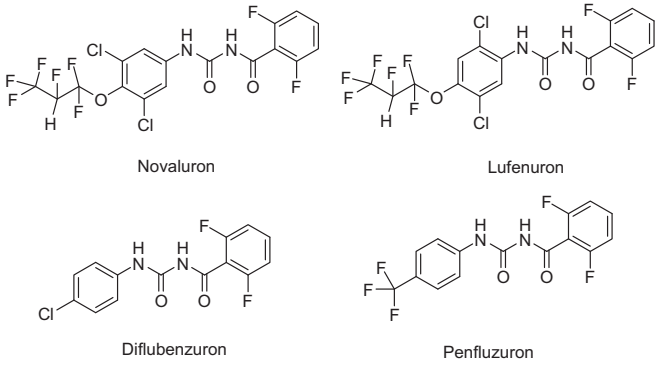


Figure 1. Representative benzoylphenyl urea insect growth regulators.

* Corresponding authors. Tel.: +91 020 25902708; fax: +91 020 25902648 (S.G.T.); tel.: +91 257 2257435; fax: +91 257 2258403 (R.S.B.).
E-mail addresses: sgtupe@yahoo.co.in (S.G. Tupe), bendrers@rediffmail.com (R.S. Bendre).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.07.017

vesicles, depolarizes the vesicle membrane through inhibition of K^+ channel, which leads to inhibition of *N*-acetylglucosamine deposition and subsequent chitin synthesis in the cuticle.[5] Chitin is also an important constituent of fungal cell wall; however, BPU compounds do not affect chitin synthesis in fungi and have been shown to be ineffective in vivo and in vitro for the control of fungal pathogens.[6,7]

Carvacrol (2-methyl-5-[1-methylethyl] phenol), a phenolic monoterpenoid is a constituent of essential oils produced by numerous aromatic plants and spices such as black cumin (*Nigella sativa* L.), marjoram (*Origanum majorana* L.), oregano (*Origanum vulgare* L.) summer savory (*Satureja hortensis* L.) and thyme (*Thymus vulgaris* L.).[8–10] The antifungal activity of carvacrol has been demonstrated against many phytopathogens[11,12] and human pathogenic fungi.[13] It causes cytoplasmic membrane damage through lesion formation and lowering of ergesterol content.[14] Rao et al. have suggested calcium burst and inhibition of TOR pathway as a mode of action for Carvacrol.[15] It is also insecticidal (less effective than BPU) and has been proved effective against different insect pests like *Thecodiplosis japonensis, Aphis craccivora,* and *Leucania separata*.[16,17]

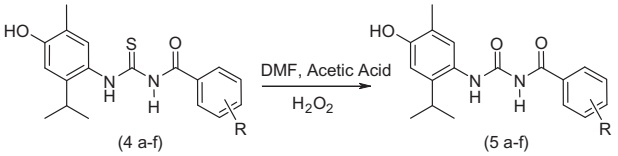
For development of better crop protection agents we envisaged hybrid molecules of carvacrol and benzoylphenyl ureas which will have dual (insecticidal and antifungal) biological activity. For this, 4-nitroso carvacrol and 4-amino carvacrol were synthesized according to previously reported procedures.[18,19] Using these derivatives, two series of compounds benzoylcarvacryl thiourea's (BCTU, **4a–f**)[20] and benzoylcarvacryl urea's (BCU, **5a–f**)[21] were synthesized (Schemes 1 and 2).

All compounds were obtained as pure solids. Characterization of a representative compound from both BCTU[22] and BCU[23] series is discussed. The ^{1}H NMR spectrum of compound **4a** (BCTU) displayed a doublet at δ 1.22, integrating for six protons which was due to –CH group attached to it. A singlet at δ 2.24, integrating for three protons was due to methyl group on carvacrol ring. A proton at CH adjacent to two methyl groups appeared as septet at δ 3.05. Also, the two aromatic protons of carvacrol ring appeared separately as two singlets at δ 6.67 and 7.27. The two aromatic equivalent protons appeared as triplet at δ 7.53. A proton at para position appeared separately as triplet at δ 7.66. Two ortho aromatic protons appeared as doublets at δ 7.97. The hydroxyl proton appeared as singlet at δ 9.34. The remaining two protons attached to nitrogen atom appeared as singlets at δ 11.49 and δ 12.11 respectively. The % of C: 65.83, H: 6.14 and N: 8.53 were well in agreement with the calculated values. LC–MS spectrum exhibited characteristic peak at 329 for $[M+H]^+$ ion clearly matching with its molecular weight 328.44.

The ^{1}H NMR spectrum of compound **5a** (BCU) displayed a doublet at δ 1.13, integrating for six protons which was due to –CH group attached to it. A singlet at δ 2.46, for six protons was of two methyl groups on carvacrol ring. A proton adjacent to two methyl groups appeared as multiplate at δ 3.35. Also, the two protons of carvacrol ring appeared separately as two singlets at δ 6.69 and 7.30. The two equivalent aromatic protons at the meta position appeared as triplet at δ 7.59 and two protons at orthro position appeared as doublets at δ 7.99. A para position proton appeared separately as triplet at δ 7.50. The hydroxyl proton appeared as singlet at δ 9.13 and the remaining two protons attached to nitrogen atom were appeared as singlet at δ 10.36 and δ 10.93, respectively. The % of C: 69.21, H: 6.45 and N: 8.97 were well in agreement with the calculated values. LC–MS spectrum exhibited the characteristic peak at 313.20 for $[M+H]^+$ ion clearly matching with its molecular weight 312.45. To get single crystal, acetone solution of **4c** was subjected to slow evaporation. The molecular structure of **4c**, that is, 4Cl–BCTU was unambiguously confirmed by X-ray crystallography (Fig. 2). CCDC-805770 contains the supplementary crystallographic data for this compound. The data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

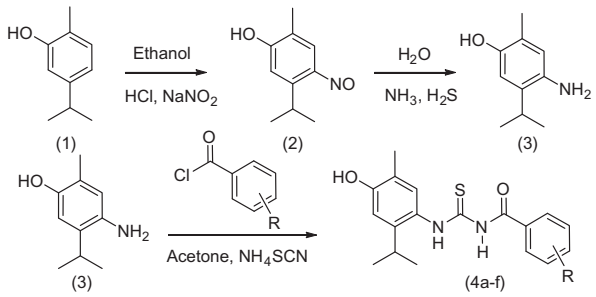
All the newly synthesized BCU and BCTU derivatives were screened for insect growth regulatory (IGR) activity against red cotton bug, *Dysdercus koenigii*. Simplicity of rearing in the laboratory and sensitivity to morphogenic compounds makes *D. koenigii,* the insect of choice for the investigation. The test compounds were dissolved in acetone (1 mg/ml). The required volume of the test solutions were then topically applied with the help of microlitre syringe to the dorsal abdominal region of same aged 5th instar nymphs. The concentrations of the compounds tested were 10, 15, 20, 30, 40 and 50 μg/nymph. The treated nymphs were placed back in the jars after the acetone had evaporated. At least three replicates (10 insects per replicate) were used for each dose of a compound. A parallel control group of nymphs treated only with acetone was set up. The bioactivities of test compounds were determined by its effects on mortality (toxic/insecticidal effect), moulting and growth (growth inhibiting/regulating activity).[24] Based on the % mortality data, LD_{50} values (lethal dose μg/nymph) were calculated using statistical computer program (Indostat Services, Hyderabad).

The LD_{50} of BCTU derivatives for 5th instar nymphs were found to be in the range 11.3–23.6 μg/nymph, whereas for BCU derivatives the LD_{50} range was 9.5–21.5 μg/nymph (Table 1). All



Where, R: 5a = H, 5b = 2-Cl, 5c = 4-Cl, 5d = 2-F, 5e = 4-F, 5f = 2,6-F

Scheme 2. Synthesis of benzoylcarvacryl urea derivatives.



Where, R: 5a = H, 5b = 2-Cl, 5c = 4-Cl, 5d = 2-F, 5e = 4-F, 5f = 2,6-F

Scheme 1. Synthesis of benzoylcarvacryl thiourea derivatives.

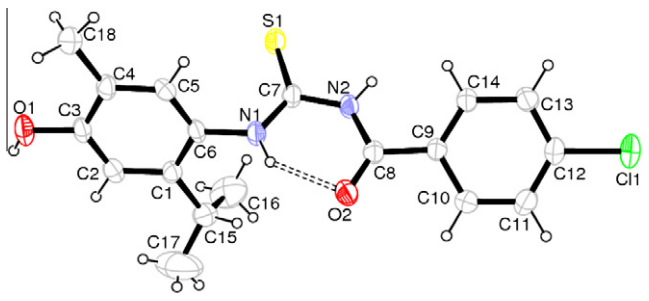


Figure 2. Molecular structure of 4Cl–BCTU (*ORTEP* diagram drawn at 30% ellipsoidal probability).

Table 1
Insecticidal activity of BCTU and BCU derivatives against *Dysdercus koenigii*

Compound	Chi Square value (χ^2)	**Regression equation**	**LD_{50}**	Fiducial limit	
				Min	**Max**
4a	2.72	0.345 + 3.389*x*	23.6	21.7	25.7
4b	3.55	3.136 + 1.675*x*	13.0	10.9	15.3
4c	1.91	2.458 + 1.928*x*	20.8	18.0	24.0
4d	2.73	3.511 + 1.354*x*	12.6	10.2	15.5
4e	2.73	3.510 + 1.354*x*	12.6	10.2	15.5
4f	7.32	3.347 + 1.569*x*	11.3	09.4	13.7
5a	3.33	2.090 + 2.186*x*	21.5	18.8	24.4
5b	5.45	2.518 + 2.046*x*	16.4	14.3	18.7
5c	3.49	2.497 + 1.996*x*	18.0	15.7	20.7
5d	0.75	3.339 + 1.469*x*	12.5	10.3	15.1
5e	5.85	2.601 + 2.045*x*	14.9	13.0	17.1
5f	1.37	3.898 + 1.269*x*	09.5	07.2	12.6
Lufenuron	1.90	3.507 + 1.566*x*	09.0	07.5	10.7
Carvacrol	5.88	2.701 + 1.510*x*	33.3	26.7	41.4

Acetone at highest concentration mortality was <15%

chi-square values were not significant ($\alpha = 0.05$) indicating good fit of regression lines. The percentage of abnormal adults was found to be increased with increase in the treatment dose. High doses between 20 and 50 μg/nymph showed deformities such as smaller body size, crumpled wings and deformed legs (abnormal adults) in some of the emerging adult population. 2,6-Fluoro derivatives (**5f**) showed the highest activity amongst respective series. Fluoro substitutes BCTU, BCU compounds exhibited better IGR activity than corresponding chloro substitute. For both the series, Cl or F groups at ortho position conferred better activity than its substitution at para position. The LD_{50} of carvacrol was 33.3 μg/nymph, while its incorporation in a BPU had significant effect as evident from the comparable activity (<13 μg/nymph) of the derivatives **4b**, **4d**, **4e**, 4f and **5d**, 5f with the standard BPU lufenuron (9.0 μg/nymph).

The purified final compounds were evaluated for antifungal susceptibility testing by microbroth dilution method according to the recommendations of the National Committee for Clinical Laboratory standards (NCCLS).[25] The antifungal activity was tested using the plant pathogenic fungal strains *Magnporthe grisea, Fusarium oxysporum, Dreschlera oryzae* and food spoilage yeasts *Debaromyces hansenii, Pichia membranifaciens.* Rice is the host for *M. grisea and D. oryzae,* whereas *F. oxysporum* infests diverse plants including tomato, tobacco, legumes, cucurbits, sweet potatoes and banana. *D. hansenii* and *P. membranifaciens* occur on grapes and are common wine spoilage yeasts. The antifungal activities of the tested compounds are given in Table 2 as Minimum Inhibitory Concentration (MIC) values. MIC was defined as the lowest concentration exhibiting >90% inhibition of visible growth compared to growth of the control.

As seen from table 2, BCTU **4c**, **4d**, **4e**, 4f and BCU **5b**, 5c showed antifungal activity against these fungi. BCTU derivatives **4a**, 4b and BCU **5a**, **5d**, **5e**, 5f were ineffective in controlling the growth of the phytopathogens and food spoilage yeasts at the highest concentration tested. Secondly, the thiourea derivatives were more effective than urea derivatives. The results of **4c**, 5c indicated that presence of chloride group at para position enhanced the antifungal activity. No in vitro antifungal activity was observed for lufenuron, which is in agreement with previous reports.[6,7] As expected, carvacrol showed good antifungal activity against all the tested organisms. The hydroxyl group of carvacrol has been shown to have a special role in the antimicrobial action of carvacrol.[26]

There are many reports on the use of carvacrol for the control of human fungal pathogens such as *Candida albicans, Aspergillus niger*, *Microsporum canis* etc.[13,27] Carvacrol is a US Food and Drug Administration approved safe food additive, and used as a flavouring agent in different foods.[28] Though initially developed as insecticides, benzoylphenyl urea compounds were reported to possess potent antitumor activity and are in clinical development for cancer treatment.[29,30] Few reports have indicated the potential of lufenuron for the control of fungal pathogens in animals.[31,32] Therefore, the BCTU and BCU derivatives were checked for

Table 2
Antifungal susceptibility testing of BCTU and BCU derivatives against phytopathogenic fungi

Derivative	Minimum inhibitory concentration (MIC in μg/ml)				
	Phytopathogenic fungi			Food spoilage yeasts	
	M. grisea	*F. oxysporum*	*D. oryzae*	*D. hansenii*	*P. membranifaciens*
4a	>512	>512	>512	>512	512
4b	>512	>512	>512	>512	512
4c	256	128	>512	256	512
4d	256	128	>512	256	>512
4e	256	128	256	256	512
4f	>512	>512	256	128	>512
5a	>512	>512	>512	>512	>512
5b	>512	512	256	128	256
5c	512	256	128	128	256
5d	>512	>512	>512	>512	>512
5e	>512	>512	>512	>512	>512
5f	>512	>512	>512	>512	>512
Lufenuron	>512	>512	>512	>512	>512
Carvacrol	128	64	64	128	128

Table 3
Antifungal susceptibility testing of BCTU and BCU derivatives against human fungal pathogens

Compound	Growth Inhibitory concentration in μg/ml							
	C. albicans NCIM 3557	*C. albicans*	*C. albicans* NCIM 3471	*C. glabrata* NCIM 3237	*C. neoformans*	*C. neoformans* NCIM 3541	*C. neoformans* NCIM 3542	*C. neoformans* NCIM 3378
4a	128	32	>512	32	64	16	32	128
4b	64	64	>512	64	128	32	64	>512
4c	64	16	32	32	64	8	32	32
ad	128	128	>512	128	32	64	128	512
4e	64	64	>512	64	16	32	64	128
4f	256	64	>512	64	512	32	64	128
5a	512	>512	>512	>512	>512	>512	256	>512
5b	128	256	>512	>512	256	256	128	128
5c	64	32	>512	32	64	32	32	32
5d	>512	16	32	16	>512	<4	16	16
5e	>512	16	32	16	>512	<4	16	16
5f	>512	16	32	16	>512	<4	16	16
Lufenuron	>512	>512	>512	>512	>512	>512	>512	>512
Carvacrol	128	128	256	128	128	128	128	128

antifungal activity against different strains of human pathogens *C. albicans*, *Candida glabrata* and *Cryptococcus neoformans* (Table 3). *C. albicans* and *C. neoformans* were isolates from clinical samples whereas other strains and *C. glabrata* were procured from National Centre for Industrially Important Microorganisms (NCIM), Pune, India. All the BCTU derivatives showed potent antifungal activity against these human pathogens. From BCU series, **5d**, **5e** and **5f** were most effective, whereas compounds **5a** and **5b** showed weak or no antifungal activity against the tested strains. For most of the compounds the activity was better than carvacrol against human pathogens.

As stated earlier, carvacrol affects the cell membranes and results in depletion of sterols. Therefore, major concern of employing these newly synthesized compounds as crop protection or antifungal agents is their potential toxicity to mammalian cells. Hence, cellular toxicity of the compounds was checked by haemolysis assay as described previously.[33] The concentrations tested were in the range of 4–1000 μg/ml. The concentration causing 50% haemolysis (HC_{50}) for all the BCTU, BCU compounds and lufenuron was >1000 μg/ml. Maximum haemolysis observed was 17% for compound **4e** at 1000 μg/ml concentration. At MIC concentrations for all the derivatives, the haemolysis was negligible (<2%). The HC_{50} values for carvacrol and a similarly acting antifungal drug Amphoterecin B were 250 and 8 μg/ml, respectively. The antifungal activity and haemolysis results indicated that the synthesized compounds are better and safer than BPU's and carvacrol.

In conclusion, two series of BCTU and BCU derivatives were synthesized by structurally modifying carvacrol and introducing benzoylphenyl urea linkage. Derivatives **4b**, **4d**, **4e**, 4f and **5d**, 5f showed comparable Insect growth regulator activity with the standard BPU lufenuron against *D. koenigii*. Most of the compounds demonstrated potent antifungal activity against human pathogens and potent to moderate activity against different phytopathogens and food spoilage yeasts. All the compounds were non-haemolytic. The synthesized compounds have a potential application in agriculture as safer and broad spectrum crop protection agents. After comprehensive evaluation, they may also be used for the control of fungal pathogens in veterinary and human healthcare.

Acknowledgments

Mr. Umesh Pete and Ratnamala Bendre are thankful to University Grants Commission, New Delhi, India, for the financial assistance. Santosh Tupe and Preeti Chaudhary thanks Council of Scientific and Industrial Research, India for the research fellowship.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.07.017.

References and notes

1. Meunier, B. *Acc. Chem. Res.* **2007**, *41*, 69.
2. Graf, F. *Parasitol. Today* **1993**, *9*, 471.
3. Fournet, F.; Sannier, C.; Money, N. *J. Am. Mos. Ctrl. Assoc.* **1993**, *4*, 426.
4. Sannino, A.; Bandini, M. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 2729.
5. Matsumura, F. *Pestic. Biochem. Physiol.* **2010**, *97*, 133.
6. Johnson, S. M.; Zimmermann, C. R.; Kerekes, K. M.; Davidson, A.; Pappagianis, D. *Med. Mycol.* **1999**, *37*, 441.
7. Scotty, N. C.; Evans, T. J.; Giuliano, E.; Johnson, P. J.; Rottinghaus, G. E.; Fothergill, A. W.; Cutler, T. J. *J. Vet. Intern. Med.* **2005**, *19*, 878.
8. Enomoto, S.; Asano, R.; Iwahori, Y.; Narui, T.; Okada, Y. *Biol. Pharm. Bull.* **2001**, *24*, 307.
9. Skocibusic, M.; Besic, N. *Phytother. Res.* **2004**, *18*, 967.
10. Vicenzi, M.; Stammati, A.; Vicenzi, A.; Silano, M. *Fitoterapia* **2004**, *75*, 801.
11. Mueller-Riebau, F.; Berger, B.; Yegen, O. Agric *J. Food Chem.* **1995**, *43*, 2262.
12. Kordali, S.; Cakir, A.; Ozer, H.; Cakmakci, R.; Kesdek, M.; Mete, E. *Bioresour. Technol.* **2008**, *99*, 8788.
13. Chami, N.; Chami, F.; Bennis, S.; Trouillas, J.; Remmal, A. *Braz. J. Infect. Dis.* **2004**, *8*, 217.
14. Pinto, E.; Pina-Vaz, C.; Salgueiro, L.; Goncalves, M. J.; Costa-de-Oliveira, S.; Cavaleiro, C.; Palmeira, A.; Rodrigues, A.; Martinez-de-Oliveira, J. *J. Med. Microbiol.* **2006**, *55*, 1367.
15. Rao, A.; Zhang, Y.; Muend, S.; Rao, R. *Antimicrob. Agents Chemother.* **2010**, *54*, 5062.
16. Lee, S. G.; Kim, S. I.; Ahn, Y. J.; Kim, J. B.; Lee, B. Y. *Pestic. Sci.* **1997**, *49*, 119.
17. Tang, X.; Chen, S.; Wang, L. *Nat. Prod. Res.* **2011**, *25*, 320.
18. Shah, H.; Vashi, S.; Mehta, S. *Indian J. Che.* **1995**, *34B*, 802.
19. Kremers, E.; Wakeman, N.; Hixon, R. *Org. Syn.* **1941**, *1*, 511.
20. General synthetic procedure for the synthesis of **4a–f**: To a hot, vigorously stirred solution of ammonium thiocyanate (0.84 g, 0.011 mol) in dry acetone (20 mL), substituted benzoyl chloride (0.011 mol) in dry acetone (10 mL) was added drop wise. The reaction mixture was stirred on a hot plate for 1.5 h and it was added to an equimolar quantity of the 4-aminocarvacrol (1.82 g, 0.011 mol) in dry acetone (10 mL). The mixture was refluxed for 5–6 h at 55 °C. The solvent was then removed under reduced pressure and the reaction mixture was diluted with ice cold water (50 mL) to afford the product. The separated solid was purified by recrystallization from hexane–ethyl acetate mixture. Yields obtained are about 70 to 80%.
21. General synthetic procedure for the synthesis of **5a–f**: Respective thioureas (0.0033 mol) were dissolved in DMF and 85 % (15 mL) formic acid and 30% hydrogen peroxide (50 mL) were slowly added to the solution. The reaction mixture was stirred overnight and poured onto crushed ice. The precipitate was collected, dried and recrystallized from hexane–ethyl acetate mixture to afford the product.
22. *Data for 1-benzoyl-3-(4-hydroxy-2-methyl-5-isopropylphenyl) thiourea (**4a**):* White solid, mp 208 °C, ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.22 (d, J = 6.87 Hz, 6 H, $CH(CH_3)_2$), 2.24 (s, 3 H, CH_3), 3.05 (m, 1 H, $CH(CH_3)_3$), 5.06 (bs, 1 H, OH), 6.77 (s, 1 H, H-Ar), 7.27 (s, 1 H, H-Ar), 7.55 (t, 1 H, H-Ar), 7.64 (t, 2 H, H-Ar), 7.91 (d, J = 7.32 Hz, 2 H, H-Ar), 9.26 (bs, 1 H, NH), 11.98 (brs, 1 H, NH) ppm. LC–MS calcd for $C_{18}H_{21}N_2O_2S$ $[M+H]^+$ 329.44 , found 329.00. $C_{18}H_{20}N_2O_2S$ (328.44): C, 65.83; H, 6.14; N, 8.53; S, 9.76; found C, 66.22; H, 6.17; N, 8.49.

23. Data for 1-benzoyl-3-(4-hydroxy-2-methyl-5-isopropylphenyl) urea (5a): White solid, mp 242 °C, ^{1}H NMR (300 MHz, DMSO-d_6, 25 °C): δ = 1.13 (d, J = 6.86 Hz, 6 H, $CH(CH_3)_3$), 2.46 (s, 3 H, CH_3), 3.35 (m, 1 H, $CH(CH_3)_2$), 6.69 (s,1 H, H-Ar), 7.30 (s, 1 H, H-Ar), 7.50 (t, 1 H, H-Ar), 7.59 (t, 2 H, H-Ar), 7.99(d, 2 H, H-Ar), 9.13 (s, 1 H,OH), 10.36 (brs, 1 H, NH), 10.93 (brs, 1 H, NH) ppm. LC–MS calcd. for $C_{18}H_{21}N_2O_3$ $[M+H]^+$ 313.37, found 313.20. $C_{18}H_{20}N_2O_3$ (312.37): C, 69.21; H, 6.45; N, 8.97; found C, 68.54; H, 5.77; N, 8.98.
24. Banerjee, S.; Magdum, S.; Kalena, P.; Banerji, A. *J. Appl. Ent.* **2001**, *125*, 25.
25. (a) Wayne, PA. National Committee for Clinical Laboratory Standard: Document M38-A.1998.; (b) Approved standard M27-A. 1997. National Committee for Clinical Laboratory Standards.
26. Veldhuizen, E. J. A.; Tjeerdsma-van bokhoven, J. L. M.; Zweijtzer, C. S.; Burt, A.; Haagsman, P. *J. Agric. Food Chem.* **1874**, *2006*, 54.
27. Soković, M.; Tzakou, O.; Pitarokili, D.; Couladis, M. *Mol. Nutr. Food Res.* **2002**, *46*, 317.
28. Code of Federal Regulations Title 21 – Food and Drugs http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?FR=172.515. Accessed April 6, 2012.
29. Hess, M. B.; Parker, N. A.; Purswell, B. J.; Dascanio, J. D. *J. Am. Vet. Med. Assoc.* **2002**, *221*, 266.
30. Dubuis, E.; Lucas, D. *Vet. Rec.* **2003**, *152*, 651.
31. Okada, H.; Kato, M.; Koyanagi, T.; Mizuno, K. *Chem. Pharm. Bull. (Tokyo)* **1999**, *47*, 430.
32. Messersmith, W.; Rudek, M.; Baker, S., et al *Eur. J. Cancer* **2007**, *43*, 78.
33. Sajjad, M.; Khan, A.; Ahmad, I. *Appl. Microbiol. Biotechnol.* **2011**, *1083*, 90.