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## Semisynthetic Neoclerodanes as Kappa Opioid Receptor Probes

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### Abstract

Modification of the furan ring of salvinorin A (**1**), the main active component of *Salvia divinorum*, has resulted in novel neoclerodane diterpenes with opioid receptor affinity and activity.

Conversion of the furan ring to an aldehyde at the C-12 position (**5**) has allowed for the synthesis of analogues with new carbon-carbon bonds at that position. Previous methods for forming these bonds, such as Grignard and Stille conditions, have met with limited success. We report a palladium catalyzed Liebeskind-Srogl cross-coupling reaction of a thioester and a boronic acid that occurs at neutral pH and ambient temperature to produce ketone analogs at C-12. To the best of our knowledge, this is the first reported usage of the Liebeskind-Srogl reaction to diversify a natural product scaffold. We also describe a one-step protocol for the conversion of **1** to 12-*epi-1* (**3**) through microwave irradiation. Previously, this synthetically challenging process has required multiple steps. Additionally, we report in this study that alkene **9** and aromatic analogues **12**, **19**, **23**, **25**, and **26** were discovered to retain affinity and selectivity at kappa opioid receptors (KOP). Finally, we report that the furan-2-yl analog of **1** (**31**) has similar affinity to **1**. Collectively, these findings suggest that different aromatic groups appended directly to the decalin core may be well tolerated by KOP receptors, and may generate further ligands with affinity and activity at KOP receptors.

### 1. Introduction

Psychoactive natural products have played a vital role in the development of treatments for a number of diseases and have advanced our knowledge of basic biological processes in the central nervous system (CNS).<sup>1</sup> For example, the endogenous opioid and cannabinoid receptor systems were discovered through the systematic study of psychoactive natural products. In addition to the discovery of endogenous opioid and cannabinoid receptor

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**Supporting Information Available** X-ray diffraction analysis of **3**, as well as HPLC analysis of compounds **3**, **8**, **9**, and **12** – **31**.

systems, the investigation of psychoactive natural products has also led to the development of useful biological probes that have advanced our knowledge of the underlying causes of many CNS disorders.<sup>2</sup> Furthermore, structural modifications of such natural products have led to the development of effective medications to treat a number of CNS disorders.<sup>3</sup>

Presently, combinatorial-based approaches and genomics have largely replaced natural products screening in CNS drug discovery. Even with these advances in technology, CNS drugs entering clinical development have only approximately a 7% probability of reaching the marketplace, as compared to an industry average of 15%.<sup>4-5</sup> Thus, it is imperative to find and explore new chemical scaffolds for CNS drug discovery and development, as well as for potential probes that can be used to further enhance our understanding of neurobiology.

One recent example of a psychoactive natural product is the neoclerodane diterpene, salvinorin A (**1**) (Figure 1).<sup>4-7</sup> This natural product is the main active component of the hallucinogenic mint plant *Salvia divinorum* Epling & Játiva (Lamiaceae). Historically, *S. divinorum* has been used by the Mazatec Indians of Oaxaca, Mexico in ethnomedicinal practice.<sup>8</sup> *S. divinorum* is notorious in the public eye for its use as a recreational drug.<sup>9, 10</sup> Due to its hallucinogenic effects, several countries and states have scheduled *S. divinorum* as a schedule I controlled substance. In 2002, the site of action of **1** was identified as opioid (KOP) receptors where **1** acts as a high efficacy agonist.<sup>4</sup> Furthermore, **1** appears to have unique properties as a KOP receptor ligand that includes its high efficacy in particular transduction systems and a reduced propensity to cause receptor desensitization.<sup>11, 12</sup> These findings suggest that **1** offers a new chemotype for the development of functionally selective KOP ligands as biological probes.<sup>13</sup> Additionally, **1** is structurally distinct from other known opioid ligands such as U50,488 (**2**) in that it does not contain a basic nitrogen; a feature traditional opioid pharmacology believed to be required.<sup>14-18</sup>

Currently available KOP receptor ligands, which have become of interest due to the interaction of the KOP/dynorphin system with major neuropsychiatric disorders and addiction, suffer from several therapeutic limitations. First, KOP agonists have been shown to potentiate cocaine reward and produce psychotomimesis, sedation, and nausea.<sup>19-22</sup> Thus, high efficacy agonism at KOP results in behavioral toxicity and preclinical models support its involvement in endogenous pathophysiology (e.g., anxiety, depression, and addiction). Therefore, partial agonism or antagonism at KOP receptors is a valuable target for medications development. Second, almost all currently available KOP antagonists have a slow onset of action and are extremely long in duration of action.<sup>23, 24</sup> This makes these agents difficult to use in translational and pharmacokinetic models. Thus, there is a pressing need to develop novel agents and biological probes devoid of these limitations.

One approach to overcoming the limitations of currently available KOP ligands is to identify and develop new chemical scaffolds with improved drug-like characteristics.<sup>24-27</sup> Another approach is to modify a KOP selective natural product, such as **1**, into a partial agonist or antagonist through systematic structure-activity relationship studies. To this end, our present investigation looked to answer three questions: (1) what is the effect of configuration of the C-12 position on opioid affinity and activity; (2) can the affinity and/or activity be enhanced from the addition of a suitable group off an aryl ketone analogue; and (3) what is the effect of altering the position of the oxygen atom in the furan ring. Our results are described herein.

## 2. Chemistry

Salvinorin A (**1**) was isolated from *S. divinorum* and we developed a one-step protocol for the conversion of **1** to 12-*epi*-**1** (**3**) through microwave irradiation (Scheme 1). 2,4,4,6-Tetrabromocyclohexa-2,5-dienone and **1** were dissolved in anhydrous, degassed dichloromethane and irradiated in the microwave for 30 minutes at 130 °C. Purification via column chromatography afforded **3** and **4**<sup>28</sup> in 39% and 30% yields, respectively. Selective olefination of **1** was envisioned by introduction of an aldehyde at C-12, exploiting the reactivity of the aldehyde relative to the other four carbonyls present in **1** (Scheme 2). Aldehyde **5** was obtained following our previously published methods.<sup>29</sup> Several different olefination reactions were attempted including Wittig and Horner-Wadsworth-Emmons conditions. These methods were ineffective due to low yields, difficulties in purification, and ultimately aldehyde degradation. In the end, success was found using Julia-Kocienski olefination conditions. These conditions are applicable for use with base-sensitive aldehydes and are milder than previously attempted olefination conditions. To obtain the needed Julia-Kocienski precursor, synthesis was initiated with 3-furaldehyde that was reduced with NaBH<sub>4</sub> to yield the corresponding alcohol **6a** in 88% yield. Alcohol **6a** was then displaced with bromine in 45% yield (**6b**) and treated with ammonium heptamolybdate tetrahydrate-peroxide complex to afford the Julia-Kocienski precursor **7** in 86% yield.<sup>30</sup> The reaction of **5** and **7** in the presence of KHMDS afforded alkenes **8** and **9** in overall reaction yield of 12%. The individual isomers were isolated by flash column chromatography followed by additional HPLC purification in a 2:1 ratio of **8** to **9**.

Additional furan-modified ketone analogues of **1** were synthesized as described in Scheme 3. Many different methods to form carbon-carbon bonds are known in the scientific literature. However, many of these methods are not suitable to highly functionalized or acid/base-sensitive compounds like **1**.<sup>31</sup> In our hands, Grignard reactions using aldehyde **5** were met with limited success due to poor solubility of **5** in THF and ether at low temperatures and resulted in low yields (0 – 20%). Béguin and coworkers were able to synthesize several ketone derivatives through *in situ* formation of the acid chloride followed by Stille coupling of stannanes in low to moderate yields (7 – 57%).<sup>32</sup> This procedure is also limited by the availability and difficulties associated with stannane reagents. To overcome these challenges, an investigation into the suitability of the Liebeskind-Srogl reaction was undertaken.<sup>33, 34</sup> The Liebeskind-Srogl reaction is a palladium catalyzed cross-coupling reaction of a thioester and boronic acid that requires a stoichiometric amount of copper (I) additive.<sup>27, 28</sup> This reaction occurs at neutral pH and room temperature and has previously been utilized on base sensitive substrates.<sup>35, 36</sup> Additionally, an increasing number of boronic acids are commercially available, which further contributes to the convenience of analogue synthesis. With a promising reactivity profile, the thiophenol ester at the C-12 position was synthesized using the same CDMT and *N*-methylmorpholine conditions previously developed with substitution of ethanethiol for thiophenol.<sup>29</sup> Thioester **11** was synthesized in 62% yield. Using bis(dibenzylideneacetone)palladium (0), copper (I) thiophene carboxylate, and triethylphosphite in anhydrous THF, coupling of thioester **11** and desired boronic acids synthesized ketone derivatives **12** – **29** in 29 – 87% yields. Ketone analogue **30** was synthesized as an intermediate towards the synthesis of a furan-2-yl analogue of salvinorin A (Scheme 4). To access this compound, ketone analogue **30** was refluxed with SeO<sub>2</sub> in bromobenzene.<sup>37</sup> The initial thought was to isolate the dicarbonyl and use previously developed conditions to cyclize to the furan-2-yl analogue in a two-step procedure.<sup>38</sup> Fortuitously, cyclization also occurred under oxidative conditions to afford analogue **31** in 18% yield from **30**. To the best of our knowledge, this is the first report using the Liebeskind-Srogl reaction to diversify a natural product scaffold.

### 3. Results and Discussion

The synthesized compounds were evaluated for affinity and efficacy at opioid receptors (Table 1).<sup>39</sup> It was thought that the analogues prepared would provide greater insight into the potential use of modified neoclerodanes as opioid receptor probes. Our investigation initially began by addressing the effect of configuration at the C-12 position has on opioid affinity and activity. It was envisioned that this alteration would further investigate the amount of structural flexibility tolerated at the binding site of KOP receptors for neoclerodanes. Inversion of this center is synthetically challenging as **1** is a highly functionalized neoclerodane diterpene and has shown sensitivity to both acids and bases,<sup>31</sup> however, **3** was synthesized by Béguin and coworkers in several steps and 19% overall yield.<sup>32</sup> NMR and independent X-ray crystallographic data (see supplemental material) were consistent with the previous report. The mechanism for the apparent bond breaking and reforming has not been fully elucidated and is currently under investigation. Compound **3** demonstrated similar affinity at KOP receptors to **1** ( $K_i = 7.4 \pm 0.7$  nM vs.  $K_i = 17 \pm 1$  nM). In our hands, **3** had a 2-fold loss in affinity relative to **1** however, Béguin and coworkers found **3** to have a greater loss in affinity relative to **1**.<sup>32</sup>

To facilitate a greater understanding of the steric demands of the furan ring, analogues **8** and **9** were prepared. It was envisioned that the synthesis of these analogues would explore the influence of the additional steric bulk in the furan binding site, as well as the preferred orientation of the oxygen atom in the furan ring by constraining its rotation with the addition of the olefin. It was found that the *cis* analogue **9** had almost 40-fold higher affinity for the KOP receptor compared to the *trans* analogue **8** ( $K_i = 30 \pm 4$  nM vs.  $K_i = 1,120 \pm 30$  nM). This finding suggests that size plays a key role in the binding of the furan ring substituent given that the extended *trans* conformation (**8**) has weaker affinity compared to the *cis* orientation (**9**). In addition, it appears that distance from the decalin core plays an important role in the bonding of the furanyl oxygen of **1**.

Previous investigations have suggested that modification or substitution of the furan ring is tolerated but often diminishes affinity and efficacy at KOP receptors.<sup>29, 32, 39</sup> To further explore the potential replacement of the furan ring with other substituents, a series of aryl ketone derivatives were synthesized. Our intention was to identify a more desirable hydrogen bond interaction with the carbonyl and its KOP receptor binding site while potentially accessing another favorable interaction in the binding pocket through judicious choice of a substituent on the aryl ring. Initially, phenyl analogue **12**<sup>32</sup> was prepared and found to have decreased affinity compared to **1** ( $K_i = 70 \pm 3$  nM vs.  $K_i = 7.4 \pm 0.7$  nM). Based on the <100 nM affinity and the potential of this scaffold to have greater metabolic stability than furan containing salvinorin A, investigations into the effects of substitutions at the *ortho*, *meta*, and *para* positions were undertaken. Given the many potential possibilities, we elected to selectively probe the effect of a methoxy group and a phenolic group. Our rationale was that we could quickly gauge the role of electron donating properties, as well as the possible effect of hydrogen bond donating compared to hydrogen bond accepting properties on affinity. Introduction of a methoxy group (**13** – **15**) was found to generally decrease affinity compared to **12**. Of these substitutions, we identified that incorporation of a *m*-methoxy group (**14**) was best tolerated ( $K_i = 290 \pm 10$  nM). Similarly, we found that introduction of a phenol group (**16** – **18**) resulted in generally decreased affinity compared to **12**. However, we found that a *m*-phenolic group was best tolerated and had comparable affinity to **12** ( $K_i = 110 \pm 5$  nM vs.  $K_i = 70 \pm 3$  nM). Given that *meta* substitution was best tolerated, we decided to explore additional *meta* substituents.

We first focused on preparing carboxamide analogue (**19**) as a nonclassical bioisosteric replacement for the phenol group. Previously, Wentland and coworkers found that this

structural modification resulted in high affinity analogues at opioid receptors when the 8-position hydroxyl of cyclazocine was modified, as well as the 3-position of the morphinan nucleus.<sup>40, 41</sup> Remarkably, carboxamide (**19**) was identified as having increased affinity approximately 3-fold compared to **17** ( $K_i = 40 \pm 1$  nM vs.  $110 \pm 5$  nM). Given this finding, we sought to further explore the nature of the increased affinity. Thus we prepared *m*-cyano (**20**), *m*-trifluoromethoxy (**21**), and *m*-trifluoromethyl (**22**). The replacement of the carboxamido group with a cyano moiety decreased affinity 6-fold compared to **19** ( $K_i = 220 \pm 10$  nM vs.  $K_i = 40 \pm 1$  nM). Substitution with a trifluoromethoxy or a trifluoromethyl group also decreased affinity compared to **19** ( $K_i > 10,000$  nM and  $K_i = 2,260 \pm 120$  nM, respectively vs.  $K_i = 40 \pm 1$  nM). These latter findings suggest that the electron withdrawing nature of the carboxamido group is not responsible for the increase in affinity. However, additional analogues will need to be prepared to more definitively prove this point.

The effect of replacing the phenyl group of **12** was also investigated. These analogues were designed to probe the potential of replacing the benzene ring with several different bioisosteric replacements as well as the necessity of an aromatic ring. The substitution of the benzene ring with a 2-furan (**23**)<sup>32</sup> and 3-furan (**24**) ketone was probed. These changes were well tolerated as **23** and **24** were found to have similar affinity to **12** ( $K_i = 80$  nM and  $K_i = 150 \pm 10$  nM, respectively vs.  $K_i = 70 \pm 3$  nM). Given this success, we probed additional replacements of the benzene ring. The substitutions of the phenyl ring with a 2-thiophene (**25**)<sup>32</sup> and 3-thiophene (**26**) were found to increase affinity at KOP receptors compared to **12** (**25**:  $K_i = 36 \pm 2$  nM, **26**:  $K_i = 31 \pm 3$  nM vs. **12**:  $K_i = 70 \pm 3$  nM). We then sought to further explore size requirements for the aromatic substituent. To this end, we annulated an additional benzene ring onto **23** (**27**), **25** (**28**), and **26** (**29**). Unfortunately, these changes were not well tolerated and affinity was considerably decreased (**27**:  $K_i > 10,000$  nM; **28**:  $K_i = 9,210$  nM and **29**:  $K_i > 10,000$  nM). Finally, the necessity of the phenyl was probed by replacing it with a *cis*-propenyl group (**30**). This change was not well tolerated and led to a 4-fold decrease in affinity compared to **12** ( $K_i = 320 \pm 20$  vs.  $K_i = 70 \pm 3$  nM). This latter finding is interesting and suggests that an aromatic ring may not be completely necessary for affinity. However, additional alkyl analogues will need to be prepared to confirm this hypothesis. Collectively, these results suggest that affinity of **12** may be enhanced by the introduction of *m*-carboxamido group (**19**) or through bioisosteric replacement of the phenyl ring (**23**, **25**, and **26**).

Interestingly, **23** was found previously to have reduced affinity compared to the present investigation ( $K_i > 1,000$  nM vs.  $K = 80$  nM).<sup>32</sup> The previous report suggested that the lack of affinity seen by **23** might be the result of instability under the assay conditions.<sup>32</sup> However, we saw no evidence of stability issues and this may explain the increase in affinity. Another possibility may be the presence of trace impurities not seen in the present investigation. One potential source of impurities is tin byproducts as the previous investigation used Stille coupling conditions.<sup>32</sup> Our present investigation did not use this type of coupling favoring a more “green” method.

Next, we sought to determine the effect of removing the ketone spacer in **23** (**31**). Our other motivation was this analogue would provide information on the importance of the position of the oxygen in the furan ring of **1** on opioid receptor affinity. Several molecular modeling studies have suggested that the oxygen of the furan ring is directly participating in a hydrogen bond with the KOP receptor.<sup>4, 42</sup> It was envisioned that significant changes in affinity between **1** and **31** would validate the importance of the hydrogen bond between the furanyl oxygen and the KOP receptor. The removal of the ketone spacer resulted in a 9-fold increase in affinity at KOP receptors compared to **23** ( $K_i = 8.7 \pm 0.4$  nM vs.  $K_i = 80 \pm 4$  nM). This finding suggests that other modifications to the furan ring of **1** may also be



tolerated. While synthetic methods exist for their preparation,<sup>43, 44</sup> their use for structure-activity relationship studies have not been described to date.

Surprisingly, **31** was found to have almost identical affinity at KOP receptors compared to **1** ( $K_i = 8.7 \pm 0.4$  nM vs.  $K_i = 7.4 \pm 0.7$  nM, respectively). This would suggest that a hydrogen bond exists from the furanyl oxygen of **31** to the same residue on the KOP receptor as the furanyl oxygen of **1**. Due to the lack of **1** – KOP receptor co-crystal structure, one way to investigate the possibility of a hydrogen bond is through mutation of residues of the KOP receptor thought to be involved in the binding of **1** and observation of the changes to affinity. However, this is beyond the scope of the present investigation.

Selected analogues were then evaluated for their efficacy at KOP receptors using the [<sup>35</sup>S]GTP- $\gamma$ -S assay (Table 2). 12-*Epi*-**1**(**3**) was found to be 18-fold less potent than the **1** ( $EC_{50} = 720 \pm 97$ ,  $E_{max} = 109 \pm 4\%$  vs.  $EC_{50} = 40 \pm 6$  nM,  $E_{max} = 124 \pm 6\%$ ). Despite its high affinity, **9** was found to have reduced potency as an agonist in the [<sup>35</sup>S]GTP- $\gamma$ -S assay compared to **1** ( $EC_{50} = 1,370 \pm 180$  nM vs.  $EC_{50} = 40 \pm 6$  nM). The reasons for this large discrepancy are unclear but may be due to poor solubility under the assay conditions. Phenyl ketone **12** was found to be a partial agonist compared to **1** ( $E_{max} = 74 \pm 6$  vs.  $E_{max} = 124 \pm 6$ ). However, its reduced potency ( $EC_{50} = 1370 \pm 180$  nM vs.  $EC_{50} = 40 \pm 6$  nM) inhibits its utility as a pharmacological probe. Carboxamide **19** was found to have 18-fold reduced potency in the [<sup>35</sup>S]GTP- $\gamma$ -S read assay compared to **1** ( $EC_{50} = 730 \pm 90$  nM vs.  $EC_{50} = 40 \pm 6$  nM). While there was little difference in affinity for KOP receptors, **26** was found to be approximately 3-fold more potent than **25** ( $EC_{50} = 620 \pm 50$  nM vs.  $EC_{50} = 1,630 \pm 23$  nM) as an agonist. The most potent compound identified in the [<sup>35</sup>S]GTP- $\gamma$ -S assay was 2-furanyl analogue **31** ( $EC_{50} = 140 \pm 10$  nM). Compound **31** was found to be 3-fold less potent than **1** as an agonist.

Given these results, we elected to further profile **1**, **19** and **31** as KOP agonists using the fluorescent Ca<sup>2+</sup> mobilization assay (Table 3). While this particular assay is a more artificial system, it was envisioned that an additional assay would provide insight into the pharmacological properties of these neoclerodanes. In particular, it was thought that this assay would provide further validation of the partial agonist character of **19** and **31** when compared to **1** in the [<sup>35</sup>S]GTP- $\gamma$ -S assay. As seen in the [<sup>35</sup>S]GTP- $\gamma$ -S assay, carboxamide **19** was found to have reduced potency compared to **1** ( $EC_{50} = 75.4 \pm 21$  nM vs.  $EC_{50} = 6.11 \pm 0.04$  nM). However, **19** was found to possess identical efficacy compared to **1** ( $E_{max} = 97 \pm 2$  vs.  $E_{max} = 97 \pm 5$ ). This finding suggests that the partial agonism seen in the [<sup>35</sup>S]GTP- $\gamma$ -S assay may not be readily translatable to other assays. However, this supposition needs further testing. Also as seen previously, the movement of the furanyl oxygen from the 3-position (**1**) to the 2-position (**31**) resulted in a 2-fold decrease in potency ( $EC_{50} = 12.2 \pm 4.4$  nM vs.  $EC_{50} = 6.11 \pm 0.04$  nM). Moreover, **31** was found to have similar efficacy to **1** ( $E_{max} = 97 \pm 2$  vs.  $E_{max} = 97 \pm 8$ ). Collectively these results suggest that the use of several different assays will be necessary to best determine partial agonist activity for KOP receptors.

Finally, we sought to explore the effects of **3** in nonhuman primates (*Macaca mulatta*) using a neuroendocrine biomarker assay compared to **1**.<sup>45</sup> This assay has been previously used to determine potency and apparent efficacy of structurally diverse KOP ligands, and is of translational value since it can be used in rodents, non-human primates, and humans.<sup>46, 47</sup> Previous reports have described **3** as a partial agonist when evaluated in the [<sup>35</sup>S]GTP- $\gamma$ -S assay ( $EC_{50} = 41 \pm 6$ ,  $E_{max} = 73 \pm 6$  compared to **2**).<sup>32</sup> A more recent study describes **3** as a full agonist using the  $\beta$ -arrestin 2 DiscoverRx assay ( $EC_{50} = 382 \pm 85$ ,  $E_{max} = 126 \pm 10$  compared to **1**).<sup>48</sup> The differences in the agonist efficacy seen for **3** are likely due to differences in receptor expression level, G-protein signaling, and other cell type dependent

changes. As seen in Figure 2, the dose-effect curve for **3** is parallel to that of **1** in this assay, but is rightward-shifted by approximately 10-fold (ED<sub>50</sub> values from mean log dose-effect curves were 0.038 and 0.0037 mg/kg, for **3** and **1**, respectively). Therefore, while **3** has lower potency than **1**, there is no clear indication that it is acting as a partial agonist in this *in vivo* assay, up to the largest dose that could be studied due to solubility limits.

#### 4. Conclusions

In efforts to further elucidate the pharmacophore and structure-activity relationships of neoclerodanes at opioid receptors, several furan-modified analogues were synthesized and evaluated. We have developed a one-step protocol for the conversion of **1** to 12-*epi*-**1** (**3**) through microwave irradiation. This methodology should be valuable for further research into the structure-activity relationships of neoclerodanes. We have described to the best of our knowledge, the first reported use of the Liebeskind-Srogl reaction to modify a natural product. Our results show that the insertion of a *cis* alkene spacer to **1** (**9**) is better tolerated sterically at the binding site compared to a *trans* alkene spacer (**8**). Affinity for KOP receptors can be maintained by replacing the furan ring with a phenyl ketone (**12**). Additional structure-activity relationship studies have shown that affinity of **12** may be enhanced by the introduction of *m*-carboxamido group (**19**) or through bioisosteric replacement of the phenyl ring (**23**, **25**, and **26**). Surprisingly, **31** was found to have almost identical affinity at KOP receptors compared to **1** ( $K_i = 8.7 \pm 0.4$  nM vs.  $K_i = 7.4 \pm 0.7$  nM, respectively). This would suggest that a hydrogen bond exists from the furanyl oxygen of **31** to the same residue on the KOP receptor as the furanyl oxygen of **1**. This finding also suggests that other modifications to the furan ring of **1** may be well tolerated by KOP receptors. Collectively, our results provide further evidence that modification of the furan ring is tolerated and produces analogues with affinity and activity at KOP receptors. Further evaluation of analogue **31** is currently underway and will be reported in due course.

#### 5. Experimental Section

Unless otherwise indicated, all reagents were purchased from commercial suppliers and were used without further purification. Melting points were determined on a Thomas-Hoover capillary melting apparatus. NMR spectra were recorded on a Bruker DRX-400 with qnp probe or a Bruker AV-500 with cryoprobe using values in ppm (TMS as internal standard) and *J* (Hz) assignments of <sup>1</sup>H resonance coupling. High resolution mass spectrometry data were collected on either a LCT Premier (Waters Corp., Milford, MA) time of flight mass spectrometer or an Agilent 6890 N gas chromatograph in conjunction with a quarto Micro GC mass spectrometer (Micromass Ltd, Manchester UK). Thin-layer chromatography (TLC) was performed on 0.25 mm plates Analtech GHLF silica gel plates using ethyl acetate/*n*-hexanes, in 1:1 ratio as the solvent system unless otherwise noted. Spots on TLC were visualized by UV (254 or 365 nm), if applicable, and phosphomolybdic acid in ethanol. Column chromatography was performed with Silica Gel (32 – 63 μ particle size) from MP Biomedical (Solon, OH). Analytical HPLC was carried out on an Agilent 1100 Series Capillary HPLC system with diode array detection at 209.4 nm on an Agilent Eclipse XDB-C18 column (250 × 10 mm, 5 μm) with isocratic elution in 60% CH<sub>3</sub>CN/40% H<sub>2</sub>O at a flow rate of 5.0 mL/min unless otherwise noted, or on a Phenomenex Luna C18 column (250 × 10 mm, 5 μm) with isocratic elution in 60% CH<sub>3</sub>CN/40% H<sub>2</sub>O unless otherwise noted. The systematic name for salvinorin A (**1**) is (2*S*,4*aR*,6*aR*,7*R*,9*S*,10*aS*,10*bR*)-methyl 9-acetoxy-2-(furan-3-yl)-6*a*,10*b*-dimethyl-4,10-dioxo-dodecahydro-1*H*-benzo[*f*]iso-chromene-7-carboxylate. Salvinorin A was isolated from *S. divinorum* as previously described.<sup>49</sup>

### 5.1. Preparation of (2R,4aR,6aR,7R,9S,10aS,10bR)-methyl 9-acetoxy-2-(furan-3-yl)-6a,10b-dimethyl-4,10-dioxododecahydro-1H-benzo[f]isochromene-7-carboxylate (**3**)

In a 20 mL microwave tube, a solution of **1** (100 mg, 0.231 mmol, 1 equiv) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (15 mL), 2,4,4,6-tetrabromocyclohexa-2,5-dienone (113.7 mg, 0.277 mmol, 1.2 equiv) was added. The tube was sealed and irradiated in the microwave for 30 minutes at 130 °C. Solvent was removed under reduced pressure and the resulting residue was purified via column chromatography using ethyl acetate, MTBE, and *n*-hexanes to afford 29 mg (39% yield borsm) as a white solid. HPLC *t* = 11.463 min; purity = 96.07%. The <sup>1</sup>H and <sup>13</sup>C spectra in CDCl<sub>3</sub>, melting point, and HRMS of **3** were as previously reported.<sup>32</sup> X-ray diffraction quality crystals were prepared by recrystallizing **3** from a mixture of EtOAc/ *n*-hexanes.

### 5.2. Preparation of (4aR,6aR,7R,9S,10aS,10bR)-methyl 9-acetoxy-2-((E)-2-(furan-3-yl)vinyl)-6a,10bdimethyl-4,10-dioxododecahydro-1H-benzo[f]isochromene-7-carboxylate (**8**) and (4aR,6aR,7R,9S,10aS,10bR)-methyl 9-acetoxy-2-((Z)-2-(furan-3-yl)vinyl)-6a,10b-dimethyl-4,10-dioxododecahydro-1H-benzo[f]isochromene-7-carboxylate (**9**)

KHMDS (1.64 mL of a 0.5 M solution in toluene, 0.83 mmol) was added in a dropwise manner to a cooled (−78 °C) solution of **7**<sup>30</sup>(263 mg, 0.91 mmol) in THF (9 mL). After 30 min, a cooled solution of aldehyde **5** (300 mg, 0.761 mmol) in THF (20 mL) was added in a dropwise manner over 3 minutes. The reaction mixture was stirred at −78 °C for 2 hours or until completion and then it was diluted with EtOAc (9 mL) and quenched by the addition of H<sub>2</sub>O (10 mL). After warming to room temperature over 30 min, the phases were separated and the aqueous phase was extracted with EtOAc (2 × 10 mL). The combined organic extracts were washed with brine (2 × 10 mL) and dried over Na<sub>2</sub>SO<sub>4</sub>. Filtration and concentration under reduced pressure afforded **8** and **9** as a 2:1 mixture, which was separated by flash column chromatography to afford, in order of elution, major isomer **8** as a white solid and the minor isomer **9** also as a white solid. The minor isomer was further purified by HPLC (40% CH<sub>3</sub>CN/60% H<sub>2</sub>O) on an Agilent Eclipse XDB-C18 column (250 × 10 mm, 5 μm) at a flow rate of 5 mL/min.

**8**: Isolated as an off-white solid (25 mg, 7.2% yield) of *trans* isomer after purification; mp = 192 – 194 °C; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.45 (s, 1H), 7.39 (s, 1H), 6.51 (d, *J* = 13.0 Hz, 2H), 5.88 (dd, *J* = 6.3, 15.8 Hz, 1H), 5.15 (d, *J* = 9.7 Hz, 2H), 3.76 (s, 3H), 2.77 (d, *J* = 8.1 Hz, 1H), 2.43 (d, *J* = 8.3 Hz, 1H), 2.34 (d, *J* = 9.9 Hz, 2H), 2.20 (s, 3H), 2.18 (s, 1H), 2.05 (s, 1H), 1.84 (s, 1H), 1.63 (s, 3H), 1.45 (s, 3H), 1.14 (s, 3H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 201.85, 171.38, 171.06, 169.84, 143.57, 141.07, 126.66, 122.82, 121.74, 107.15, 77.40, 74.92, 63.96, 53.46, 51.82, 51.17, 42.89, 41.96, 38.05, 35.17, 30.56, 20.40, 17.95, 16.19, 15.04. HRMS (*m/z*): [M+Na] calcd for C<sub>25</sub>H<sub>30</sub>O<sub>8</sub>Na, 481.1838; found, 481.1832. HPLC *t*<sub>R</sub> = 8.832 min; purity = 98.21%.

**9**: Isolated as a white solid (15 mg, 4.3% yield) of *cis* isomer after purification; mp = 182 – 184 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.52 (s, 1H), 7.45 (s, 1H), 6.45 (s, 1H), 6.40 (d, *J* = 11.2 Hz, 1H), 5.54 (d, *J* = 11.2 Hz, 1H), 5.47 – 5.41 (m, 1H), 5.20 – 5.15 (m, 1H), 3.76 (s, 3H), 2.81 – 2.75 (m, 1H), 2.34 (d, *J* = 9.9 Hz, 4H), 2.20 (s, 3H), 2.18 (s, 1H), 2.09 – 2.04 (m, 1H), 1.84 – 1.79 (m, 1H), 1.63 (s, 3H), 1.45 (s, 3H), 1.14 (s, 3H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 202.14, 171.81, 171.47, 170.21, 143.76, 142.04, 128.49, 124.02, 121.01, 110.94, 75.23, 74.12, 64.36, 53.84, 52.23, 51.65, 43.04, 42.30, 38.44, 35.70, 30.99, 20.83, 18.36, 16.65, 15.52. HRMS (*m/z*): [M+Na] calcd for C<sub>25</sub>H<sub>30</sub>O<sub>8</sub>Na, 481.1838; found, 481.1830. HPLC *t*<sub>R</sub> = 8.691 min; purity = 100%.



### 5.3. Preparation of (2*S*,4*aR*,6*aR*,7*R*,9*S*,10*aS*,10*bR*)-methyl 9-acetoxy-6*a*,10*b*-dimethyl-4,10-dioxo-2-(phenyl-thiocarbonyl)dodecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (**11**)

To an oven dried round bottom flask under argon was added acid **10**<sup>28</sup> (0.64 g, 1.57 mmol, 1 equiv), CDMT (0.84 g, 4.72 mmol, 3 equiv), *N*-methylmorpholine (1.04 mL, 9.44 mmol, 6 equiv) followed by the addition of anhydrous THF (20 mL). After 1 hour, thiophenol (0.48 mL, 4.72 mmol, 3 equiv) was added and reaction stirred at room temperature for 48 hours. The reaction was quenched with H<sub>2</sub>O (25 mL) and extracted with Et<sub>2</sub>O (3 × 30 mL). The combined Et<sub>2</sub>O portion was washed with saturated aqueous NaHCO<sub>3</sub> (3 × 20 mL), 2*N* HCl (3 × 20 mL), brine (3 × 20 mL), and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The solvent was evaporated *in vacuo* and the resulting residue purified by flash column chromatography on silica gel using mixtures of EtOAc/*n*-hexanes and triturated in EtOAc/*n*-hexanes to afford 0.4912 g (62% yield) of **11** as a white solid, mp 136 – 140 °C; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.47 – 7.38 (m, 5H), 5.14 (dd, *J* = 8.6, 11.6 Hz, 1H), 5.09 (dd, *J* = 7.2, 9.6 Hz, 1H), 3.72 (s, 3H), 2.74 (dd, *J* = 5.2, 11.6 Hz, 1H), 2.65 (dd, *J* = 7.2, 13.7 Hz, 1H), 2.37 – 2.22 (m, 2H), 2.22 – 2.10 (m, 6H), 1.84 – 1.74 (m, 1H), 1.74 – 1.50 (m, 3H), 1.39 (s, 3H), 1.09 (s, 3H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 201.80, 197.76, 171.71, 170.01, 169.87, 134.91, 130.09, 129.62, 126.07, 80.51, 75.03, 64.39, 53.56, 52.21, 50.64, 42.17, 39.52, 37.96, 35.68, 30.89, 20.77, 18.38, 16.38, 16.20. HRMS (*m/z*): [M+H] calcd for C<sub>26</sub>H<sub>31</sub>O<sub>8</sub>S, 503.1740; found 503.1720. HPLC *t*<sub>R</sub> = 8.507 min; purity = >99.99%.

### 5.4. General Procedure for Boronic Acid Coupling

A solution of **11** (1 equiv), appropriate boronic acid (3 equiv), copper (I) thiophene-2-carboxylate (1.5 equiv), and bis(dibenzylideneacetone)palladium(0) (5 mol %) in anhydrous THF under argon was stirred at room temperature. To the stirring solution, triethylphosphite (20 mol %) was added. The reaction mixture was stirred at room temperature and monitored by TLC until complete. Reactions were typically complete in 2 – 5 hours. The mixture was then washed with saturated aqueous NaHCO<sub>3</sub> (3 × 15 mL), brine (15 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The solvent was evaporated *in vacuo* and the resulting residue purified by flash column chromatography on silica gel using mixtures of EtOAc/*n*-hexanes and triturated in EtOAc/*n*-hexanes to afford the desired product.

#### 5.4.1. (2*S*,4*aR*,6*aR*,7*R*,9*S*,10*aS*,10*bR*)-methyl 9-acetoxy-2-benzoyl-6*a*,10*b*-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (**12**)

—Compound **12** was synthesized from compound **11** using the general procedure and phenylboronic acid to afford 0.221 g (82.4%) isolated as an off-white solid, mp = 164 – 167 °C. HRMS (*m/z*): [M+Na] calcd for C<sub>26</sub>H<sub>30</sub>O<sub>8</sub>Na, 493.1838; found 493.1819. HPLC *t*<sub>R</sub> = 6.003 min; purity = 95.36%. The <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were in agreement with that previously reported.<sup>32</sup>

#### 5.4.2. (2*S*,4*aR*,6*aR*,7*R*,9*S*,10*aS*,10*bR*)-methyl 9-acetoxy-2-(2-methoxybenzoyl)-6*a*,10*b*-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (**13**)

—Compound **13** was synthesized from compound **11** using the general procedure and 2-methoxyphenylboronic acid to afford 0.082 g (66.2%) isolated as a white solid, mp = 186 – 188 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.79 (dd, *J* = 1.8, 7.8 Hz, 1H), 7.56 – 7.47 (m, 1H), 7.07 – 6.99 (m, 1H), 6.96 (d, *J* = 8.2 Hz, 1H), 5.89 (t, *J* = 8.1 Hz, 1H), 5.10 – 5.02 (m, 1H), 3.92 (s, 3H), 3.69 (s, 3H), 2.75 – 2.63 (m, 2H), 2.30 – 2.19 (m, 2H), 2.16 – 2.06 (m, 6H), 1.72 (dt, *J* = 3.2, 13.3 Hz, 1H), 1.68 – 1.46 (m, 3H), 1.40 (s, 3H), 1.05 (s, 3H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 202.11, 197.37, 171.83, 171.72, 169.99, 159.03, 135.43, 131.80, 124.34, 121.47, 112.15, 79.32, 75.08, 65.20, 55.96, 53.51, 52.16, 49.69, 42.24, 38.20, 38.08, 35.76, 30.96, 20.84, 18.51, 16.90, 16.26. HRMS (*m/z*): [M+NH<sub>4</sub>] calcd for C<sub>27</sub>H<sub>36</sub>O<sub>9</sub>N, 518.2390; found 518.2369. HPLC *t*<sub>R</sub> = 6.040 min; purity = >99.99%

**5.4.3. (2S,4aR,6aR,7R,9S,10aS,10bR)-methyl 9-acetoxy-2-(3-methoxybenzoyl)-6a,10b-dimethyl-4,10-dioxododecahydro-1H-benzo[*f*]isochromene-7-carboxylate (14)**—Compound **14** was synthesized from compound **11** using the general procedure and 3-methoxyphenylboronic acid to afford 0.093 g (60.9%) isolated as a white solid, mp = 162 – 164 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.48 – 7.43 (m, 2H), 7.40 (dd, *J* = 5.8, 10.3 Hz, 1H), 7.16 (ddd, *J* = 1.1, 2.6, 8.1 Hz, 1H), 5.86 (t, *J* = 8.3 Hz, 1H), 5.16 – 5.06 (m, 1H), 3.86 (s, 3H), 3.72 (s, 3H), 2.77 – 2.61 (m, 2H), 2.28 (dd, *J* = 7.1, 13.5 Hz, 2H), 2.20 – 2.09 (m, 6H), 1.80 – 1.73 (m, 1H), 1.67 (dt, *J* = 8.0, 14.9 Hz, 1H), 1.62 – 1.52 (m, 2H), 1.46 (s, 3H), 1.08 (s, 3H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 202.16, 195.34, 171.78, 171.14, 170.04, 160.31, 134.96, 130.32, 121.55, 121.08, 113.35, 75.51, 75.04, 65.02, 55.72, 53.45, 52.18, 49.49, 42.19, 38.59, 37.92, 35.79, 30.89, 20.80, 18.46, 16.93, 16.21. HRMS (*m/z*): [M+H] calcd for C<sub>27</sub>H<sub>33</sub>O<sub>9</sub>, 501.2125; found 501.2112. HPLC *t*<sub>R</sub> = 6.217 min; purity = 99.03%.

**5.4.4. (2S,4aR,6aR,7R,9S,10aS,10bR)-methyl 9-acetoxy-2-(4-methoxybenzoyl)-6a,10b-dimethyl-4,10-dioxododecahydro-1H-benzo[*f*]isochromene-7-carboxylate (15)**—Compound **15** was synthesized from compound **11** using the general procedure and 4-methoxyphenylboronic acid to afford 0.069 g (50.7%) isolated as a white solid, mp = 117 – 120 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.90 – 7.85 (m, 2H), 6.97 – 6.91 (m, 2H), 5.81 (t, *J* = 8.2 Hz, 1H), 5.14 – 5.01 (m, 1H), 3.87 (d, *J* = 7.1 Hz, 3H), 3.69 (s, 3H), 2.74 – 2.65 (m, 1H), 2.62 (dd, *J* = 8.7, 13.6 Hz, 1H), 2.25 (dd, *J* = 6.7, 13.8 Hz, 2H), 2.20 – 2.05 (m, 6H), 1.75 – 1.47 (m, 4H), 1.43 (s, 3H), 1.05 (s, 3H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 202.25, 193.81, 171.82, 171.36, 170.07, 164.65, 131.56, 126.54, 114.55, 75.19, 75.08, 65.08, 55.81, 53.45, 52.18, 49.37, 42.21, 38.59, 37.93, 35.73, 30.90, 20.81, 18.47, 17.08, 16.20. HRMS (*m/z*): [M+NH<sub>4</sub>] calcd for C<sub>27</sub>H<sub>36</sub>O<sub>9</sub>N, 518.2390; found 518.2379. HPLC *t*<sub>R</sub> = 6.247 min; purity = 96.68%.

**5.4.5. (2S,4aR,6aR,7R,9S,10aS,10bR)-methyl 9-acetoxy-2-(2-hydroxybenzoyl)-6a,10b-dimethyl-4,10-dioxododecahydro-1H-benzo[*f*]isochromene-7-carboxylate (16)**—Compound **16** was synthesized from compound **11** using the general procedure and 2-hydroxybenzeneboronic acid to afford 0.090 g (44.0%) isolated as a white solid, mp = 200 – 203 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 11.73 (s, 1H), 7.58 (d, *J* = 8.1 Hz, 1H), 7.53 (t, *J* = 7.8 Hz, 1H), 7.03 (d, *J* = 8.5 Hz, 1H), 6.95 (t, *J* = 7.6 Hz, 1H), 5.92 (t, *J* = 8.4 Hz, 1H), 5.10 (t, *J* = 10.0 Hz, 1H), 3.72 (s, 3H), 2.72 (dd, *J* = 8.7, 13.8 Hz, 2H), 2.28 (dd, *J* = 6.8, 13.4 Hz, 2H), 2.23 – 2.09 (m, 6H), 1.84 – 1.56 (m, 4H), 1.47 (s, 3H), 1.09 (s, 3H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 202.20, 200.58, 171.74, 170.98, 170.06, 163.70, 137.98, 129.64, 119.97, 119.25, 116.55, 75.08, 74.43, 64.96, 53.46, 52.22, 49.49, 42.23, 38.94, 37.93, 35.83, 30.88, 20.79, 18.49, 16.82, 16.22. HRMS (*m/z*): [M – H] calcd for C<sub>26</sub>H<sub>29</sub>O<sub>9</sub>, 485.1812; found 485.1808. HPLC *t*<sub>R</sub> = 6.986 min; purity = 96.16%.

**5.4.6. (2S,4aR,6aR,7R,9S,10aS,10bR)-methyl 9-acetoxy-2-(3-hydroxybenzoyl)-6a,10b-dimethyl-4,10-dioxododecahydro-1H-benzo[*f*]isochromene-7-carboxylate (17)**—Compound **17** was synthesized from compound **11** using the general procedure and 3-hydroxyphenylboronic acid to afford 0.033 g (28.9%) isolated as a white solid, mp = 131 – 135 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.23 (t, *J* = 7.9 Hz, 1H), 7.16 – 7.12 (m, 1H), 7.10 (d, *J* = 7.8 Hz, 1H), 6.96 (s, 1H), 6.92 (dd, *J* = 1.9, 8.1 Hz, 1H), 5.78 (dd, *J* = 6.9, 10.2 Hz, 1H), 5.17 (dd, *J* = 8.0, 12.1 Hz, 1H), 3.73 (d, *J* = 2.0, 3H), 3.00 – 2.89 (m, 2H), 2.82 (dd, *J* = 10.3, 13.4 Hz, 1H), 2.37 – 2.25 (m, 2H), 2.21 (s, 3H), 2.19 – 2.09 (m, 2H), 1.84 (d, *J* = 9.8 Hz, 1H), 1.78 – 1.62 (m, 3H), 1.48 (s, 3H), 1.07 (s, 3H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 202.84, 196.51, 172.04, 171.73, 171.59, 157.11, 133.45, 130.47, 122.70, 120.61, 114.06, 76.42, 75.37, 63.95, 52.94, 52.19, 48.83, 42.71,

38.75, 37.79, 35.85, 30.81, 21.00, 18.82, 17.22, 16.13. HRMS ( $m/z$ ): [M-H] calcd for C<sub>26</sub>H<sub>29</sub>O<sub>9</sub>, 485.1812; found 485.1801. HPLC  $t_R$  = 4.286 min; purity = 99.66%.

**5.4.7. (2S,4aR,6aR,7R,9S,10aS,10bR)-methyl 9-acetoxy-2-(4-hydroxybenzoyl)-6a,10b-dimethyl-4,10-dioxododecahydro-1H-benzo[*f*]isochromene-7-carboxylate (18)**—

Compound **18** was synthesized from compound **11** using the general procedure and 4-hydroxyphenylboronic acid to afford 0.092 g (68.0%) isolated as a white solid, mp = 232 – 236 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.82 (d,  $J$  = 8.8 Hz, 2H), 6.85 (d,  $J$  = 8.8 Hz, 2H), 6.16 (s, 1H), 5.80 (t,  $J$  = 8.3 Hz, 1H), 5.13 – 5.08 (m, 1H), 3.72 (s, 3H), 2.72 (d,  $J$  = 7.7 Hz, 1H), 2.62 (dd,  $J$  = 8.7, 13.6 Hz, 1H), 2.30 (d,  $J$  = 10.7 Hz, 2H), 2.20 (s, 1H), 2.17 (s, 5H), 1.79 – 1.52 (m, 4H), 1.45 (s, 3H), 1.08 (s, 3H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 202.27, 193.59, 171.80, 171.60, 170.41, 161.34, 131.84, 126.60, 116.09, 77.44, 75.25, 65.01, 53.45, 52.21, 49.43, 42.25, 38.48, 37.94, 35.71, 30.88, 20.85, 18.46, 17.06, 16.23. HRMS ( $m/z$ ): [M-H] calcd for C<sub>26</sub>H<sub>29</sub>O<sub>9</sub>, 485.1812; found 485.1783. HPLC  $t_R$  = 4.624 min; purity = 95.63%.

**5.4.8. (2S,4aR,6aR,7R,9S,10aS,10bR)-methyl 9-acetoxy-2-(3-carbamoylbenzoyl)-6a,10b-dimethyl-4,10-dioxododecahydro-1H-benzo[*f*]isochromene-7-carboxylate (19)**—

Compound **19** was synthesized from compound **11** using the general procedure and 3-aminocarbonylphenylboronic acid to afford 0.062 g (37.8%) isolated as a white solid, mp = 148 – 152 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.30 (s, 1H), 8.07 (d,  $J$  = 7.8 Hz, 1H), 8.02 (d,  $J$  = 7.9 Hz, 1H), 7.58 (t,  $J$  = 7.8 Hz, 1H), 6.52 – 6.31 (m, 1H), 5.88 (t,  $J$  = 8.4 Hz, 1H), 5.84 – 5.65 (m, 1H), 5.18 – 5.11 (m, 1H), 3.72 (s, 3H), 2.82 – 2.73 (m, 1H), 2.65 (dd,  $J$  = 8.4, 13.6 Hz, 1H), 2.28 (dd,  $J$  = 7.3, 13.3 Hz, 3H), 2.20 – 2.09 (m, 5H), 1.78 (d,  $J$  = 13.0 Hz, 1H), 1.75 – 1.54 (m, 3H), 1.47 (s, 3H), 1.09 (s, 3H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 202.32, 194.95, 171.81, 170.95, 170.22, 168.05, 134.59, 133.96, 133.38, 132.26, 129.75, 127.79, 75.66, 75.18, 64.74, 53.40, 52.20, 49.71, 42.24, 38.11, 37.91, 35.78, 30.90, 20.82, 18.49, 16.87, 16.27. HRMS ( $m/z$ ): [M+Na] calcd for C<sub>27</sub>H<sub>31</sub>NO<sub>9</sub>Na, 536.1897; found 536.1901. HPLC  $t_R$  = 3.465 min; purity = 96.22% using 40% CH<sub>3</sub>CN:60% H<sub>2</sub>O as the mobile phase.

**5.4.9. (2S,4aR,6aR,7R,9S,10aS,10bR)-methyl 9-acetoxy-2-(3-cyanobenzoyl)-6a,10b-dimethyl-4,10-dioxododecahydro-1H-benzo[*f*]isochromene-7-carboxylate (20)**—

Compound **20** was synthesized from compound **11** using the general procedure and 3-cyanophenylboronic acid to afford 0.055 g (43.9%) isolated as a white solid, mp = 124 – 127 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.25 (t,  $J$  = 1.4 Hz, 1H), 8.18 – 8.14 (m, 1H), 7.92 – 7.88 (m, 1H), 7.66 (t,  $J$  = 7.8 Hz, 1H), 5.79 (t,  $J$  = 8.3 Hz, 1H), 5.18 – 5.10 (m, 1H), 3.72 (s, 3H), 2.80 – 2.71 (m, 1H), 2.58 (dd,  $J$  = 7.9, 13.7 Hz, 1H), 2.35 – 2.26 (m, 2H), 2.21 (s, 1H), 2.20 – 2.08 (m, 5H), 1.82 – 1.52 (m, 4H), 1.48 (s, 3H), 1.10 (s, 3H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 202.11, 193.43, 171.71, 170.42, 170.09, 137.18, 134.85, 133.13, 132.96, 130.28, 117.76, 113.99, 75.65, 75.05, 64.78, 53.49, 52.22, 50.01, 42.18, 37.90, 37.64, 35.72, 30.88, 20.80, 18.41, 16.77, 16.30. HRMS ( $m/z$ ): [M-H] calcd for C<sub>27</sub>H<sub>28</sub>NO<sub>8</sub>, 494.1815; found 494.1806. HPLC  $t_R$  = 6.510 min; purity = 95.94%.

**5.4.10. (2S,4aR,6aR,7R,9S,10aS,10bR)-methyl 9-acetoxy-6a,10b-dimethyl-4,10-dioxo-2-(3-(trifluoromethoxy)benzoyl)dodecahydro-1H-benzo[*f*]isochromene-7-carboxylate (21)**—

Compound **21** was synthesized from compound **11** using the general procedure and 3-(trifluoromethoxy)-phenylboronic acid to afford 0.038 g (24.3%) isolated as a white solid, mp = 92 – 94 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.86 – 7.80 (m, 2H), 7.56 (t,  $J$  = 8.0 Hz, 1H), 7.48 (d,  $J$  = 7.2 Hz, 1H), 5.81 (t,  $J$  = 8.3 Hz, 1H), 5.16 – 5.09 (m, 1H), 3.72 (s, 3H), 2.78 – 2.69 (m, 1H), 2.62 (dd,  $J$  = 8.3, 13.6 Hz, 1H), 2.29 (dd,  $J$  = 7.2, 13.5 Hz, 2H), 2.19 (s, 1H), 2.18 – 2.09 (m, 5H), 1.77 (d,  $J$  = 13.2

Hz, 1H), 1.71 – 1.53 (m, 3H), 1.47 (s, 3H), 1.09 (s, 3H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 202.15, 193.97, 171.75, 170.72, 170.07, 150.00 (q, <sup>3</sup>J<sub>CF</sub> = 1.97), 135.62, 130.91, 127.43, 126.77, 121.56, 120.56 (q, J<sub>CF</sub> = 258.18), 75.62, 75.06, 64.91, 53.48, 52.21, 49.77, 42.19, 38.03, 37.91, 35.76, 30.89, 20.79, 18.44, 16.88, 16.27. HRMS (*m/z*): [M+Na] calcd for C<sub>27</sub>H<sub>29</sub>F<sub>3</sub>O<sub>9</sub>Na, 577.1661; found 577.1621. HPLC *t*<sub>R</sub> = 15.925 min; purity = 97.74%.

**5.4.11. (2*S*,4*aR*,6*aR*,7*R*,9*S*,10*aS*,10*bR*)-methyl 9-acetoxy-6*a*,10*b*-dimethyl-4,10-dioxo-2-(3-(trifluoromethyl)benzoyl)dodecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (22)**—Compound **22** was synthesized from compound **11** using the general procedure and 3-(trifluoromethyl)phenylboronic acid to afford 0.044 g (38.8%) isolated as an off-white solid, mp = 94 – 97 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.23 (s, 1H), 8.10 (d, *J* = 7.9 Hz, 1H), 7.88 (d, *J* = 7.8 Hz, 1H), 7.66 (t, *J* = 7.8 Hz, 1H), 5.84 (t, *J* = 8.3 Hz, 1H), 5.16 – 5.10 (m, 1H), 3.72 (s, 3H), 2.79 – 2.70 (m, 1H), 2.61 (dd, *J* = 8.2, 13.6 Hz, 1H), 2.29 (dd, *J* = 7.5, 13.5 Hz, 2H), 2.20 (s, 1H), 2.19 – 2.08 (m, 5H), 1.77 (d, *J* = 13.2 Hz, 1H), 1.75 – 1.53 (m, 3H), 1.48 (s, 3H), 1.09 (s, 3H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 202.15, 194.16, 171.74, 170.66, 170.08, 134.49, 132.33, 132.05 (q, <sup>2</sup>J<sub>CF</sub> = 33.11 Hz), 130.87 (q, <sup>3</sup>J<sub>CF</sub> = 3.41 Hz), 129.97, 126.18 (q, <sup>3</sup>J<sub>CF</sub> = 3.84 Hz), 123.63 (q, J<sub>CF</sub> = 272.85 Hz), 75.56, 75.06, 64.91, 53.49, 52.22, 49.84, 42.20, 37.92, 37.86, 35.75, 30.90, 20.80, 18.44, 16.90, 16.28. HRMS (*m/z*): [M+Na] calcd for C<sub>27</sub>H<sub>29</sub>F<sub>3</sub>O<sub>8</sub>Na, 561.1712; found 561.1670. HPLC *t*<sub>R</sub> = 5.799 min; purity = 95.0% as determined using a Phenomenex Luna column (250 × 4.5 mm, 5 μm).

**5.4.12. (2*S*,4*aR*,6*aR*,7*R*,9*S*,10*aS*,10*bR*)-methyl 9-acetoxy-2-(furan-2-carbonyl)-6*a*,10*b*-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (23)**—Compound **23** was synthesized from compound **11** using the general procedure and 2-furanylboronic acid to afford 0.109 g (84.6%) isolated as a white solid, mp = 207 – 209 °C. HRMS (*m/z*): [M+H] calcd for C<sub>24</sub>H<sub>29</sub>O<sub>9</sub>, 461.1812; found 461.1796. HPLC *t*<sub>R</sub> = 13.29 min; purity = 97.83% using 40% CH<sub>3</sub>CN:60% H<sub>2</sub>O as the mobile phase. The <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were in agreement with that previously reported.<sup>32</sup>

**5.4.13. (2*S*,4*aR*,6*aR*,7*R*,9*S*,10*aS*,10*bR*)-methyl 9-acetoxy-2-(furan-3-carbonyl)-6*a*,10*b*-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (24)**—Compound **24** was synthesized from compound **11** using the general procedure and furan-3-boronic acid to afford 0.106 g (67.5%) isolated as a white solid, mp = 159 – 161 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.20 (dd, *J* = 0.8, 1.3 Hz, 1H), 7.44 (dd, *J* = 1.4, 1.9 Hz, 1H), 6.79 (dd, *J* = 0.8, 1.9 Hz, 1H), 5.28 (t, *J* = 8.0 Hz, 1H), 5.17 – 5.07 (m, 1H), 3.69 (s, 3H), 2.71 (dd, *J* = 5.4, 11.4 Hz, 1H), 2.54 (dd, *J* = 7.8, 13.8 Hz, 1H), 2.33 – 2.23 (m, 2H), 2.18 (s, 1H), 2.14 (s, 3H), 2.10 – 2.01 (m, 2H), 1.79 – 1.70 (m, 2H), 1.69 – 1.58 (m, 1H), 1.55 – 1.47 (m, 1H), 1.40 (s, 3H), 1.06 (s, 3H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 202.05, 191.00, 171.77, 170.83, 170.06, 149.28, 144.44, 124.23, 109.43, 78.51, 75.08, 64.82, 53.52, 52.19, 50.08, 42.18, 38.25, 37.92, 35.57, 30.90, 20.80, 18.40, 16.97, 16.32. HRMS (*m/z*): [M+H] calcd for C<sub>24</sub>H<sub>29</sub>O<sub>9</sub>, 461.1812; found 461.1796. HPLC *t*<sub>R</sub> = 5.105 min; purity = 99.67%.

**5.4.14. (2*S*,4*aR*,6*aR*,7*R*,9*S*,10*aS*,10*bR*)-methyl 9-acetoxy-6*a*,10*b*-dimethyl-4,10-dioxo-2-(thiophene-2-carbonyl)dodecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (25)**—Compound **25** was synthesized from compound **11** using the general procedure and 2-thiopheneboronic acid to afford 0.036 g (36.4%) isolated as an off-white solid, mp = 188 – 190 °C. HRMS (*m/z*): [M+Na] calcd for C<sub>24</sub>H<sub>28</sub>O<sub>8</sub>SNa, 499.1403; found 499.1380. HPLC *t*<sub>R</sub> = 5.733 min; purity = 96.12%. The <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were in agreement with that previously reported.<sup>32</sup>

**5.4.15. (2S,4aR,6aR,7R,9S,10aS,10bR)-methyl 9-acetoxy-6a,10b-dimethyl-4,10-dioxo-2-(thiophene-3-carbonyl)dodecahydro-1H-benzo[f]isochromene-7-carboxylate (26)**—Compound **26** was synthesized from compound **11** using the general procedure and 3-thiopheneboronic acid to afford 0.081 g (74.3%) isolated as an off-white solid, mp = 170 – 173 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.20 (dd, *J* = 1.2, 2.8 Hz, 1H), 7.56 (dd, *J* = 1.2, 5.1 Hz, 1H), 7.37 (dd, *J* = 2.9, 5.1 Hz, 1H), 5.60 (t, *J* = 8.1 Hz, 1H), 5.17 – 5.08 (m, 1H), 3.72 (s, 3H), 2.78 – 2.67 (m, 1H), 2.61 (dd, *J* = 8.3, 13.7 Hz, 1H), 2.29 (dd, *J* = 7.5, 13.5 Hz, 2H), 2.20 (s, 1H), 2.19 – 2.06 (m, 5H), 1.71 (ddd, *J* = 12.5, 22.2, 34.2 Hz, 3H), 1.59 – 1.49 (m, 1H), 1.45 (s, 3H), 1.08 (s, 3H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 202.17, 189.63, 171.78, 171.01, 170.06, 138.44, 134.68, 127.63, 127.21, 77.09, 75.08, 64.96, 53.49, 52.19, 49.71, 42.20, 38.30, 37.92, 35.69, 30.90, 20.81, 18.44, 17.05, 16.27. HRMS (*m/z*): [M+Na] calcd for C<sub>24</sub>H<sub>28</sub>O<sub>8</sub>SNa, 499.1403; found 499.1393. HPLC *t*<sub>R</sub> = 5.657 min; purity = 99.78%.

**5.4.16. (2S,4aR,6aR,7R,9S,10aS,10bR)-methyl 9-acetoxy-2-(benzofuran-2-carbonyl)-6a,10b-dimethyl-4,10-dioxododecahydro-1H-benzo[f]isochromene-7-carboxylate (27)**—Compound **27** was synthesized from compound **11** using the general procedure and 2-benzofuranylboronic acid to afford 0.090 g (77.2%) isolated as a white solid, mp = 228 – 232 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.74 (d, *J* = 7.8 Hz, 1H), 7.71 (d, *J* = 0.9 Hz, 1H), 7.60 (dd, *J* = 0.8, 8.5 Hz, 1H), 7.55 – 7.50 (m, 1H), 7.38 – 7.31 (m, 1H), 5.75 (t, *J* = 8.3 Hz, 1H), 5.16 – 5.08 (m, 1H), 3.72 (s, 3H), 2.77 – 2.67 (m, 2H), 2.29 (dd, *J* = 7.5, 13.5 Hz, 2H), 2.25 – 2.18 (m, 2H), 2.18 – 2.11 (m, 4H), 1.81 – 1.60 (m, 4H), 1.48 (s, 3H), 1.10 (s, 3H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 202.15, 186.33, 171.78, 170.86, 170.04, 156.20, 149.86, 129.49, 126.98, 124.56, 123.93, 116.49, 112.90, 76.49, 75.07, 64.86, 53.49, 52.20, 49.92, 42.22, 38.22, 37.95, 35.77, 30.92, 20.80, 18.45, 16.78, 16.29. HRMS (*m/z*): [M+Na] calcd for C<sub>28</sub>H<sub>30</sub>O<sub>9</sub>Na, 533.1788; found 533.1797. HPLC *t*<sub>R</sub> = 7.558 min; purity = 99.33%.

**5.4.17. (2S,4aR,6aR,7R,9S,10aS,10bR)-methyl 9-acetoxy-2-(benzo[b]thiophene-2-carbonyl)-6a,10bdimethyl-4,10-dioxododecahydro-1H-benzo[f]isochromene-7-carboxylate (28)**—Compound **28** was synthesized from compound **11** using the general procedure and thianaphthene-2-boronic acid to afford 0.095 g (78.8%) isolated as a white solid, mp = 127 – 130 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.10 (s, 1H), 7.93 (d, *J* = 8.0 Hz, 1H), 7.88 (d, *J* = 8.1 Hz, 1H), 7.54 – 7.46 (m, 1H), 7.46 – 7.40 (m, 1H), 5.74 (t, *J* = 8.2 Hz, 1H), 5.19 – 5.09 (m, 1H), 3.72 (s, 3H), 2.79 – 2.71 (m, 1H), 2.67 (dd, *J* = 8.1, 13.7 Hz, 1H), 2.34 – 2.25 (m, 2H), 2.25 – 2.18 (m, 2H), 2.17 – 2.08 (m, 4H), 1.85 – 1.73 (m, 2H), 1.73 – 1.63 (m, 1H), 1.63 – 1.53 (m, 1H), 1.48 (s, 3H), 1.09 (s, 3H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 202.22, 190.04, 171.79, 170.84, 170.01, 143.20, 139.72, 139.13, 131.67, 128.45, 126.81, 125.56, 123.11, 76.90, 75.08, 64.83, 53.46, 52.19, 49.81, 42.21, 38.59, 37.90, 35.75, 30.91, 20.79, 18.44, 16.98, 16.29. HRMS (*m/z*): [M+Na] calcd for C<sub>28</sub>H<sub>30</sub>O<sub>8</sub>SNa, 549.1559; found 549.1573. HPLC *t*<sub>R</sub> = 9.577 min; purity = 98.44%.

**5.4.18. (2S,4aR,6aR,7R,9S,10aS,10bR)-methyl 9-acetoxy-2-(benzo[b]thiophene-3-carbonyl)-6a,10bdimethyl-4,10-dioxododecahydro-1H-benzo[f]isochromene-7-carboxylate (29)**—Compound **29** was synthesized from compound **11** using the general procedure and 1-benzothiophen-3-ylboronic acid to afford 0.065g (23.3%) isolated as an off-white solid, mp = 133 – 136 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.72 (d, *J* = 7.9 Hz, 1H), 8.43 (s, 1H), 7.89 (d, *J* = 7.9 Hz, 1H), 7.56 – 7.50 (m, 1H), 7.46 (dd, *J* = 4.1, 11.1 Hz, 1H), 5.77 (t, *J* = 8.2 Hz, 1H), 5.18 – 5.08 (m, 1H), 3.72 (s, 3H), 2.77 – 2.70 (m, 1H), 2.65 (dd, *J* = 8.3, 13.7 Hz, 1H), 2.29 (dd, *J* = 7.2, 13.5 Hz, 2H), 2.26 – 2.18 (m, 2H), 2.13 (d, *J* = 16.9 Hz, 4H), 1.84 – 1.53 (m, 4H), 1.47 (s, 3H), 1.09 (s,



3H).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ )  $\delta$  202.27, 190.09, 171.80, 171.13, 170.04, 139.79, 139.45, 136.99, 131.61, 126.46, 126.16, 125.67, 122.57, 76.76, 75.12, 64.94, 53.50, 52.19, 49.68, 42.23, 38.51, 37.97, 35.68, 30.91, 20.79, 18.46, 17.06, 16.26. HRMS ( $m/z$ ):  $[\text{M}+\text{H}]$  calcd for  $\text{C}_{28}\text{H}_{31}\text{O}_8\text{S}$ , 527.1740; found 527.1742. HPLC  $t_R$  = 6.64 min; purity = 96.84%.

**5.4.19. (2S,4aR,6aR,7R,9S,10aS,10bR)-methyl 9-acetoxy-2-((Z)-but-2-enoyl)-6a,10b-dimethyl-4,10-dioxododecahydro-1H-benzo[f]isochromene-7-carboxylate (30)**—Compound **30** was synthesized from compound **11** using the general procedure and *cis*-1-propen-1-ylboronic acid to afford 0.195 g (72.7%) isolated as a white solid, mp = 153 – 155 °C.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  6.48 (dq,  $J$  = 7.3, 11.4 Hz, 1H), 6.33 (dd,  $J$  = 1.7, 11.4 Hz, 1H), 5.19 – 5.11 (m, 1H), 4.92 (dd,  $J$  = 7.4, 9.3 Hz, 1H), 3.72 (s, 3H), 2.73 (dd,  $J$  = 5.8, 11.1 Hz, 1H), 2.52 (dd,  $J$  = 7.4, 13.7 Hz, 1H), 2.33 – 2.25 (m, 2H), 2.17 (dd,  $J$  = 2.0, 7.0 Hz, 7H), 2.10 (dd,  $J$  = 3.4, 14.3 Hz, 1H), 2.00 (dd,  $J$  = 3.2, 11.8 Hz, 1H), 1.76 (dd,  $J$  = 3.3, 13.3 Hz, 1H), 1.65 (dd,  $J$  = 12.6, 27.3 Hz, 1H), 1.59 – 1.48 (m, 2H), 1.38 (s, 3H), 1.08 (s, 3H).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ )  $\delta$  201.98, 196.49, 171.76, 170.83, 170.10, 149.06, 122.82, 79.95, 75.10, 64.78, 53.60, 52.20, 50.37, 42.21, 38.05, 37.94, 35.56, 30.91, 20.81, 18.40, 16.76, 16.43, 16.35. HRMS ( $m/z$ ):  $[\text{M}+\text{Na}]$  calcd for  $\text{C}_{23}\text{H}_{30}\text{O}_8\text{Na}$ , 457.1838; found 457.1859. HPLC  $t_R$  = 15.253 min; purity = 95.43% using 40%  $\text{CH}_3\text{CN}$ :60%  $\text{H}_2\text{O}$  as the mobile phase.

#### 5.5. Preparation of (2S,4aR,6aR,7R,9S,10aS,10bR)-methyl 9-acetoxy-2-(furan-2-yl)-6a,10b-dimethyl-4,10-di-oxododecahydro-1H-benzo[f]isochromene-7-carboxylate (31)

Alkene **30** (0.108 g, 0.249 mmol) was dissolved in bromobenzene (15 mL) followed by the addition of selenium (IV) oxide (0.083 g, 0.746 mmol). Reaction mixture was heated at 160°C and monitored by TLC. The solvent was evaporated *in vacuo* and the resulting residue purified by flash column chromatography on silica gel using mixtures of EtOAc/*n*-hexanes and triturated in EtOAc/*n*-hexanes to yield 0.195 g (18.1%) of **31** as a white solid, mp 198 – 200 °C;  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  7.39 (dd,  $J$  = 0.8, 1.7 Hz, 1H), 6.36 – 6.31 (m, 2H), 5.52 (dd,  $J$  = 5.6, 11.6 Hz, 1H), 5.18 – 5.11 (m, 1H), 3.73 (s, 3H), 2.81 – 2.72 (m, 1H), 2.45 (dd,  $J$  = 5.6, 13.6 Hz, 1H), 2.36 – 2.27 (m, 2H), 2.23 (s, 1H), 2.20 – 2.12 (m, 5H), 1.93 – 1.85 (m, 1H), 1.83 – 1.76 (m, 1H), 1.71 – 1.59 (m, 2H), 1.44 (s, 3H), 1.11 (s, 3H).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ )  $\delta$  202.17, 171.79, 171.07, 170.20, 151.41, 143.38, 110.66, 109.27, 75.27, 72.01, 64.30, 53.78, 52.21, 51.25, 42.33, 40.52, 38.36, 35.51, 30.95, 20.79, 18.36, 16.53, 15.28. HRMS ( $m/z$ ):  $[\text{M}+\text{Na}]$  calcd for  $\text{C}_{23}\text{H}_{28}\text{O}_8\text{Na}$ , 455.1682; found, 455.1695. HPLC  $t_R$  = 24.103 min; purity = 97.44% using 40%  $\text{CH}_3\text{CN}$ :60%  $\text{H}_2\text{O}$  as the mobile phase.

#### 5.6. Opioid Binding Assay.<sup>50</sup>

Recombinant Chinese Hamster Ovary (CHO) cells (hMOP-CHO, hDOP-CHO and hKOP-CHO) were produced by stable transfection with the respective human opioid receptor cDNA, and provided by Dr. Larry Toll (SRI International, CA). The cells were grown on plastic flasks in DMEM (90%) (hDOP-CHO and hKOP-CHO) or DMEM/ F-12 (45%/ 45%) medium (hMOP-CHO) containing 10% FetalClone II (HyClone) and Geneticin (G-418: 0.10–0.2 mg/ml) (Invitrogen) under 95% air/5%  $\text{CO}_2$  at 37° C. Cell monolayers were harvested and frozen at –80 °C. The hKOP-CHO, hMOP-CHO and hDOP-CHO cells are used for opioid binding experiments. For the [ $^{35}\text{S}$ ]GTP- $\gamma$ -S binding experiments, we use hKOP-CHO and hMOP-CHO cells for assaying KOP and MOP receptor function. We use the NG108–15 neuroblastoma×glioma cell for the DOP [ $^{35}\text{S}$ ]GTP- $\gamma$ -S binding assay. We used [ $^3\text{H}$ ][D-Ala<sup>2</sup>-MePhe<sup>4</sup>,Gly-ol<sup>5</sup>]enkephalin ([ $^3\text{H}$ ]DAMGO, SA=44–48 Ci/mmol) to label MOP, [ $^3\text{H}$ ][D-Ala<sup>2</sup>,D-Leu<sup>5</sup>]enkephalin ([ $^3\text{H}$ ]DADLE, SA=40–50 Ci/mmol) to label DOP receptors and [ $^3\text{H}$ ](–)-U69,593 (SA=50 Ci/mmol) to label KOP receptor binding sites. On the day of the assay, cell pellets were thawed on ice for 15 minutes then homogenized with a

polytron in 10 mL/pellet of ice-cold 10mM Tris-HCl, pH 7.4. Membranes were then centrifuged at  $30,000 \times g$  for 10 minutes, resuspended in 10 ml/pellet ice-cold 10mM Tris-HCl, pH 7.4 and again centrifuged  $30,000 \times g$  for 10 min. Membranes were then resuspended in 25 °C 50 mM Tris-HCl, pH 7.4 (~100 mL/pellet hMOP-CHO, 50 ml/pellet hDOP-CHO and 120 ml/pellet hKOP-CHO). All assays took place in 50 mM Tris-HCl, pH 7.4, with a protease inhibitor cocktail [bacitracin (100  $\mu\text{g}/\text{mL}$ , bestatin (10  $\mu\text{g}/\text{mL}$ ), leupeptin (4  $\mu\text{g}/\text{mL}$ ) and chymostatin (2  $\mu\text{g}/\text{mL}$ )], in a final assay volume of 1.0 mL. All drug dilution curves were made up with buffer containing 1 mg/mL BSA. Nonspecific binding was determined using 20  $\mu\text{M}$  levallorphan ( $[^3\text{H}]\text{DAMGO}$  and  $[^3\text{H}]\text{DADLE}$ ) and 1  $\mu\text{M}$  (-)-U69,593 (for  $[^3\text{H}]\text{U69,593}$  binding).  $[^3\text{H}]\text{Radioligands}$  were used at ~2 nM concentrations. Triplicate samples were filtered with Brandel Cell Harvesters (Biomedical Research & Development Inc., Gaithersburg, MD), over Whatman GF/B filters, after a 2 hr incubation at 25 °C. The filters were punched into 24-well plates to which was added 0.6 ml of LSC-cocktail (Cytoscint). Samples were counted, after an overnight extraction, in a Trilux liquid scintillation counter at 44% efficiency. Opioid binding assays had ~30  $\mu\text{g}$  protein per assay tube. Inhibition curves were generated by displacing a single concentration of radioligand by 10 concentrations of drug.

### 5.7. $[^{35}\text{S}]\text{GTP-}\gamma\text{-S}$ Binding Assay

The  $[^{35}\text{S}]\text{GTP-}\gamma\text{-S}$  assays were conducted as described elsewhere.<sup>50</sup> In this description, buffer "A" is 50 mM Tris-HCl, pH 7.4, containing 100 mM NaCl, 10 mM  $\text{MgCl}_2$ , 1 mM EDTA and buffer "B" is buffer A plus 1.67 mM DTT and 0.15% BSA. On the day of the assay, cells were thawed on ice for 15 min and homogenized using a polytron in 50 mM Tris-HCl, pH 7.4, containing 4  $\mu\text{g}/\text{mL}$  leupeptin, 2  $\mu\text{g}/\text{mL}$  chymostatin, 10  $\mu\text{g}/\text{mL}$  bestatin and 100  $\mu\text{g}/\text{mL}$  bacitracin. The homogenate was centrifuged at  $30,000 \times g$  for 10 min at 4 °C, and the supernatant discarded. The membrane pellets were resuspended in buffer B and used for  $[^{35}\text{S}]\text{GTP-}\gamma\text{-S}$  binding assays.  $[^{35}\text{S}]\text{GTP-}\gamma\text{-S}$  binding was determined as described previously. Briefly, test tubes received the following additions: 50  $\mu\text{L}$  buffer A plus 0.1% BSA, 50  $\mu\text{L}$  GDP in buffer A/0.1% BSA (final concentration = 40  $\mu\text{M}$ ), 50  $\mu\text{L}$  drug in buffer A/0.1% BSA, 50  $\mu\text{L}$   $[^{35}\text{S}]\text{GTP-}\gamma\text{-S}$  in buffer A/0.1% BSA (final concentration = 50 pM), and 300  $\mu\text{L}$  of cell membranes (50  $\mu\text{g}$  of protein) in buffer B. The final concentrations of reagents in the  $[^{35}\text{S}]\text{GTP-}\gamma\text{-S}$  binding assays were: 50 mM Tris-HCl, pH 7.4, containing 100 mM NaCl, 10 mM  $\text{MgCl}_2$ , 1 mM EDTA, 1 mM DTT, 40  $\mu\text{M}$  GDP and 0.1% BSA. Incubations proceeded for 3 hr at 25 °C. Nonspecific binding was determined using GTP- $\gamma\text{-S}$  (40  $\mu\text{M}$ ). Bound and free  $[^{35}\text{S}]\text{GTP-}\gamma\text{-S}$  were separated by vacuum filtration (Brandel) through GF/B filters. The filters were punched into 24-well plates to which was added 0.6 mL LSC-cocktail (Cytoscint). Samples were counted, after an overnight extraction, in a Trilux liquid scintillation counter at 27% efficiency.

### 5.8. Fluorescent $\text{Ca}^{2+}$ Mobilization Assay.<sup>51</sup>

All cells were maintained in F-12 nutrient medium (Ham), supplemented with 10% fetal bovine serum (FBS), 1% penicillin and streptomycin (p/s), and 0.2% normocin. Cell culture supplies were from Invitrogen (Carlsbad, CA) unless otherwise specified. CHO cells stably expressing KOP R- $\text{G}\alpha_{\text{q}16}$  were removed from their flasks using Versene and quenched with the Ham media, centrifuged and re-suspended in media. Cells were counted with a Cellometer Auto T4 (Nexcelom Bioscience, Lawrence, MA) and 30,000 cells were transferred to each well of a black Costar 96-well optical bottom plate (Corning Corporation, Corning, NY). Each plate was incubated at 37 °C overnight to confluence. The culture media was removed from the plates and cells were subsequently loaded with a fluorescent calcium probe (Calcium 5 dye, Molecular Devices, Sunnyvale, CA) in an HBSS-based buffer containing 20 mM HEPES, 0.25% BSA, 1% DMSO, and 10  $\mu\text{M}$  probenecid (Sigma) in a total volume of 225  $\mu\text{L}$ . Cells were incubated at 37°C for 1 h and then

stimulated with DAMGO, U69,593 or test compounds at various concentrations using a Flexstation 3 plate-reader, which automatically added 25  $\mu$ L of the compounds at 10X concentration to each well after reading baseline values for  $\sim$ 17 sec. Agonist-mediated change in fluorescence (485 nm excitation, 525 nm emission) was monitored in each well at 1.52 sec intervals for 60 sec and reported for each well. Data were collected using Softmax version 4.8 (MDS Analytical Technologies) and analyzed using Prism software (GraphPad, La Jolla, CA). Nonlinear regression analysis was performed to fit data and obtain maximum response ( $\text{max}$ ),  $\text{EC}_{50}$ , correlation coefficient ( $r^2$ ) and other parameters. All experiments were performed at least 3 times to ensure reproducibility and data reported as mean  $\pm$  standard error, unless noted otherwise.

### 5.9. Neuroendocrine biomarker assay.<sup>45</sup>

Chair-trained male rhesus monkeys (*Macaca mulatta*) were tested after extensive prior exposure to the experimental situation. Approximately 15 min following catheter placement, two baseline blood samples of approximately 2 mL were collected, 5 min apart from each other (defined as  $-10$  and  $-5$  min, relative to the onset of dosing), and kept at room temperature until the time of spinning (3000 rpm at 4  $^{\circ}$ C) and serum separation. Experiments were carried out with a cumulative dosing procedure, where doses of **1** or **3** were administered i.v. in increasing 0.5 log unit steps, every 20 min, and a blood sample was taken 15 min after each dose. Data for **1** were from a previous determination under identical experimental conditions.<sup>45</sup> Serum samples were kept at  $-40$   $^{\circ}$ C until the time of analysis, typically within 2 weeks of collection. Samples were analyzed in duplicate with a standard human prolactin immunoradiometric kit (Siemens Medical Solutions Diagnostics, Los Angeles CA), following manufacturer's instructions. Procedures were reviewed and approved by the Rockefeller University Animal Care and Use Committee, in accordance with the Guide on the Care and Use of Animals (National Academy Press, 1996). Data are presented graphically as ng/ml (i.e., drug effect – mean pre-injection baseline per subject). Linear regression analyses were calculated from mean dose-effect curve points spanning the  $\text{ED}_{50}$  point.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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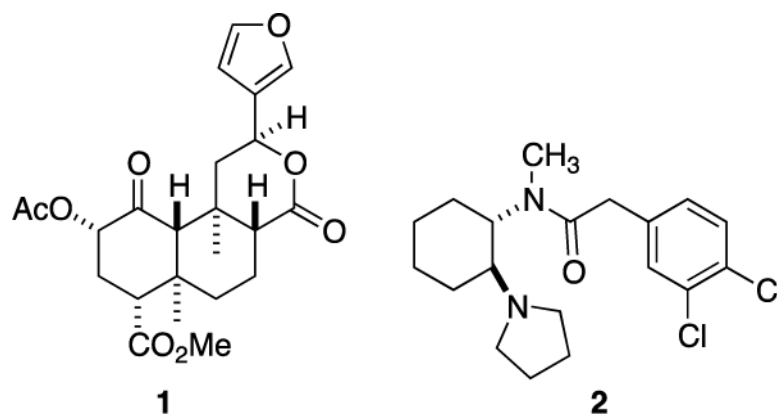
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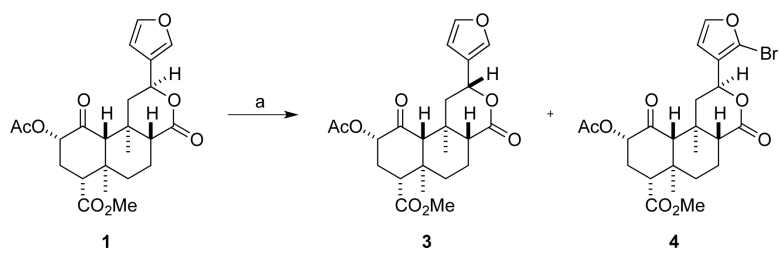
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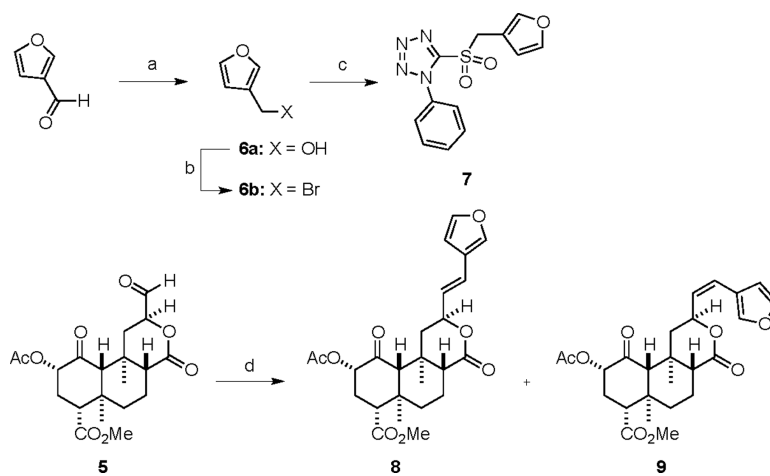




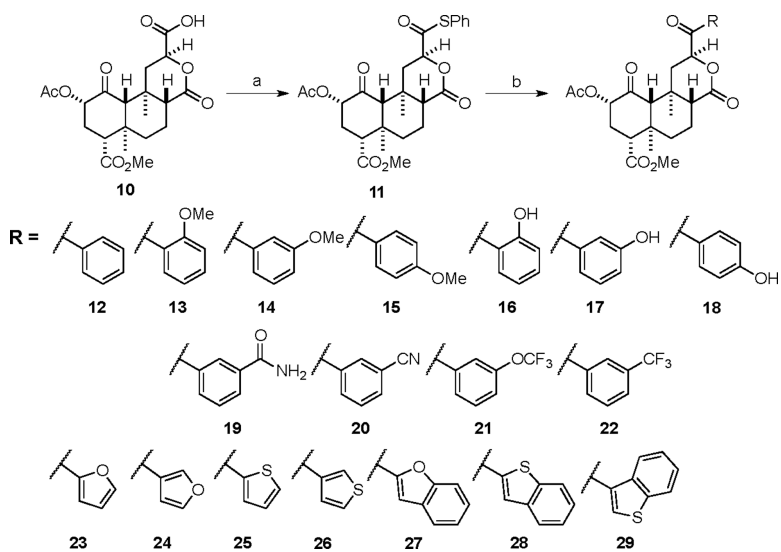
**Figure 1.**  
Structures of salvinorin A (1) and U50,488 (2).

**Scheme 1.**

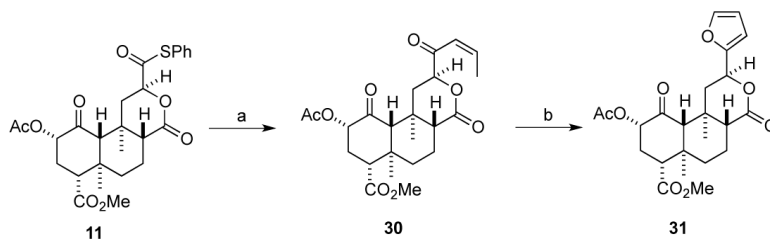
<sup>a</sup>Reagents and Conditions: a) 2,4,4,6-Tetrabromo-2,5-cyclohexadienone, CH<sub>2</sub>Cl<sub>2</sub>, 130 °C, 30 min MW.

**Scheme 2.**

*<sup>a</sup>Reagents and Conditions:* a)  $\text{NaBH}_4$ ,  $\text{I}_2$ , THF,  $0\text{ }^\circ\text{C}$ ; b)  $\text{PBr}_3$ , THF,  $0\text{ }^\circ\text{C}$ ; c) 1-phenyl-1*H*-tetrazole-5-thiol,  $\text{Et}_3\text{N}$ ,  $\text{H}_2\text{O}_2$ ,  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ ,  $\text{CH}_2\text{Cl}_2$ ; d) **7**, KHMDS, THF,  $-78\text{ }^\circ\text{C}$

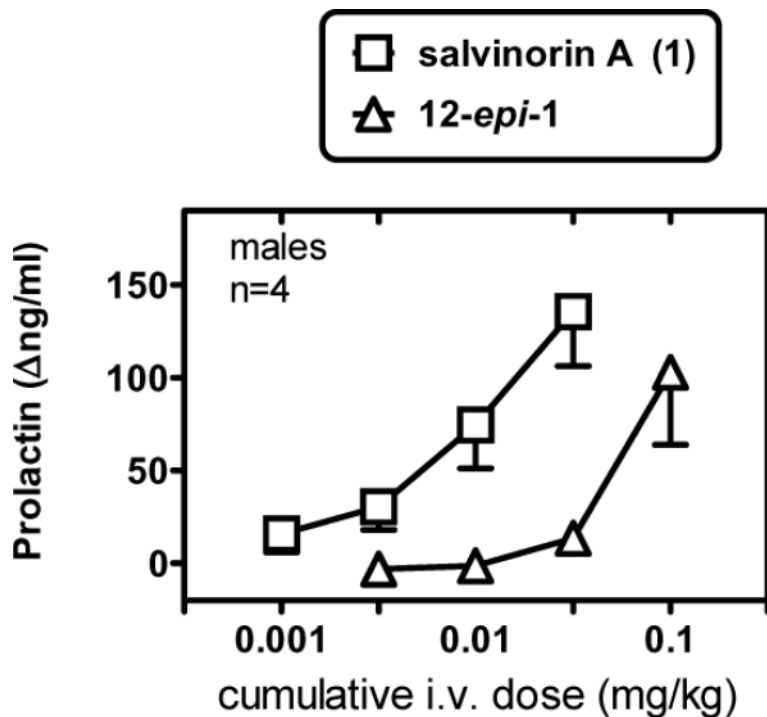
**Scheme 3.**

<sup>a</sup>Reagents and Conditions: a) CDMT, NMM, thiophenol, CH<sub>2</sub>Cl<sub>2</sub>; b) Appropriate boronic acid, copper thiophene-2-carboxylate (CuTC), Pd(dba)<sub>2</sub>, P(OEt)<sub>3</sub>, THF

**Scheme 4.**

<sup>a</sup>Reagents and Conditions: a) *cis*-1-Propen-1-ylboronic acid, CuTC, Pd(dba)<sub>2</sub>, P(OEt)<sub>3</sub>, THF; b) SeO<sub>2</sub>, Bromobenzene, reflux





**Figure 2.** Dose-effect curve in a neuroendocrine biomarker assay for the effects of i.v. **1** and 12-epi-**1** (**3**) on serum prolactin levels in male subjects. Data for **1** were from a previous determination under identical experimental conditions.<sup>45</sup> Abscissa, dose of **1** or **3**. Ordinate, serum prolactin levels, expressed as change from individual preinjection baseline (nanograms per milliliter; mean  $\pm$  SEM).

**Table 1**Opioid receptor binding affinities of neoclerodanes **1**, **3**, **9**, **10**, and **12 – 31**.

Cmpd	$K_i \pm$ SD, nM		
	MOP	DOP	KOP
<b>1</b> <sup>a</sup>	1,370 ± 130	>10,000	7.4 ± 0.7
<b>3</b>	> 10,000 (72) <sup>c</sup>	> 10,000 (74) <sup>c</sup>	17 ± 1 <sup>a</sup>
<b>8</b>	> 10,000 (78) <sup>c</sup>	> 10,000 (66) <sup>c</sup>	1,120 ± 30 <sup>a</sup>
<b>9</b>	2,600 ± 140 <sup>a</sup>	> 10,000 (94) <sup>c</sup>	30 ± 4 <sup>a</sup>
<b>12</b>	1,490 ± 160 <sup>a</sup>	> 10,000 (100) <sup>c</sup>	70 ± 3 <sup>a</sup>
<b>13</b>	> 10,000 (53) <sup>c</sup>	> 10,000 (87) <sup>b</sup>	1,070 ± 80 <sup>a</sup>
<b>14</b>	3,090 ± 260 <sup>a</sup>	> 10,000 (77) <sup>b</sup>	290 ± 10 <sup>a</sup>
<b>15</b>	> 10,000 (74) <sup>c</sup>	> 10,000 (81) <sup>b</sup>	> 10,000
<b>16</b>	3,600 ± 40 <sup>a</sup>	> 10,000 (94) <sup>c</sup>	500 ± 20 <sup>a</sup>
<b>17</b>	> 1,000 (55) <sup>b</sup>	> 10,000 (80) <sup>c</sup>	110 ± 5 <sup>a</sup>
<b>18</b>	> 10,000 (93) <sup>c</sup>	> 10,000 (100) <sup>c</sup>	8,470 ± 900 <sup>a</sup>
<b>19</b>	>10,000 (84) <sup>b</sup>	> 10,000 <sup>b</sup> (52)	40 ± 1 <sup>a</sup>
<b>20</b>	>1,000 (61) <sup>b</sup>	>10,000 (68) <sup>b</sup>	220 ± 10 <sup>a</sup>
<b>21</b>	4,800 ± 400 <sup>a</sup>	>1,000 (37) <sup>b</sup>	>10,000 (54) <sup>b</sup>
<b>22</b>	3,400 ± 150 <sup>a</sup>	>1,000 (46) <sup>b</sup>	2,260 ± 120 <sup>a</sup>
<b>23</b>	9,800 ± 2,500 <sup>a</sup>	>10,000 (91) <sup>c</sup>	80 ± 4 <sup>a</sup>
<b>24</b>	> 10,000 (66) <sup>c</sup>	> 10,000 (95) <sup>c</sup> <sub>xs</sub>	150 ± 10 <sup>a</sup>
<b>25</b>	> 10,000 (65) <sup>c</sup>	> 10,000 (99) <sup>c</sup>	36 ± 2 <sup>a</sup>
<b>26</b>	2,530 ± 250 <sup>a</sup>	> 10,000 (88) <sup>c</sup>	31 ± 3 <sup>a</sup>
<b>27</b>	> 10,000 (100) <sup>c</sup>	> 10,000 (91) <sup>c</sup>	> 10,000 (92) <sup>c</sup>
<b>28</b>	> 10,000 (58) <sup>c</sup>	> 10,000 (95) <sup>c</sup>	9,210 ± 680 <sup>a</sup>
<b>29</b>	>1,000 (60) <sup>b</sup>	>1,000 (56) <sup>b</sup>	>10,000 (64) <sup>b</sup>
<b>30</b>	>1,700 <sup>a</sup>	>5,000 <sup>a</sup>	320 ± 20 <sup>a</sup>
<b>31</b>	> 1,000 (81) <sup>b</sup>	>10,000 (99) <sup>b</sup>	8.7 ± 0.4 <sup>a</sup>

<sup>a</sup>  $K_i$  values ( $\pm$ SD) were determined from full dose-response curves (n=3) as described in methods.<sup>b</sup> Results are showing the percent of control produced by the 1  $\mu$ M test drug.<sup>c</sup> Results are showing the percent of control produced by the 10  $\mu$ M test drug.

**Table 2**Stimulation of [<sup>35</sup>S]GTP- $\gamma$ -S binding to human KOP receptors.

Cmpd	KOP EC <sub>50</sub> ± SD <sup>a</sup>	KOP E <sub>max</sub> ± SD <sup>b</sup>
<b>1</b>	40 ± 6	124 ± 6
<b>3</b>	720 ± 97	109 ± 4
<b>9</b>	1,370 ± 180	105 ± 4
<b>12</b>	2,280 ± 550	74 ± 6
<b>19</b>	730 ± 90	92 ± 4
<b>23</b>	2,130 ± 310	92 ± 4
<b>25</b>	1,630 ± 230	95 ± 4
<b>26</b>	620 ± 50	88 ± 2
<b>31</b>	140 ± 10	104 ± 2

<sup>a</sup>EC<sub>50</sub> = Effective concentration for 50% maximal response.<sup>b</sup>E<sub>max</sub> is % which compound stimulates binding compared to (-)-U50,488 at KOP receptors.

**Table 3**Opioid receptor activity measured in the fluorescent Ca<sup>2+</sup> mobilization assay.

Cmpd	KOP EC <sub>50</sub> ± SD <sup>a</sup>	KOP E <sub>max</sub> ± SD <sup>b</sup> %
U69,593 <sup>c</sup>	12.5 ± 5.4	100
<b>1</b> <sup>d</sup>	6.11 ± 0.04	97 ± 5
<b>19</b> <sup>c</sup>	75.4 ± 21	97 ± 2
<b>31</b> <sup>c</sup>	12.2 ± 4.4	97 ± 8

<sup>a</sup>EC<sub>50</sub> = Effective dose for 50% maximal response.<sup>b</sup>E<sub>max</sub> is % which compound stimulates binding compared to (–)-U69,593 at KOP receptors.<sup>c</sup>n = 3<sup>d</sup>n = 2