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Synthesis of a novel series of 2,3,4-trisubstituted oxazolidines designed by isosteric replacement or rigidification of the structure and cytotoxic evaluation

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We have previously reported on a study of structure-activity relationship in a series of 2,3,4-substituted oxazolidines recently discovered by our group varying the substituent at ring or stereochemistry of the oxazolidine ring. We discovered the cytotoxic and pro-apoptotic potential of compounds 1-2 with a good selectivity against cancer cell lines. In the present study we describe the synthesis and cytotoxic evaluation against cancer cell lines (HL60, JURKAT, MDA-MB-231 and LNCaP) of a series of oxazolidines designed by isosteric replacement or rigidification of the oxymethylene spacer of compounds 1-2. Alkenes 3-4 retained the activity against MDA-MB-231 cells and they were more active on HL60, JURKAT and LNCaP cells. Concerning LNCaP cells, E-isomer 4 was at least 7 times and about 3 times more potent than lead 1 and Z-isomer 3, respectively. Compound 4 exerted significant activity against LNCaP with IC$_{50}$ in low micromolar range (11 µM) without affecting VERO cells and PBMC proliferation (IC$_{50}>100$ µM) indicating low toxicity to normal cells.

Introduction

Cancer is the leading cause of death worldwide. In addition, the number of new cases is increasing mainly due to population growth and aging. Breast and prostate cancer are the most common types that affect women and men, respectively. As the successful cancer treatment remains a challenging goal, research into novel, selective and less toxic chemotherapeutic agents is gathering pace. There is a great need to develop alternative and more effective therapies to improve both life expectancy and quality of patient’s life.

As a part of an ongoing project aimed at the development of new anticancer compounds we have previously reported a study of structure activity relationships in a series of 2,3,4-trisubstituted oxazolidines recently discovered by our group (Fig. 1). In this study, we prepared 25 compounds to evaluate the importance of ring substituent and stereochemistry of oxazolidine on the antiproliferative activity against cancer cell lines.

It was observed that hydrophobic and electron withdrawing COOCH$_3$ or NO$_2$ group is important for the activity of this class of compounds. The unsubstituted compounds (X = H) were inactive against cancer cell lines. The presence of hydrophilic and electron withdrawing COOH or hydrophobic and electron donor OMe results in poor activity. Regarding the substituent position, a substituent at 3 or 4-position is important for the activity. All ortho-substituted compounds were inactive. The S isomers were generally more active than their enantiomers. In some cases, S isomers were 10 times more potent. Finally, it was not observed significant activity difference between S isomers compounds bearing COOCH$_3$ and NO$_2$ at 3 or 4-position. However, para-substituted compounds appear to be more selective than meta against cancer cells. With this in mind, we decided to carry out further study to evaluate the importance of the oxymethylene spacer between benzene and oxazolidine rings of compounds 1 or 2 in order to obtain more potent and selective compounds (Fig. 2). This series was planned by rigidification of the structure (compounds 3-5) or isosteric replacement (compounds 6-7). In most cases NO$_2$ was chosen as X group due to its intrinsic stability and synthetic viability. In the case of compound 6, the chosen synthetic route demanded COOCH$_3$ group.

Preliminary mechanism of action evaluation showed that compounds 1 and 2 were able to induce DNA fragmentation at 50 µM in HL60 cells. In the case of compound 1, about 90% of cells had fragmented DNA while compound 2 led to DNA fragmentation in about 40% of cells. This indicated that compound 1 has pro-apoptotic potential. Although the molecular
target was not identified, it is an important finding because *apoptosis* is one of the most important pathways used to discover new anticancer drugs.\(^{14,15}\) Despite different mechanisms of action, several important currently marketed anticancer drugs are able to trigger *apoptosis* in cancer cells (i.e. cisplatin and doxorubicin).\(^{16,17}\) Thus, it’s expected that compounds that modulate this pathway are promising hit compounds for the development of new anticancer drugs. So, we intended to evaluate in this study the cytotoxicity of this new series of oxazolidines against cancer cell lines (HL60, JURKAT, MDA-MB-231 and LNCaP) and the pro-apoptotic potential of the most potent compounds.

### Results and discussion

The strategy for the synthesis of olefins 3-4 was based on the retrosynthetic analysis shown in Fig. 3. The disconnection of double bond into two fragments offers two possibilities. In strategy 1, the new phosphonium salt 8 and 4-nitrobenzaldehyde 9 are the potential precursors, while in strategy 2 the disconnection furnishes the known Garner’s aldehyde 10 and 4-nitrobenzyl phosphonium salt 11. Take into account that generally substituted benzaldehydes are cheap commercially available compounds and substituted benzyl phosphonium salts are expensive or not available, it seemed reasonable to adopt strategy 1. Thus, we aimed to prepare the novel compound 8.

Initially, the key intermediate alcohol 15 was prepared as previously reported (Fig. 4).\(^{13}\) In brief, commercially available \(\text{D}-\text{serine}\) 12 was protected with \(\text{tert}\)-butoxycarbonyl and carboxylic acid was converted into methyl ester by treatment with methyl iodide and potassium carbonate to give 13 in 78\% overall yield.\(^{18,19}\) Next, acetonide formation of 13 was carried out using 2,2-dimethoxypropane (DMP) and BF\(_3\)OEt\(_2\) to afford acetonide 14 in 77\% yield.\(^{20}\) Lastly, methyl ester of acetonide 14 was reduced to alcohol 15 using NaBH\(_4\) in 85\% yield.\(^{23}\)

With 15 in hand, we proceeded to the synthesis of compound 8. Treatment of alcohol 15 with imidazole, iodine and PPh\(_3\) gave 16 in 61\% yield.\(^{22}\) Unfortunately, this modest yield was obtained only using 200 mg of starting material. When we scaled up to 400 mg, the yield decreases to about 45\%. This could be explained by the instability of compound 16. It was observed that storage of 16 at room temperature lead to the formation of degradation products. Next, compound 16 was reacted with PPh\(_3\) in toluene at 90 \(^\circ\)C.\(^{23}\) Usually, phosphonium salts precipitate during the reaction. In this case, precipitate formation was not observed. Indeed, TLC on silica gel revealed large amount of starting material after 24 h and formation of at least 3 polar byproducts. It is possible that steric hindrance at electrophilic carbon of 16 (CH\(_2\)I) results in poor reactivity. Thus, we decided to abandon our initial synthetic strategy and proceed to the second strategy. Our initial focus was on the synthesis of known Garner’s aldehyde 10. Treatment of 14 with DIBAL under classical Garner conditions gave 10 in only 40\% yield.\(^{19}\) We recovered 40\% of starting material using this method. Besides, small amount of alcohol 15 (8\%) was obtained. Unfortunately, aldehyde 10 and ester 14 had very similar \(R_f\) values using many solvent mixtures. Thus, it was difficult to purify the desirable product 10. We tried to increase the yield by using excess DIBAL and adding it very slowly but in all the cases we obtained similar yields. In order to prepare 10 in good yield, a second method was carried out. Alcohol 15 was oxidized under Swern conditions to give 10 in virtually quantitative yield.\(^{24}\) Compound 10 was obtained in good purity with this method without using column chromatography. Next, we prepared benzyl phosphonium salt 11 (Fig. 5).
Fig. 4 Preparation of key intermediates. Reagents and conditions: a) Boc₂O, NaOH, t-BuOH/H₂O (1:1); b) CH₃I, K₂CO₃, DMF; c) DMP, BF₃·OEt₂, acetone; d) NaBH₄, THF/MeOH (7:3), 0 ºC → reflux; e) Imidazole, I₂, PPh₃, toluene; f) PPh₃, toluene, 90 ºC; g) DIBAL, Toluene, -78 ºC; h) DMSO, (COCl)₂, Et₃N, CH₂Cl₂, -78 ºC → 0 ºC; i) LiOH, acetone/H₂O (7:3).

Fig. 5 Preparation of olefins 3, 4 and 21. Reagents and conditions: a) NaBH₄, THF/MeOH (7:3), -15 ºC → r. t.; b) Imidazole, I₂, PPh₃, toluene; c) PPh₃, toluene; d) I- BuLi 1.6 M in hexanes, THF; II-10 in THF.

Benzaldehyde 17 was reduced to benzyl alcohol 18 using NaBH₄ in 88% yield. Next, benzyl alcohol 18 was converted into iodide 19 by treatment with imidazole, iodine and PPh₃ in 78% yield. Treatment of iodide 19 with PPh₃ in toluene gave phosphonium salt 11 in 90% yield. With 10 and 11 in hand, we were finally in conditions to prepare olefins 3 and 4. Treatment of phosphonium salt 11 with BuLi followed by addition of Garner’s aldehyde 10 gave olefins 3 and 4 in 70% yield as isomer mixture which were separated by preparative TLC (Z/E 6:4). Some controversy about coupling constants (J) in ¹H NMR spectra was found in early-published studies. E-olefin unsubstituted (X = H) with R configuration was previously reported in the literature. Pellicciari’s group reported that JCH=CH was 15.8 Hz for this compound while Raghavan’s group found 12.2 Hz. In our case, JCH=CH for E- and Z-olefin (4 and 3, X = NO₂, S isomer) were 16.0 and 11.6 Hz, respectively (Fig. 6). These values are similar to the reported by Pellicciari’s group.

In order to prepare amide analogue 5, initially methyl ester of acetonide 14 was hydrolyzed in alkaline condition to give 20 in 95% yield (Fig. 4). Initial attempt to couple 20 with 4-nitroaniline to give 5 was carried out using NHS and EDC. Unfortunately, this mild and useful method did not lead to product formation. We believe that delocalization of nonbonding electron pair in 4-nitroaniline results in low nucleophilicity. Thus, we employed a better electrophile to accomplish this reaction. Carboxylic acid of 20 was activated with benzyl chloroformate and triethylamine followed by addition of 4-nitroaniline to give 5 in 57% yield (Fig. 7). Compound 6 was synthesized from Z/E olefin mixture 21. Initially, this mixture was prepared from phosphonium salt 25 and Garner’s aldehyde 10 under Wittig conditions in 68% yield (Fig. 5). Isomer mixture 21 was resistant to reduction with H₂ and catalytic Pd-C at room temperature and 1 atm. After testing different parameters including Pd-C ratio, temperature and pressure, we found that this reduction could be carried out using 1:1 Pd-C (10% w/w)/substrate at 55 ºC and 55 atm to give 6 in...
**Fig. 6** Expansion of $^1$H NMR spectrum (400 MHz, CDCl$_3$, 45 ºC) of olefins (3-4). Olefin protons (CH=CH) were shown. **A** - E-olefin spectrum. **B** - Z-olefin spectrum.

**Fig. 7** Preparation of compounds 5, 6 and 7. Reagents and conditions: a) I - Benzyl chloroformate, Et$_3$N, CH$_2$Cl$_2$, -15 ºC; II - 4-Nitroaniline, -15 ºC → r. t.; b) H$_2$, Pd-C, THF, 55 ºC, 55 atm; c) 4-Nitrothiophenol, PPh$_3$, DIAD, toluene, 80 ºC.

50% yield (Fig. 7). Finally, we prepared sulfur isoster 7 (Fig. 7). Treatment of alcohol 15 with 4-nitrothiophenol under Mitsunobu conditions provided sulfur isoster in 31% yield.

The antiproliferative activity of compounds 1-7 was assessed on four human cancer cells lines, namely, HL60 promyelocytic leukemia, JURKAT T cell leukemia, MDA-MB-231 breast carcinoma cells and LNCaP prostate adenocarcinoma cells.

Cytotoxic effects on normal cells were evaluated using VERO African green monkey kidney cells and PBMC peripheral blood mononuclear cells. The results are summarized in Table 1 and expressed as the concentration of drug inhibiting cell growth by 50% (IC$_{50}$).

Olefins 3-4 were equally potent than lead 1 against MDA-MB231 cells. However, 3-4 were more active than 1 against HL60, JURKAT and LNCaP cells. Concerning LNCaP cell line (which is a cell line derived from a lymph node metastasis of a prostate adenocarcinoma), compounds 3 and 4 were the most active (IC$_{50}$ = 27 and 11 µM) in this series. It is worth noting that lead compounds were inactive against this line (IC$_{50}$ >80 µM) and E-olefin 4 was at least 7 times more potent than lead 1. Besides, prostate is the most common cancer in men, thus there is a great need to develop new alternatives for this cancer type. In view of our previous results on the importance of the aromatic ring for the potency of this class, it was expected that alkenes E and Z would have different activity against cancer cells since ring is positioned in opposite sides in these isomers. However, this effect was noted only on LNCaP cells. In this case, rigidification of the structure using double bond spacer enhances the activity. E-isomer was about 3 times more potent than Z-isomer. Other interesting fact is the great selectivity for cancer cells.
showed good selectivity for these cancer cell lines with selectivity index of > 9 for LNCaP cells. Furthermore, 4 possesses drug-like physicochemical properties (MW = 348, 7 H-acceptor, 0 H-donors, 5 freely rotatable bonds, clogP = 4.6). The conformationally restricted amide 5 was inactive against all cancer cells. Probably, amide 5 adopts an unfavorable conformation to bind with molecular target.

Comparing compound 6 with lead 2, it was observed that 6 is less active against JURKAT and MDA-MB-231 cells and equally potent against HL60 cells. However, compound 6 showed a relevant activity against LNCaP cell line (IC_{50} = 37 µM) while lead 2 was inactive against this cell line (IC_{50} >80 µM). Thus, isosteric replacement of -O- with -CH_2- seems to be favorable on LNCaP cells. It appears that oxygen is not involved in H-bond and methylene fairly changes electron density of ring. This could partially explain the good results obtained with alkynes 3-4 on LNCaP cells. Finally, isosteric replacement with sulfur was not tolerated. Compound 7 was inactive against these four cell lines. The larger size of sulfur as compared to oxygen or oxidation at sulfur may be responsible for the inactivity of compound 7. This steric effect is in accordance with previous observations that ortho-substituted compounds are inactive.

Compounds 1, 3 and 4 were incubated at 50 µM with HL60 or MDA-MB231 cells. After 24 h, DNA fragmentation was evaluated (Fig. 8). Significant increases in DNA fragmentation were detected after treatment with all compounds at 50 µM in HL60 cells. However, corresponding increases were not observed in MDA-MB231 cells. Concerning HL60 cells, compound 1 induced more DNA fragmentation in comparison with 3 and 4. Thus, -OCH_2- spacer is favorable to enhance pro-apoptotic potential. In comparing isomers 3-4, isomer Z 3 had more pro-apoptotic potential than E 4 in this case. It’s possible that these compounds are involved in the same pathway for HL60 cells. Nevertheless, apoptotic pathway does not appear to be important in MDA-MB231 cells. Thus, apparently this class modulates more than one pathway.

**Conclusions**

In summary, we described herein the synthesis and cytotoxic evaluation of a series of analogues of chiral oxazolidine 1 and 2 designed by isosteric replacement or rigidification of the oxymethylene spacer. Introduction of double bond was well tolerated in almost all cases. Alkene E 4 had a relevant activity.
against LNCaP with IC\textsubscript{50} value of 11 \textmu M without affecting Vero or PBMC cell proliferation. It was about 3 times more active than Z-isomer on this cancer cell line. Besides, compound 4 has drug-like physicochemical properties. Thus, compound 4 has potential for further development as an anticancer agent. Rigidification using amide or isosteric replacement did not enhance the activity of this class and will not be considered for further modifications.

Notes and references
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Graphical Abstract

Synthesis of a novel series of 2,3,4-trisubstituted oxazolidines designed by isosteric replacement or rigidification of the structure and cytotoxic evaluation

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Rigidification of the structure of 2,3,4-trisubstituted oxazolidines enhances the activity against LNCaP cells without affecting normal cells proliferation.