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β′-Hydroxy-α,β-unsaturated ketones: A new pharmacophore for the design of anticancer drugs

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Abstract—A series of β′-hydroxy-α,β-unsaturated ketones were prepared by means of an iron(III) catalyzed domino process. The in vitro antiproliferative activities were examined in the human solid tumor cell lines A2780, SW1573, and WiDr. The results showed that β′-hydroxy-α,β-unsaturated ketones were more potent than α,β-unsaturated ketones. The best activity profiles were obtained for the derivatives bearing cyclic or branched substituents on the side chains.

The β′-hydroxy-α,β-unsaturated ketone fragment is a common structural feature present in diverse natural products such as the *Alnus firma* ketol yashabushiketol (**1**),[1] persenones A (**2**) and B (**3**) from avocado (*Persea americana*),[2] or the *Streptomyces* produced antifungal macrocyclic antibiotic 3874 H3 (Fig. 1).[3] Persenones A (**2**) and B (**3**) are unique antioxidants that preferentially suppress radical generation. In particular, persenone A (**2**) is an effective inhibitor of both nitric oxide and superoxide generation in cell culture systems.[4] Persenone A (**2**) at a concentration of 20 μM almost completely suppressed both inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) protein expression.[5] In a parallel study, two important structural findings for the activity were disclosed.[6] The double bond conjugated to the carbonyl group showed an important factor for the activity. To the contrary, the absolute configuration of the secondary hydroxyl group was not a critical factor. Thus, persenones A (**2**) and B (**3**) appear as possible food agents to prevent diseases such as cancer.[7]

Due to our interest in the discovery of novel pharmacophores for the development of anticancer compounds, we explored the possibility of β′-hydroxy-α,β-unsaturated ketones as antitumor agents.

In this article, we present a systematic study on the synthesis and growth inhibitory activity of diverse α,β-unsaturated ketone derivatives obtained through iron(III) chloride catalysis. As a model system to study the biological activity, the representative human solid tumor cells A2780 (ovarian cancer), SW1573 (non-small cell

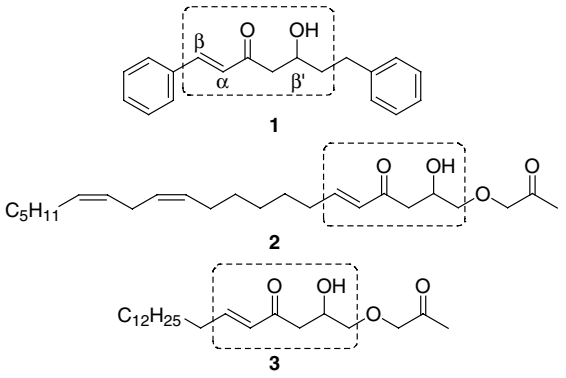

Figure 1. Structures of natural products with a β′-hydroxy-α, β-unsaturated ketone fragment.

Keywords: Unsaturated ketones; Solid tumors; Drug design; Structure–activity relationships; Anticancer drugs.
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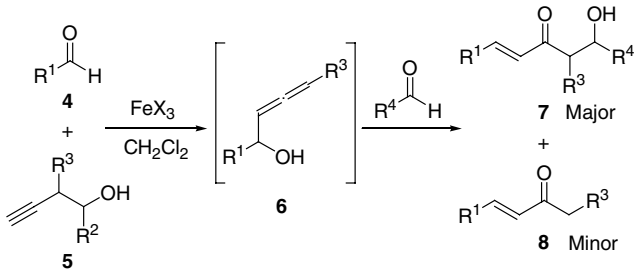
lung cancer, NSCLC), and WiDr (colon cancer) were selected. A structure–activity relationship is also discussed.

We have reported recently a novel 2-oxonia-[3,3]-sigmatropic rearrangement as competitive alternative pathway to the alkyne Prins cyclization resulting in the addition of secondary homopropargylic alcohols **4** to aldehydes **5** catalyzed by iron(III).[8] The procedure is depicted in Scheme 1. This method represented an alternative to the previously reported procedures employing vanadium[9] and indium,[10] which required the preparation of the appropriate allenol **6**.

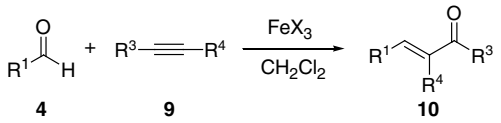
The method tolerated a wide range of aliphatic and aromatic aldehydes. The process produced in moderate to good yield (40–70%) a mixture of derivatives **7** and **8**. The β′-hydroxy-α,β-unsaturated ketone **7** was the major product. With the exception of the Lewis acid catalyst, any attempt to control the final balance of the obtained products (**7**:**8**) by changing experimental conditions was fruitless. Thus, when iron(III) bromide was used instead of the usual iron(III) chloride the process became completely chemoselective, being the β′-hydroxy-α,β-unsaturated ketone **7** obtained as a single product.

If this reaction were run with two diverse aldehydes (R^1 and R^4 different) with varying reactivity, a cross-over domino process took place.[8] Similarly, the nature of the Lewis acid catalyst is key for the outcome of the reaction. When iron(III) bromide was used as the catalyst only the β′-hydroxy-α,β-unsaturated ketone **7** was obtained, although in modest yields (**7g**, 25% yield). However, we should keep in mind that in this domino process, three consecutive chemical events take place in one-pot reaction with an average yield of 70–80%. Overall, these domino processes run in a regioselective and efficient manner. Domino processes have received great attention from the chemical community because they address fundamental principles of synthetic efficiency and reaction processing.[11]

An additional functional-diversity point on the synthesis of α,β-unsaturated ketones was obtained by the iron(III) promoted stereoselective coupling of alkynes and aldehydes (Scheme 2).[12] The method allowed us to obtain ketones of the general structure **10** with diverse substituents at the α vinylic position (R^4). The reaction worked well with both aliphatic and aromatic alkynes, whilst



Scheme 1. Iron(III) promoted coupling of homopropargylic alcohols and aldehydes.



Scheme 2. Iron(III) promoted coupling of alkynes and aldehydes.

aromatic aldehydes did not react. The coupling between terminal alkynes and aliphatic aldehydes led to a mixture of (*E*,*Z*)-1,5-dihalo-1,4-dienes and disubstituted (*E*)-α,β-unsaturated ketones **10**.

On the other hand, the reaction of non-terminal aromatic acetylenes **9** with aldehydes gave the trisubstituted (*E*)-α,β-unsaturated ketones **10** as the exclusive product. Thus, derivatives **10b**, **10d**, and **10e** were obtained in 80%, 68%, and 65% yield, respectively. The procedure was not valid for aliphatic and unsaturated skipped alkynes since mixtures were produced. To the contrary, the procedure was compatible with homopropargylic alcohol although modest yields were obtained (**10c**, 33% yield).

The series of 16 products reported in this study is shown in Table 1.[13] Compounds **7a**–**i** and **8a**–**b** were obtained according to the method shown in Scheme 1. The remaining derivatives **10a**–**e** were synthesized via the strategy depicted in Scheme 2.

The lipophilicities of this series of compounds were calculated to correlate their values with the antitumor activity. In this study, lipophilicity is given as *C*log *P* and the values (Table 1) were calculated using the computer program *C*log *P*®.[14] This program is designed to determine the partition coefficient of the non-ionized form of a given compound. In a recent comparative study, *C*log *P*® appeared the most accurate predictor of *C*log *P* values.[15]

In addition to lipophilicity, the in vitro anticancer activity was evaluated using the National Cancer Institute (NCI) protocol.[16] In this method, for each drug a dose–response curve is generated and three levels of effect can be calculated, when possible. The effect is defined as percentage of growth (PG), where 50% growth inhibition (GI_{50}), total growth inhibition (TGI) and 50 % cell killing (LC_{50}) represent the drug concentration at which PG is +50, 0, and −50, respectively.

We screened growth inhibition and cytotoxicity against the human solid tumor cell lines A2780, SW1573, and WiDr after 48 h of drug exposure using the sulforhodamine B (SRB) assay.[17] The resulting biological activities for each compound expressed as GI_{50} are reported in Table 1.

The *C*log *P* values obtained for the majority of compounds were in the range 2.63–5.52. Only derivative **7c** showed a larger *C*log *P* value of 9.46. On the other hand, the growth inhibition results allowed us to classify the compounds in three groups according to their anticancer activity profile.

Table 1. Lipophilicity and in vitro antiproliferative activity against human solid tumor cells[a]

Compound	*C*log *P*[b]	Substitutent			Cell line		
		R^1	R^3	R^4	A2780	SW1573	WiDr
7a	5.52	*n*-Hex	H	*n*-Hex	26 (±2.0)	25 (±4.2)	31 (±6.2)
7b	4.47	*c*-Hex	H	*c*-Hex	1.8 (±1.0)	3.1 (±0.7)	2.9 (±1.5)
7c	9.46	$(CH_2)_{10}Br$	H	$(CH_2)_{10}Br$	16 (±2.0)	30 (±6.4)	32 (±8.0)
7d	3.14	*i*-Bu	H	*i*-Bu	17 (±1.8)	27 (±4.9)	21 (±1.0)
7e	2.88	*t*-Bu	H	*t*-Bu	9.9 (±4.8)	26 (±4.1)	59 (±14)
7f	3.01	Ph	H	Ph	3.3 (±1.8)	4.3 (±1.3)	14 (±0.8)
7g	2.95	Ph	H	*i*-Bu	2.6 (±0.7)	13 (±3.4)	16 (±6.3)
7h	3.45	*p*-MePh	H	*i*-Bu	3.9 (±2.5)	12 (±5.8)	15 (±5.0)
7i	4.12	*p*-BrPh	Me	*i*-Bu	5.5 (±3.6)	11 (±2.1)	12 (±5.3)
8a	3.16	*n*-Hex	H		91 (±13)	79 (±37)	56 (±38)
8b	2.63	*c*-Hex	H		21 (±7.4)	30 (±4.0)	23 (±4.1)
10a	4.21	*i*-Bu	Ph	H	20 (±3.1)	31 (±1.1)	22 (±11)
10b	4.52	*i*-Bu	Ph	Me	>100	>100	>100
10c	3.08	*i*-Bu	Ph	CH_2OH	18 (±2.5)	24 (±3.3)	19 (±8.7)
10d	5.24	*i*-Bu	Ph	Ph	21 (±6.2)	33 (±4.0)	21 (±8.8)
10e	5.18	*c*-Hex	Ph	Me	28 (±4.5)	36 (±5.7)	41 (±25)

[a] Values representing GI_{50} are given in μM and are means of two to four experiments, standard deviation given in parentheses.
[b] Ref. 14.

A first group is comprised of compounds **7b** and **7f–i**. These products gave GI_{50} values in the range 1.8–5.5, 2.8–13, and 2.9–16 μM against A2780, SW1573, and WiDr cells, respectively. The most potent compound evaluated was **7b** that showed GI_{50} values in the low micromolar range against all cell lines (Table 1). The activity profile of this derivative was similar in the three cancer cell lines. This is an interesting result, since conventional and investigational anticancer drugs showed that colon cancer cells were more drug resistant than ovarian cancer cells.[18] The set of derivatives **7a**, **7c–e**, **8b**, **10a**, and **10c–e** exhibited a slightly decreased activity. The GI_{50} values for these products were in the range 16–59 μM. Finally, compounds **8a** and **10b** were included in the inactive group. The derivatives demonstrated a significant decrease in activity as shown by the GI_{50} values (GI_{50} >50 μM).

The observation that the vast majority of the derivatives evaluated in this study present antitumor activity in all cell lines is consistent with our hypothesis considering the α,β-unsaturated ketone fragment as a privileged scaffold with the substituents modulating the biological activity. In view of these results, it appears that the in vitro biological activity of these α,β-unsaturated ketones does not correlate with the calculated *C*log *P* values. However, the substitution pattern and steric hindrance in both side chains are important.

Analysis of the obtained dose–response parameters provides the following structure–activity relationships. In general, those compounds having a β′-hydroxy-α,β-unsaturated ketone fragment (**7a–i**, **10c**) showed superior activity than those with only the α,β-unsaturated ketone fragment (**8a–b**, **10a–b**, and **10d–e**). The most potent derivative **7b** bears two cyclohexyl groups. Compounds with cyclic (R^1 and R^4 = *c*-Hex **7b**, Ph **7f**) or branched (R^1 and R^4 = *t*-Bu **7e**) substituents on the side chains were more active than the corresponding linear derivatives (R^1 and R^4 = *n*-Hex **7a**, $(CH_2)_{10}Br$ **7c**, and *i*-Bu **7d**). The antiproliferative activities were in the order *c*-Hex > Ph > *t*-Bu > *n*-alkyl ≈ *i*-Bu. For the cross-over products **7g–i** no significant difference in activity was observed with respect to the parent derivative **7f** against ovarian and colon cells. However, the NSCLC cell line was more sensitive to **7f** than to **7g–i**.

Although the experiments are preliminary, we found that these synthetic derivatives induce considerably growth inhibition in a panel of three diverse human solid tumor cells and did not show cytotoxicity at the maximum concentration test. Therefore, these β′-hydroxy-α,β-unsaturated ketones may be considered cytostatic drugs. Cytostatic drugs are interesting in antiangiogenic therapy because they do not kill cells in the same way that cytotoxic drugs do; rather, they prevent them from growing and dividing. Targeting angiogenesis is radically different from conventional chemotherapy and represents a changing view on the curability of cancer that may completely revolutionize cancer treatment.

In conclusion, we have prepared a series of α,β-unsaturated ketones and evaluated their ability to inhibit tumor cell growth. The results show that β′-hydroxy-α,β-unsaturated ketones are promising scaffolds for the design of new anticancer drugs. Based on these results, it is anticipated that these compounds will be active against both sensitive and resistant solid tumors.

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