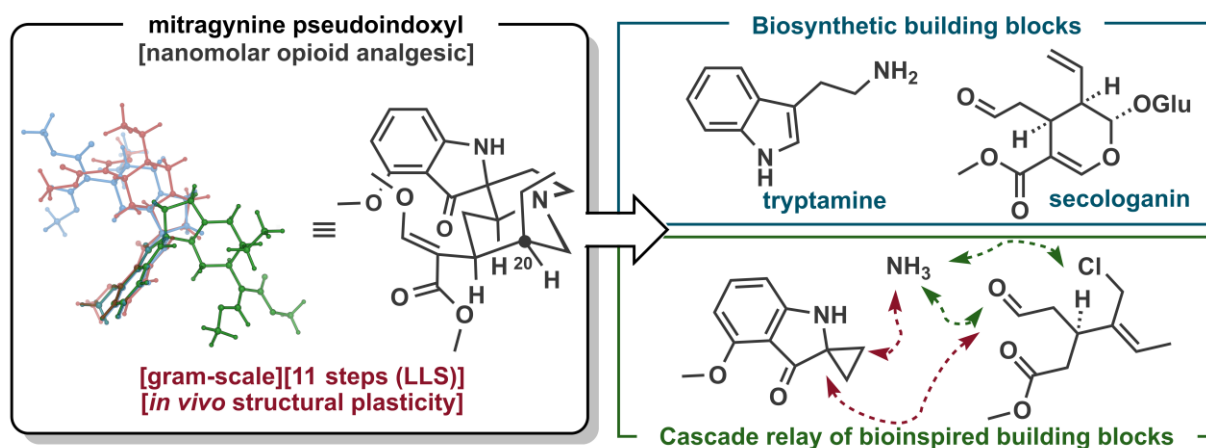# Gram-scale syntheses of kratom pseudoindoxyl metabolites: Illuminating the dynamic stereochemistry and the resulting structural plasticity of mitragynine pseudoindoxyl

Péter Angyal, Kristóf Hegedüs, Bence Balázs Mészáros, János Daru, Ádám Dudás, Anna Rita Galambos, Nariman Essmat, Mahmoud Al-Khrasani*, Szilárd Varga*, and Tibor Soós*

Abstract

Mitragynine pseudoindoxyl has recently attracted increasing attention as a promising analgesic alternative for pain management with considerably fewer side effects. Here, we describe the first enantioselective and scalable total synthesis of this natural product in addition to its C-20 epimeric congener, speciogynine pseudoindoxyl. The characteristic spiro-5-5-6 tricyclic system of these alkaloids is formed via a protecting group-free cascade relay process in which oxidized tryptamine and secologanin analogs are used. Furthermore, we uncovered that mitragynine pseudoindoxyl exists and acts not as a single molecular entity but as a dynamic ensemble of stereoisomers in protic environments, thus, it exhibits structural plasticity in biological systems. Accordingly, these synthetic, structural, and biological studies provide a basis for the planned design of mitragynine pseudoindoxyl analogs, which can guide the development of next-generation analgesics.

Gram-scale syntheses of kratom pseudoindoxyl metabolites: Illuminating the dynamic stereochemistry and the resulting structural plasticity of mitragynine pseudoindoxyl

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Abstract

Mitragynine pseudoindoxyl has recently attracted increasing attention as a promising analgesic alternative for pain management with considerably fewer side effects. Here, we describe the first enantioselective and scalable total synthesis of this natural product in addition to its C-20 epimeric congener, speciogynine pseudoindoxyl. The characteristic spiro-5-5-6 tricyclic system of these alkaloids is formed via a protecting group-free cascade relay process in which oxidized tryptamine and secologanin analogs are used. Furthermore, we uncovered that mitragynine pseudoindoxyl exists and acts not as a single molecular entity but as a dynamic ensemble of stereoisomers in protic environments, thus, it exhibits structural plasticity in biological systems. Accordingly, these synthetic, structural, and biological studies provide a basis for the planned design of mitragynine pseudoindoxyl analogs, which can guide the development of next-generation analgesics.

Main text

INTRODUCTION

Opioids targeting μ-opioid receptors (MORs) play a fundamental role in the management of moderate-to-severe acute and chronic pain. Although opioid agonists are still the most effective analgesics, there is a growing concern about the safety of their long-term administration due to various adverse effects, such as respiratory

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depression, constipation, and addiction. Additionally, the current upsurge in opioid abuse, coupled with the spread of illicit synthetic opioids, has reached an epidemic level in the United States,[1] wreaking havoc on public health, society, and the economy. In addition to introducing new legislation and public education, the development of safer analgesics is a major goal in pain management.[2]

In this context, alkaloids from *Mitragyna speciosa* (commonly known as kratom) have recently emerged as a promising analgesic alternative for pain management with considerably fewer side effects than those associated with opioids.[3] Kratom is a tropical tree-like herb native to Southeast Asia that has been used for centuries for its distinctive psychotropic properties.[4] Kratom has been noted to have dose-dependent stimulant and opioid-like effects, and its illicit use to treat chronic pain and drug dependence is on the rise globally (>15 million estimated kratom users in the US alone).[5] The predominant natural products in kratom are indole alkaloids, mainly corynanthe-type monoterpenoids. While these mitragyna alkaloids are structurally significantly different from the morphine skeleton, they do display antinociceptive effects, which are supported by their ability to bind to opioid (i.e., MORs, DORs, KORs, NORs)[6] and other receptors (e.g., serotonin receptors)[7]. Systematic investigations have also shown that the opioid agonistic effect of *Mitragyna speciosa* cannot be fully explained by that of its main alkaloid, mitragynine (**1**) (230 nM for MOR) (**Fig. 1**). Finally, more potent analgesics, which were the oxidative metabolites of mitragynine, were identified in *in vitro* assays. Accordingly, the main alkaloid metabolizes *in vivo*[8-10] into 7-OH-mitragynine (**2**) (37 nM for MOR), which is followed by a subsequent 1,2-semipinacol rearrangement in human plasma to the more potent (by two orders of magnitude) mitragynine pseudoindoxyl (**3**) (0.8 nM for MOR). Nevertheless, hindering the completeness of the oxidative metabolic picture of mitragynine (**1**), another metabolite in addition to mitragynine pseudoindoxyl (**3**) was also detected in the semipinacol rearrangement step, but its structure and possible opioid effect have not been determined to date due to its low occurrence in human plasma.[9] Most importantly, however, animal studies have shown that mitragynine pseudoindoxyl (**3**) may avoid some of the key problems of current opioid therapies, as antinociceptive tolerance, physical dependence, and respiratory depression were not observed.[11] To gain insight into the underlying molecular mechanism of its distinct signaling profile, high-resolution cryo-electron microscopy (cryo-EM) structural studies on the MOR-Gi1 complex with **3** and molecular dynamics simulations have been recently performed.[12]
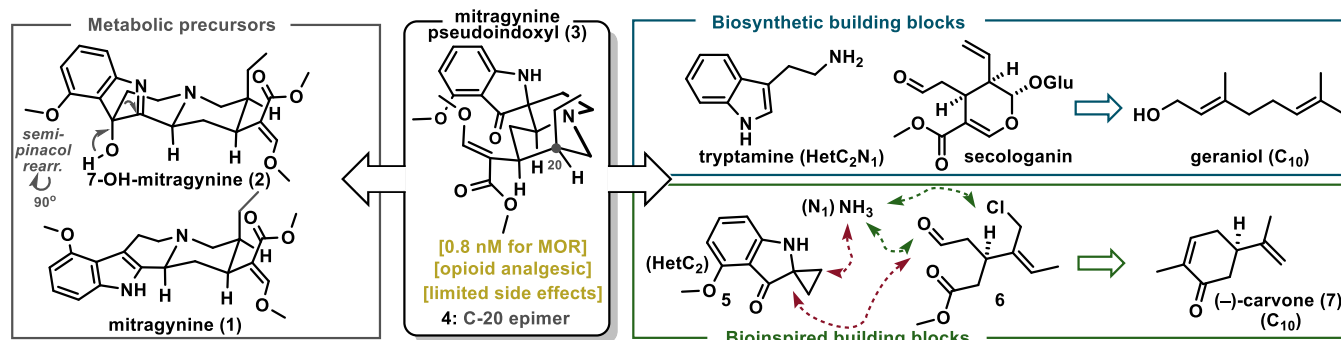
Fig. 1. Introduction and comparison of distinct pathways toward pseudoindoxyl metabolites of kratom alkaloids

Although the syntheses of kratom's major indole alkaloids[4] and their oxindole derivatives[13] have already received considerable attention from the synthetic community, total syntheses of pseudoindoxyl derivatives of mitragynine (**3**) and kratom's other minor alkaloids (e.g., the recently reported speciogynine derivative **4**)[14,15] have not yet been revealed[16]. Thus far, access to mitragynine's oxidative metabolites **2** and **3** has relied exclusively on biomimetic semisynthesis from mitragynine (**1**).[11] Although these and related studies demonstrated the power of late-stage functionalization strategies (e.g., site-selective aromatic C-H functionalization),[17,18] the attainable chemical space did not allow extensive structure–activity relationship (SAR) studies or deep-seated modifications on this unique pseudoindoxyl scaffold, thus hampering further advances in drug development.[19,20]

Given the enormous therapeutic potential and the lack of an enabling synthetic platform for SAR studies, we aimed to develop a scalable[21] route to pseudoindoxyl opioid **3**. Of all the possible disconnections of its spiro-tetracyclic backbone, the one that maximizes convergence and skeletal simplification and whose forward synthesis is concise and potentially scalable was chosen. **Fig. 1** illustrates the process through which we disconnected mitragynine pseudoindoxyl (**3**) to two subunits, a dubbed "oxidized tryptamine" fragment **5** and a secologanin analog[22] **6** that was anticipated to arise from (-)-carvone (**7**)[23]. A cascade process was then devised that would (i.) strictly avoid the use of protecting groups and harness the natural reactivities of the two key building blocks and (ii.) forge the CD ring system and the critical chiral spirocyclic center. Furthermore, our strategy was based on a chiral center derived from (-)-carvone (**7**), of which its stereochemical information could be propagated to diastereoselectively adjust two other stereocenters. The decision to implement this diastereoselective cascade relay was also underpinned by the computational analysis of the possible diastereomers of **3** (see SI for details). It turned out that the thermodynamically most stable stereostructure was that of **3**, which was also the product of the biosynthetic pathway.[24] Finally, we believed that our secologanin analog could further broaden the synthetic landscape, as it could provide access to both diastereomeric kratom pseudoindoxyls of the corynanthe-type, *allo* (mitragynine) and *normal* (speciogynine) isomers, by selective hydrogenation of its olefinic bond.[25,26] As such, this synthetic study was

designed to mimic the manner through which nature assembles mitragynine pseudoindoxyl (**3**), including rapid complexity generation via cascade reactions of tryptamine and secologanin analogs, step- and redox economy, divergency and protecting group-free assembly.[27] Herein, we report the first total synthesis of mitragynine pseudoindoxyl (**3**) and its diastereomer, speciogynine pseudoindoxyl (**4**).

RESULTS AND DISCUSSION

Our investigation began with the construction of the chiral secologanin-like building block **6** using (-)-carvone (**7**) as a chiral terpenoid starting material. To this end, we developed an operationally simple and scalable synthetic route that features a relay of chemoselective transformations[28] (**Fig. 2**). First, the homologation of (-)-carvone (**7**) was achieved in a two-step procedure: ene-type chlorination[29] of the electron-rich double bond with trichloroisocyanuric acid (TCICA) was followed by a copper-catalyzed C-C forming step to form homocarvone (**8**). Notably, the latter step was selective, as no 1,2- or 1,4-addition products were observed under optimized conditions. Then, two orthogonal oxidations were carried out; a nucleophilic epoxidation selectively transformed the electron-deficient double bond into an epoxide, and a second ene-type chlorination formed allylic chlorine **9** with high (*Z*) selectivity presumably due to syn-pentane strain.[30] After obtaining the desired intermediate **9** in an operationally simple way with a useful yield (39% yield over 4 steps, avg. 79%), two C-C bond cleavages remained to successfully remodel the initial terpene skeleton[31] and achieve the open chain C-9 carbon core of the envisioned secologanin building block **6**. To prepare for these C-C bond-breaking reactions, a mild acidic hydrolyzation of the epoxide moiety was carried out at room temperature, which retained the sensitive allylic chlorine moiety intact. Then, the resulting mixture of diastereomeric ketone diols was exposed to an excess of sodium periodate to effect the required C-C cleavages and provide the open-chain carboxylic acid **10**. The handling of this multifunctional and reactive molecule **10**, however, turned out to be challenging due to the presence of electrophilic and nucleophilic sites combined with its apparent ability to form cyclic acetals or esters. After careful experimentations, a simple ester formation by diazomethane was found to address this problem, as it promoted clean methylation to complete the sequence without the need for further purifications (71% yield over 3 steps, avg. 89%). In summary, a scalable, protecting group-free route to secologanin analog **6** was achieved from (-)-carvone (**7**) in only seven steps. Each of the reaction steps was performed on a dekagram scale, and only one purification step was involved, attesting to the robustness and potential further scalability of the sequence.

Then, we focused on synthesizing the spirocyclic cyclopropane-indoxyl building block **5**. Initial attempts to accomplish the synthesis of **5** on the gram scale were unsuccessful due to the high reactivity and sensitivity of this compound (see SI for details); at best, only milligrams of spiro-pseudoindoxyl derivative **5** were obtained. Upon

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extensive experimentation, we found an alternative synthetic tactic using mild enough conditions to provide **5** in sufficient quantities. As shown in **Fig. 2**, commercially available aniline **11** was used to construct the reactive spirocyclic building block **5** in five steps. The synergistic effect[32] of the meta-positioned directing groups of **11** in directed ortho metalation resulted in the construction of the contiguously functionalized 1,2,3-aromatic system **12** after acylation of the lithiated species with **13**, with a 60% yield on a decagram scale. Next, **12** was converted to anilino-α−bromo compound **14** via a dibromination/base-induced ring closure/acidic hydrolyzation sequence with a 67% yield, which set the stage for the last spiro-pseudoindoxyl formation step. Then, after screening a variety of different reagents (see SI for details), KHMDS was found to promote the formation of the desired tryptamine analog **5** with good overall efficiency on a gram scale.
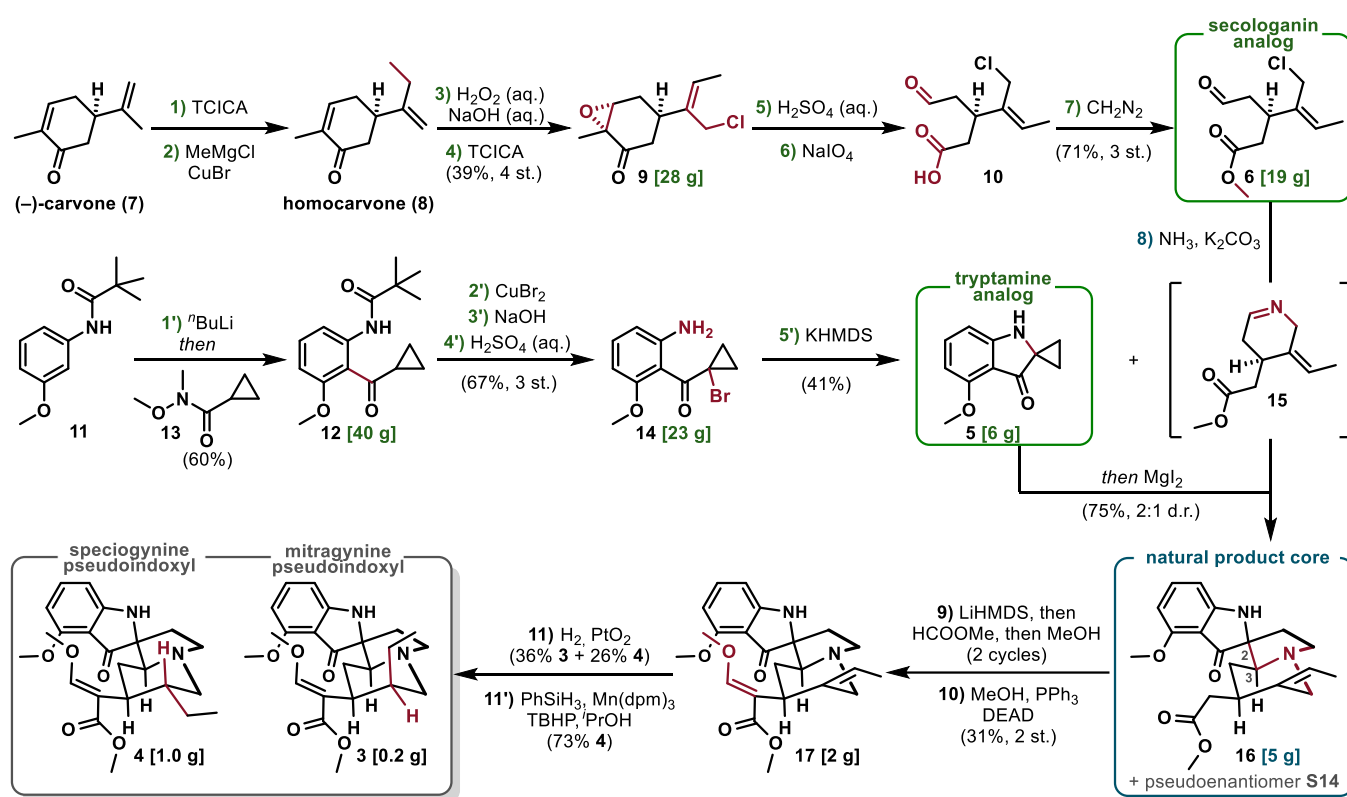

Fig. 2. Bioinspired synthesis to form pseudoindoxyl metabolites on a gram scale. Reagents and conditions: 1) TCICA (0.33 eq.), CHCl₃, 5 to 25 °C; 2) CuBr (0.25 eq.), MeMgCl (1.0 eq.), THF, -65 °C; 3) H₂O₂ (aq.) (1.3 eq.), NaOH (aq.) (0.27 eq.), MeOH, -10 to 0 °C; 4) TCICA (0.24 eq.), CHCl₃, 5 to 23 °C; 5) 50% H₂SO₄ (aq.), THF, 5 to 23 °C; 6) NaIO₄ (3.0 eq.), THF/H₂O, 5 to 23 °C; 7) CH₂N₂, Et₂O, 5 to 10 °C; 1') *ⁿ*BuLi (2.2 eq.) *then* **13** (1.0 eq.), THF, 5 to 25 °C; 2') CuBr₂ (2.2 eq.), EtOAc, reflux; 3') NaOH (2.0 eq.), MeOH, 25 °C; 4') 25% H₂SO₄ (aq.), MeOH, 65 °C; 5') KHMDS (2.0 eq.), THF, 5 to 23 °C; 8) **6** (2.2 eq.), NH₃ (1 atm), K₂CO₃ (exc.), CaCl₂ (exc.), CH₂Cl₂, 23 °C, *then* the addition of **5** (1 eq.) and MgI₂ (1 eq.) in THF, 23 °C; 9) LiHMDS (5.0 eq.), *then* HCOOMe (10 eq.), THF, -40 °C, *then* the addition of MeOH, 23 °C (2 cycles); 10) MeOH (2.0 eq.), DEAD (1.0 eq.), PPh₃ (1.0 eq.), THF, 23 °C; 11) H₂ (1 atm), PtO₂•H₂O (50 m/m%), NaHCO₃ (exc.), TFE, 23 °C; 11') PhSiH₃ (3.0 eq.), Mn(dpm)₃ (0.25 eq.), TBHP in decane (1.0 eq.), *ⁱ*PrOH, 23 °C. TCICA=trichloroisocyanuric acid, THF=tetrahydrofuran, KHMDS=potassium bis(trimethylsilyl)amide, exc.=excess, LiHMDS=lithium bis(trimethylsilyl)amide, DEAD=diethyl azodicarboxylate, TFE=2,2,2-trifluoroethanol, Mn(dpm)₃=tris(2,2,6,6-tetramethyl-3,5-heptanedionato)manganese(III), TBHP=tert-butyl hydroperoxide.

With ample quantities of the two building blocks **5** and **6**, we began to explore the key cascade relay sequence needed to construct the spirocyclic-tetracyclic core found in mitragynine pseudoindoxyl (**3**). As both bioinspired analogs were endowed with reactive functionalities, it seemed less apparent that they could be woven efficiently and selectively together without using protecting groups. Nevertheless, we reasoned that the functional group incompatibility could be addressed by developing a cascade relay sequence and leveraging a heterocyclic intermediate derived from secologanin analog **6** in a cascade process. This fleeting cyclic imino compound **15** was considered to be a suitable substrate for the ring-opening and Mannich reaction cascade with **5** to produce the spirocyclic-tetracyclic core of **3**. However, this cascade-relay strategy was dependent on the feasibility of producing the unstable cyclic imine intermediate **15**. Although the synthesis and isolation of **15** were difficult due to the formation of oligomeric and polymeric side products upon synthesis and storage, we were able to find appropriate basic and anhydrous conditions to form **15** with consistently high conversions and selectivity on a multigram scale (see SI for an NMR study). Then, without an aqueous workup or removal of solvent, this unstable cyclic imine **15** was carried onwards for the ensuing cascade reaction with the cyclopropyl building block **5**. This formal [3+2] cycloaddition step[33,34] to generate a spirocyclic 5-5 ring system was previously demonstrated on similar oxindole systems; thus, we elected conditions used by Carreira (e.g., MgI$_2$ bifunctional Lewis acid) in our experimental design. Of the possible four diastereomers, the desired isomer **16** was obtained in a 50% yield, with its pseudoenantiomer **S14** (see SI for the elucidated structure) in 25% yield as an inseparable mixture (5 g scale). Although the installation of the two stereocenters was not fully diastereoselective, we anticipated that the forward reaction conditions would concomitantly epimerize those C-2 and C-3 stereocenters into the desired configurations. At least two important features of this cascade relay were noteworthy: i) Our imine formation/alkylation tactic enabled the facile synthesis of cyclic imines, which usually requires extensive redox and protecting group manipulations;[35] and ii) The overall cascade sequence enabled rapid complexity generation and good convergence, as 4 bonds, 2 rings and 2 stereocenters were formed from two comparably complex building blocks.[36]

With the pentacyclic core of mitragynine pseudoindoxyl (**3**), two seemingly straightforward tasks remained: the installation of the methyl enol ether moiety onto the acetyl side chain and stereoselective reduction of the alkene. To this end, an α-formylation protocol was adapted to our substrate; this protocol does not require preventive but only transient protection of the pseudoindoxyl NH. Accordingly, the double deprotonation of **16** with excess LiHMDS was followed by the addition of methyl formate and quenching with methanol; this produced the alpha-formylated product as a mixture of isomers (see SI for a detailed study) and ensured the deprotection of the transient *N*-formyl group. Then, the selective methylation of the enol tautomer was explored using commonly used alkylation protocols (see SI

for details). However, these methods were plagued with poor and inconsistent yields due to *N*-methylated side-product formation. To circumvent this problem, we used the Mitsunobu reaction, which allowed chemoselective *O*-alkylation and afforded **17** in good yield and quantity (gram scale). Finally, the stereoselective reductions of **17** were addressed to complete the total synthesis of the targeted mitragynine metabolite **3**, alongside its recently reported diastereomer, speciogynine pseudoindoxyl (**4**). Many conditions and catalysts were screened to maximize the diastereoselectivity of the reduction process in both possible directions. We found that the use of Adam's catalyst was necessary to provide our primary target, the *allo*-type mitragynine pseudoindoxyl (**3**), with modest diastereoselectivity. To accomplish the selective reduction of **17** to diastereoisomer **4**, a radical stepwise reduction was chosen to provide the thermodynamically more stable diastereoisomeric product. The HAT-type reduction, pioneered by Shenvi[37], afforded the "normal-type" speciogynine pseudoindoxyl (**4**) with a 73% yield in a diastereoselective manner.

Although our primary goal was to access kratom's atypical opioid **3** in a scalable manner, this endeavor also provided new insights into its molecular properties. During the optimization of the last reduction step (see SI for details), we noticed that this natural metabolite was labile under various reaction conditions and isolations, which was also manifested in diminished yields. Prompted by these observations and having a useful quantity of **17**, we aimed to thoroughly study the last step's reaction mixture and isolate minor or even trace components. These efforts ultimately resulted in the identification of two unknown diastereomers of mitragynine pseudoindoxyl (**18** in 5% yield and **19** in 4% yield), which most likely arose from a retro-Mannich/Mannich-type rearrangement of the spiro-pseudoindoxyl moiety (**Fig. 3A**). Then, we identified conditions and methods that allowed the isolation and structural elucidation of those stereoisomers. The NMR structure elucidation of these isomers showed that they are spirocyclic diastereomers with *allo* and *epiallo* configurations; thus, the latter stereoisomer is the not-yet-known spiroindoxyl derivative of speciociliatine. Next, it was found that aprotic solvents impeded and polar protic solvents facilitated the isomerization and shifted the equilibrium (e.g., **3**:**18**:**19** = ca. 6:1:3 in HFIP, 23 °C). Most importantly, we found that the thermodynamic equilibrium of the three stereoisomers (**3**:**18**:**19** = ca. 7:2:1) could be reached within 20 min at 37 °C in Krebs-Ringer solution, which mimics physiological conditions (**Fig. 3B**). Additional insight was obtained from the computational assessment of the relative free energies of the (protonated) isomers. First, it confirmed the experimental relative thermodynamic stabilities; second, it explained the observed experimental difference between protic and aprotic media, i.e. protonation of the tertiary amine was found to reduce the energy difference between the isomers (see SI for details).

While analogous and kinetically more stable spiro-oxindoles are known to exist in at least two isomeric forms[38], only one structural isomer has been observed for spiro-pseudoindoxyl systems to date.[39] Although Takayama hypothesized the formation of four possible stereoisomers 30 years ago in their pioneering work, only one pseudoindoxyl diastereomer was isolated after the 1,2-semipinacol rearrangement of the biogenetic precursor 7-OH-mitragynine (**2**).[40]
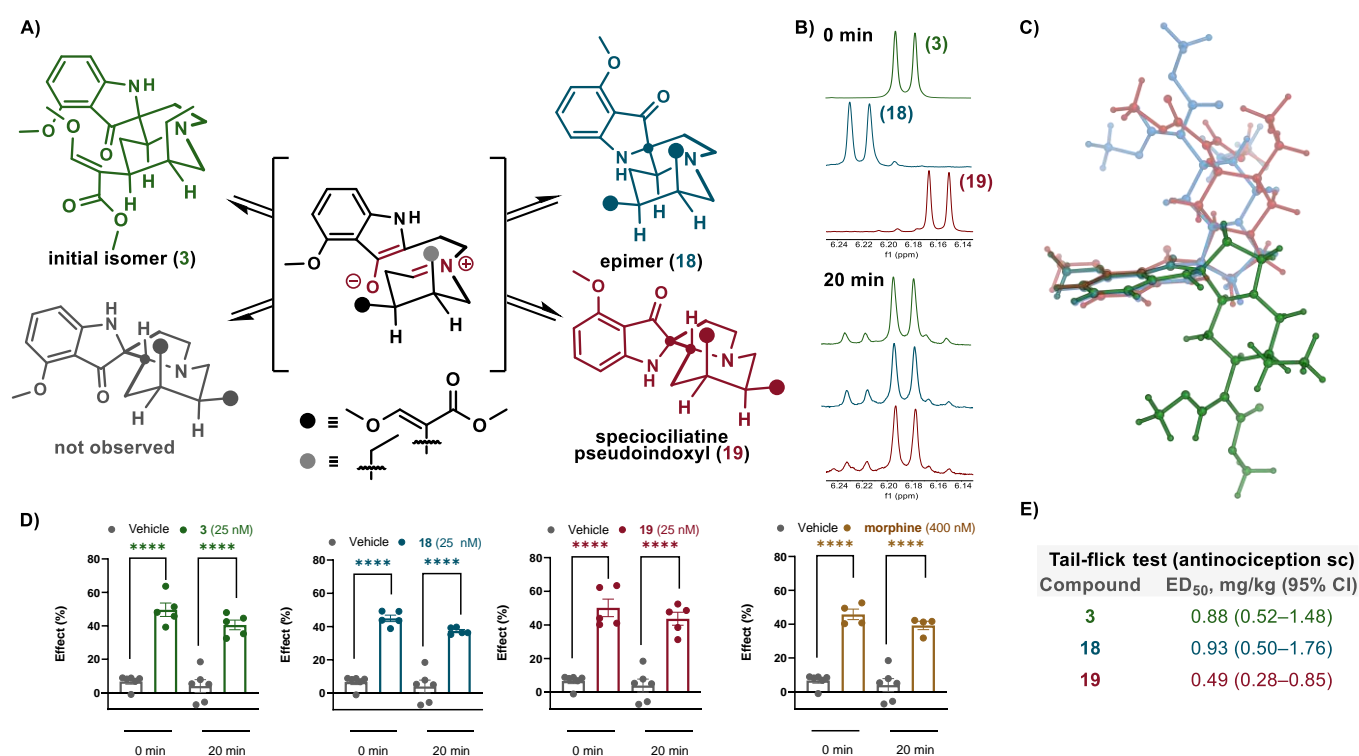

Fig. 3. Isomerization of mitragynine pseudoindoxyl (3) and related studies. A) Retro-Mannich/Mannich isomerization and structure of the isomers obtained; **B)** NMR spectra: Single isomers **3**, **18** and **19** were introduced to physiological-like media, and their isomerization was monitored with ^{1}H-NMR measurements, as illustrated by the shifts of characteristic aromatic signals. (starting materials, 0 min **(up)** vs. 37 °C, pH=7.4, 20 min **(down)**); **C)** Superimposed structures of the calculated lowest energy conformations of **3** (green), **18** (blue), and **19** (red); **D)** Results of the *in vitro* mouse vas deferens bioassay comparing the effect of single isomers: Test or reference compounds were added to the MVD immediately or 20 min after solution making. The effect on MVD smooth muscle contractions was assessed after 10 min. Statistical significance was accepted based on one-way ANOVA with Tukey's multiple comparison test, $p < 0.05$ (**** $p < 0.0001$). Data are presented as the mean ± S.E.M. of 5-6 tissues. **E)** Results of the *in vivo* radiant heat tail flick assay in mice comparing the effect of single isomers: The antinociceptive effect of test compounds was assessed 20, 30, 60 and 120 min after s.c. administration at various doses (see SI for details). The antinociceptive potencies (ED_{50}) were calculated from linear dose response curves. The ED_{50} values and 95% confidence intervals were calculated at the most effective time point, namely, 20 min after s.c. administration.

A critical question that remained at this stage concerned the opioid activity of these stereoisomers. Our computational studies of the stereoisomers suggested that the shapes of these molecules are significantly different (**Fig. 3C**); thus, a profound difference in their biological activities might be expected. Nevertheless, on the basis of

the observation of the rapid stereoisomerization phenomenon, we anticipated that all stereoisomers might show the same activity in biological systems. Thus, we initiated comparative *in vitro* and *in vivo* studies for each single stereoisomer. First, we evaluated the opioid-receptor-mediated effects of these isomers (**3**, **18**, and **19**) in the mouse vas deferens (MVD) assay[41,42] (**Fig. 3D**). All compounds showed naloxone-sensitive inhibitory effects on MVD muscle contractions, with significantly higher potency than morphine. Furthermore, Student's t-test showed no difference in the inhibitory effect between stereoisomers administered immediately after solution preparation or 20 min later. Accordingly, this study suggests that the opioid effect of those isomers is related to their dynamic stereochemical equilibrium. Next, we examined the acute analgesic effect of these isomers. Thus, a thermal pain model, the radiant-heat tail-flick assay[11,43], was selected and performed in the same mouse strain used for MVD experiments (**Fig. 3E**). Every experiment comprised five series of measurements, one before and four after subcutaneous drug administration, carried out at 20-, 30-, 60- and 120-minute intervals. The antinociceptive effect was assessed 20 min after s.c. drug administration since the antinociception of the compounds peaked at that time (see SI, **Fig. S35**). Importantly, there was no significant difference between the stereoisomers' ED_{50} values, indicated by the overlap in 84% confidence intervals. Accordingly, we found that mitragynine pseudoindoxyl (**3**) actually exists and acts as a dynamic ensemble of stereoisomers in the protic environment and, what is perhaps even more important, in biological systems. This finding has many important implications that can shape future drug developments: i.) the elusive, unidentified 7-OH-mitragynine metabolite in human plasma described by McCurdy[9] is likely to be the above stereoisomer(s) of **3**; ii.) mitragynine pseudoindoxyl displays structural plasticity, and the evolving molecular ensemble could target additional receptors; iii.) the local biological environment (e.g., pH, metal ions) can alter the potency of the pseudoindoxyl ensemble due to the shift of the equilibrium of stereoisomers; iv.) and the oxidative metabolism of speciociliatine to its pseudoindoxyl derivative (the C-3 epimer of mitragynine)[44] is predicted to result in the same stereomeric mixture as described herein, which also implies an unusual convergent metabolic pathway for kratom's alkaloids.

CONCLUSIONS

In summary, the first scalable and chiral pool-based synthetic routes to members of kratom pseudoindoxyls have been developed by using oxidized tryptamine and secologanin analogs. The highly convergent strategy, the deliberate minimization of protecting groups, and late-stage divergency allowed the rapid construction of targeted natural products **3** and **4** on a gram scale. As an unexpected spin-off of the synthetic efforts, we discovered that mitragynine pseudoindoxyl (**3**) exists and acts not as a single molecular entity but as a dynamic ensemble of stereoisomers in protic environments and, what is perhaps even more important, in biological systems. Accordingly,

these synthetic, structural, and biological studies provide a basis for future functional modifications of mitragynine pseudoindoxyl (**3**), which can guide the development of next-generation analgesics.

Acknowledgments

Author contributions

Sz.V., T.S., and P.A. conceived the project. Sz.V. and T.S. directed the synthetic part of the research. P.A. and K.H. designed and carried out the synthetic experiments. B.B.M. and Sz.V. executed structural elucidations. B.B.M. and J.D. designed and performed the DFT calculations. Á.D. prepared references for biological evaluation. M.A–K. designed and directed the biological part of the research. A.R.G. and N.E. carried out the biological assays. All authors contributed to the writing of the manuscript.

Competing interests

The authors declare no competing interests.

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