A New Family of Rigid Dienone Musks Challenges the Perceptive Range of the Human Olfactory Receptor OR5AN1

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Dedicated to the memory of Professor Georg Fráter
(∗ 27 September 1941; † 25 June 2019)

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Abstract A new family of dienone musks was discovered by alkylation of different aldehydes with but-3-en-1-yn-1-yllithium and subsequent domino reaction of a Saucy–Marbet transfer vinylation–Claisen rear-

rangement with an intramolecular Diels–Alder reaction, and concluding Lewis acid catalyzed double-bond isomerization. The newly synthesized dienone structures possess pleasant musk odors displaying fatty, slightly fruity and green facets. Although the dienone musks were predicted in silico to bind to the OR5AN1 receptor based on QM/MM calculations, they were found to be inactive in the in vitro assay. The latter results suggest that the OR5AN1 receptor is not the prime musk receptor but primarily responsible for the animalic character of certain macrocyclic ketones and nitro musks.

Key words fragrances, musk odorants, odorant receptors, olfactory properties, structure–activity relationships

Like no other perfumery material, musks embody seductive sensuality, irresistible attraction and erogenous magnetism, attributes that define a perfume: Musks make us dream.1 Abstracted from the complex odor of Tonquin musk (Moschus moschiferus L.) tincture with its principle odorant (R)-(−)-muscone (1, Figure 1), musks come in many different molecular shapes and fragrant tonalities.2 However, in terms of odor, there are two main subfamilies: the animalic-powdery type with the nitro arene musk ketone (2) and (R)-(−)-muscone (1) being most representative, and the musky-floral type with the macrolide Thibetolide/Exaltolide (3) as well as the polycyclic aromatic musk (PCM) Galaxolide (4) as major representatives. The more recent fourth class of linear alicyclic musks, with Sylkolide (5)3 as example, also belongs to the musky-floral family. In principle, this is also true for the latest class of musk odorants that derived from 6 as principal lead structure discovered during derivatization of carotol. 4 This fifth class features a dienone motif with the terminal (E)-double bonds substituted by two bulky moieties, as exemplified in compounds 7–10.5,6 The steric bulk on the γ-carbon atom is most important for the muskiness, as demonstrated in 7,5 but the sila-derivative 11 in comparison with 7 indicated that the size of the TMS group already slightly exceeds the optimal dimensions of the binding pocket, as indicated by the in-
increased odor perception threshold (th). The γ,δ-double bond outweighs the contribution of the α,β-double bond, but both are indispensable for a musk odor sensation. Whereas linear alicyclic musks display fruity side notes in the direction of raspberry as in the case of Sylkolide (5) or pear for Helvetolide, the dienone musks tend towards green aspects as in 10 and 11 or earthy facets as manifest in the beetroot character of 9.

Since tandem sigmatropic rearrangements of vinyl propargyl systems with allenyl intramolecular Diels–Alder (IMDA) reactions offer facile access to tetrahydro-pyrones with allenylic IMDA. An Alder (IMDA) reaction that combines a propargyl Claisen rearrangement with an allenyl IMDA. As outlined in Scheme 1, 2,2,5-trimethyl-4-hexenal (13), easily available for instance by enolate alkylation of isobutyraldehyde with prenyl halides, was reacted with but-3-en-1-yn-1-yl lithium to provide the dienylene alcohol 15 in 96% yield. In the presence of trace amounts of p-toluensulfonic acid monohydrate, Saucy–Marbet reaction of 15 with isopropenyl methyl ether (16) then furnished in an efficient domino reaction consisting of [3,3]-sigmatropic rearrangement of 17 and subsequent IMDA of the allenyl intermediate 18, the 2,6,7,7a-tetrahydro-1H-inden-4-yl ketone 19 in 85% yield. This unconjugated dienone 19 was devoid of any musk character and possessed a woody-green odor with carrot-like earthy aspects instead. We were however much delighted to find the sought-after musk note upon isomerization of the dienone system of 19 with aluminum trichloride, which afforded in 51% yield the new conjugated dienone musk 12 with tetrasubstituted γ,δ-double bond and an encouraging threshold of 2.9 ng/L air.

Although slightly weaker than the representatives 6–10, this new musk 12 is with C16H24O (232.37 u) both heavier and more rigid in structure than these leads. Since the sequence delineated in Scheme 1 allowed for easy variation of the substitution patterns, we investigated the structure–odor correlation of this new family with the derivatives summarized in Figure 2.

While the gem-dimethyl cyclopentyl substituent of 9 is at a different position in 12, it is essential nonetheless: The nor structure 20 is greener and weaker (th 21 ng/L air) but still musky, and so is the ethyl derivative 21 (th 40 ng/L air), though it is less green. Interestingly, the isopropyl derivative 22 (th 25 ng/L air) is somewhat stronger again than the ethyl derivative 21, and this goes together with a pronounced green tonality. In contrast, the spiro[cyclohexane-1,2′-inden] system 23 definitely exceeds the boundaries of the musk receptor and thus, 23 is devoid of any musk char-

| Figure 1 | The five different classes of musk odorants: Macrocyclic ketones 1, nitro musks 2, macrocyclic lactones 3, polycyclic musks (PCM) 4, linear alicyclic musks 5, and the most recently discovered dienone musks 6–12 |

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acter. Its gem-diethyl seco-structure 24 is completely odorless even. Similarly, the additional methyl substituent on the cyclohexyl ring in 25 eradicated the muskiness of 12, and the compound smelled green, aromatic, and fruity in the direction of clary sage. Incorporating one cyclopentenyl methyl group as methylene unit into the ring enlarges the system to the 3,4,5,6,7,8-hexahydronaphthalen-1(2H)-ylidene, which is musky-green and comparable with 20–22 in terms of intensity. Adding another methyl group at C-7, however, culminated in the most intense dienone musk 27 of this new series, possessing a pleasant musk odor with fatty, slightly fruity and again green facets. With an odor threshold of 0.4 ng/L air it is almost as intense as the most potent dienone musk 9 (0.26 ng/L air) reported so far. Compound 28 finally demonstrates that the bulky substructures on the γ- and δ- carbon atom of the dienone backbone should be trans-configured, since it is, with its two adjacent gem-dimethyl groups in the γ,δ-annulating cyclopentenyl ring, completely odorless.

Since this new family of dienone musks 12 (2.9 ng/L air) and 20–27 is structurally rather rigid and conformationally confined, it should be ideally suited to characterize the spectrum of activity of the OR5AN1 receptor, which was considered to be the prime human musk receptor.13 Ahmed et al.14 had reported a positive correlation between EC50 values and calculated QM/MM binding energies of a series of musk odorants to OR5AN1. These binding energies were obtained from docking into a homology model of OR5AN1 followed by QM/MM optimization. We first reconstructed the published protocol with open-source tools, and validated it using the homology model and ten compounds from the original reference14 (for details see the Supporting Information). Our results reproduced the correlation between the QM/MM binding energies and the experimental EC50 values as observed previously14 (cf. the Supporting Information). Subsequently, we calculated the QM/MM binding energy of novel compounds 9, 12, 20–23, 26–27 by using the same protocol (Table 1). For all compounds, the important hydrogen bond with Tyr260 and the hydrophobic interactions with the surrounding Phe residues as described previously14 were established, and the resulting QM/MM binding energies were found to be in a similar range as reported for the known musk odorants.14 As an example, the top poses of the lead compound 9 and the new musk 12 are shown in Figure 3. Interestingly, although the two odorants display the same key interactions, the dienone moieties do not superimpose. This observation can be explained by the relatively large binding pocket, which allows for different binding modes to form a hydrogen bond with Tyr260 with only small differences in binding energy.

Based on the calculations, all dienone musks 9, 12, 20–23 and 26–27 were predicted to bind to the OR5AN1 musk receptor with QM/MM binding energies \( E_{\text{bind}} \) comparable to those of the reference musks reported previously.14 To test this prediction, the OR5AN1 receptor was expressed in HEK293 cells along with RTP15 and a luciferase reporter gene according to ref.14. While the results from the literature were confirmed, including the weak activation by Thibetolide/Exaltolide (3), no activation of OR5AN1 by the dienone musks 9, 12, 20–23, 26–27 (Table 1) was observed (only compounds >10% relative efficacy were considered activators). Since, however, the dienones do smell musky, other receptors must be involved. Recently, the OR5A2 receptor was reported to be activated by a diverse range of musk odorants,15 indicating that OR5A2 might be the prime musk receptor. However, when we transfected OR5A2 along with

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**Figure 2** Olfactory properties of the new dienone musks 20–28

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**Figure 3** Top binding poses (QM/MM geometry optimized) of compounds 9 (cyan) and 12 (yellow) with the homology model of OR5AN1. Key residues in the binding pocket (Tyr260, Phe105, Phe194, Phe207, Phe252) are shown in color. In addition, the hydrogen bond between the carbonyl group of the ligands and Tyr260 is indicated.
RTP1S and RTP2 into HEK293 cells, no activation of OR5A2 by ambrettolide, musk ketone, or muscone was observed, suggesting that OR5A2 is not functionally expressed in our cell system.

The QM/MM binding energies predicted that the dienone musks \(9, 12, 20-23, 26-27\) bind to OR5AN1 whereas the in vitro experiments showed no activation of this receptor with these compounds (Table 1). To elucidate the reasons behind this discrepancy, we calculated the binding energy of five additional odorants, which were reported previously to not activate OR5AN1.\(^{14}\) For all these inactive compounds, including Galaxolide (4; Table 1), the calculated energy values were again in a similar range to those of the active compounds; i.e., they would be predicted to be active (cf. Supporting Information). These results taken together indicate that the published computational workflow,\(^{14}\) which was validated only against active compounds, is not able to discriminate between odorants that activate OR5AN1 and those that do not. A discussion on the possible causes of this result and on the limitations of the computational workflow is included in the Supporting Information.

In summary, we have discovered a new family of dienone musks\(^{16-18}\) that does not activate the OR5AN1 receptor in vitro, although computational models predicted it would. Since only animalic-powdery musks such as ketones 1 and 2 activate OR5AN1 with significant efficacy, we believe this odorant receptor to be responsible for the animalic rather than the musk character. The prime musk receptor is then yet to be discovered and characterized, but the newly synthesized rigid dienone musks will help to shed light on which receptor that is.

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### Table 1  Comparison of Calculated QM/MM Binding Energy \(E_{\text{bind}}\) [kcal/mol] Profiles, Measured \(EC_{50}\) [\(\mu\text{M}\)], and Relative Efficacy [%] for the Human OR5AN1 Musk Receptor

<table>
<thead>
<tr>
<th>Compound</th>
<th>(E_{\text{bind}}) [kcal/mol] (OR5AN1)</th>
<th>(EC_{50}) (OR5AN1)(^a)</th>
<th>Relative efficacy [%] (OR5AN1)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 (Thibetolide/Exaltolide)</td>
<td>–53.1</td>
<td>7.4</td>
<td>16</td>
</tr>
<tr>
<td>4 (Galaxolide)</td>
<td>–45.5</td>
<td>n.i.</td>
<td>6</td>
</tr>
<tr>
<td>Cyclohexadecanone</td>
<td>–45.4</td>
<td>9.4</td>
<td>52</td>
</tr>
<tr>
<td>Cyclohexadec-5-en-1-one (Velvione)</td>
<td>–45.9</td>
<td>6.4</td>
<td>48</td>
</tr>
<tr>
<td>1-(tert-Butyl)-3,5-dimethyl-2,4,6-trinitrobenzene (musk xylene)</td>
<td>–48.1</td>
<td>1.0</td>
<td>73</td>
</tr>
<tr>
<td>1-(tert-Butyl)-3,4,5-trimethyl-2,6-dinitrobenzene (musk tibetene)</td>
<td>–55.9</td>
<td>0.88</td>
<td>100</td>
</tr>
<tr>
<td>Dienone musk 9</td>
<td>–45.3</td>
<td>n.i.</td>
<td>8</td>
</tr>
<tr>
<td>Dienone musk 12</td>
<td>–49.0</td>
<td>n.i.</td>
<td>7</td>
</tr>
<tr>
<td>Dienone musk 20</td>
<td>–41.8</td>
<td>n.i.</td>
<td>6</td>
</tr>
<tr>
<td>Dienone musk 21</td>
<td>–41.7</td>
<td>n.i.</td>
<td>7</td>
</tr>
<tr>
<td>Dienone musk 22</td>
<td>–45.7</td>
<td>n.i.</td>
<td>7</td>
</tr>
<tr>
<td>Dienone musk 23</td>
<td>–53.4</td>
<td>n.i.</td>
<td>7</td>
</tr>
<tr>
<td>Dienone musk 26</td>
<td>–50.9</td>
<td>n.i.</td>
<td>7</td>
</tr>
<tr>
<td>Dienone musk 27</td>
<td>–48.5</td>
<td>n.i.</td>
<td>7</td>
</tr>
</tbody>
</table>

\(^a\) n.i. = no induction.

\(^b\) Relative activation to musk tibetene (= 100%).
Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/s-0040-1708009.

References and Notes

(16) Saucy–Marbet/IMDA Reaction to Ketone 19: A 100-mL autoclave was charged with a mixture of propargyl alcohol 15 (2.69 g, 14.0 mmol), isopropenyl methyl ether (16, 4 equiv, 4.04 g, 56.0 mmol), p-toluenesulfonic acid monohydrate (2 mg) and toluene (40 mL). The reaction mixture was heated to 150 °C for 24 h, prior to removal of the solvent and purification by flash chromatography (PE/MTBE = 95:5, Rf 0.44) and bulb-to-bulb distillation, which furnished the tetrahydroindenyl ketone 19 (2.8 g, 85%, b.p. 152 °C/0.18 mbar) as a colorless odoriferous liquid.
(17) Isomerization of 19 to the Dienone Musk 12: At 0 °C, AlCl3 (0.1 equiv, 58 mg, 0.43 mmol) was added to a solution of ketone 19 (1.0 g, 4.3 mmol) in dichloromethane (20 mL). The resulting mixture was stirred for 2 h at this temperature, and then allowed to warm to room temperature. After stirring overnight, quenching with 1M aq. HCl, extraction with dichloromethane (3 × 30 mL), and purification by flash chromatography (PE/MTBE = 95:5, Rf 0.43) with subsequent bulb-to-bulb distillation provided the (E)-configured dienone musk 12 (510 mg, 51%, b.p. 140 °C/0.14 mbar) as pale-yellow liquid. 1H NMR (400 MHz, CDCl3): δ = 5.82 (2 H, 5-H2), 2.98 (td (d = 6.5 Hz, 2 H, 5′-H2), 2.30 (s, 2 H, 1′-H4), 2.19 (s, 3 H, 1-H3), 1.55 (t, J = 6.5 Hz, 2 H, 6′-H2), 1.09 (s, 6 H, Me2C-2′), 1.02 (s, 6 H, Me2C-7′) ppm. 13C NMR (100 MHz, CDCl3): δ = 199.2 (s, C-2), 159.8 (s, C-7′a), 151.7 (s, C-4′), 132.9 (s, C-3′a), 118.1 (d, C-3), 48.0 (t, C-1′), 46.7 (t, C-3′), 37.6 (t, C-6′), 36.9 (s, C-2′), 32.7 (s, C-7′), 32.0 (q, C-1), 29.7 (2q, Me2C-2′), 26.3 (2q, Me2C-7′), 24.4 (t, C-5′) ppm. Odor description: musky, slightly fatty, slightly green. Odor threshold (th): 2.9 ng/L air.
(18) In vitro OR5AN1 Assay: HEK293T cells were grown in DMEM medium containing FBS (9%). The cells were seeded in 96-well plates (10,000 cells/well). After 24 h, the cells were transfected with plasmids for the expression of human OR5AN1, a human RPT1S variant (V227I), and a cAMP-responsive element luciferase reporter using Lipofectamine 2000 according to the manufacturer’s instructions. For each plate pcDNA3.1(+)-Lucy-FLAG-rho-OR5AN1 (0.625 µg), pcDNA3.1(+)-RPT1S-V227I (1 µg) and pG4A-29 (1 µg) were transfected. The cells were stimulated with the musks (dissolved in growth medium) 18 h post transfection. After 4 h, the cells were lysed, and the luciferase activity was measured. Efficacy was determined as maximal activation over the full dose-response curve. EC50 values (conc. for 50% efficacy) were determined by the Graph pad prism program v 6.09 with four-parameter curve fit. Relative efficacy was calculated in relation to the most active compound (musk tibetene = 100%). Compounds with maximal efficacy <10% were considered inactive.

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