Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 24, pp. 3091-3094, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)10147-0

SYNTHESIS AND CELL GROWTH INHIBITORY PROPERTIES OF SUBSTITUTED *(E)*-1-PHENYLBUT-1-EN-3-ONES

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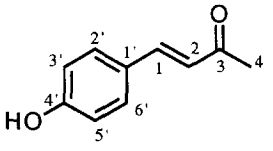
Abstract: A series of (*E*)-1-phenylbut-1-en-3-ones, based on the naturally occurring (*E*)-1-(4′-hydroxyphenyl)but-1-en-3-one [IC_{50} (K562) 60 μM], was synthesised and screened for cytotoxic activity against the K562 human leukaemia cell line. (*E*)-1-(Pentafluorophenyl)but-1-en-3-one [IC_{50} (K562) 1.8 μM] was found to be over 30-fold more active than **1**. © 1997 Elsevier Science Ltd.

Whilst many clinically important anticancer drugs have historically been developed from natural products the use of natural products as lead compounds continues to be important in drug discovery.[1] The chances of success are significantly increased if the source of the natural products has already been used as a medicine. We knew that the dried whole plant of *Scutellaria barbata* D. Don (*Labiatae*) is used in Traditional Chinese Medicine as an anti-inflammatory, an antitumour agent and a diuretic.[2] In our search for new anticancer drugs we isolated the α,β-unsaturated ketone, (*E*)-1-(4′-hydroxyphenyl)but-1-en-3-one **1** from *Scutellaria barbata* D. Don and found it to possess moderate antitumour activity [IC_{50} (K562) 60 μM].[3] In order to determine the features important for this anticancer activity a range of phenylbutenones has been synthesised and evaluated for their ability to inhibit cell growth *in vitro*. Herein we describe the synthesis of various substituted phenylbutenones and the assays used to determine their antitumour activity in an *in vitro* cell culture system (MTT assay).



E-1-(4′-hydroxyphenyl)but-1-en-3-one **1**

The growth inhibitory activities of the ketone **1** and its analogues described later were determined in the K562 human chronic myelogenous leukaemia cell line using the MTT assay. This assay is based on the reduction of the yellow coloured 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) by mitochondrial dehydrogenases of metabolically active cells to a purple-blue formazan, as detailed by Edmondson *et al.*[4] The IC_{50} concentration was calculated with reference to a standard curve constructed for control cells and represents the concentration which results in a 50% decrease in cell growth after five days incubation.

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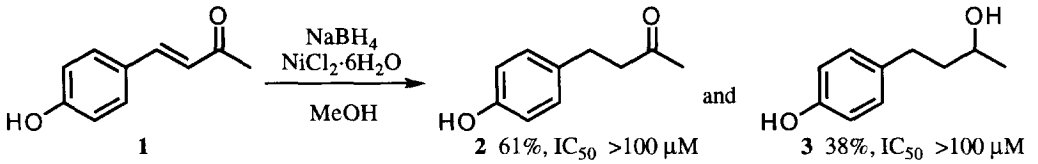
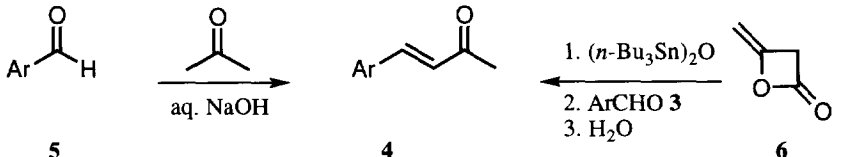
We first reduced the natural product itself, prepared in large quantity by the method of Donnelly and Higuchi,[5] using sodium borohydride in the presence of nickel(II) chloride (scheme 1).[6] This method usually gives high levels of conjugate reduction, however probably due to the use of excess sodium borohydride we isolated a mixture of **2** and **3** corresponding to both 1,2 and 1,4-reduction. Neither of these compounds inhibited cell growth indicating that the olefinic bond is a key feature that, in part, determines antitumour activity.



1 **2** 61%, IC_{50} >100 μM **3** 38%, IC_{50} >100 μM

Scheme 1

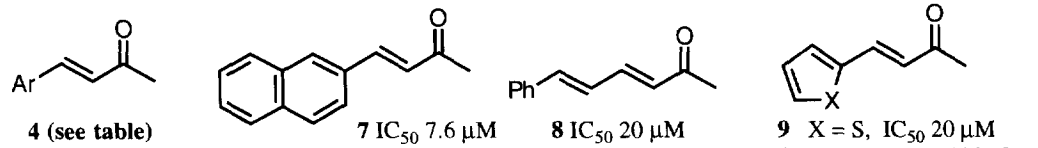
We therefore prepared a series of substituted *trans*-phenylbutenones **4** by the Claisen Schmidt based catalysed aldol condensation of the appropriate substituted benzaldehyde **5** with acetone. This was most conveniently achieved using sodium hydroxide as the base (Scheme 2, method A[7]). Alternatively when this method failed to provide the α,β-unsaturated ketone we used the method of Shibata *et al.*[8] whereby diketene **6** is reacted with the substituted benzaldehyde **5** and bis(tri-*n*-butyltin)oxide (Scheme 2, method B[9]).



5 **Method A** **4** **Method B** **6**

Scheme 2

The isolated yields[10] of *trans*-phenylbutenones **4** obtained in this manner were only moderate but do not reflect any great inefficiency in the reactions. Loss of material was due to the need to perform lengthy chromatographic purification to ensure that all traces of tin-containing impurities were removed, as these would undoubtedly interfere with any measure of cytotoxicity. Both methods for the preparation of phenylbutenones **4** are particularly attractive since they specifically generate the (*E*)-isomer and use substituted benzaldehydes as the starting materials, a large number of which are commercially available and inexpensive. Inspection of the ^1H nmr spectra clearly indicated that the substituted phenylbutenones made by both methods A and B were both geometrically pure and were configured *trans* ($J_{H\alpha\text{-}H\beta}$ *ca.* 15-16 Hz).



4 (see table) **7** IC_{50} 7.6 μM **8** IC_{50} 20 μM **9** X = S, IC_{50} 20 μM **10** X = O, IC_{50} >100 μM

Scheme 3

The results of the cell growth inhibition of the substituted phenylbutenone (IC_{50} values in the K562 cell line) are shown in the table. It was gratifying to find that most of the analogues are more active than the lead compound **1**. Substitution on the phenyl ring markedly alters the growth inhibitory properties of the molecule. Electron withdrawing groups confer increased activity. This can be best demonstrated by comparing the nitro

substituted compounds (**4b** and **4c**, IC_{50} 2.6 μM) with the unsubstituted ring (**4v**, IC_{50} 17 μM). We then prepared the pentafluorophenyl substituted analogue, hoping that the electron deficient ring would lead to a similar level of growth inhibition. This hope was well founded as ketone **4a** has an IC_{50} of 1.9 μM clearly demonstrating the well appreciated value of substituting hydrogen with fluorine in drug candidates.[11] Indeed this material was the most active of the ketones screened. In general, the ketones that possessed electron donating groups in the *para* position **4x–4e'** were less active than the unsubstituted analogue **4v**. However, the trimethoxy substituted analogues **4p** (IC_{50} 9.3 μM) and **4u** (IC_{50} 17 μM) were found to be more active than the corresponding mono and di-methoxy substituted derivatives **4y** (IC_{50} 34 μM), **4x** (IC_{50} 30 μM) and **4d'** (IC_{50} 79 μM). In **4p** the two *ortho* groups probably force the ketone to adopt a conformation in which the olefin π-bond and the phenyl group are not fully co-planar. Additionally in **4u** the electron donating ability of the *para* methoxyl group is greatly diminished by the presence of the other two methoxyl substituents. Overall there appears to be a rough correlation between the electronic properties of the phenyl group and the value of the IC_{50}; the most active analogues have strongly electron withdrawing groups present on the aromatic ring. It is therefore tempting to speculate that the mode of action involves some type of alkylation possibly *via* a Michael addition which would be accelerated by electron withdrawing groups present on the ring. A similar Michael addition is thought to be the origin of the cytotoxicity of many naturally occurring sesquiterpene lactones that act *via* non-specific alkylation of enzymes.[12] Work to elucidate the mode of action of the most active analogues **4a-4d** is underway. The analogues **9** and **10** (Scheme 3) incorporating a thiophene and furan ring are both inactive. However, the naphthyl butenone **7** (IC_{50} 7.6 μM) showed a 3-fold improved activity over **4v**. The phenylhexadienone **8** was still active but only moderately so.

Table : Cell growth inhibitory properties of the phenylbutenones **4** against the K562 cell line.

4	Ar	Yield (%)‡	IC_{50}(μM)
a	C_6F_5	15 (B)	1.9
b	4-$NO_2C_6H_4$	65 (B)	2.6
c	3-$NO_2C_6H_4$	23 (B)	2.6
d	3-Br-4-FC_6H_3	9(B)	2.9
e	4-(F_3C)C_6H_4	†	3.3
f	3-(F_3C)C_6H_4	†	3.5
g	5-Br-2-FC_6H_3	20	5.3
h	4-BrC_6H_4	†	5.6
i	3,5-$(MeO)_2C_6H_3$	77 (A)	5.8
j	4-FC_6H_4	†	6.1
k	4-Br-2-FC_6H_3	26 (B)	7.4
l	4-MeC_6H_4	†	7.5
m	2,4-$Cl_2C_6H_3$	†	7.9
n	4-ClC_6H_4	†	8.3
o	3,4-$(BnO)_2C_6H_3$	69 (A)	8.4
p	2,4,6-$(MeO)_3C_6H_2$	82 (A)	9.3
q	2-OH-3-$(MeO)C_6H_3$	27 (A)	9.9
r	4-$(BnO)C_6H_4$	76 (A)	10.3
s	3,4-$(OCH_2O)C_6H_3$	82 (A)	12
t	4-$(MeS)C_6H_4$	26 (A)	15
u	3,4,5-$(MeO)_3C_6H_2$	64 (A)	17
v	C_6H_5	26 (A)	17
w	4-(^iPr)C_6H_4	16 (A)	21
x	2,4-$(MeO)_2C_6H_3$	72 (A)	30
y	3,4-$(MeO)_2C_6H_3$	63 (A)	34
z	3-HO-4-$(MeO)C_6H_3$	72 (A)	42
a'	4-HO-3,5-$(MeO)_2C_6H_2$	33 (A)	56
b'	4-HO-3-$(MeO)C_6H_3$	40 (A)	68
c'	4-(Me_2N)-2-$(MeO)C_6H_3$	29 (A)	68
d'	4-$MeOC_6H_4$	71 (A)	79
e'	4-(Me_2N)C_6H_4	72 (A)	90

‡ method; † commercially available (Maybridge Chemical Company)

Although previous structure-activity relationships of a series of phenylbutenones have shown that these compounds possess anti-inflammatory[13,14] and antibacterial[15] activity and can scavenge both superoxide and hydroxyl radicals,[16] this is the first report of the promising antitumour activity of such a simple class of compounds.

Acknowledgements

We thank the Cancer Research Campaign and the Chemistry Department of UMIST for support of this work and Sally Haran and Tim Ward of the PICR Cell Culture Unit for maintaining the cell line.

References and Notes

1 *'Human medicinal agents from plants'*, eds. Kinghorn, A. D.; Balandrin, M. F.; ACS symposium series, 534, American Chemical Society, Washington, DC, **1993**.

2 Qian, B. *Clinical Effects of Anticancer Chinese Medicine*, Shanghai Translation Publishing House, **1987**.

3 Ducki, S.; Hadfield, J. A.; Lawrence, N. J.; Liu, C.-Y.; McGown, A. T.; Zhang, X. G. *Planta Medica*, **1996**, *62*, 185-186.

4 Edmondson, J. M.; Armstrong, L. S.; Martinez, A. O. *J. Tissue Culture Methods*, **1988**, *11*, 15-17.

5 Higuchi, R., Donnelly, D. M. X. *Phytochemistry*, **1977**, *16*, 1587-1590.

6 Abe, N.; Fujisaki, F.; Sumoto, K.; Miyano, S. *Chem. Pharm. Bull.*, **1991**, *39*, 1167-1170; Dhawan, D.; Grover, S. K. *Synth. Commun.*, **1992**, *22*, 2405-2409.

7 **Method A:** A mixture of the substituted benzaldehyde **5** (10 mmol), acetone (20 ml) and 10% aqueous sodium hydroxide (20 ml) was left to stand at ambient temperature overnight. Water was added and the mixture was extracted with chloroform, dried over anhydrous magnesium sulfate, filtered, evaporated *in vacuo* and recrystallised to give the *trans*-phenylbutenone **4**.

8 Shibata, I.; Nishio, M.; Baba, A.; Matsuda, H. *Chem. Lett.*, **1993**, 1219-1222.

9 **Method B:** Diketene (2 mmol) **6** and bis-(tri-*n*-butyltin)oxide (2 mmol) were stirred in dry THF (2 ml) under a nitrogen atmosphere at 0 °C for 15 mins. The substituted benzaldehyde **5** (2 mmol) in dry THF (2 ml) and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1)-pyrimidinone (2 mmol) were added and the mixture was stirred for a further 4 hours at 40 °C. Aqueous methanol was added and the mixture was evaporated. The residue was purified by chromatography (silica) and recrystallised from hexane to give the *trans*-phenylbutenone **4**.

10 All compounds were fully characterised and gave the expected spectral and analytical data; Microanalysis confirmed their chemical purity (values for C and H within 0.3%).

11 *'Fluorine in Medicine in the 21st Century'*, Conference papers, UMIST, UK, April 18-21, **1994**.

12 Beekman, A. C.; Woerdenbag, H. J.; van Uden, W.; Pras, N.; Konings, A. W. T.; Wikström, H. V.; and Schmidt, T. J. *J. Nat. Prod.*, **1997**, *60*, 252-257.

13 Elias, G.; Roa, M. N. A. *Eur. J. Med. Chem.*, **1988**, *23*, 379-380.

14 Jagetia, G. C., Jacob, P. S., Rao, M. N. A. *Mutation Research*, **1994**, *306*, 71-80.

15 Elias, G.; Roa, M. N. A. *Ind. J. Pharm. Sci.*, **1988**, *50*, 339-341.

16 Saldanha, L. A.; Elias, G.; Rao, M. N. A. *Arzneim.-Forsch./Drug Res.*, **1990**, *40*, 89-91.

(*Received in Belgium* 15 *September* 1997; *accepted* 3 *November* 1997)