Towards resolving the resveratrol conundrum: Synthesis and in vivo pharmacokinetic evaluation of BCP-resveratrol

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ABSTRACT: Over the last few decades, resveratrol has gained significance due to its impressive array of biological activities; however, its true potential as a drug has been severely constrained by its poor bioavailability. Indeed, several studies have implicated this bioavailability trait as a major road-block to resveratrol’s potential clinical applications. To mitigate this pharmacokinetic issue, we envisioned a tactical bioisosteric modification of resveratrol to bicyclo[1.1.1]pentane (BCP) resveratrol. Relying on the beneficial bioisosteric potential demonstrated by the BCP-scaffold, we hypothesized that BCP-resveratrol would have an inherently better in vivo PK profile as compared to its natural counterpart. To validate such a hypothesis, it was necessary to secure a synthetic access to this novel structure. Herein we describe the first synthesis of BCP-resveratrol and disclose its PK properties.

Over the past two decades, resveratrol has become a topic of intense research in fields such as biology, chemistry and medical chemistry. Indeed, the compound has shown many biological activities including antioxidant, anticancer, antidiabetic, cardioprotective, and even antiaging properties. Importantly, these modern findings are in line with the traditional role of this compound, as an active principle, in Japanese and Chinese folk medicine used to treat ailments related to liver, skin, heart and lipid metabolism. However, despite this impressive array of biological and medicinal properties, poor bioavailability of resveratrol has severely restricted its application in human therapeutics. In fact, several studies on resveratrol, and its formulations, in different rodent animal species and humans have repeatedly emphasized on this fact. Clearly, a resolution to this pharmacokinetic issue may hold the key to unlock the true medicinal potential of resveratrol and thereby facilitate its clinical applications.

A closer analysis of the low bioavailability of resveratrol reveals that its low plasma concentrations after oral administration is an outcome of rapid first-pass metabolism to glucuronide and sulfate conjugates. An obvious, and reported, approach to optimize the absorption and metabolism properties of resveratrol can be seen in manipulation of its phenolic functional groups. However, literature implication of the 4-OH group on the ring A of resveratrol (1, see Figure 1) in inhibition and cell proliferation properties, warranted caution in designing of SAR studies. Indeed, the task of retaining the 4-OH group while eliminating the phenolic ring is challenging. To face this daunting task we decided to mandate the deployment of contemporary bioisostere tactics. Specifically, we were keen on deploying bicyclo[1.1.1]pentane (BCP) scaffold, a known bioisostere of 1,4-disubstituted aryl ring systems, as a replacement of the phenolic ring A in resveratrol (see Figure 1).

Our hypothesis was supported by strong literature precedents that allowed us to reasonably rationalize BCP-resveratrol (2) as a potential contender of the parent compound. On that premise, the broad range of biological activities showcased by resveratrol makes 2 a strong candidate for biological evaluation against all the successful targets reported for the natural product. However, we opined that performing an in vivo PK study on 2 first, to prove or disprove its in vivo PK advantage over 1, was essential to justify such an extensive effort. Additionally, given our previous experience with the synthesis of BCP derivatives, we were cognizant of the challenges offered by the non-trivial synthetic behavior of this uniquely strained scaffold. In this Letter, we now report the first synthesis of BCP derivative 2, and also disclose its PK studies.

Our retrosynthetic protocol for synthesis of BCP-resveratrol is shown in Scheme 1. We first dissected the target, 2, at the olefin to give us the aldehyde 4 as a reasonable starting point. In a forward sense, a Wittig reaction between the ylide 3 and 4 would generate the target compound. Next, we focused on the synthesis of 4 and envisioned it to originate from 9 via the application of classical reactions such as the Weinreb ketone synthesis, and the Baeyer Villiger rearrangement. Thus, the onward synthesis would commence with the formation of the Weinreb amide from commercially available 9 to generate 8. The amide in 8 could then be subjected to a stoichiometric amount of phenyl magnesium bromide to furnish the ketone 7. Baeyer Villiger oxidation of the latter would result in for-
mation of the bis ester 6 which in turn could be hydrolyzed to furnish 5. Reduction of the carboxylic acid in the latter would give the aldehyde 4.

Scheme 1. Retrosynthetic analysis of 2.

In line with our retrosynthetic plan, we could access the bis ester 6 in a straightforward fashion. However, despite several attempts, hydrolysis of 6 proved to be difficult and resulted in a complex mixture of decomposition products (see Scheme 2). To test the viability of sequential hydrolysis, we also attempted hydrolysis of the carboxylic acid 10. However, that attempt led to a similar outcome. It is possible that the hydroxide in intermediate I, by the virtue of the electron-withdrawing effect of the carbonyl function in 6 or 10, results in the ring-opening and subsequent disintegration of the BCP scaffold.

Scheme 2. Hydrolysis attempts on 6 and 10.

Reagents and conditions: (i) 1:1 N NaOH:EtOH, rt, 1 h.

Above results indicated a need for a partial modification of our original retrosynthetic plan. To that end, we planned to postpone the liberation of the hydroxyl function to the last step of our planned synthetic sequence. However, to endorse this late-stage modification, especially in the light of our failed hydrolysis attempts, we planned to study the deprotection of alcohol on a substrate with a non-carbonyl functional group at the 3-position of the BCP ring. To that end, the aryl-BCP-benzoate (12) was identified as an appropriate model substrate. The aryl derivative was promptly synthesized from 10 by first converting its carboxylic acid to perester 11, and then subjecting the latter to the homolytic aromatic substitution conditions to afford 12 (see Scheme 3).

Frustratingly, hydrolysis attempts on 12 resulted in decomposition. Fortunately however, we could secure the tertiary alcohol in 13 by treating 12 with MeLi.LiBr at a low temperature.

Equipped with a potential protocol to complete our endgame, we then ventured into the synthesis of BCP-resveratrol. Our sequence commenced with the reduction of the carboxylic acid in 9 to give 14. Next, the primary alcohol in 14 was concealed as a TBS ether, and the ester was subjected to basic hydrolysis conditions to expose the acid, and ultimately yield 15. The carboxylic acid in 15 was transformed into the ketone, 16, by employing the Weinreb protocol outlined above. Bayer Villiger oxidation of the ketone in 16, followed by TBAF promoted removal of the TBS group gave the primary alcohol 17. The latter could then be easily oxidized to the aldehyde 18. Notably, this reaction sequence could be repeated on gram scale to generate aldehyde 18 in an overall yield of 30%.

Scheme 4. Synthesis of the key intermediate 18.

Reactions and conditions: (i) Oxalyl chloride, cat. DMF, DCM, rt, 1 h; (ii) NaBH₄, MeCN, rt, 1 h; (iii) TBSCI, imidazole, DCM, rt, 2 h, 89%; (iv) NaOH, MeOH, 60 °C, 1 h, 81%; (v) CH₃NH₂.HCl, EDCI, cat. HOBt, DIMEA, DCM, rt, 16 h; (vi) 3 M PhMgBr in THF, THF, 0 °C to rt, 66% (two steps); (vii) m-CPBA (77%), DCM, 50 °C, 27 h, 85%; (viii) 1 M TBAF in THF, THF, rt, 2 h, 92%; (ix) Dess–Martin periodinane, rt, 1 h, 84%. EDCI = 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide, HOBt = 1-hydroxybenzotriazole, DIPEA = N,N-diisopropylethylamine.

The endgame of the synthesis commenced with the Wittig olefination of 18 with the phosphine salt to secure 19 as a mixture of predominantly E olefin (E/Z > 19:1 by ¹H NMR). Finally, by employing our conditions for removal of the benzoyl function, we secured the BCP-resveratrol (2) in a good yield of 80%.

Scheme 5. Completion of the synthesis of 2.

Reactions and conditions: (i) lithium 5-((triphenyl-l5-phosphanylidene)methyl)benzene-1,3-bis(olate), THF, -78 °C to 0 °C, 1.5 h, 31%; (ii) MeLi.LiBr in ether, THF, 0 °C to rt, 1.5 h, 80%.

Having BCP-resveratrol (2) in hand, we could experimentally determine, and compare, its key in vitro parameters such as thermodynamic solubility and lipophilicity with resveratrol (1). Thus, the thermodynamic solubility at pH 7.4 was found to be 619±64 µg/ml and 19±2 µg/ml for 2 and 1 respectively. Lipophilicity, measured as LogD (pH 7.4), was found to be 1.9 and 2.9 for 2 and 1 respectively. Interestingly, the trend of higher solubility and lower lipophilicity of 2 as compared to 1, was in line with Stepan’s seminal findings on the use of BCP.
Encouraged by these observations, we tested 1 and 2 against available cancer cell lines; MDA-MB-231 (a metastatic breast cancer cell line), MiaPaCa2 (Pancreatic cancer cell line), and SUDHL10 (B cell lymphoma cell line) (see Table 1). We were pleased to observe that both the compounds showed similar activities in all three cases.

**Table 1**: Biological activities of resveratrol and BCP-resveratrol in selected cancer cell lines.

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>% inhibition of cell proliferation at 30µM</th>
<th>CC₅₀ (µM)</th>
<th>Puromycin (reference compound)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA-MB-231</td>
<td>20</td>
<td>22</td>
<td>0.48</td>
</tr>
<tr>
<td>Mia-PaCa2</td>
<td>30</td>
<td>30</td>
<td>0.20</td>
</tr>
<tr>
<td>SU-DHL-10</td>
<td>46</td>
<td>41</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Next, we dedicated our attention to determining the PK properties of BCP-resveratrol. At that end, we were cognizant of a few studies on resveratrol wherein a sensitive bioanalytical method such as LC-MS/MS was employed. Based on this, we decided to perform in vivo PK studies of BCP-resveratrol in rat and then attempt the bioanalysis using LC-MS/MS. Our final task was to equate the outcome of our studies with the reported PK data on resveratrol. For this comparison, we identified the work reported by Asensi and co-workers as most suitable. Thus, during the course of their extensive investigations on the anti-cancer and PK properties of resveratrol, the authors have reported resveratrol to reach plasma levels of 273 ng/mL after oral administration at 20 mg/kg, with Tₘ₅ₐₓ at 5 min. Additionally, in a more recent study, Kapetanovic and co-workers carried out similar experiments in rats, albeit with a much higher dose of 50 mg/kg delivered every 24 h for 14 days. Interestingly, they registered a Cₘ₅ₐₓ of 76.7 ng/mL on day 1 and that of 176 ng/mL on day 14 prove that the plasma levels of 1 do not increase significantly despite repetitive dosing.

Pleasingly, our studies revealed that BCP-resveratrol achieved Cₘ₅ₐₓ of 942 ng/mL, a 3-fold increase over resveratrol; and an AUC₀-ₙₐₙ of 587 ng.h/mL, a 10-fold increase over the reported values for resveratrol (for PK parameters see Table 2). Moreover, whereas the plasma levels of resveratrol seemed to deplete beyond the scope of reasonable measurement after an hour in Asensi’s study, BCP-resveratrol concentration could be easily measured up to 4 h with the mean plasma concentrations observed (in three rats) to be 85 ng/mL at this time point.

**Table 2**: Comparison of PK parameters of BCP-resveratrol with reported values of resveratrol.

<table>
<thead>
<tr>
<th>PK parameter</th>
<th>BCP-resveratrol</th>
<th>Resveratrol (estimated from the published data)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cₘ₅ₐₓ (ng/mL)</td>
<td>942</td>
<td>273</td>
</tr>
<tr>
<td>Tₘ₅ₐₓ (h)</td>
<td>0.067</td>
<td>0.083</td>
</tr>
<tr>
<td>AUC₀-ₙₐₙ (ng.h/mL)</td>
<td>587</td>
<td>47.5</td>
</tr>
<tr>
<td>t₁/₂ (h)</td>
<td>2.6</td>
<td>0.19</td>
</tr>
<tr>
<td>CL/F (L/h/kg)</td>
<td>21.8</td>
<td>409</td>
</tr>
</tbody>
</table>

Mean Cₘ₅ₐₓ of BCP-resveratrol was 942 ng/mL, 5 minutes after oral administration of 20 mg/kg. Mean AUC₀-ₙₐₙ of BCP-resveratrol was 587 ng.h/mL.

Clearly, a comparison of the reported PK studies on resveratrol with our finding on BCP-resveratrol (see Figure 2) suggests that the latter has an unambiguous PK advantage, in both absorption and metabolic stability, over resveratrol.

Additionally, we also performed in vitro metabolic stability tests on both BCP-resveratrol and resveratrol (see Table 3). In human hepatocytes study we observed that the former had >3-fold higher metabolic stability. In accordance with our in vivo studies, BCP-resveratrol was seen to have significantly more metabolic stability than the natural product in rat hepatocytes.

**Table 3**: Metabolic stability studies, of resveratrol and BCP-resveratrol, in human and rat hepatocytes.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Human hepatocytes</th>
<th>Rat hepatocytes</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>t₁/₂ (min) Mean ± SD</td>
<td>CL₁,h, app (µl/min/10⁶ cells) Mean ± SD</td>
</tr>
<tr>
<td>BCP-resveratrol</td>
<td>90.4± 11.7</td>
<td>7.7 ± 1.0</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>23.2 ± 1.6</td>
<td>29.9 ± 2.1</td>
</tr>
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</table>

It is also worth reporting that formation of glucuronide and sulfate conjugates in human hepatocytes studies was significantly higher for resveratrol compared to BCP-resveratrol (Figure 3), thus confirming the efficiency of the phenyl ring replacement.
Given the aforementioned bioisoteric properties of BCP motif, it would be interesting to compare its applications in pharmaceutical and other fields. It is also worth mentioning that, the strategic disposition of the BCP motif in 2 and 23 can render these building-blocks, and the scaffolds employing them, the cogency to mitigate intellectual property and compound attrition related issues. Lastly, our in vivo PK studies on 2 strongly justify its biological testing in many of the targets that have shown promise for resveratrol. We hope that this report will initiate such enquiries.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

SYNTHESIS DETAILS, CHARACTERIZATION DATA FOR ALL NEW COMPOUNDS, AND PK PROTOCOLS (PDF)

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. Project leader, V. A; Synthetic chemists Y. L., Y. T., V. A; Biological studies V. P.

Notes

The authors declare no competing financial interest.

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(18) For experimental details see Supporting Information.


(23) While our manuscript was being reviewed by this journal, the synthesis of BCP derivative of tyrosine was reported; see: Auberson, Y. P.; Brocklehurst, C.; Furegati, M.; Fessard, T. C.; Koch, G.; Vecchia, G.; Briard, E. Improving nonspecific binding and solubility: Bicycloalkyl groups and cubanes as para-phenyl bioisosteres. ChemMedChem. DOI: 10.1002/cmdc.201700082.