Natural Product Reports

REVIEW

Polyunsaturated fatty acid amides from the *Zanthoxylum genus* – from culinary curiosities to unique tools for chemical biology

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Douglas J. Cullen,[b] Lydia Bowman,[b] Jason J. Chruma,*[a] and Patrick H. Toy*[b]

Covering up to February 2017

The pericarps of several species from the *Zanthoxylum genus*, a.k.a. the "prickly ash", have long been used for culinary purposes throughout Asia, most notably in the Sichuan (previously Szechuan) cuisine of Southwestern China, due to the unique tingling and numbing orosensations arising from a collection of polyunsaturated fatty acid amide (alkamide) constituents. The past decade has experienced dramatically increased academic and industrial interest in these pungent *Zanthoxylum*-derived alkamides, with a concomitant explosion in studies aimed at elucidating the specific biochemical mechanisms behind several medically-relevant biological activities exhibited by the natural products. This rapid increase in interest is partially feuled by advances in organic synthesis reported within the past few years that finally have allowed for the production of diastereomerically-pure *Zanthoxylum* alkamides and related analogs in multigram quantities. Herein is a comprehensive review of the discovery, total synthesis, and biological evalution of *Zanthoxylum*-derived polyunsaturated fatty acid amides and synthetic analogues. Critical insights into how chemical synthesis can further benefit future chemical biology efforts in the field are also provided.

1. Introduction

In essentially every quadrant of the globe, local cultures have made use of plants from the *Zanthoxylum* genus (a.k.a. "prickly ash" or "toothache tree") of the Rutaceae family. Extracts from the bark and berries of *Z. clava-herculis* (Southern prickly ash) and *Z. americanum* (Northern prickly ash) have long been incorporated into traditional Native American herbal remedies.[1] Likewise, several *Zanthoxylum* species are essential components in traditional Nigerian medicine for the treatment of a wide range of conditions.[2] The pulverized peppercorns from the Japanese *Z. piperitum*, referred to locally as sanshō (山椒), is both an essential culinary spice for broiled eel (kabayaki unagi) and a vital component in a traditional Japanese therapy (Kampo) for treating digestive disorders.[3,4] Most notably, the pericarps from the Chinese species *Z. bungeanum* (red huajiao, 花椒) and *Z. schinifolium* (green huajiao or tengjiao, 藤椒) are synonymous with the cuisine of

[a] *Key Laboratory of Green Chemistry & Technology (MOE), College of Chemistry and Sino-British Materials Research Institute, College of Physical Science & Technology, Sichuan University, No. 29, Wangjiang Road, Chengdu, Sichuan 610064, P. R. of China*
[b] *Department of Chemistry, The University of Hong Kong, Pokfulam Road, Hong Kong, P. R. of China*

Sichuan (formerly *Szechuan*) Province, collectively being referred to internationally as "Sichuan peppercorn".[5] This universal interest for both culinary and medicinal applications is a direct result of a variety of predominantly pungent polyunsaturated fatty acid amides (alkamides), several of which are produced exclusively by members of the *Zanthoxylum* genus. These polyunsaturated fatty acid amide natural products have long held the interest of the synthetic organic community, but it has only been within the past few years that procedures were described for the multigram-scale synthesis of *cis*-olefin-containing *Zanthoxylum* alkamides as single diastereomers. Likewise, concerted international efforts toward the exploration of *Zanthoxylum* alkamides and related synthetic analogues as chemical tools to probe and elucidate biological processes and activities are predominantly confined within the last decade. In contrast to previous reports surveying *Zanthoxylum* natural products[6] or alkamides in general,[7] this review aims to be a comprehensive collection of the discoveries, syntheses and biological evaluations of *Zanthoxylum*-derived alkamides and synthetic analogues (up through early 2017). Critical insight as to how chemical synthesis can further advance biomolecular studies of these natural products also will be provided.

2. Isolation and characterization

2.1. Sanshools and hydroxy-sanshools

The sanshools are the predominant class of long-chained polyunsaturated amides produced by the *Zanthoxylum* genus (Fig. 1). These alkamide natural products are characterized by the presence of three conjugated double bonds at the Δ-end of the fatty acid chain. The nomenclature of the sanshools is such that the term "sanshool" refers to the non-functionalized isobutylamide group, with "hydroxy-sanshool" referring to the (2-hydroxy)isobutylamide congeners.

Although the pungent components found in the *Zanthoxylum* genus had previously been investigated chemically,[8,9] it was not until 1955 that the first sanshool structure, α-sanshool (**1**, previously referred to as either echinacein[10] or neoherculin[11,12]) was correctly identified as (2*E*,6*Z*,8*E*,10*E*)-*N*-(isobutyl)dodeca-2,6,8,10-tetraenamide.[11,12] While most commonly associated

Douglas J. Cullen recently completed a MChem degree at the University of Edinburgh. His final year of research, conducted under the supervision of Dr. Stephen P. Thomas, concerned manganese-catalysed coupling reactions. Previously he was an exchange student at the University of Hong Kong during the 2015-16 academic year, where he worked on sanshool synthesis in the research group of Dr. Patrick H. Toy.

Lydia Bowman currently is a student at the University of Edinburgh. She was an exchange student at the University of Hong Kong during the 2016-17 academic year, where she worked on the synthesis of various fatty acid amides structurally related to the sanshools in the research group of Dr. Patrick H. Toy. She expects to graduate with a MChem degree in the near future.

Jason J. Chruma received a BS degree in Chemistry from the University of Arizona (w/ Prof. Robin Polt) and a PhD from the University of Pennsylvania (w/ Prof. Amos B. Smith, III). After completing an NIH post-doctoral fellowship with Prof. Ronald C. Breslow at Columbia University, he began an independent career in 2005 as an Assistant Professor at the University of Virginia. In 2012, he moved to the College of Chemistry at Sichuan University where he is currently a Professor and Assistant to Dean. His research is focused on the development of new methods for the synthesis of alkaloids and other bioactive nitrogen-containing organic frameworks. Additionally, he has served as a scientific consultant to craft breweries in China on the use of Sichuan peppercorn in their products. In February 2017, he completed a one-week visitorship at the University of Hong Kong.

Patrick H. Toy received a BS degree in Chemistry from the Ohio State University, and a PhD degree from Wayne State University under the supervision of Prof. Martin Newcomb for research into the mechanisms of enzyme-catalysed hydrocarbon hydroxylation reactions. He was a post-doctoral researcher at the Scripps Research Institute in the research group of Prof. Kim D. Janda, where he investigated polymers for solid-phase synthesis and catalyst/reagent immobilization. Following a short stint as a medicinal chemistry research scientist at Wyeth-Ayerst Research (now a part of Pfizer), he began his independent academic career at the University of Hong Kong. A beer-fuelled conversation with his students during a Sichuan hot pot dinner inspired their interest in the sanshools and related fatty acid amides.

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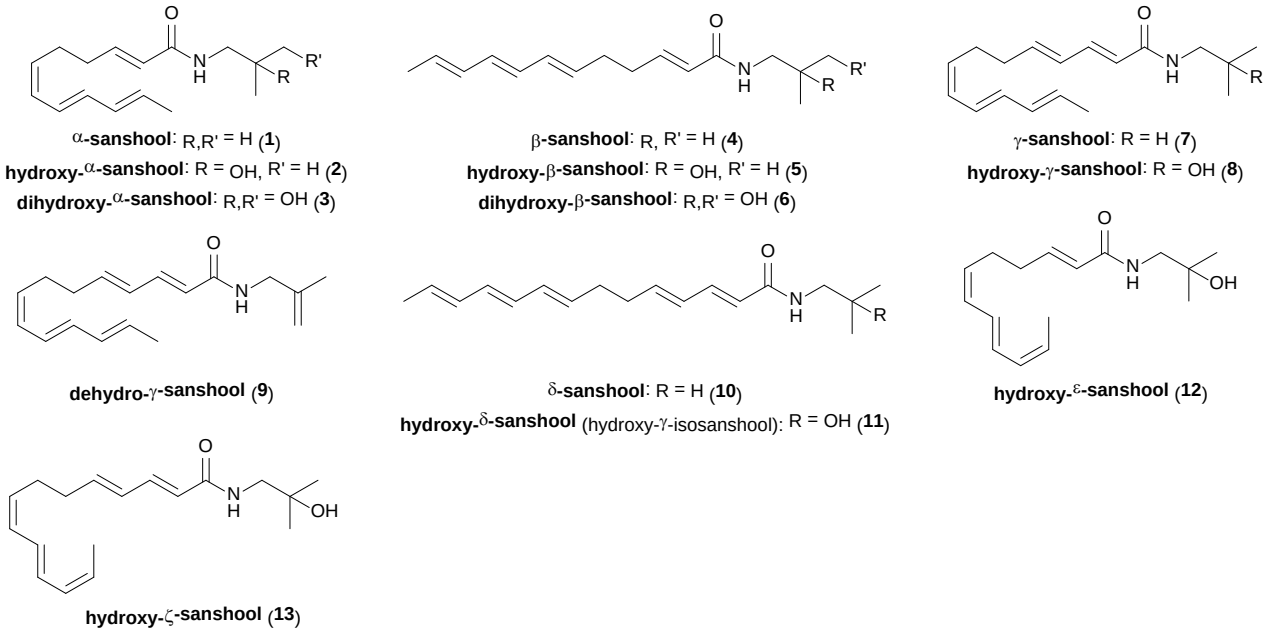


Fig. 1. Sanshools.

with the *Zanthoxylum* genus, alkamide **1** also has been isolated from *Echinacea angustifolia*.[10] Crombie and Tayler later reported the successful extraction and isolation of **1** from *Z. piperitum* DC and a subsequent stereomutation to form β-sanshool (**4**), as well as confirming the respective stereochemistries of each polyene.[13] Longer chain sanshools were reported later. γ-Sansool (**7**) and hydroxy-γ-sanshool (**8**) were both first isolated from the pericarps of *Z. ailanthoides* by Yasuda and co-workers.[14] Yasuda's group was particularly active in the field of sanshool research as they were also the first to report the isolation of hydroxy-α-sanshool (**2**)[15] and hydroxy-β-sanshool (**5**).[16] Moreover, they determined that the all-(*E*) polyene **5** could be generated from the *cis*-alkene **2** by irradiation of the latter with UV light in the presence of iodine.[16]

The distribution of the alkamides within the plant suggests that the hydroxylation of the isobutylamine region occurs within the fruit, whereas the sanshool compounds are present throughout the plant, particularly in the bark.[15–17] The corresponding all-(*E*) C_{14}-isomer, hydroxy-δ-sanshool (**11**, occasionally referred to as hydroxy-γ-isosanshool)[18–20] was isolated in 1988,[13] and the dehydroxylated congener δ-sanshool (**10**) was reported nine years later.[14] Derivatives of the sanshools with distinct functional variations have also been reported, including dihydroxylated derivatives of α- and β-sanshool (**3** & **6**)[20–23] and a dehydrated derivative of hydroxy-γ-sanshool, dehydro-γ- sanshool (**9**).[23,24] Bryant and Mezine described hydroxy-ε-sanshool (**12**) when conducting investigations into the neurological activity of the sanshool family.[25] The structure of this substance was proposed by comparison with data published independently by the groups of both Yasuda[15] and Kashiwada.[26] Alkamide **12** also was proposed as a product of isolation in 2008,[21] once again using comparison of spectra to those reported in the Yasuda and Kashiwada papers. A conclusive isolation and structural analysis for **12**, however, was not reported until 2014 by Bader and co-workers,[18] who noted that **12** was not, in fact, reported by either Yasuda or Kashiwada. Bader's group was also responsible for the isolation from *Z. piperitum* pericarps (using supercritical fluid extraction) and structural elucidation of the rare and unstable hydroxy-ζ-sanshool (**13**) in the same report.

2.2. Bungeanools

Dried pericarps from *Z. bungeanum* MAXIM, referred to locally as red huajiao (花椒) and internationally as Sichuan peppercorn, are ubiquitous in authentic Sichuan cuisine due to their distinctive taste and the unique numbing and tingling sensations that they impart from the collection of alkamides present.[5] As the name suggests, the bungeanools were first isolated from the pericarps of this particular *Zanthoxylum* species (Fig. 2). Structurally, the bungeanools are essentially

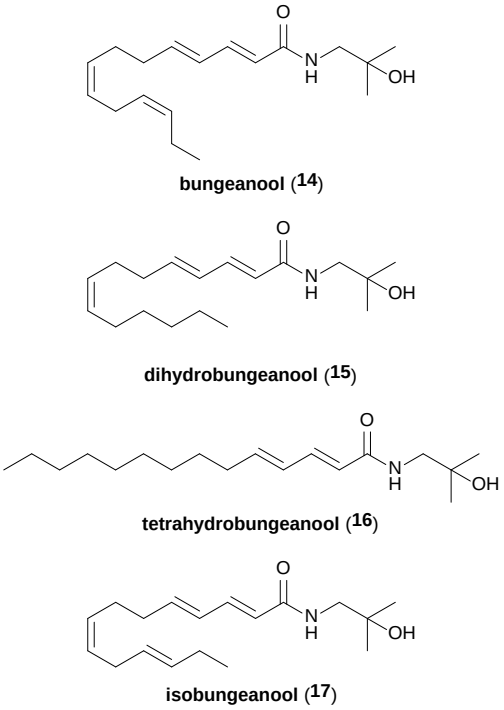


Fig. 2. Bungeanools.

partially saturated variants of hydroxy-γ-sanshool (**8**). The first extraction of the bungeanools was achieved by successive silica gel column chromatography, followed by HPLC purification of a chloroform extract of fresh *Z. bungeanum* pericarps.[16] In addition to hydroxy-sanshools **2**, **4**, **7**, and **11**, this procedure also afforded two new alkamides as unstable colourless syrups. Specifically, the (*E,E,Z,*Z)-tetraene bungeanool (**14**) and the (*E,E,Z,E*)-diastereomer isobungeanool (**17**) were isolated in 0.016 and 0.008% yields, respectively. These alkamide natural products were also recovered, along with two new compounds, dihydrobungeanool (**15**) and tetrahydrobungeanool (**16**), from 95% ethanolic extracts of the air-dried pericarps of the same species.[24] Methanolic extracts of *Z. bungeanum* pericarps have also yielded **14**.[27]

The pericarps from *Z. schinifolium* are also used quite extensively in Sichuan cuisine. One very common use for these peppercorns, as well as those from *Z. bungeanum*, is to extract their flavour components and pungent alkamides into edible oils. Methanol extracts from 16 commercial variants of these fragrant and numbing oils all afforded bungeanools **14**, **16**, and **17**.[19] Members of the bungeanool family have also been extracted from other *Zanthoxylum* species such as *Z. integrifoliolum*.[28]

2.3. Lanyuamides

The lanyuamides are another family of *Zanthoxylum* alkamides closely related in structure to those previously mentioned (Fig. 3). All six members of the lanyuamide family reported to date consist of a 12- or 14-carbon unsaturated (and frequently oxidized) fatty acid chain generally linked to isobutylamine via an amide bond. The initial discovery of the lanyuamides,

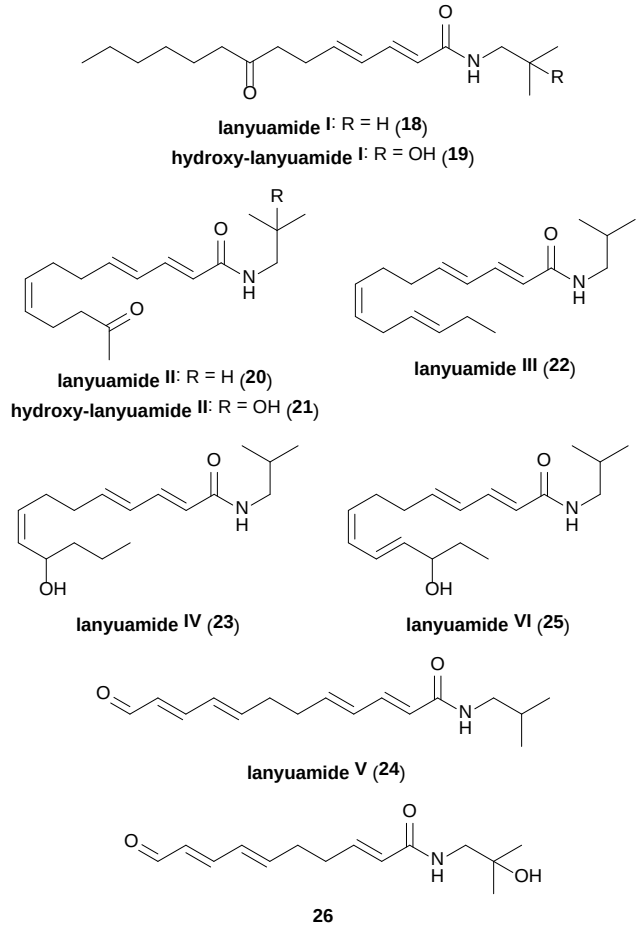


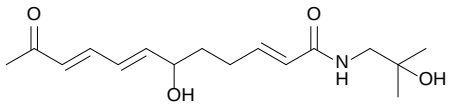
Fig. 3. Lanyuamides.

specifically lanyuamides I-III (**18**, **20**, **22**) originated from a methanolic extract of the dried fruits of *Z. integrifoliolum*.[28] While lanyuamide III (**22**) is effectively the dehydroxylated variant of isobungeanool (**17**), lanyuamides I and II (**18** and **20**, respectively) represent the first reported examples of ketone-containing *Zanthoxylum*-derived alkamides. In a following report, the three related partially-oxidized polyunsaturated fatty acid isobutyl amides lanyuamides IV-VI (**23**-**25**) were isolated and characterized from a methanolic extract of the dried flowers of *Z. integrifoliolum*.[29] Aldehyde **24** is the only member of the lanyuamides that possesses a C_{12} fatty acid component and resembles the product of oxidative cleavage of the terminal olefin in δ-sanshool (**10**). In 2012, Zhou and co-workers isolated a similar as-of-yet unnamed aldehyde-containing C_{10} alkamide (**26**) from *Z. bungeanum* pericarps.[27] The remaining two members of the lanyuamides, **23** and **25**, contain a single alcohol moiety. Both **23** and **25** possess one chirality centre and they exhibit levorotatory optical activity, but the exact stereochemical configurations for these two alkamides have not been assigned conclusively. While it is known that the sanshools and bungeanools are sensitive to both alcoholic solvents and oxidation of the fatty acid chain,[30] the lanyuamides appear to be genuine natural products and not artefacts of the isolation process. The (2-hydroxy)isobutyl-

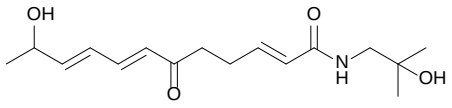
amide variants of lanyuamide I and lanyuamide II also have been reported.[31] Specifically, hydroxylated alkamides **19** and **21** were isolated as colourless gels from a methanolic extract of the root bark of *Z. ailanthoides*, which is sometimes referred to as "Japanese prickly ash".

2.4. ZP-amides

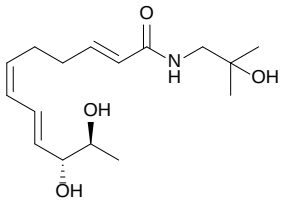
Just as the lanyuamides can be described as partially oxidized versions of the C_{14}-fatty acid chain-containing γ- and δ-sanshools, the ZP-amides exhibit a similar relation to the C_{12} alkamides hydroxy-α-sanshool and hydroxy-β-sanshool (Fig. 4). ZP-amides A-E (**27**-**31**) were first reported by Hitano and co-workers as unstable colourless syrups isolated from the pericarps of *Z. piperitum* fruits.[32] Even though all of the ZP-amides isolated in this original report (**27**-**31**) contain at least one stereogenic centre, none of them exhibit any optical activity suggesting that they all were isolated as racemic compounds. The relative stereochemistry of the *syn*-diol unit in ZP-amide D (**30**) was determined by treatment with acetone and analysis of the resulting dimethyl ketal by rotating-frame



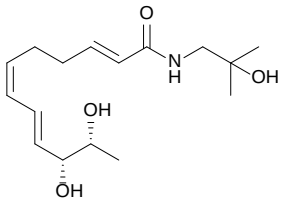
ZP-amide A (**27**)



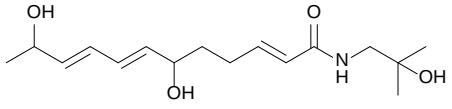
ZP-amide B (**28**)



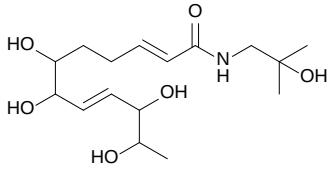
ZP-amide C (**29**)



ZP-amide D (**30**)



ZP-amide E (**31**)



ZP-amide F (**32**)

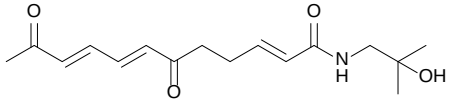


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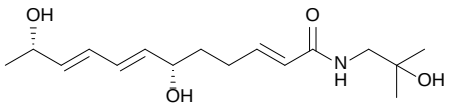
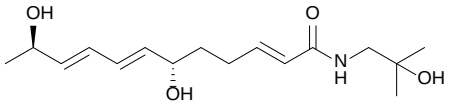
Fig. 4. ZP-amides.



***syn*-ZP-amide E** (**31a**)



***anti*-ZP-amide E1** (**31b**)

Fig. 5. Proposed renaming for the two diastereomers of **30**.

Overhauser spectroscopy (ROESY). The relative stereochemistry in *anti*-diol ZP-amide C (**29**) was thus established by comparison to the *syn*-diol **30**. In the original isolation paper, Hitano and co-workers isolated ZP-amide E (**31**) as two distinct racemic compounds, presumably *syn* and *anti* diastereomers, and labelled them collectively as ZP-amides E and F. Unfortunately, later reports by other researchers in the field simply referred to these two diastereomeric compounds collectively as "ZP-amide E".[33–35] To further complicate matters, Kim and co-workers isolated a new pentahydroxylated member of the ZP-amide family (**32**) as a colourless wax from *Z. piperitum* pericarps and titled it ZP-amide F,[34] without recognizing that Hitano and co-workers had already used this name to refer to one of the diastereomers of **31**. To alleviate this conflict in the literature, we propose that the two possible diastereostereomers for compound **31** be referred to as *syn*-ZP-amide E (**31a**) and *anti*-ZP-amide E (**31b**), as shown in Fig. 5, and the polyhydroxylated alkamide **32** reported by Kim and co-workers retain the name ZP-amide F. Unlike other members of the ZP-amide family, polyol **32** did exhibit modest optical activity, suggesting that it is not a racemic mixture. Unfortunately, the relative and absolute configuration of this unique alkamide has yet to be established conclusively. Finally, in addition to ZP-amide A, Huang and co-workers reported that the related unnamed diketone **33** could be isolated from the dried pericarps of *Z. bungeanum*.[27]

2.5. Qinbunamides

As part of a recent campaign to discover unique natural products capable of enhancing neurotrophic activity, the groups of Zhang and Gao screened isolates from ethanolic extracts of *Z. bungeanum* pericarps and accordingly introduced what might be the most controversial members of the *Zanthoxylum* alkamide family: the qinbunamides (Fig. 6).[35] As with ZP-amides A-E, all three members of the qinbunamides contain at least one chirality centre but lack any optical activity, suggesting that they were isolated as racemic mixtures. Qinbunamide A (**34**) and qinbunamide B (**35**) are unique in that they both contain a very unusual ethyl ether moiety. Given that 1) the sanshools are known to be unstable compounds sensitive to oxygen, light, and hydroxylated solvents[30] and 2) ethanol was used as the initial extraction solvent, it is extremely tempting to conclude that **34** and **35** are simply artefacts of

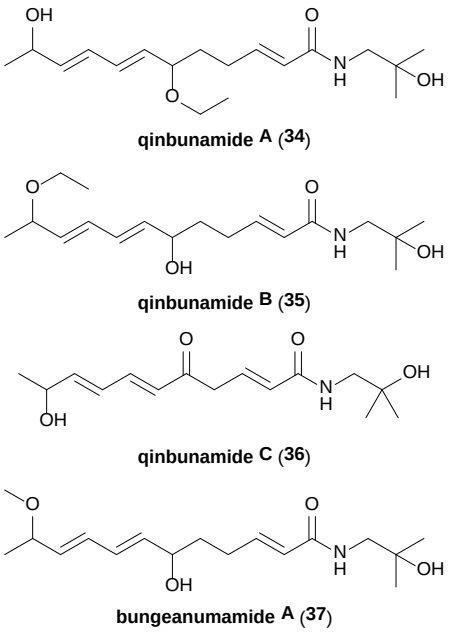


Fig. 6. Qinbunamides and bungeanumamide A

isolation. The related compound bungeanumamide A (**37**), which is the methyl ether analogue of qinbunamide B (**35**), has been reported twice, both from methanolic (rather than ethanolic) extracts of *Zanthoxylum* pericarps,[34,36] thus furthering the suspicion that these ether-containing alkamides are artefacts of isolation. Incomplete and circumstantial evidence that **34** and **35** are genuine natural products and not artefacts was provided in the initial isolation report,[35] but this issue currently remains unresolved. The last member of the qinbunamide family, qinbunamide C (**36**), is less controversial but no less unique. In addition to possessing a ketone and a hydroxyl moiety, **36** is the only *Zanthoxylum* alkamide reported to date with a C_{11} fatty acid chain.

2.6. Timuramides

The timuramides (Fig. 7) were originally reported by Beutler and co-workers in 2013 as novel alkamides isolated from methanolic extracts of dried pericarps from Nepalese *Z. armatum* (referred to locally as "timur").[33] Timuramide A (**38**) and timuramide B (**39**) both possess an unusual endoperoxide group suggesting a formal [4+2] cycloaddition between oxygen and either hydroxy-α-sanshool (**2**) or hydroxy-β-sanshool (**5**), respectively. Both **38** and **39** exhibit optical activity, indicating that they are single enantiomers. Unfortunately, attempts to elucidate the absolute configuration of **38**, such as via selective hydrogenolysis of the endoperoxide moiety and conversion of the resulting diol to the corresponding bis-Mosher's ester, did not yield fruit. The other two members of the timuramide family, timuramides C (**40**) and D (**41**), also appear to be oxidized metabolites of hydroxy-α-sanshool (**2**) in which either the terminal or distal alkene in the conjugated triene tail of **2** is cleaved oxidatively to the corresponding carboxylic acid, respectively. Currently, timuramide C (**40**) is the only member of the timuramide family to be found as a metabolite in other *Zanthoxylum* species, specifically from the pericarps of Korean *Z. piperitum*.[34]

2.7. Zanthoamides

The latest addition to the family of *Zanthoxylum*-derived polyunsaturated fatty acid amides are the zanthoamides, reported from the groups of Wei and Gao in 2016 as products isolated from *Z. bungeanum* pericarps (Fig. 8).[37] Zanthoamides A (**42**) and B (**43**) are essentially C_{14}-analogues of the partially-oxidized C_{12}-chain alkamides ZP-amide A (**27**) and ZP-amide E (**31**), respectively. Similarly, zanthoamide C (**44**) is the all-*trans* olefin variation of ZP-amides C (**29**) and/or D (**30**). Zanthoamides A-C all contain at least one stereogenic centre, but are considered naturally racemic compounds due to their lack of optical activities. The relative stereochemistries of diols **43** and **44** were not determined and

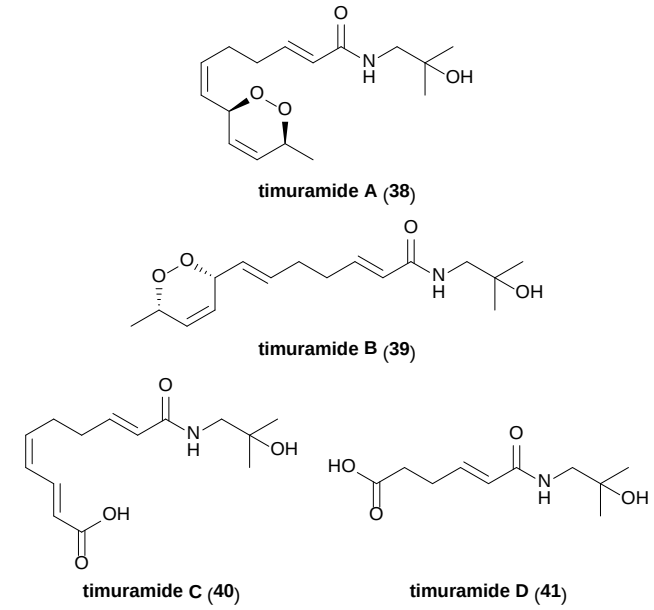


Fig. 7. Timuramides

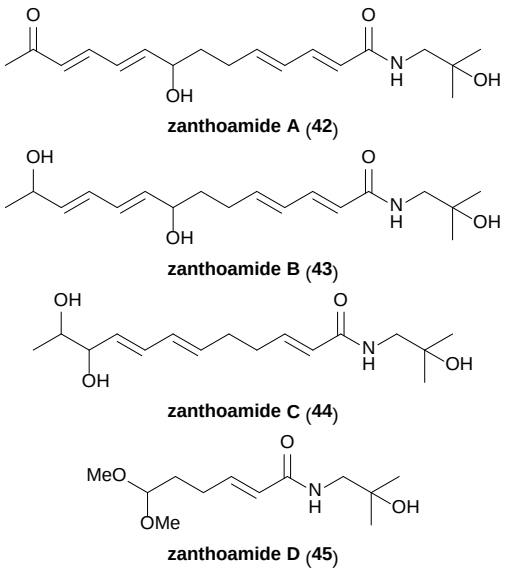
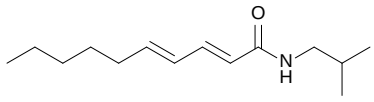


Fig. 8. Zanthoamides

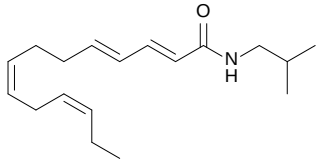
could be mixtures of diastereomers, as was the case with the analogous ZP-amides. The final member of the family is the truncated alkamide zanthoamide D (**45**). Given the extensive use of methanol in the isolation and purification process, it is possible that the unusual dimethylacetal group in **45** is an artefact and the actual natural product is the corresponding aldehyde, representing a reduced form of carboxylic acid timuramide D (**41**). If this is indeed the case, then lanyuamide V (**24**) and trienal **26** would not be the only *Zanthoxylum* alkamides that possess an aldehyde moiety.

2.8. Related polyunsaturated amides

The alkamides described in the previous sections are those most associated with the *Zanthoxylum* genus, but these plants also produce a number of other polyunsaturated fatty acid amides common to the Rutaceae and other plant families (Fig 9). For example, the C_{10} fatty acid isobutyl amide pellitorine (**46**), which is a metabolite found in several different plant families,[38] has been isolated from the roots, pericarps, and/or stem bark from *Z. tessmannii*,[39] *Z. zanthoxyloides*,[40] *Z. achtoum*,[41] and *Z. heitzii*.[42,43] Additionally, the C_{14} tetraene alkamide hazaleamide (**47**), which is the dehydroxylated derivative of bungeanool **14**, was first isolated from the bark of *Fagara rhetza* (Rutaceae), an Indonesian medicinal plant,[44] but it was also extracted from the dried fruits of Taiwanese *Z. integrifoliolum.*[28] For the sake of this review, focus will be maintained on those alkamides most associated to the *Zanthoxylum* genus, specifically those discussed in Sections 2.1-7.

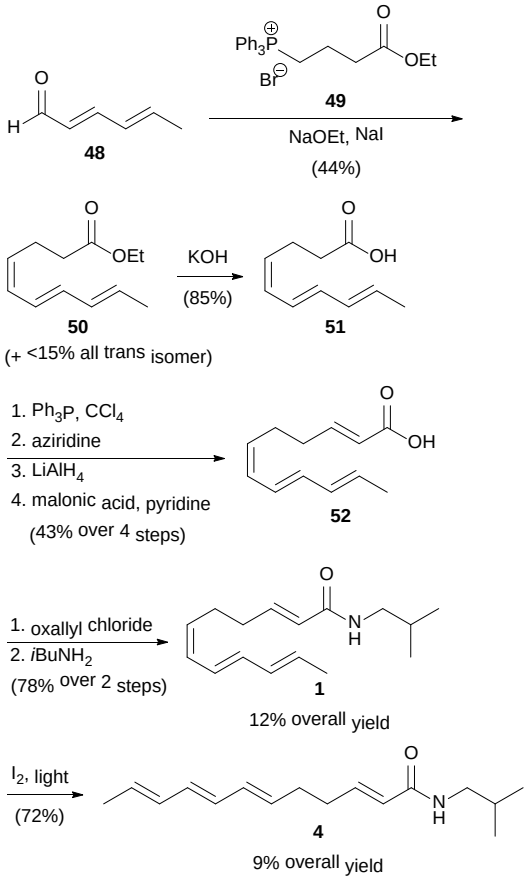


pellitorine (**46**)



hazaleamide (**47**)

Fig. 9. Related alkamides found in *Zanthoxylum* species



Scheme 1. Sonnet synthesis of α-sanshool (**1**) and β-sanshool (**4**).[45]

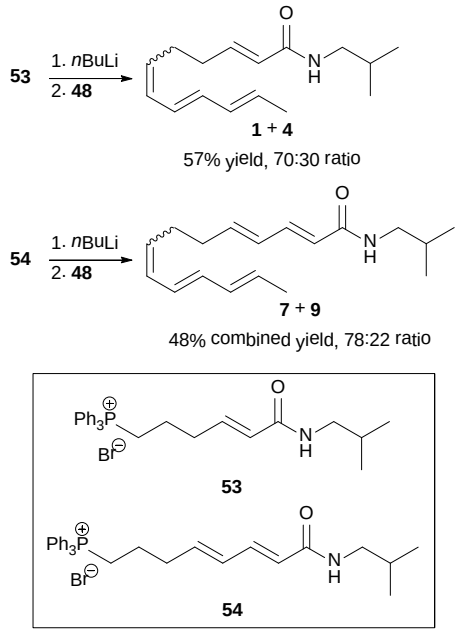
3. Synthetic studies

3.1. Early synthetic studies and challenges

The first reported chemical synthesis of any of the *Zanthoxylum* alkamides presented above appeared in 1969, when Sonnet described the preparation of α-sanshool (**1**) and its isomerization into β-sanshool (**4**).[45] As outlined in Scheme 1, a linear synthetic route was used starting with sorbaldehyde (**48**). This was olefinated using a Wittig reaction with phosphonium salt **49** to produce predominantly the *Z*-alkene containing ester **50**, which was subsequently hydrolysed to the corresponding carboxylic acid **51**. Transformation of **51** into **52** was accomplished by a 4-step sequence of: 1) activation of the carboxylic acid group, 2) aziridide formation, 3) reduction to the corresponding aldehyde, and 4) Doebner-Miller condensation. Carboxylic acid **52** was converted into **1** (12% overall yield) by reaction with oxalyl chloride to generate the corresponding acid chloride, followed by treatment with isobutyl amine. Finally, *Z*-alkene **1** was converted into the corresponding *E*-olefin **4** in 72% yield (9% overall yield) by treatment with iodine in visible light. While successful, this work suffers from the fact that many of the compounds synthesized were reported to be mixtures of alkene isomers. Due to the limitations of analytical chemistry at the time, however, the details of these mixtures were not adequately discussed.

Some years later, Crombie and Fisher reported the synthesis of mixtures of α-sanshool (**1**) and β-sanshool (**4**), and γ-sansool (**7**) and δ-sanshool (**9**) via routes that also relied on Wittig reactions using aldehyde **48** as a starting material (Scheme 2).[46,47] The reactions using phosphonium salts **53** and **54** (the synthesis of which required numerous steps from simple starting materials) afforded mixtures of **1** and **4**, and **7** and **9**, respectively. Unfortunately, no discussion regarding the separation of these mixtures into their individual components was provided.

While these early synthetic studies are noteworthy, it is clear that they are, by modern standards, lacking in terms of compound characterization data provided and/or final product



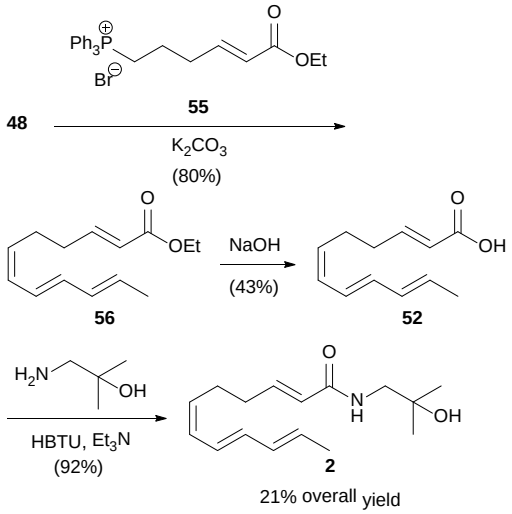
Scheme 2. Crombie and Fisher synthesis of mixtures of α-sanshool (**1**) and β-sanshool (**4**) and γ-sanshool (**7**) and δ-sanshool (**9**).[46,47]

purity. Nowadays well characterized pure compound samples are desired for applied biological studies, and more recent synthetic efforts have been directed towards producing such sanshool samples.

3.2. Toy syntheses of sanshools and hydroxy-sanshools

Much more recently, Toy and co-workers initiated studies towards the synthesis of both the sanshools and hydroxy-sanshools. In their work, carboxylic acid synthetic intermediates that can be converted directly into either of these classes of compounds were targeted.

3.2.1. Hydroxy-α-sanshool. Their first efforts in this area were directed towards the synthesis of hydroxy-α-sanshool (**2**).[48] As outlined in Scheme 3, aldehyde **48** was also employed as a starting material, as was a Wittig reaction to install the *Z*-olefin moiety in a somewhat more convergent route than the one used by Sonnet. A critical difference with the previous studies is that ester-containing phosphonium salt **55** was used to produce **56**, whereas Crombie and Fisher used amide **53** to directly produce a mixture of **1** and **4**. While this route was less direct to the target, the advantage of making this change was that hydrolysis of **56** afforded carboxylic acid **52** which can be recrystallized to isomeric purity, as demonstrated previously by Sonnet.[39] Finally conversion of pure **52** into **2** in 21% overall yield was achieved using standard peptide coupling reaction conditions. Importantly, this synthetic strategy finally allowed for the generation of multi-gram quantities of diastereomerically pure (*E,Z,E,E*)-tetraene **52** and the corresponding alkamide **2**, as is required for in-depth biological studies.



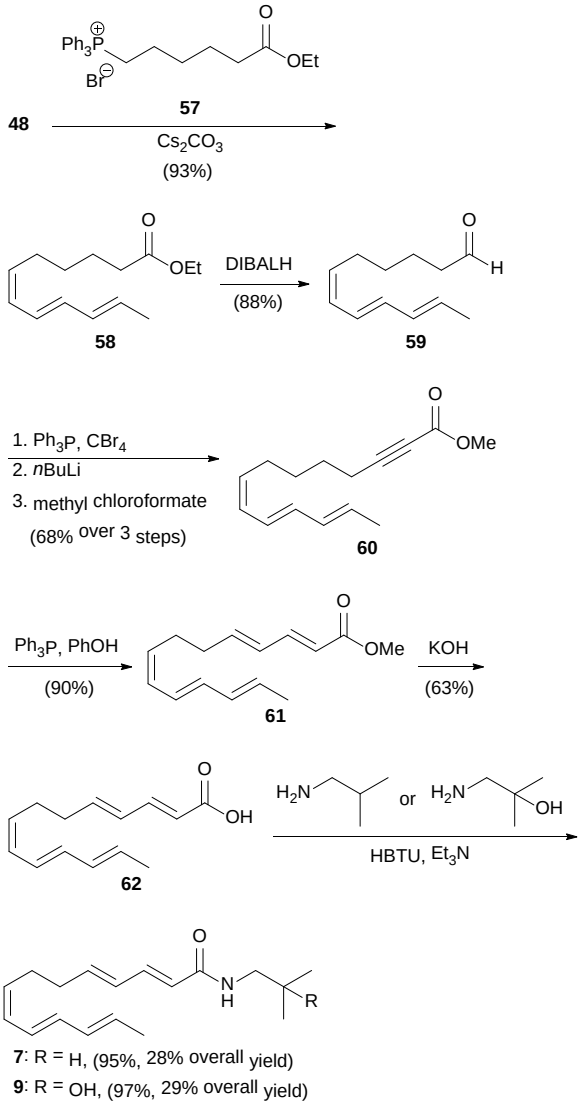
Scheme 3. Toy group synthesis of hydroxy-α-sanshool (**2**).[48]

3.2.2. γ-Sanshool and hydroxy-γ-sanshool. More recently Toy and co-workers focused their attention on the synthesis of γ-sanshool (**7**) and hydroxy-γ-sanshool (**8**).[49] As before, the preparation of this pair of compounds started with aldehyde **48** and a Wittig reaction, this time using phosphonium salt **56**, to install the *Z*-alkene group in **58** (Scheme 4). Reduction of the ester moiety of **58** to the corresponding aldehyde afforded **59**, which in turn was subjected to the Corey-Fuchs protocol for chain extending alkyne formation to produce methyl ester **60**. This key alkynoate intermediate was then isomerized stereoselectively under organocatalytic conditions to the corresponding conjugate *E*,*E*-dienoate **61** without impacting the critical *Z*-alkene group. Subsequent hydrolysis of the methyl ester group of **61** afforded the final synthetic intermediate, carboxylic acid **62**. Fortunately, this C_{14}-fatty acid was a solid material that could be readily recrystallized to remove any stereochemical isomeric impurities introduced by the starting material **48** and the Wittig reaction used to form **58**. Finally, treatment of **62** with the appropriate amines under standard peptide coupling reaction conditions afforded pure **7** and **8** in 28% and 29% overall yield, respectively. As with their previous strategy toward **2**, Toy and co-workers' approach to **7** and **8** is unique in that it allows for the production of the *Zanthoxylum* alkamides in multi-gram quantities as diastereomerically pure alkene isomers from very simple and widely available starting materials.

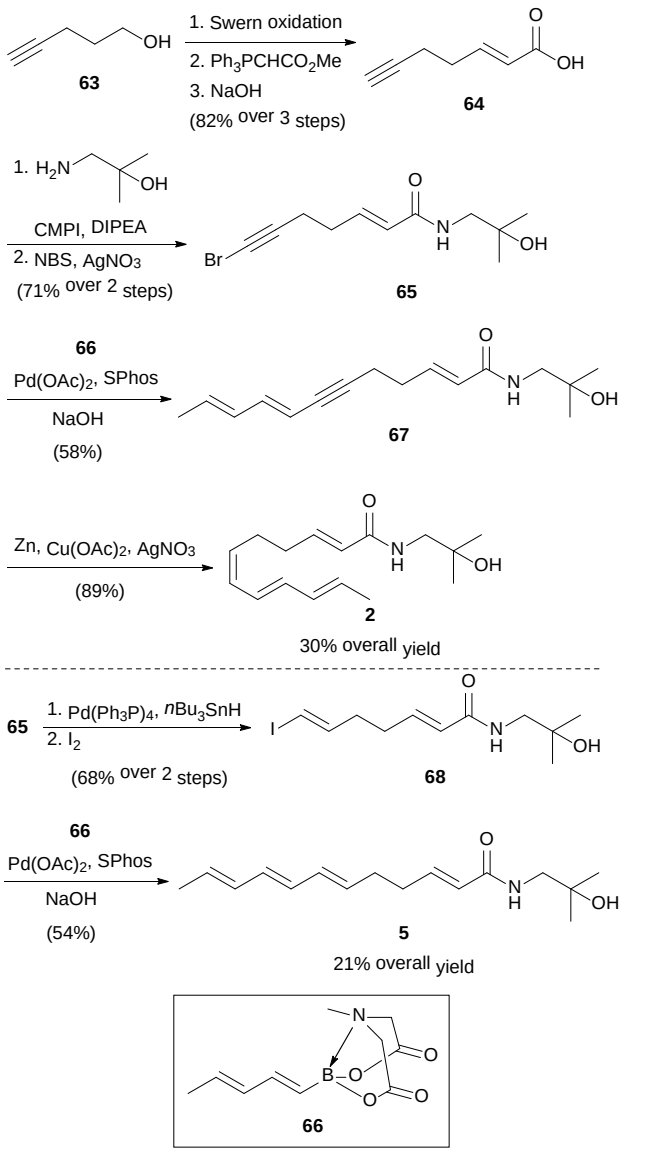
3.3. Igarashi syntheses of sanshools and hydroxy-sanshools

At essentially the same time as the Toy group was pursuing their studies, Igarashi and co-workers were also investigating the synthesis of some of the sanshools and hydroxy-sanshools.

3.3.1. Suzuki-Miyaura coupling strategy. In their initial report, they described the use of a Suzuki-Miyaura coupling strategy for the synthesis of both hydroxy-α-sanshool (**2**) and hydroxy-β-sanshool (**5**).[50] As shown in Scheme 5, pent-4-yn-1-ol (**63**) was used as the starting material, and this was converted by a multistep procedure



Scheme 4. Toy group synthesis of γ-sanshool (**7**) and hydroxy-γ-sanshool (**8**).[49]

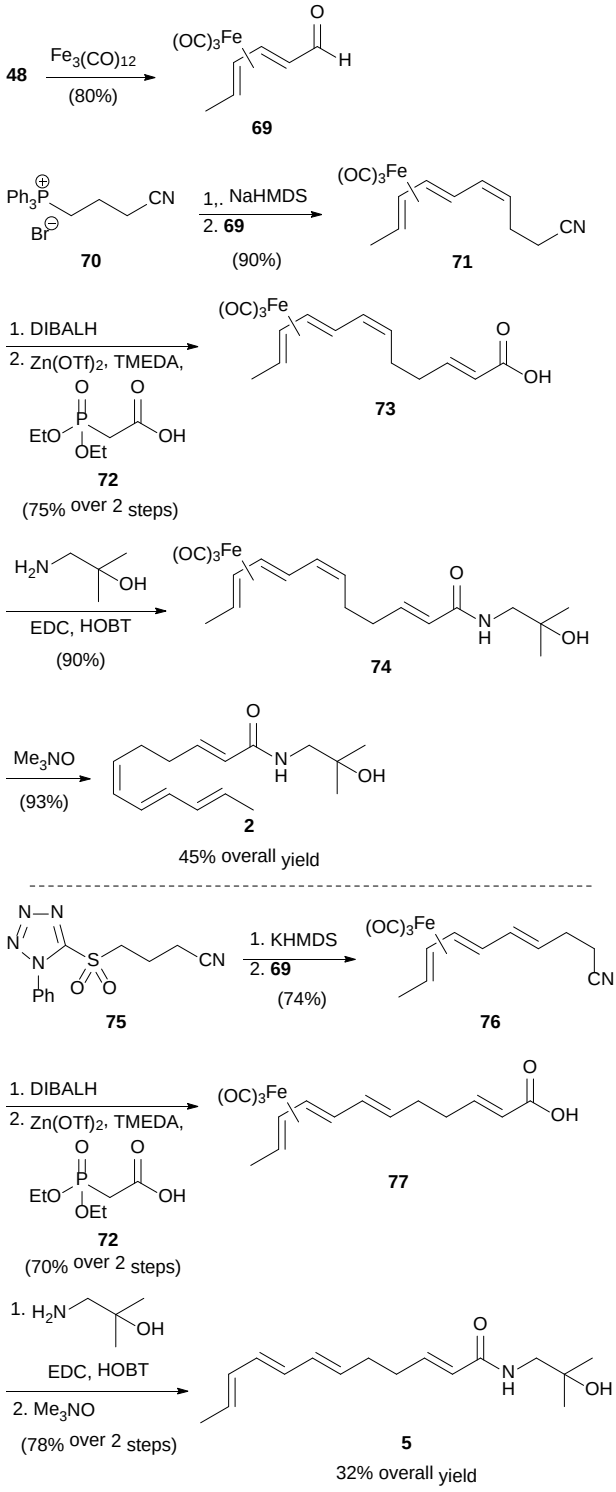


Scheme 5. Igarashi synthesis of hydroxy-α-sanshool (**2**) and hydroxy-β-sanshool (**5**).[50]

into carboxylic acid **64**. Amide formation and bromination of **64** afforded key intermediate bromoalkyne **65**, which could either be subjected to Suzuki-Miyaura coupling with MIDA boronate **66** to produce **67**, or reduced and iodinated to afford vinyl iodide **68**. Finally, chemo- and stereoselective reduction of the alkyne moiety in **67** afforded (*E,Z,E,E)*-tetraene **2** in 30% overall yield, whereas Suzuki-Miyaura coupling of **68** with **66** produced the all-*trans* congener **5** in 21% overall yield.

3.3.2. Iron-carbonyl complexation strategy. Subsequently, Igarashi and co-workers reported a second generation synthesis of hydroxy-α-sanshool (**2**) and hydroxy-β-sanshool (**5**), along with a first-generation synthesis of γ-sanshool (**7**), using an iron-carbonyl complexation strategy.[51] The rationale for using iron-carbonyl complexes in this work was to stabilize the synthetic intermediates; such complexes were indeed found to be stable solid materials. As outlined in Scheme 6, aldehyde **48** was again used as the starting material and its iron-carbonyl complex **69** was subjected to a Wittig reaction with phosphonium salt **70** to form **71**. Reduction of the nitrile group of **71** to the corresponding aldehyde was followed by a Horner-Wadsworth-Emmons (HWE) olefination reaction with phosphonate **72** to afford **73**. Conversion to amide **74** followed by decomplexation of the iron moiety furnished alkamide **2** in 45% overall yield. For the corresponding synthesis of **5**, Julia-Kocienski olefination of **69** with sulfoxide **75** afforded **76**, which was further transformed into all-*trans* tetraene alkamide **5** using the same reactions as before via the intermediate carboxylic acid **77**. Finally, amidation and decomplexation of **77** afforded **5** in 32% overall yield.

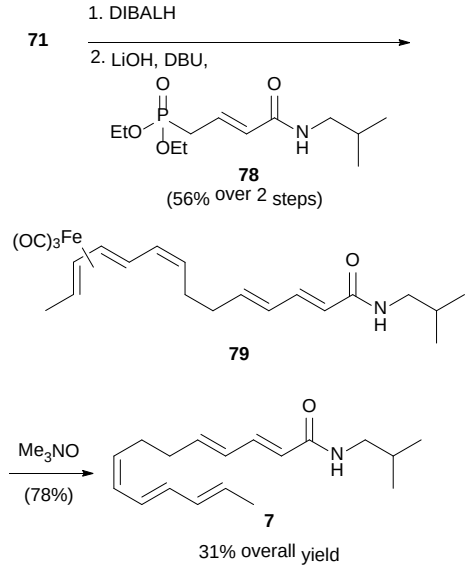
As shown in Scheme 7, the synthesis of **7** was accomplished by once again reducing the nitrile group of **71** to the corresponding aldehyde followed by a HWE reaction with phosphonate **78** to afford polyene **79**. Decomplexaiton of the iron complex in **79** produced alkamide **7** in 31% overall yield.



Scheme 6. Igarashi synthesis of hydroxy-α-sanshool (**2**) and hydroxy-β-sanshool (**5**).[51]

3.4. Mugnaini syntheses of δ-sanshool

More recently, Mugnaini and Corelli described two syntheses of δ-sanshool (**10**).[52] In their first generation synthesis they used alcohol **80** as the starting material (Scheme 8). This was first oxidized to aldehyde **81**, which was subsequently subjected to a HWE olefination reaction with phosphonate **78**



Scheme 7. Igarashi synthesis γ-sanshool (**7**).[51]

to afford diene **82.** Deprotection of the alcohol group of **82** was followed by its oxidation to yield aldehyde **83**. Finally, a second HWE olefination reaction of **83** with phosphonate **84** afforded **10** in 7% overall yield.

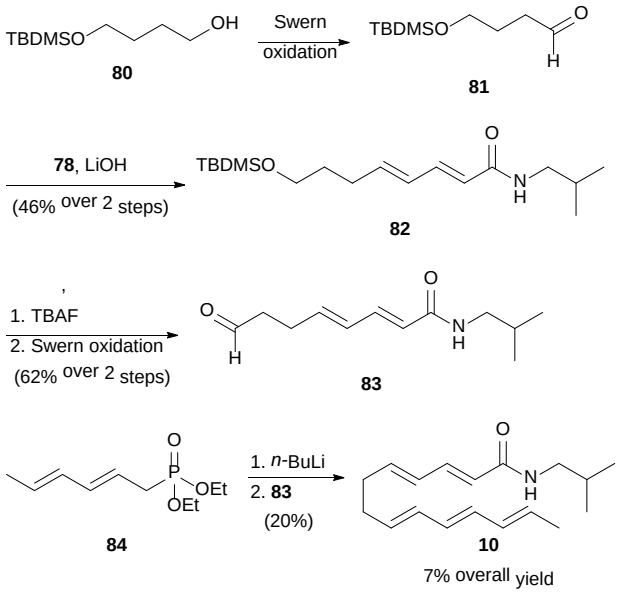
As outlined in Scheme 9, in their second generation synthesis of **10**, Mugnaini and Corelli used the same building blocks, but switched the order of HWE olefination reactions used to combine them. Thus, starting aldehyde **81** was reacted with phosphonate **84** to afford (*E*,*E*,*E*)-triene **85**. Deprotection of the alcohol group of **85** followed by its oxidation afforded intermediate aldehyde **86**, which was subjected to the second HWE olefination with phosphonate **78** to produce **10** in 17% overall yield.

3.5. Givaudan synthesis of bungeanool

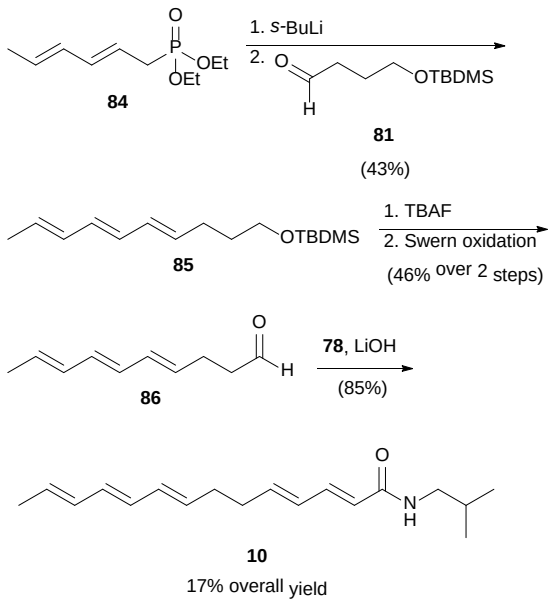
The only other naturally occurring alkamide discussed above in Section 2 to be synthesized chemically is bungeanool (**14**). This was reported by researchers at Givaudan in 2003 and their route is outlined in Scheme 10.[53] Propargyl bromide **87** was coupled with alkynol **88** to form diynol **87**. Selective partial reduction of the alkyne groups of **87** to the corresponding *Z*-alkenes was followed by oxidation of the alcohol to afford aldehyde **90**. This intermediate was then subjected to a HWE olefination reaction with phosphonate **91** to produce ester **92**. Finally, **92** was converted into **14** by the reaction sequence of: 1) ester hydrolysis, 2) activation of the resulting carboxylic acid, and 3) exposure to the required amine. Unfortunately, only the yields for the formation of **92** and **14** were reported, so it is not possible to evaluate the overall efficiency of this synthesis.

3.6. Design and synthesis of novel alkamides inspired by hydroxy-α-sanshool

In addition to the diversity of *Zanthoxylum* alkamides available from natural sources, a significant collection of synthetic

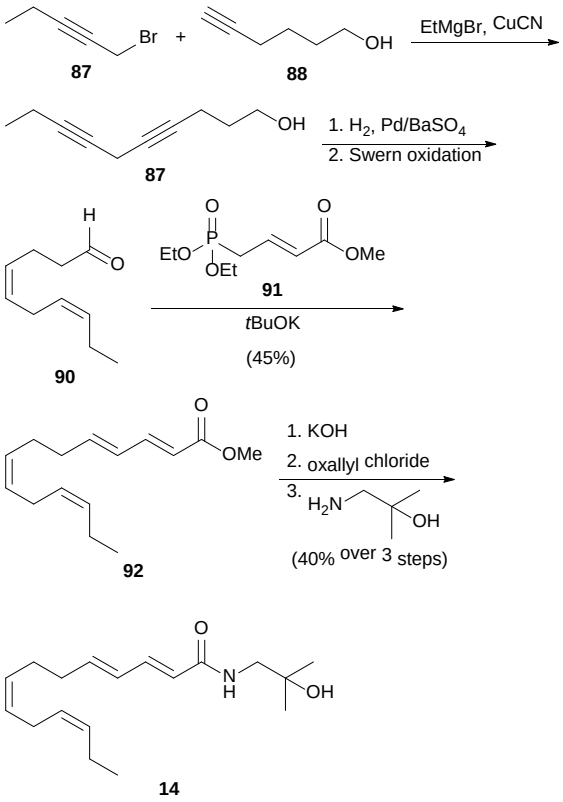


Scheme 8. Mugnaini and Corelli synthesis of δ-sanshool (**10**).[52]



Scheme 9. Mugnaini and Corelli second generation synthesis of δ-sanshool (**10**).[52]

derivatives also have been reported. The first concerted efforts in this regard were described by the group at Givaudan when they reported their synthesis of bungeanool (**14**).[53] Specifically, they constructed a small library of synthetic alkamides related to the sanshools and bungeanools in an attempt to identify the critical structural components responsible for the pungency of these natural products (Figure 10). The initial series of synthetic sanshool derivatives (**93-98**) involved truncation, elongation, or modification of the polyunsaturated tail. These compounds, coupled with natural *Zanthoxylum* alkamides, led to a proposal of the minimal structure required for the paresthetic (tingling) effect associated with certain hydroxy-sanshools, e.g. **2** and **8**. (*E*,*E*,*Z*)-Trieneamides **99** and **100** were



Scheme 10. Givaudan synthesis of bungeanool (**14**).[53]

then generated to test this model (see Section 4.1 for more details). Finally, a series of isobutyl and (2-hydroxy)isobutyl amides of polyunsaturated acids containing an aromatic ring (**101-107**) and a small collection of cinnamamides (**103a-i**) were constructed in the hopes of identifying a simplified and more stable alkamide with similar orosensation properties to hydroxy-α-sanshool (**2**).

Six years after these seminal structure-activity relationship studies, researchers at Nestlé reported their own collection of synthetic hydroxy-α-sanshool analogues in an attempt to elucidate the critical structural features responsible for the compound's ability to activate transient receptor potential (TRP) channels TRPV1 and TRPA1 (see section 4.2 for more details).[54] As shown in Figure 11, these derivatives fell into three categories: 1) (2-hydroxy)isobutyl amides of four different C_{12} fatty acid tails containing between zero to two double bonds (**108-111**), 2) amides between (*Z*)-dodec-5-enoic acid and either methyl L-serinate (**112**), dimethyl L-glutamate (**113**), methyl L-glycinate (**114**), or methyl L-alanate (**115**), and 3) amides between L-alanine and either (2*E*,6*Z*)-dodeca-2,6-dienoic acid (**116**), linolenic acid (**117**), or palmitoleic acid (**118**). Finally, in their recent synthesis of δ-sanshool (**10**, see section 3.4), Mugnaini and Corelli also generated four unique analogues of the natural product.[52] Tetraeneamides **119** and **120** were both obtained in low yield by HWE olefination between the previously described aldehyde **83** and the requisite diethylphosphinates (Scheme 11A). Similarly, cyclopropyl amide **121** and isopropylamide **122** were

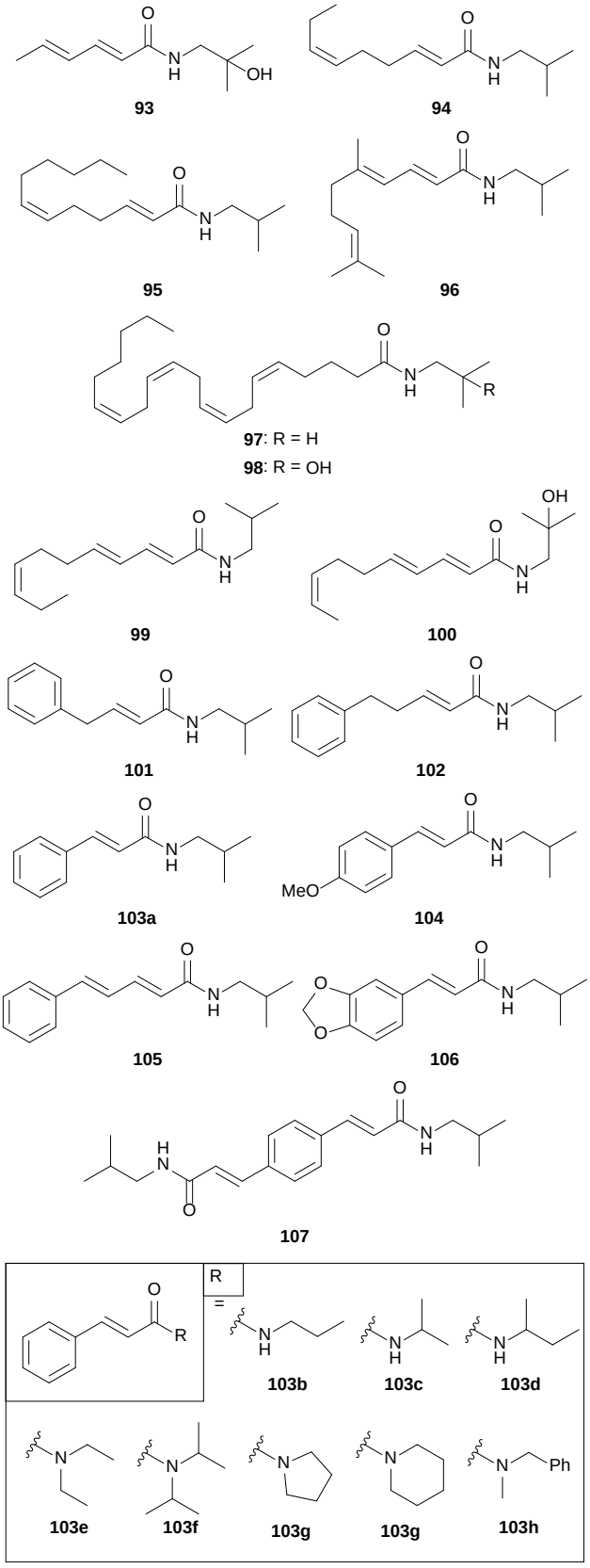


Fig. 10. Synthetic derivatives of the sanshools and bungeanools.[53]

generated via significantly higher yielding (E)-selective olefination reactions with the conjugated triene aldehyde **86** (Scheme 11B).

4. Biological activity and structure-activity relationships

Throughout the world, members of the *Zanthoxylum* genus have been incorporated into traditional medicines and herbal remedies for a wide range of medical indications. Similarly, throughout the scientific literature, a wide variety of biological effects have been attributed to *Zanthoxylum*-based extracts, including antioxidant, antibacterial, anticancer, anti-inflammatory and hepatoprotective. Unfortunately, there appears to be significant confusion as to the source of these observed biologically activities. Several studies, particularly those associated with antibacterial activity, involve the essential oils derived from *Zanthoxylum* fruits;[55] it is critical to

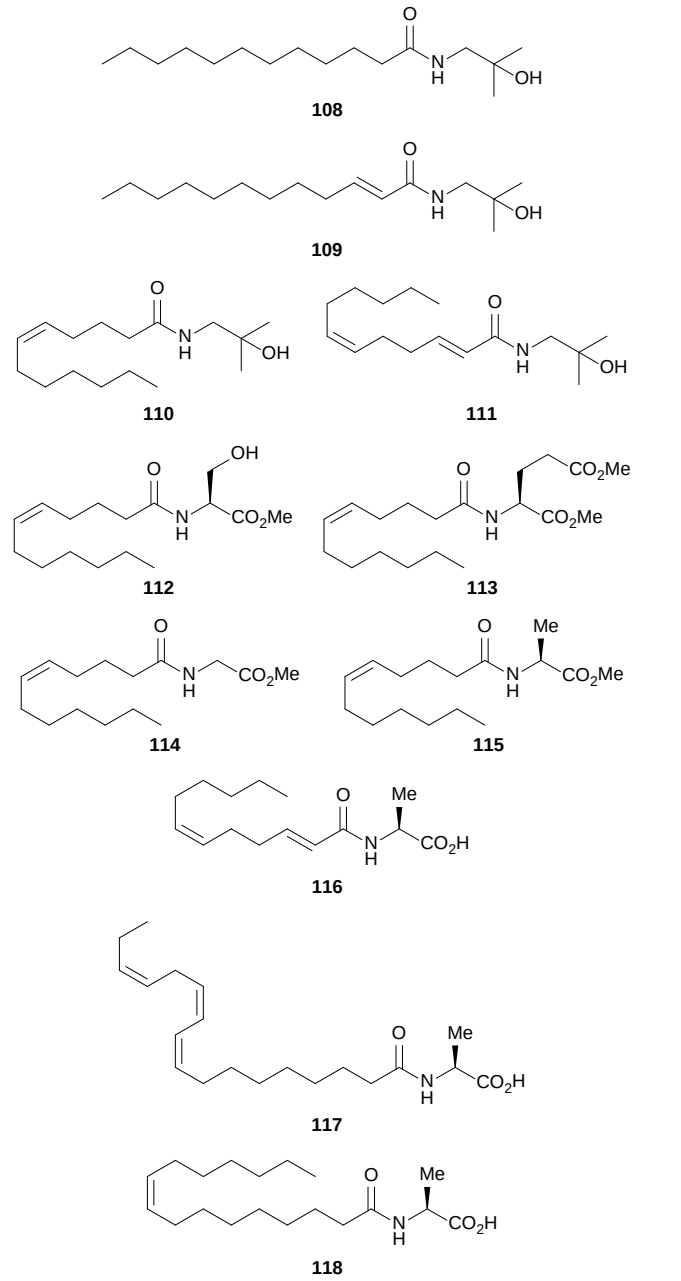
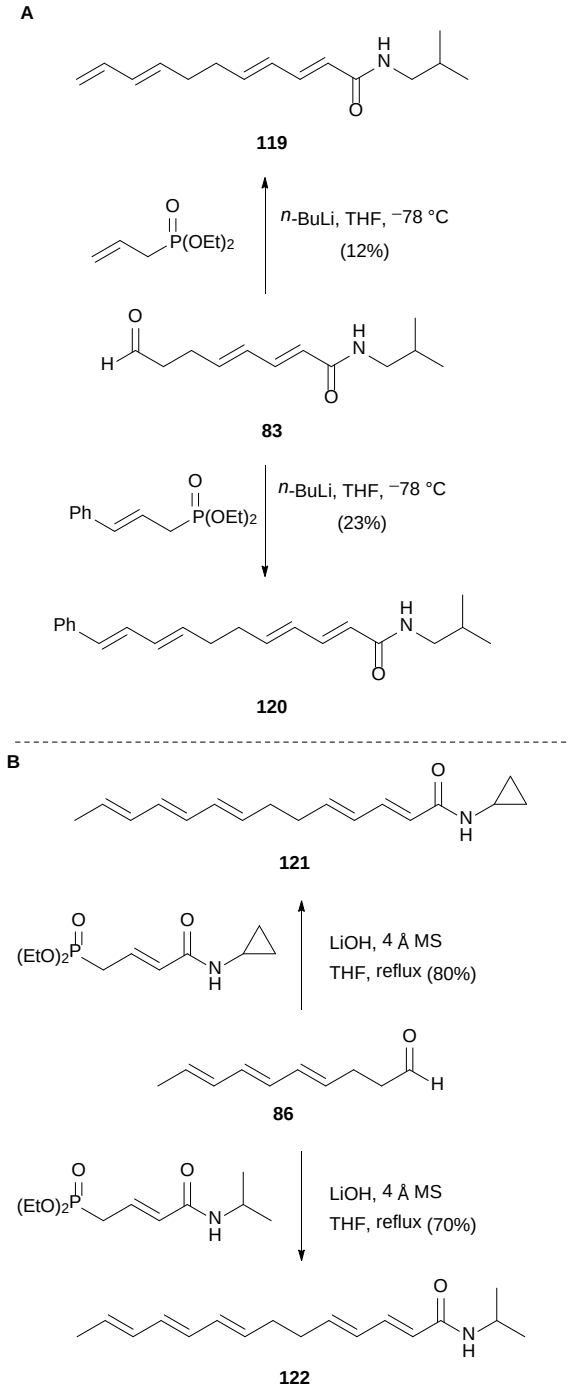


Fig. 11. Synthetic sanshool derivatives.[54]



Scheme 11. A. Synthesis of δ-sanshool tetraene analogues **119** and **120**. B. Synthesis of δ-sanshool amide derivatives **121** and **122**.[52]

emphasize that *these essential oils do not contain any alkamides*![30,55] Accordingly, authors are strongly encouraged to no longer attribute the biological activities observed with *Zanthoxylum* essential oils in previous or future studies to alkamide natural products. That being said, many of the demonstrated biological activities associated with traditional remedies containing *Zanthoxylum*-based components can be attributed to the pungent alkamide components present. Some of these traditional *Zanthoxylum* extract-based medicines are manufactured in pharmaceutical-grade and are being evaluated in modern clinical trials in both Japan and the United States, with substantial associated pharmacokinetic studies on the critical alkamide components.[56–58] The following section surveys studies performed with the aim of understanding the various biological activities known to be associated with *Zanthoxylum* alkamides.

4.1. Orosensation

The most studied biological activity for the *Zanthoxylum* alkamides is their impact on orosensation. Early studies simply refer to the general property in the oral cavity as "pungency", but at least three unique types of orosensation are now associated with *Zanthoxylum* alkamides: "burning", paresthetic (tingling) and anaesthetic (numbing). As will be discussed in the next section, the specific biochemical mechanisms governing these unique orosensations are still hotly contested. Significant structure-activity relationship studies with naturally-produced *Zanthoxylum* alkamides and synthetic derivatives, on the other hand, have delineated the fundamental structural components requisite for the various orosensation activities. Prior to the work from Hofmann's group in 2014,[15] orosensation studies employed solutions of *Zanthoxylum* alkamides or *Zanthoxylum* extracts containing ethanol, potentially leading to several false-negative results, particularly in regard to anaesthetic and burning properties. Nevertheless, it has long been established that one of the most abundant alkamides in Sichuan peppercorn (huajiao) and Japanese pepper (sanshō), hydroxy-α-sanshool (**2**), is also most responsible for the oral paresthetic sensation experienced upon consumption of these spices.

In 2003, Galopin and co-workers at Givaudan were the first to propose a minimal structure required for the tingling effect,[53] although the details of their orosensation studies were not provided and they simply used the qualitative descriptors of "pungent" and "not pungent" to refer to various *Zanthoxylum* alkamides and synthetic derivatives. Preliminary studies compared natural products α-sanshool (**1**), hydroxy-α-sanshool (**2**), hydroxy-γ-sanshool (**8**), bungeanool (**14**) and tetrahydrobungeanool (**16**) with synthetic derivatives **93**-**98** (*cf*. Figure 10). From this initial collection, only alkamides **1**, **2**, **8**, **14**, and **95** proved to be "pungent", leading the authors first to conclude that the (CH=*Z*=CH-CH_2-CH_2-CH=*E*=CH) and *N*-isobutylcarboxamide motifs common to all of these compounds were most critical for the associated pungency (Figure 12). Surprisingly, synthetic derivative **94**, which possesses both of these motifs, did not demonstrate any noticeable pungency suggesting that the proposed minimal structure alone is not sufficient. Two more synthetic compounds, white powder **99** and colourless oil **100**, were then synthesized to further refine the model. Both of these synthetic analogues proved to be pungent, leading the authors to conclude that for noticeable pungency the compounds must possess, in addition to the minimal structure, at least two of three optional features: 1) a hydroxyl group on the 2-position of the isobutylamide, 2) extended conjugation to the amide carbonyl, or 3) a $\geq C_2$ chain after the critical *cis* olefin (Figure 12,

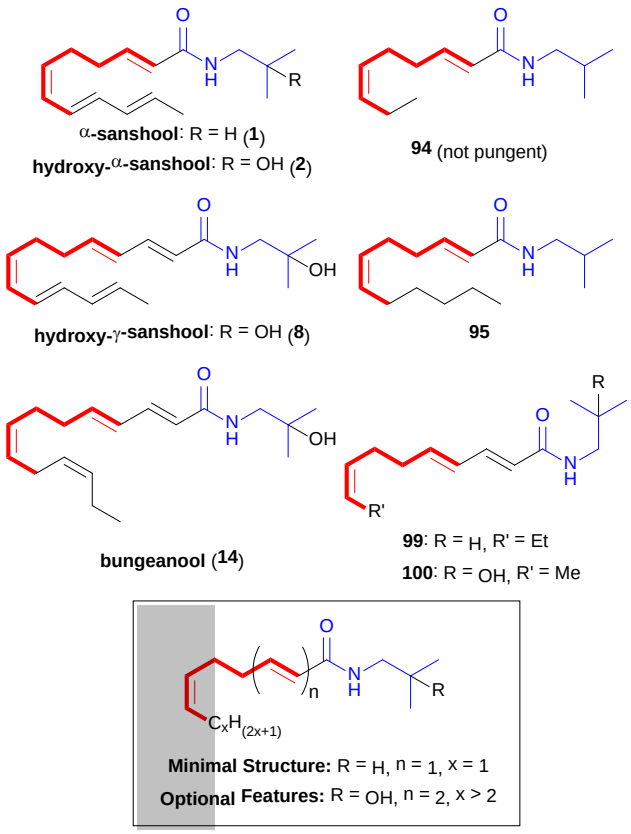


Fig. 12. The proposed minimal structure for pungency in natural *Zanthoxylum* alkamides and synthetic derivatives involves an (*E*,*Z*) isolated diene unit (*bold red*) and an *N*-isobutylamide (blue). At least two of three optional features also must be present.[53]

inset). In the hopes of identifying a simplified and stabilized analogue of α-sanshool, Galopin and co-workers also synthesized and tested a collection of aromatic alkamides (**111**–**107**, *cf.* Figure 10). Unfortunately, only one compound from this series, *N*-isobutylcinnamamide (**103a**), demonstrated any noticeable pungency.

In 2005, Sugai and co-workers evaluated the orosensation activities and tastes of four sanshools (α-, β-, γ-, and δ-) and two hydroxy-sanshools (α- and β-), all of which were isolated and purified from dried *Z. piperitum* pericarps.[59] Unlike previous studies,[53] these efforts distinguished between the capsaicin-like burning pungencies (even assigning Scoville unit values for each compound) and the numbing and tingling oral sensations; results from these investigations are summarized in Table 1. A key and unique distinction from these investigations is that the pungency of the *N*-isobutylamide sanshools (**1**, **4**, **7**, **10**) is significantly characterized as "burning", whereas the hydroxylated congeners (**2** and **5**) are exclusively tingling and/or numbing. It should be noted, however, that the burning sensation associated with the sanshools is still significantly less than that experienced from capsaicin (12,000,000 SU). Another noteworthy observation from this investigation is that, contrary to previous studies that simply identify β-sanshool (**4**) and hydroxy-β-sanshool (**5**) as "not pungent",[53] these two all-*trans* alkamides were noted to exhibit anaesthetic activity.

Table 1. Orosensory activities, Scoville unit (SU) values and tastes for six *Zanthoxylum* alkamides[59]

Alkamide	Oral sensation	SU (mL/g)	Taste
α-sanshool (**1**)	burning, tingling, numbing	80,000	*n.d.*
β-sanshool (**4**)	numbing	70,000	bitter
γ-sanshool (**7**)	burning, numbing	110,000	fresh, bitter
δ-sanshool (**10**)	burning, numbing	110,000	fresh
hydroxy-α-sanshool (**2**)	tingling, numbing	26,000	*n.d.*
hydroxy-β-sanshool (**5**)	numbing, astringent	13,000	bitter

The most thorough and reliable orosensation studies on *Zanthoxylum* alkamides are those reported by Bader and co-workers in 2014.[18] Notable aspects from these investigations are 1) a modified "half-tongue" test[60] was employed in which the compounds were administered to the tongues of the tasting panels by pre-absorption onto a square of filter paper instead of as a solution containing ethanol, thus minimizing false-negative results, and 2) tingling (paresthetic) and numbing (anaesthetic) orosensory recognition for each alkamide examined was quantitated by establishing threshold concentrations. For these studies, eight pure *Zanthoxylum* alkamides were obtained by supercritical fluid extraction from dried *Z. piperitum* pods followed by extensive HPLC purification. Specifically, the pungencies of hydroxy-α-sanshool (**2**), hydroxy-β-sanshool (**5**), hydroxy-γ-sanshool (**8**), hydroxy-δ-sanshool (**11**, referred to as hydroxy-γ-isosanshool), hydroxy-ε-sanshool (**12**), hydroxy-ζ-sanshool (**13**), bungeanool (**14**), and isobungeanool (**17**) were all explored; the results from these investigations are summarized in Table 2. All six of the *Zanthoxylum* alkamides investigated that possessed the critical structural motifs proposed by Galopin and co-workers (**2**, **8**, **12-14**, **17**)[53] elicited pronounced and roughly equipotent tingling oral sensations. In accord with the previous report from Sugai and co-workers,[59] the sensory panellists all reported a potent and persistent numbing/anaesthetic oral impression from the all-*trans* alkamides **5** and **11**. This combination of potent paresthetic (from *cis*-olefin containing *N*-(2-hydroxy)isobutyl alkamides) and anaesthetic (from the all-*trans* polyene congeners) activities adequately characterizes the unique tingling and numbing orosensation

Table 2. Orosensory activities and recognition thresholds for several *Zanthoxylum* alkamides[18]

Alkamide	Oral sensation	Threshold concentration
hydroxy-α-sanshool (**2**)	paresthetic	8.3 nmol/cm^2
hydroxy-β-sanshool (**5**)	*anaesthetic*	3.9 nmol/cm^2
hydroxy-γ-sanshool (**8**)	paresthetic	6.8 nmol/cm^2
hydroxy-δ-sanshool (**11**)	*anaesthetic*	7.1 nmol/cm^2
hydroxy-ε-sanshool (**12**)	paresthetic	4.2 nmol/cm^2
hydroxy-ζ-sanshool (**13**)	paresthetic	7.0 nmol/cm^2
bungeanool (**14**)	paresthetic	3.5 nmol/cm^2
isobungeanool (**17**)	paresthetic	3.5 nmol/cm^2

experienced upon eating fresh Sichuan peppercorns. Given that the paresthetic hydroxy-α-sanshool (**2**) is known to isomerize to the anaesthetic all-*trans* tetraene **5** over time, especially in the presence of UV light,[16,30] this observation could also serve as an explanation as to why aged Sichuan peppercorns tend to lose their unique mouth-tingling capabilities while maintaining their tongue-numbing characteristics.

4.2. Excitation of sensory neurons

The first reported studies aimed at determining the peripheral basis for the tingling paresthesia induced by *Zanthoxylum* alkamides were described by Bryant and Mezine of the Monell Chemical Senses Center.[25] They found that hydroxy-α-sanshool (**2**) altered the levels of spontaneous activity in cool-sensitive fibres in addition to inducing activity in tactile fibres, cold nocireceptors and silent fibres that were insensitive to innocuous thermal or tactile stimuli. Furthermore, **2** induced tactile or thermal sensitivity in fibres that were initially insensitive to cooling or touch. The neuronal distribution of sensitivities to capsaicin and to **2** indicated that **2** affects neurons mediating innocuous sensations, and therefore it could indeed be useful as a model stimulus for paresthesia studies.

Later Oh and co-workers reported that **2** activates transient receptor potential ankyrin 1 (TRPA1) and TRP vaniloid 1 (TRPV1) ion channels in human embryonic kidney (HEK) cells.[61] Specifically, **2** caused Ca^{2+} influx in cells transfected with TRPV1 or TRPA1, and evoked inward currents. Furthermore, **2** evoked licking behaviour when injected into a hind paw of wild-type mice, but this effect was reduced in TRPV1-deficient mice. Thus, they concluded that TRPA1 and TRPV1 are the molecular targets of **2** in sensory neurons and that activation of these ion channels explain its pungent and tingling properties. These findings provided credence to the earlier assertion that TRPV1 activation alone could not explain the properties of **2**.[59]

This research was subsequently supported by the findings of le Coutre his collaborators.[62] They performed some simple structure-activity relationship studies and found that the *Z*-configuration of double bond at position C-6 of **2** was essential for its TRPA1 activation ability. Interestingly, no such structural requirements were found for TRPV1 activation. Additionally, they reported that activation of TRPA1 by **2** seemed to involve covalent bonding, but TRPV1 did not.

These ideas were later disputed by Julius and co-workers, who found that although **2** is an agonist of both TRPA1 and TRPV1, it binds to the two-pore potassium channels TASK-1, TASK-3 and TRESK (previously known as KCNK3, KCNK9 and KCNK18, respectively).[63] Neuron activation through a unique mechanism involving inhibition of these pH- and anaesthetic-sensitive channels was their suggested cause of the tingling effect.

These neurophysiological studies used animal- and human-derived models to demonstrate that **2** induces bursts of neuronal firing in specific cutaneous afferents through chemical response within the receptor membrane. Stuky and Bautista, and their co-workers used an *ex vivo* skin-nerve preparation to examine the pattern and intensity with which the sensory terminals of cutaneous neurons respond to **2**, and their findings suggested that it excites a distinct subset of D-hair, ultrasensitive light-touch receptors in the skin and targets uncharacterized populations of Aδ- and C-fibre nerve afferents.[64] This research indicates that mechanosensation, or tactile response, is also integral to the effect. The response was observed in animals lacking TRPA1 and TRPV1 receptors, which suggests that neither of these mediates the effects of **2**. Inhibition of both Aδ-mechanonociceptors and the activity of multiple voltage-gated sodium channel subtypes, such as Na_v 1.7, by **2** is significant since the former mediates both sharp and acute pain and inflammatory pain, and the latter is a key mediator of inflammatory mechanical pain in "fast pain" mechanosensory neurons.[65]

These studies focused on the molecular receptor mechanisms and did not directly assess how chemical receptor events could relate to the tingling effect, and it remained unclear which fibres are responsible. To address this issue Haugura and co-workers investigated the somatosensory perception of tingling caused by Sichuan pepper in humans to identify the characteristic temporal frequency and compared this to the established selectivity of tactile afferents.[66] When Sichuan pepper was applied to the lower lip of study participants, they judged the frequency of the tingling sensation on the lips by comparing this with the frequencies of mechanical vibrations applied to their right index finger. The frequency perceived of the tingling by the participants was routinely at around 50 Hz, corresponding to the range of tactile rapidly adapting 1 (RA1) afferent fibres. Furthermore, adaptation of the RA1 channel by prolonged mechanical vibration reduced the tingling frequency caused by Sichuan pepper, and this confirmed that the frequency-specific tactile channel is shared between mechanical vibration and Sichuan pepper.

More recently the same group used a perceptual interference paradigm to show that the Sichuan pepper-induced RA input indeed contributes to the human tactile processing.[67] The absolute detection thresholds for vibrotactile input were measured with and without Sichuan pepper application on the fingertip, and it was found to significantly impair detection of vibrations at 30 Hz (RA channel dominant frequency), but it did not affect detection of vibrations at 240 Hz (Pacinian-corpuscle (PC) channel dominant frequency), or vibrations at 1Hz (slowly adapting 1 (SA1) channel dominant frequency). In total these results indicate that Sichuan pepper induces a peripheral RA channel activation that is important for tactile perception, and such anomalous activation of RA channels may contribute to the tingling experience of Sichuan pepper.

4.3. Impact on gut motility

As anyone who has indulged in a traditional Sichuan-style hot pot meal is well aware, consumption of *Zanthoxylum* pericarps (e.g., Sichuan peppercorns) can dramatically increase gut motility and colonic activity. Indeed, pericarps from *Z. piperitum* (Japanese pepper or sanshō) are one of three herbal

ingredients (the other two being ginger and ginseng) in the traditional Japanese medicine daikenchuto, which has been used for centuries for the treatment of various gastro-instestinal issues.[68,69] TU-100 (sometimes referred to as TJ-100),[57,70] a pharmaceutical-grade daikenchuto formulation, is currently under investigation by the Japanese Foundation for Multidisciplinary Treatment of Cancer and the US Food and Drug Administration in patients with postoperative ileus and ischemic intestinal disorders.[67,71,72] Substantial evidence points to the *Zanthoxylum* alkamides hydroxy-α-sanshool (**2**) and, to a lesser extent, hydroxy-β-sanshool (**5**) as the primary components in daikenchuto responsible for the contraction of gut smooth muscle cells, induction of colonic motor activity, and increased defecation rates.[73–76] Additionally, γ-sanshool (**7**) has been shown to improve intestinal transit in a mouse model of postoperative ileus.[77] Interestingly, all three of these alkamides appear to exert their effects by different mechanisms. For example, Tsuchiya and co-workers demonstrated that **7** induced increase gut motility by serving as an agonist for TRPA1.[77] Kubota and co-workers, on the other hand, revealed that colonic contraction induced by **2** was not a result of stimulation of TRPA1 or TRPV1, but most likely via blockade of the two-pore domain potassium channel KCNK9 (TASK-3).[75] The colonic motor pattern induced by alkene isomer **5** was different from that observed with **2**. Both **5** and **2** are antagonists for KCNK3 (TASK-1), but only **2** can block KCNK9, in addition to KCNK18 (TRESK), providing a potential explanation for the difference in observed impact on specific colonic contraction behaviour. Both **2** and **5** exhibit substantial dose-dependent plasma concentrations after oral administration.[57,58] Additionally, direct application of these alkamides can evoke site-specific contractions within the digestive tract,[69,70] suggesting two potential pathways that these alkamides can interact with the milieu of cell types and tissues involved in the digestive process.

4.4. Nerve growth factor (NGF) potentiation

As mentioned above, the ZP-amides A-E (**27**-**31**) and qinbunamides A-C (**34**-**36**) were isolated from the pericarps of *Z. bungeanum* by Zhang and Gao in a study aimed at identifying natural products capable of promoting neurotrophic activity.[35] As part of their research they assessed the ZP-amides **27**, **29**-**31**, and qinbunamides **35**, and **36** (**28** and **34** were omitted due to lack of material) for their ability to enhance NGF-mediated neurite outgrowth in rat pheochromocytoma (PC12) cells. Cytotoxicity studies confirmed that none of the alkamides investigated had any significant toxicity in PC12 cells or HCT116 human colon cancer cells at concentrations up to 50.0 μM. When combined with NGF, these six fatty acid amides (concentration of 20 μM) all exerted an increase in neurite-bearing cells compared to both negative control (DMSO) and positive control (NGF alone) cells. Diols **29** and **30**, as well as ethyl ether **35**, exhibited the strongest potentiation of NGF activity, with each consistently showing an approximately 4% boost in neurite bearing cells (p <0.01 vs NGF).

4.5. Cancer

Several studies indicate that extracts for *Zanthoxylum* fruits, leaves, bark, and roots can exert noticeable anticancer activity. For example, extracts from *Z. piperitum* block the PAK1/cyclin D1 pathway as well as the growth of neurofibromatosis type 1 (NF1)-deficient cancer xenografts in mice.[78,80] Several alkamides isolated from Nepalese *Z. armatum* were tested in a *NF1*- and *p53*-defective murine malignant glioma tumour cell line that compared loss of cell viability with growth inhibition in a dual-reporter assay (Table 3).[33] Four of the ten *Zanthoxylum* alkamides tested demonstrated >50% growth inhibition, but only hydroxy-α-sanshool (**2**) did so without negatively impacting cell viability. The unique endo-peroxide timuramide A (**38**) also demonstrated favourable cell viability, albeit with relatively low concomitant growth inhibition activity.

Table 3. Growth inhibition activity and concomitant cell viability in a *Nf1*- and *p53*-defective murine glioma tumour cell line upon treatment with selected Zanthoxylum-derived alkamides (2.0 μg/mL)[33]

Alkamide	Growth inhibition	Cell viability
hydroxy-α-sanshool (**2**)	53%	106
hydroxy-β-sanshool (**5**)	65%	56
ZP-amide A (**27**)	52%	75
ZP-amide C (**29**)	31%	79
ZP-amide D (**30**)	56%	66
ZP-amide E (**31**)	41%	67
timuramide A (**38**)	38%	97
timuramide B (**39**)	13%	65
timuramide C (**40**)	47%	66
timuramide D (**41**)	42%	70

Extracts from the fruit of *Z. piperitum* De Candolle inhibited cell proliferation and induced JNK-dependent autophagic cell death in three different human cancer cell lines (DLD-1, HepG2, and Caco-2).[80] This is in line with previous studies which demonstrated that α-sanshool (**1**) induces apoptosis in human hepatocarcinoma HepG2 cells.[81] It is possible that several different components of the fruit extract, in addition to **1** and other alkamides, were responsible for the observed pro-apoptotic activity. It should be noted that the anticancer activity of the *Zanthoxylum* fruit extract was not universal; A549, MCF-7 and WiDr cells, in addition to normal intestinal cells, were not affected.

4.6. Diabetes

Type-I and type-II diabetes are complicated and multi-faceted metabolic disorders caused by insulin resistance or the lack of insulin secretion. The transmembrane cannabinoid (CB) receptors play important roles in a wide variety of physiological processes, including glucose-mediated insulin secretion and T-cell activation. Of the three known CB receptors, CB1 and CB2 appear to be the most influential in regards to the progress of type-I diabetes. A current hypothesis is that blockade of the CB1 receptor with concomitant activation of the CB2 receptor might be a useful

therapeutic option for the treatment of type-I diabetes. Toward this end, Dossou and co-worker screened a collection of alkamides extracted from *Z. bungeanum* pericarps with the hopes of identifying a dual CB1 antagonist/CB2 agonist.[20] The initial CB1 and CB2 inhibition (antagonism) studies are summarized in Table 4. It should be noted that while the structure of δ-sanshool (**10**) was drawn, it was incorrectly referred to as γ-sanshool (**7**) in the original publication. Personal communication with the corresponding author (R. Moaddel) confirmed that the actual compound was **10** and not **7**.† Both hydroxy-α-sanshool (**2**) and tetrahydrobungeanool (**16**) proved to be potent and selective CB2 antagonists, with the latter exhibiting greater potency and selectivity. Hydroxy-β-sanshool (**5**), on the other hand, was the most potent CB1 antagonist of the alkamides screened. Isobungeanool (**17**) and δ-sanshool (**10**) proved to be the weakest CB2 antagonists. Since the goal was to identify a dual CB1 antagonist/CB2 agonist, the extremely poor activity of **17** against CB1 precluded this compound from further investigation. The all-*trans* pentaene **10**, on the other hand, was more promising. Further investigation confirmed that **10** was an effective CB2 agonist (EC_{50} of 41.7 nM), in addition to being an acceptable CB1 antagonist (IC_{50} of 100 nM). Accordingly, the authors proposed that alkamide **10** is a potential candidate for the treatment of type-I diabetes. Additionally, very recent studies indicate that administration of *Zanthoxylum*-derived alkamides, specifically **2**, **5**, and **8**, can ameliorate pancreatic dysfunction, protein metabolism disorder, and glycolipid metabolism in streptozotocin (SZT)-induced diabetic rats.[82–84]

Table 4. Inhibition (antagonism) activity of *Z. bungeanum* alkamides on CB1 and CB2[20]

Alkamide	CB1 IC_{50} (nM)	CB2 IC_{50} (nM)	CB1/CB2
hydroxy-α-sanshool (**2**)	7400	59	1254
dihydroxy-α-sanshool (**3**)	41	168	0.24
hydroxy-β-sanshool (**5**)	9	131	0.07
hydroxy-γ-sanshool (**8**)	184	197	0.9
δ-sanshool (**10**)	100	664	0.15
hydroxy-δ-sanshool (**11**)	1392	224	6.2
tetrahydrobungeanool (**16**)	4600	2	2556
isobungeanool (**17**)	2400	9100	0.26

4.7. Other activities

Several studies have been conducted on the antiparasitic activity of *Zanthoxylum* extracts, including the antileishmanial effect of extracts from *Z. rhoifolium* and potent activity against the malaria parasite exhibited by extracts from *Z. heitzii*.[85,86] Although very little is currently known, early investigations indicate that natural products other than the alkamide components in these extracts are responsible for the observed antiparasitic activities.[85] That being said, Navarrete and Hong identified α-sanshool (**1**) as the sole anthelmintic agent from a *Z. liemannianum* stem bark extract that was able to decrease the count of intestinal nematode eggs in naturally infected sheep.[87] While low toxicity is generally associated with *Zanthoxylum* alkamides, the authors noted that intraperitoneal injection of **1** induced tonic-clonic seizures in mice.

Among several other uses, *Zanthoxylum* decoctions have been employed worldwide in traditional medicines as anti-inflammatory agents. Despite this rich history, detailed clinical investigations on the anti-inflammatory capabilities of *Zanthoxylum*-derived alkamides are severely lacking. Last year, however, Wang and co-workers demonstrated that several alkamides isolated from *Z. bungeanum* could inhibit a process associated with inflammation, namely the production of nitric oxide (NO) in LPS-stimulated RAW264.7 cells.[37] Of the alkamides tested, tetrahydrobungeanool (**16**, IC_{50} 27.1 μM), unnamed diketone **33** (IC_{50} 39.4 μM), zanthoamide A (**42**, IC_{50} 48.7 μM), and ZP-amide A (**27**, IC_{50} 49.8 μM) were the most potent inhibitors of NO production. It is worth noting that none of the twelve *Zanthoxylum* alkamides that were tested demonstrated any detectable cytotoxicity against the murine macrophages (up to 50.0 μM), nor against human colon cancer (HCT116) or human prostate cancer (PC-3) cells.

In their studies on the oral sensations exhibited by several isolated *Zanthoxylum* alkamides, Bader and co-workers also investigated the impact on salivation.[18] Interestingly, the structural differences that evoked changes in oral sensation also expressed differences in the induction of salivation. For example, the *cis*-olefin-containing alkamide hydroxy-α-sanshool (**2**), which demonstrated tingling/paresthetic activity also induced massive salivation. The tongue-numbing all-*trans* congener hydroxy-β-sanshool (**5**), on the other hand, did not show any significant impact on saliva production.

In addition to human health issues, the sanshools have recently gone under investigation for agricultural purposes. For example, Tang and co-workers reported that a collection of sanshools and hydroxy-sanshools (**1-9**, **12**) extracted from *Z. bungeanum* pericarps was able to protect rice seedlings from the damaging effects of metolachlor treatment.[23] Combination treatment of this sanshool mixture with the pesticide led to growth rates, shoot height, root length and fresh biomass close to that of non-treated controls, versus significantly lower values in all of these fields for seedlings treated with metolachlor alone. Accordingly, it is possible that *Zanthoxylum*-derived alkamides could serve as protective agents in combination with agricultural herbicides.

5. Conclusions and perspectives

As discussed in this review, the polyunsaturated fatty acid amides produced by the *Zanthoxylum* genus of trees have intrigued and benefitted human societies throughout the world. With the advancement of science, these *Zanthoxylum*-derived alkamides have also advanced from simply culinary curiosities and components of folk remedies to legitimate clinical candidates and unique molecular tools for modern biology. Current studies adequately demonstrate that isomerization of the olefin stereochemistry in these alkamides (e.g. **2** to **5**) can dramatically alter both the degree and specific nature of the observed biological activities, thus emphasizing the importance of employing diastereomerically-pure

compounds for all biological evaluations. While most studies rely on *Zanthoxylum* alkamides extracted and purified from natural sources, advances in organic synthesis now allow relatively rapid access to multi-gram quantities of some of the most intriguing members of this family of natural products as single polyolefin isomers. The *Zanthoxylum* alkamides still offer an attractive opportunity for further advances in organic synthesis and analysis. For example, five members of this family, namely lanyuamide IV (**23**), lanyuamide VI (**25**), ZP-amide F (**32**), timuramide A (**38**), and timuramide B (**39**), possess at least one chirality centre and optical activity. Nevertheless, the absolute configurations of these compounds remain unassigned. This is not without significant efforts. As mentioned in Section 2.6, Devkota and co-workers unsuccessfully attempted to determine the absolute stereochemistry of **38** by selectively reducing the unusual endo-peroxide moiety and converting the resulting diol into the corresponding bis-Mosher's ester.[33] The authors correctly note that such unsaturated diols represent a blind spot in current methods for configurational analysis. Accordingly, *de novo* total synthesis is the only strategy currently available to determine the absolute stereochemistry of the chiral *Zanthoxylum* alkamides. Whereas asymmetric syntheses of alcohols **23**, **25**, and **32** are certainly within reach using modern methods, access to the unique endo-peroxides **38** and **39** in enantiomerically-pure form from strictly synthetic means will require new methodological advances.

Organic synthesis also has a lot to offer to the biomedical evaluation of *Zanthoxylum* alkamides. Current studies have focused primarily on the compounds that are easiest to isolate and purify from natural sources in significant quantities. Accordingly, the biological activities of the less prevalent members, such as the lanyuamides, qinbunamides, and zanthoamides, are not properly explored. Chemical synthesis is required to fill in this gap by providing access to larger quantities of these underrepresented alkamides. Moreover, the collection of synthetic analogues available for structure-activity relationship studies (see Section 3.6) is still relatively limited and lacking in compounds that recapitulate the desired biological activities without suffering the same sensitivities to light, heat, and hydroxylated solvents. Success in such an endeavour would provide unique small molecule tools to help us better understand the intricacies of a variety of biological processes including digestion, diabetes, tumour growth, and nerve growth and signalling.

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7. Notes and References

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