Bioorganic & Medicinal Chemistry Letters 22 (2012) 2186–2191

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Catalysis by molecular iodine: A rapid synthesis of 1,8-dioxo-octahydroxanthenes and their evaluation as potential anticancer agents

Naveen Mulakayala [a], P.V.N.S. Murthy [b], D. Rambabu [a,b], Madhu Aeluri [a], Raju Adepu [a], G.R. Krishna [c], C. Malla Reddy [c], K.R.S. Prasad [b], M. Chaitanya [d], Chitta S. Kumar [d], M.V. Basaveswara Rao [e,*], Manojit Pal [a,*]

[a] Institute of Life Sciences, University of Hyderabad Campus, Gachibowli, Hyderabad 500 046, Andhra Pradesh, India
[b] Department of Chemistry, K L University, Vaddeswaram Guntur 522 502, Andhra Pradesh, India
[c] Department of Chemical Sciences, Indian Institute of Science Education and Research, Kolkata, West Bengal 741 252, India
[d] Department of Biochemistry, Sri Krishnadevaraya University, Anantapur 515 003, Andhra Pradesh, India
[e] Department of Chemistry, Krishna University, Machilipatnam 521 001, Andhra Pradesh, India

ARTICLE INFO

Article history:
Received 25 December 2011
Revised 26 January 2012
Accepted 27 January 2012
Available online 7 February 2012

Keywords:
1,8-Dioxo-octahydroxanthene
5,5-Dimethyl-1,3-cyclohexanedione
Iodine

ABSTRACT

Molecular iodine facilitated the reaction of 5,5-dimethyl-1,3-cyclohexanedione with aromatic aldehydes in *iso*-propanol affording a variety of 1,8-dioxo-octahydroxanthenes in high yields. Most of the compounds synthesized showed good anti-proliferative properties in vitro against three cancer cell lines and 9-(2-hydroxyphenyl)-3,3,6,6-tetramethyl-3,4,5,6,7,9-hexahydro-1*H*-xanthene-1,8(2*H*)-dione possessing a 2-hydroxy phenyl group at C-9 position was found to be promising. Further structure elaboration of the same compound and the crystal structure analysis and hydrogen bonding patterns of another compound that is, 9-(4-methoxyphenyl)-3,3,6,6-tetramethyl-3,4,5,6,7,9-hexahydro-1*H*-xanthene-1,8(2*H*)-dione prepared by using this methodology is presented.

Xanthene derivatives have attracted considerable interest because of their various pharmacological properties such as antibacterial, antiviral and anti-inflammatory activities.[1,2] Apart from their use as valuable synthetic precursors for many organic compounds[3] and dyes[4] they also found uses in laser technologies,[5a] and fluorescent materials for visualization of biomolecules.[5b] While the cancer cell cytotoxicity of xanthene derivatives **A** (Fig. 1) has been documented[6a] recently the anticancer properties of their saturated analogs for example, 1,8-dioxo-octahydroxanthenes **C** designed via **B** (Fig. 1) has not been investigated earlier. Moreover, in view of the fact that cancer is the second leading cause of death[6b] worldwide there is a need for the identification of new and more effective anticancer agents. This prompted us to synthesize and assess the anticancer potential of 1,8-dioxo-octahydroxanthenes in vitro.

One of the commonly used methods reported for the synthesis of xanthene derivatives involved the condensation of aldehyde with 1,3-cyclohexanedione or 5,5-dimethyl-1,3-cyclohexanedione. This reaction can be carried out in the presence of protonic acids[7] or a range of Lewis acids such as $InCl_3{\cdot}4H_2O$,[8] $FeCl_3{\cdot}8H_2O$,[9] $NaHSO_4$,[10] or heterogeneous catalysts, such as Dowex-50W,[11] $NaHSO_4{\cdot}SiO_2$,[12] silica sulfuric acid,[13] polyaniline *p*-toluenesulfonate,[14] PPA–SiO_2,[15] TiO_2/SO_4,[2–16] Amberlyst-15,[17] $SbCl_3/SiO_2$,[18] or Fe^{3+}-montmorillonite.[19] Among the other catalysts used include trimethylsilylchloride,[20] *p*-dodecylbenzenesulfonic acid,[21,22] triethylbenzylammonium chloride[23] or NH_2SO_3/SDS.[24] Additionally, the above condensation reaction can also be carried out in ionic liquid[25] or ethylene glycol.[26] However, some of these methodologies suffer from disadvantages, such as low yields,[14,15] prolonged reaction time,[8,12,21–23] harsh reaction conditions,[7] and the requirement of excess catalysts[20] or special apparatus.[22] Recently, iodine being an inexpensive and commercially available reagent have attracted considerable interest due to its non-hazardous nature and efficiency in various organic transformations.[27] Due to our continuing interest in the use of iodine as a reagent or catalyst[28] we have observed that 5,5-dimethyl-1,3-cyclohexanedione (**1**) undergoes smooth condensation with aromatic aldehydes (**2**) in the presence of elemental iodine to give 1,8-dioxo-octahydroxanthene derivatives (**3**) (Scheme 1) within short period of time. Herein we report our preliminary results on the development of an iodine-mediated simple and highly efficient method for synthesis of our target xanthene derivatives **C** (or **3**) and their evaluation as potential anticancer agents. To the best of our knowledge synthesis of xanthenes using such a methodology and their anticancer properties has not been disclosed earlier.

* Corresponding authors. Tel.: +91 40 6657 1500; fax: +91 40 6657 1581.
E-mail address: manojitpal@rediffmail.com (M. Pal).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2012.01.126

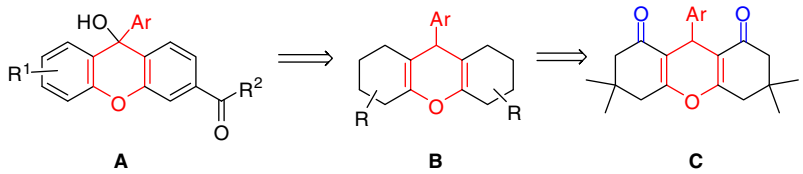
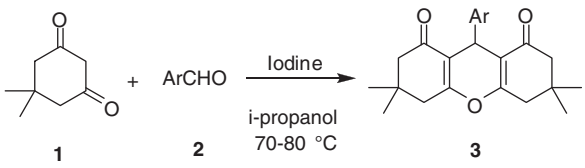


Fig. 1. Design of 1,8-dioxo-octahydroxanthenes (**C**) from the known cytotoxic xanthenes (**A**) as potential anticancer agents.

The reaction was carried out using a mixture of compound **1** (2.0 equiv) and aldehyde **2** (1.0 equiv) in the presence of iodine (0.2 equiv) in *i*-propanol (3.0 mL) at 70–80 °C. The reaction proceeded smoothly and was completed within 20 min to give the 9-aryl substituted xanthenes derivatives **3** in good to excellent yields (Table 1). A range of functionalized aromatic aldehydes including mono and disubstituted derivatives were employed in the present iodine-mediated condensation reaction. Both electron-donating groups for example, fluoro (entry 2), chloro (entry 3), bromo (entries 4, 10 and 11), methyl (entry 9), hydroxy (entries 5, 6, 10, 13 and 14) or methoxy (entries 8, 14 and 15) and electron-withdrawing group for example, nitro (entries 7 and 12) present in the aldehyde were well tolerated. All the 1,8-dioxo-octahydroxanthenes **3** prepared were well characterized by spectral (^{1}H NMR, IR and MS) data. The appearance of (i) a singlet in the region 4.6–4.8 δ for C-9 proton and two singlets in the region 0.99–1.15 δ each for two methyl hydrogens in the ^{1}H NMR spectra and (ii) IR signals in the region 1680–1660 cm^{-1} for carbonyl group characterized the compound **3**. Additionally, the molecular structure of a representative compound for example, **3i** was established unambiguously by single crystal X-ray diffraction (Fig. 2).[29] Further, crystal structure analysis was carried out to understand the packing and/or hydrogen bonding patterns in crystal of these molecule and results are summarized in the following section.

Compound **3i** crystallizes in orthorhombic Pca2 (1) space group with two molecules in the asymmetric unit (Z: 11 Z′: 0) (Fig. 3). The two *p*-methoxyphenyl rings of the molecules in the asymmetric unit are found to be not in coplanar with 3,3,6,6-tetramethyl-3,4,5,6,7,9-hexahydro-1*H*-xanthene-1,8(2*H*)-dione. The torsion angles of the *p*-methoxyphenyl ring with 3,3,6,6-tetramethyl-3,4,5,6,7,9-hexahydro-1*H*-xanthene-1,8(2*H*)-dione are (C5–C6–C7–C18 76.9 (5), C18–C7–C8–C9 −78.0(4) and C42–C31–C32–C33 77.5(4) and C29–C30–C31–C42 −77.9(5). The two molecules in the asymmetric unit form same hydrogen bonding patterns in the crystal structure. They also participated in weak Vander-Waal interactions through the keto functional group, and are stabilized by methylene interactions. These interactions propagated in 3D network packing as shown in Figure 4.

Mechanistically, the present iodine-mediated reaction seemed to proceed via in situ generation of intermediate **E** that subsequently afforded the desired product **3** (Scheme 2). Thus (i) aldol-type condensation between 1,3-cyclohexanedione (**1**) and aldehyde (**2**) followed by (ii) intramolecular Michael addition involving the hydroxyl group and enone moiety of the resulting intermediate **E** afforded the compound **3**. It is mention worthy that possible interaction of molecular iodine with carbonyl oxygen has been indicated in the literature earlier.[30] Moreover, the intermediacy of compound **E** was supported by the fact that this compound was isolated when the iodine-mediated reaction was carried out at room temperature for 10 min. No cyclic product **3** was obtained even after continuing the reaction for 12 h. However, the intermediate **E** was converted to **3** when heated to reflux in isopropanol for 20 min in the presence of iodine.



Scheme 1. Reaction of 5,5-dimethyl-1,3-cyclohexanedione (**1**) with aldehydes (**2**) in the presence of molecular iodine.

To demonstrate the further scope of this reaction one of the compound synthesized was used for further structure elaboration. Thus, the compound **3e** was propargylated[31] using propargyl bromide (Scheme 3) and the resulting alkyne **4** was then reacted with *p*-iodophenol in the presence of $(PPh_3)_2PdCl_2$ and CuI under a Sonogashira condition.

Most of the xanthene derivatives synthesized for example, **3**, **4** and **5** were tested for their anticancer properties in vitro. We evaluated our compounds for their anti-proliferative properties in vitro against a number of cancer cell lines for example, human chronic myeloid leukemia cells (K562), human colon carcinoma cells (Colo-205), and human neuroblastoma cells (IMR32). The test compounds were examined at various concentrations in a MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay and the IC_{50} values obtained for each compounds are summarized in Table 2. Harmine, a member of β-carboline family of compounds showed cytotoxicity against HL60 and K562 cell lines[32] was used as a reference compound. While most of these compounds showed inhibition of leukemia cell growth as reflected by their IC_{50} values the good results however were obtained using compounds **3b**, **3c**, **3e**, **3f**, **3g**, **3i**, **3j**, **3l**, **3n**, **3o**, **4** and **5** (IC_{50} <50 μM, Table 2). Similarly, except compounds **3i** and **3k** all other compounds (IC_{50} <50 μM, Table 2) were found to be active against colon carcinoma cells and except compounds **3c**, **3h**, **3i**, **3k**, **3l**, **3m** and **3o** rest of the compounds (IC_{50} <50 μM, Table 2) showed good activities against breast cancer cells. Overall, compound **3e** possessing a 2-hydroxy phenyl group at C-9 position was found to be promising (IC_{50} ~ 23–38 μM, Table 2) and further functionalization of the 2-hydroxy phenyl ring (e.g., compounds **3f**, **3j**, **4** and **5**) did not improve the activity.

In conclusion, we have demonstrated the efficiency of molecular iodine as a catalyst for the preparation of 1,8-dioxo-octahydroxanthenes as potential anticancer agents. Both activated and inactivated aromatic aldehydes participated well in the present condensation reaction affording the expected products in high yields. The advantages of the present method include (i) short reaction time, (ii) operational simplicity and easy handling, (iii) functional group tolerability, (iv) cleaner reaction products and (v) does not involve the use of expensive and hazardous catalysts/co-catalysts or reagents. The crystal structure analysis and hydrogen bonding patterns of one compound prepared by using this methodology are presented. Most of the compounds synthesized showed good anti-proliferative properties in vitro against three cancer cell lines and the compound **3e** possessing a 2-hydroxy phenyl group at C-9 position was found to be promising. Overall, our study suggests that 1,8-dioxo-octahydroxanthenes presented here

Table 1
Preparation of 1,8-dioxo-octahydroxanthenes (**3**) using molecular iodine[a]

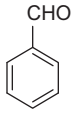
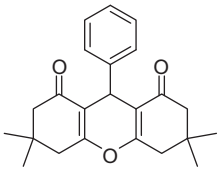
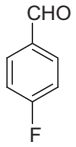
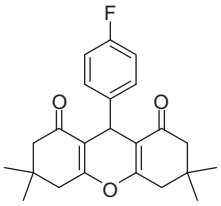
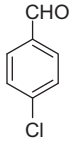
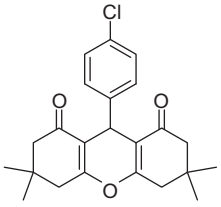
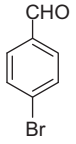
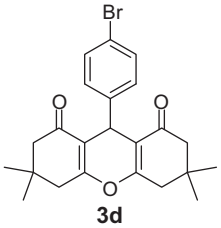
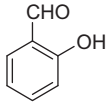
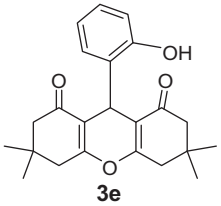
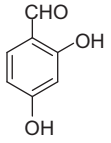
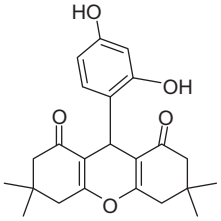
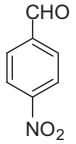
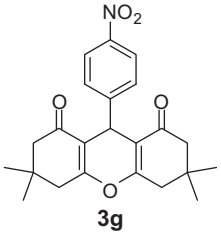
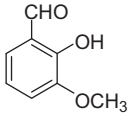
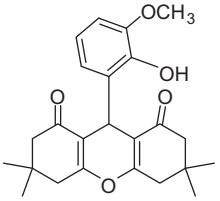
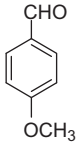
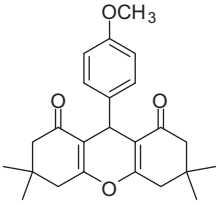
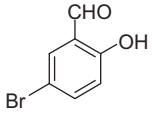
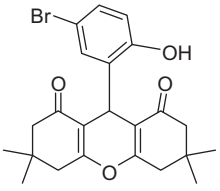
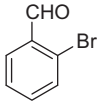
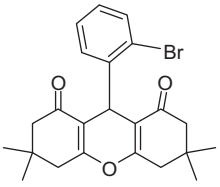
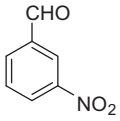
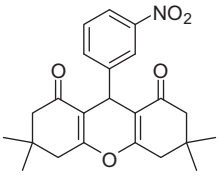
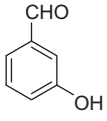
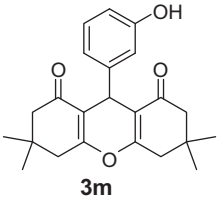
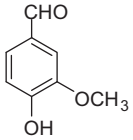
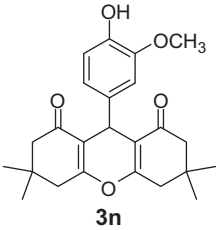
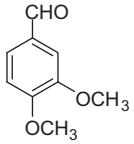
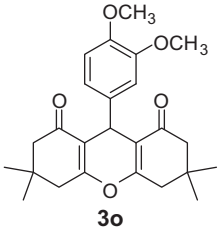
Entry	Aldehyde (**2**)	Time (min)	Product (**3**)	Yield[b] (%)	Ref.
1		18	 **3a**	90	20
2		19	 **3b**	93	8
3		17	 **3c**	91	20
4		18	 **3d**	93	20
5		16	 **3e**	95	
6		19	 **3f**	90	
7		19	 **3g**	91	8

Table 1 (*continued*)

Entry	Aldehyde (**2**)	Time (min)	Product (**3**)	Yield[b] (%)	Ref.
8		18	 **3h**	93	
9		17	 **3i**	90	8
10		19	 **3j**	94	
11		16	 **3k**	91	8
12		18	 **3l**	96	12
13		19	 **3m**	95	15
14		17	 **3n**	93	21

(*continued on next page*)

Table 1 (*continued*)

Entry	Aldehyde (**2**)	Time (min)	Product (**3**)	Yield[b] (%)	Ref.
15		20	 **3o**	92	26

[a] All the reactions were carried out using compound **1** (2.0 equiv) and aldehyde **2** (1.0 equiv) in the presence of iodine (0.2 equiv) in *i*-propanol (3.0 mL) at 70–80 °C.
[b] Isolated yield.

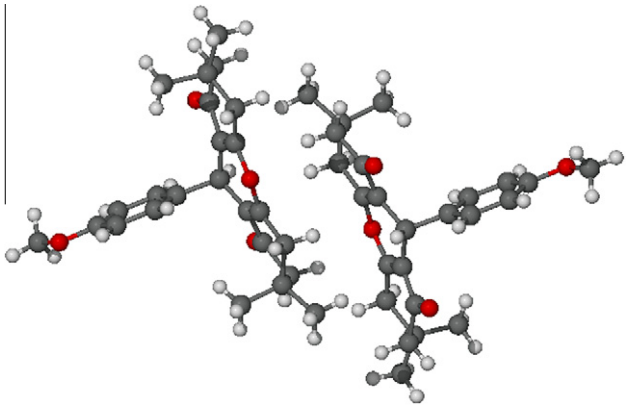


Fig. 2. ORTEP representation of the compound (**3i**) (Thermal ellipsoids are drawn at 50% probability level).

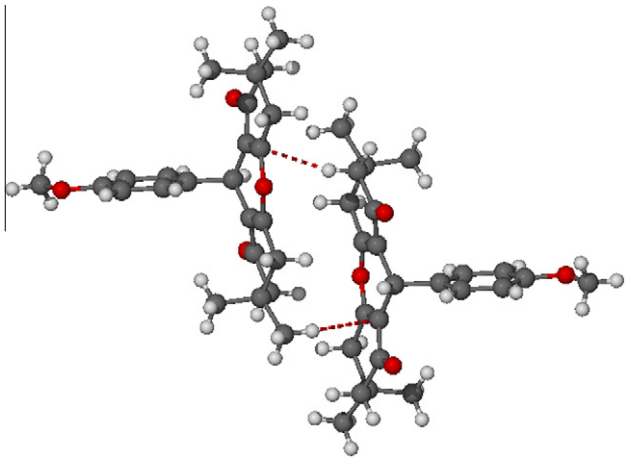


Fig. 3. Showing the hydrogen bonding patterns in two molecules of asymmetric unit in compound **(3i)**.

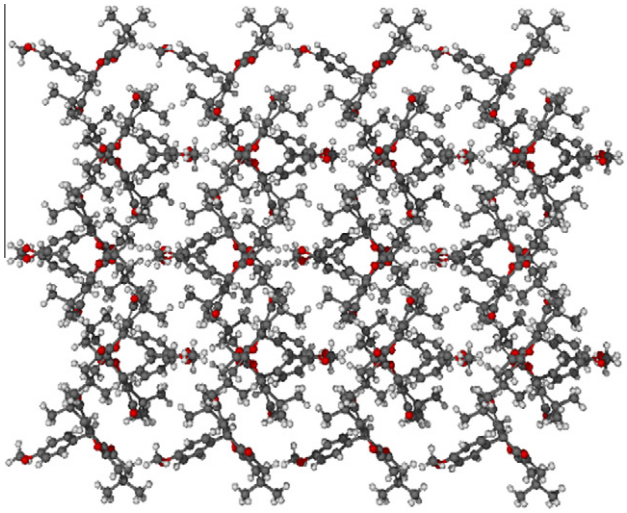
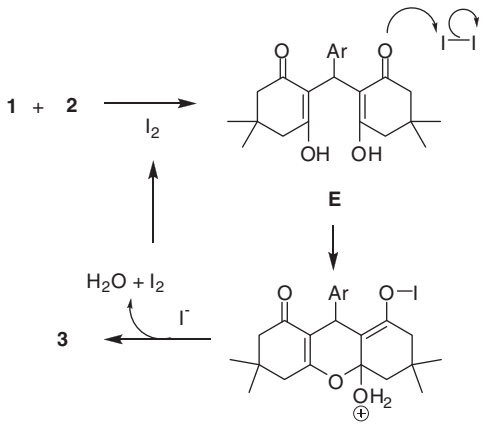
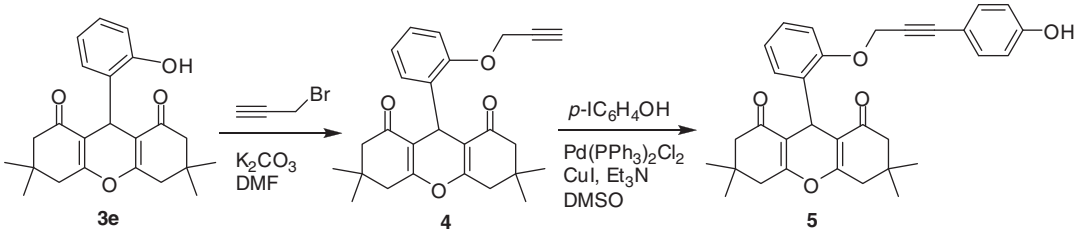


Fig. 4. (a) Showing the 3D packing patterns in compound (**3i**).



Scheme 2. Proposed reaction mechanism for the iodine-mediated reaction of 1,3-cyclohexanedione (**1**) with aldehyde (**2**).



Scheme 3. Structural elaboration of compound **3e**.

Table 2
Cytotoxic properties of compounds **3**, **4** and **5** against various cancer cell lines

Compound	IC_{50}[a,b] (μM)		
	K562	Colo-205	IMR32
3a	56	39	45
3b	35	30	42
3c	44	42	55
3d	59	42	49
3e	23	38	23
3f	36	28	41
3g	46	38	49
3h	55	41	62
3i	49	>100	53
3j	37	29	46
3k	74	>100	>100
3l	47	37	52
3m	56	41	63
3n	49	32	45
3o	45	33	56
4	34	28	42
5	28	30	35
Harmine	32	26	38

[a] IC_{50} represent the concentration of compound that causes a 50% growth inhibition to untreated cells using the MTT assay.
[b] Data represent the mean values of three independent determinations.

have medicinal value and the basic framework of this class of heterocycles could be an attractive template for the identification of novel and potential anticancer agents.

Acknowledgements

The authors thank management of Institute of Life Sciences for continuous encouragement and support.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2012.01.126.

References and notes

1. Jonathan, R. D.; Srinivas, K. R.; Glen, E. B. *Eur. J. Med. Chem.* **1988**, *23*, 111.
2. (a) Robak, J.; Gryglewski, R. J. *Pol. J. Pharmacol.* **1996**, *48*, 555; (b) Wang, H. K.; Morris-Natschke, S. L.; Lee, K. H. *Med. Res. Rev.* **1997**, *17*, 367; (c) Rukavishnikov, A. V.; Smith, M. P.; Birrell, G. B.; Keana, J. F. W.; Griffith, O. H. *Tetrahedron Lett.* **1998**, *39*, 6637.
3. Shchekotikhin, Y. M.; Nikolaeva, T. G. *Chem. Heterocycl. Compd.* **2006**, *42*, 28.
4. Hilderbrand, S. A.; Weissleder, R. *Tetrahedron Lett.* **2007**, *48*, 4383.
5. (a) Pohlers, G.; Scaiano, J. C. *Chem. Mater.* **1997**, *9*, 3222; (b) Knight, C. G.; Stephens, T. *Biochem. J.* **1989**, *258*, 683.
6. (a) Giri, R.; Goodell, J. R.; Xing, C.; Benoit, A.; Kaur, H.; Hiasa, H.; Ferguson, D. M. *Bioorg. Med. Chem.* **2010**, *18*, 1456; (b) Parkin, D. M.; Bray, F.; Ferlay, J.; Pisani, P. *Cancer J. Clin.* **2005**, *55*, 74.
7. Darviche, F.; Balalaie, S.; Chadegani, F.; Salehi, P. *Synth. Commun.* **2007**, *37*, 1059.
8. Fan, X.; Hu, X.; Zhang, X.; Wang, J. *Can. J. Chem.* **2005**, *83*, 16.
9. Fan, X.-S.; Li, Y.-Z.; Zhang, X.-Y.; Hu, X.-Y.; Wang, J.-J. *Chin. J. Org. Chem.* **2005**, *25*, 1482.
10. Ma, J.-J.; Li, J.-C.; Tang, R.-X.; Zhou, X.; Wu, Q.-H.; Wang, C.; Zhang, M.-M.; Li, Q. *Chin. J. Org. Chem.* **2007**, *27*, 640.
11. Shakibaei, G. I.; Mirzaei, P.; Bazgir, A. *Appl. Catal., A* **2007**, *325*, 188.
12. Das, B.; Thirupathi, P.; Reddy, K. R.; Ravikanth, B.; Nagarapu, L. *Catal. Commun.* **2007**, *8*, 535.
13. Seyyedhamzeh, M.; Mirzaei, P.; Bazgir, A. *Dyes Pigm.* **2008**, *86*, 836.
14. John, A.; Yadav, P. J. P.; Palaniappan, S. *J. Mol. Catal. A: Chem.* **2006**, *248*, 121.
15. Kantevari, S.; Bantu, R.; Nagarapu, L. *J. Mol. Catal. A: Chem.* **2007**, *269*, 53.
16. Jin, T.-S.; Zhang, J.-S.; Wang, A.-Q.; Li, T.-S. *Synth. Commun.* **2005**, *35*, 2339.
17. Das, B.; Thirupathi, P.; Mahender, I.; Reddy, V. S.; Rao, Y. K. *J. Mol. Catal. A: Chem.* **2006**, *247*, 233.
18. Zhang, Z.-H.; Liu, Y.-H. *Catal. Commun.* **2008**, *9*, 1715.
19. Song, G.; Wang, B.; Luo, H.; Yang, L. *Catal. Commun.* **2007**, *8*, 673.
20. Kantevari, S.; Bantu, R.; Nagarapu, L. *Arkivoc* **2006**, *xvi*, 136.
21. Jin, T.-S.; Zhang, J.-S.; Xiao, J.-C.; Wang, A.-Q.; Li, T.-S. *Synlett* **2004**, 866.
22. Jin, T.-S.; Zhang, J.-S.; Xiao, J.-C.; Wang, A.-Q.; Li, T.-S. *Ultrason. Sonochem.* **2006**, *13*, 220.
23. Shi, D.-Q.; Zhuang, Q.-Y.; Chen, J.; Wang, X.-S.; Tu, S.-J.; Hu, H.-W. *Chin. J. Org. Chem.* **2003**, *23*, 694.
24. Jin, T.-S.; Zhang, J.-S.; Wang, A.-Q.; Zhang, F.-S. *Chin. J. Org. Chem.* **2005**, *25*, 335.
25. Ma, J.-J.; Zhou, X.; Zhang, X.-H.; Wang, C.; Wang, Z.; Li, J.-C.; Li, Q. *Aust. J. Chem.* **2007**, *60*, 146.
26. Tu, S.-J.; Zhou, J.-F.; Lu, Z.-S.; Deng, X.; Shi, D.-Q.; Wang, S.-H. *Synth. Commun.* **2002**, *32*, 3063.
27. For selected references, see: (a) Yadav, J. S.; Reddy, B. V. S.; Hashim, S. R. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3025; (b) Yadav, J. S.; Reddy, B. V. S.; Premalatha, K.; Swamy, T. *Tetrahedron Lett.* **2005**, *46*, 2687; (c) Kumar, H. M. S.; Reddy, B. V. S.; Reddy, E. J.; Yadav, J. S. *Chem. Lett.* **1999**, 857; (d) Yadav, J. S.; Reddy, B. V. S.; Rao, C. V.; Chand, P. K.; Prasad, A. R. *Synlett* **2001**, 1638; (e) Wu, J.; Xia, H.-G.; Gao, K. *Org. Biomol. Chem.* **2006**, *4*, 126; (f) Varla, R.; Nuvula, S.; Adapa, S. R. *J. Org. Chem.* **2006**, *71*, 8283; For a review, see: (g) Mphahlele, M. J. *Molecules* **2009**, *14*, 4814.
28. (a) Barange, D. K.; Batchu, V. R.; Gorja, D.; Pattabiraman, V. R.; Tatini, L. K.; Babu, J. M.; Pal, M. *Tetrahedron* **2007**, *63*, 1775; (b) Venkataraman, S.; Barabge, D. K.; Pal, M. *Tetrahedron Lett.* **2006**, *47*, 7317; (c) Pal, M.; Subramanian, V.; Parasuraman, K.; Yeleswarapu, K. R. *Tetrahedron* **2003**, *59*, 9563.
29. Crystal data of **3i**: Molecular formula = $C_{24}H_{28}O_4$, Formula weight = 380.20, Crystal system = Orthorhombic, space group = Pca2(1), a = 14.145(8) Å, b = 15.162(9) Å, c = 20.708(14) Å, V = 4441.6(5) Å^3, T = 296(2) K, Z = 11, Dc = 1.678 Mg m^{-3}, μ(Mo-Kα) = 2.11 mm^{-1}, 32,863 reflections measured, 4394 independent reflections, 3712 observed reflections [$I > 2.0\sigma$ (I)], R1_obs = 0.045, Goodness of fit = 1.042. Crystallographic data (excluding structure factors) for **3i** have been deposited with the Cambridge Crystallographic Data Center as supplementary publication number CCDC 858071.
30. (a) Wang, Y.-h.; Li, L.; Chen, X.-s. *Chem. Res. Chin. Univ.* **2008**, *24*, 520. doi:10.1016/S1005-9040(08)60109-9; (b) Bandgar, B. P.; Shaikh, K. A. *Tetrahedron Lett.* **1959**, *2003*, 44.
31. Hans, R. H.; Guantai, E. M.; Lategan, C.; Smith, P. J.; Wan, B.; Franzblau, S. G.; Gut, J.; Rosenthal, P. J.; Chibale, K. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 942.
32. Jahaniani, F.; Ebrahimi, S. A.; Rahbar-Roshandel, N.; Mahmoudian, M. *Phytochemistry* **2005**, *66*, 1581.