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Review

Guineensine: Isolation, Synthesis, and Biological Activity

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Abstract: The genus *Piper* is the largest among plants of the Piperaceae family. Phytochemical studies on various *piper* species indicate the presence of bioactive compounds, with alkamides being among the most prominent. Piperine is well studied, and is usually found in abundance in most species. Guineensine is an alkamide that merits particular interest and, until now, has received less scientific attention. Therefore, in the present review, we discuss guineensine's isolation, synthesis, and pharmacological activity. Data were collected from 1974 to 2024. Databases including PubMed, Google Scholar, and Science Direct were used to retrieved information using the following keywords: guineensine, isolation, synthesis, biological activity, alkamides, *Piper* spp., pepper, and SAR. Guineensine is obtained using various isolation methods. However, it yields low amounts; therefore, its synthesis is important. In addition, guineensine exerts many biological activities. Its potential is connected to its terminal benzodioxolyl and isobutyamide groups and to the length of its unsaturated carbon chain of twelve atoms. Findings of the studies presented in this review provide substantiation regarding the scientific interest in guineensine. Isolation procedures present advantages and disadvantages, and the methods of its synthesis are efficient. Its biological activity seems promising and further studies may lead to the development of new therapeutic agents.

Keywords: alkamides; extraction; natural product; piperamide; *Piper* spp.; pepper; SAR

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1. Introduction

1.1. Piper Species and Uses in Traditional Medicine

Piperaceae is a family of plants containing unique natural components [1]. The best-known members of the family, distributed in tropical and subtropical regions, are *Piper* and *Peperomia* [2,3]. The genus *Piper* grows worldwide (America, South Asia, South Pacific, and Africa) with more than 700 species. Plants of the genus *Piper* in India, Southeast Asia, and Africa are economically important due to their use as spices and traditional medicines [4]. *Piper* is the largest genus in the Piperaceae family and has a rich ethnobotanical and ethnopharmacological history [4].

Piper spp. seeds, leaves, and roots are used in traditional medicine against a variety of ailments, including fever, coughs, colds, headaches, rheumatism, boils, and gastrointestinal disorders. They are also useful for the treatment of respiratory diseases and have gastrointestinal and liver protective properties [1]. According to Salehi et al. [3], 106 species of the genus *Piper* were discussed for their medicinal properties and are used traditionally in various tropical and subtropical regions.

The *Piper* family has been used in traditional medicine systems for thousands of years including Traditional Chinese Medicine, Ayurveda in India, and traditional medicine in Latin America and the West Indies. For example, leaves of *P. abbreviatum* are used externally by Filipinos to treat splenomegaly, and its fruits are used to treat coughs and colds [5]. *P. aduncum* is traditionally used to treat stomach pain, vaginitis, influenza, rheumatism, cough, fever, and general infections as well as coughs and colds [6,7]. The roots of *P. boehmeriifolium* are used in the Ayurvedic system of Indian medicine as a laxative, anthelmintic, and wind remedy [8]. In China, many *Piper* spp., such as *P. boehmeriifolium, P. hongkongense*, etc, are used as anti-platelet and anti-coagulant agents [9]. *P. cubeba* is listed as one of the most important plants for cancer treatment in Moroccan and Chinese traditional medicine [10]. The roots of *P. nigrum* are used by Thai people in the form of ghee, powder, enemas, and balms to treat abdominal tumours, bloating, adenitis, cancer, cholera, colds, colic, kidney stones, asthma, and headaches [11].

1.2. Alkaloids and Alkamides in Piper Species

Studies on the phytochemical profile of *Piper* spp. indicate the presence of a variety of bioactive compounds such as flavonoids and other phenolic compounds, tannins, saponins, glycosides, terpenoids, amides and alkaloids [1,2,4,12,13]. Most apparent is the presence of amide alkaloids, also called piperamides, and alkamides for which extensive research is dedicated to their biological activity [3,14–17]. Alkalmides are natural derived compounds, and products of the secondary metabolism of several medicinal plants of the Asteraceae, Solanaceae, Rutaceae, and Piperaceae families [18,19]. The alkaloid piperine is the representative compound of the genus *Piper*, and it is found in remarkable abundance in *P. nigrum* and *P. longum* fruits [20,21]. Guineensine, although found in minor quantity with respect to piperine, is an alkamide that has gained popularity among the scientific community, as is discussed in the following paragraphs.

1.3. Guineensine and Structural Classification of Fatty Acid-Derived Piperamides

Guineensine (2*E*, 4*E*, 12*E*)-13-(benzo [*d*][1,3]dioxol-5-yl)-*N*-isobutyltrideca-2,4,12-trienamide) **1** was first isolated in 1974 by Okogun et al. from fruits of *P. guineense*, collected in the western parts of Nigeria [22]. Its chemical structure was elucidated by Okwute et al. and found to contain three distinct units (Figure 1) [23]: a terminal benzodioxolyl group (from this point called as tail group), a terminal isobutylamide group (from now on called as head group), and a fatty acid-derived linker. The latter is an unsaturated twelve-carbon chain connecting the aforementioned terminal groups. No *cis-trans* isomeric forms of guineensine were isolated from *Piper* species [24]. Guineensine is a bioactive member of the piperamides family.

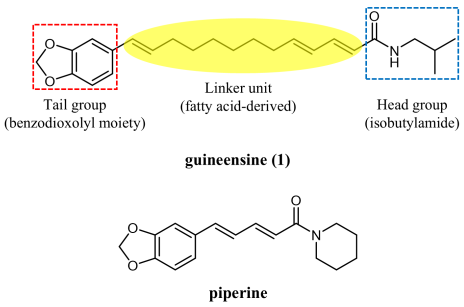
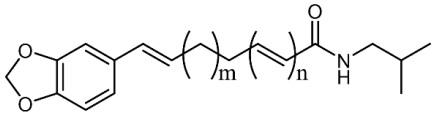


Figure 1. The structure of guineensine **1**, divided in three core parts (**up**) and the structure of the most common piperamide; piperine (**down**).

The amide alkaloids found in *Piper* species are commonly known as piperamides [25]. Among them, piperine is the most abundant pungent alkamide and is widely studied for its biological properties. [26,27]. On the other hand, guineensine belongs to a special family of fatty acid (FA)-derived piperamides sharing common structural features, along with differences with piperine (Figure 1). Apart from the obviously similar skeleton, they are both characterized by a benzodioxolyl terminal group. However, guineensine has a more complex unsaturated carbon chain with isolated double carbon bonds and an isobutyl group attached to the amidic nitrogen, instead of the conjugated carbon chain and the piperidine terminal moiety of the piperine.

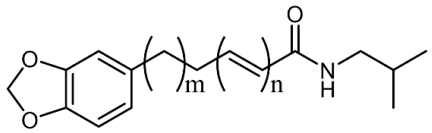
The FA piperamide family, structurally analogue to guineensine **1**, includes retrofractamides A **2** [28], B **3** (known also as pipercide) [29], C **4** [28] and D **5** [30], brachystamides B **6** [31] and D **7** [30], and piperchabamide D **8** [32] and pipgulzarine **9** [33] (Figure 2). Products **1**–**9** have a styryl double bond attached to the aryl group.



	m	n	Product
1	5	2	guineensine
2	1	2	retrofractamide A
3	3	2	retrofractamide B
4	3	1	retrofractamide C
5	2	2	retrofractamide D
6	7	2	brachystamide B
7	8	2	brachystamide D
8	5	1	piperchabamide
9	6	1	pipgulzarine

Figure 2. FA–piperamides structurally analogues to guineensine.

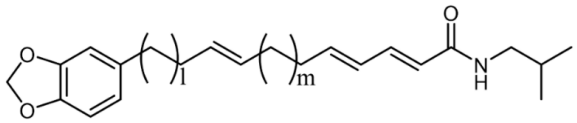
Broadly similar to the previous products are dihydropipercide **10** [34], chingchengamide A **11** [35], dihydropiperlonguminine **12** [36], pipercallosine **13** [37], and brachystamide A **14** [31] (Figure 3). These products lack the double bond adjacent to the tail aromatic group.



	m	n	Product
10	5	2	dihydropipercide
11	1	2	chingchengamide A
12	1	1	dihydropiperlongumine
13	3	2	pipericallosine
14	9	2	brachystamide A

Figure 3. FA–piperamides lacking the carbon–carbon double bond next to benzodioxolyl group.

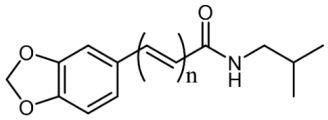
A number of isolated piperamides incorporate a methylene group between the benzodioxolyl group and the isolated C=C. Laetispicine **15** [38], ridleyamide **16** [39], and brachystamide C **17** [27] belong to this subgroup of FA piperamides (Figure 4).



	l	m	Product
15	**0**	5	laetispicine
16	**1**	5	ridleyamide
17	**0**	6	brachystamide C

Figure 4. FA–piperamides with one or more methylene units next to benzodioxolyl group.

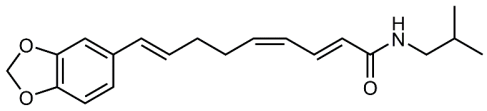
Products mentioned in this list contain one or more isolated double bonds separated by one or more saturated carbon atoms. However, it is worth noting that the natural products, fagaramide **18** [40] and piperlonguminine **19** [41], with a conjugated chain of one or two double bonds, respectively, are closer to the structure of piperine (Figure 5).



	n	Product
18	**0**	fagaramide
19	**1**	piperlonguminine

Figure 5. FA–piperamides bearing conjugated linker group.

Although the FA piperamide family is dominated by all-*E* carbon double bonds, the natural products piperlongumamide E **20** [40] and (3*Z*, 5*Z*)-*N*-isobutyl-8-(3′,4′-methylenedioxyphenyl)-heptadienamide **21** [42] contain one or more C=C with *Z* configuration (Figure 6).



piperlongumamide E (20)

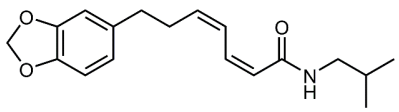


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Figure 6. FA–piperamides with *Z*-double bonds.

The above list is limited to natural products isolated from *Piper* species. More piperamides obtained by organic synthesis exist and some of them will be discussed later.

Guineensine was isolated originally from *P. guineense* from which it owes its name [22]. However, it is present in the dietary pepper species *P. nigrum* [43] and *P. longum* [44], used also in traditional medicine, as well as in the lesser-known *P. submultinerve* [45], *P. attenuatum* [46], *P. brachystachyum* [44], *P. hancei* [46], *P. retrofractum* [47], and others. As with most piperamides (excluding piperine), guineensine has not been widely studied for its biological activities. It is known for its endocannabinoid uptake inhibition and larvicidal/insecticidal properties, but additionally, it was found to exhibit other pharmacological activities, such as its antiviral, anti-inflammatory, and antiplasmodial abilities, and its inhibition of cholinesterases. The aim of this review is to critically examine the extraction and isolation techniques for compound **1**, to describe the available total syntheses, and to explore the reported biological activities.

2. Isolation Methods of Guineensine from *Piper* Species

Guineensine is collected from *Piper* spp. using different extraction procedures and several system solvents, as presented in Table 1. Fruits of the plants are used to obtain the extracts, from which guineensine is isolated via TLC and preparative HPLC methods [22,43,48–50]. Extraction methods are accompanied by strengths and weaknesses.

Table 1. Methods for isolation of guineensine.

No.	Extraction Method	Extraction Time	Yield/Extraction Solvent (s)	Pros	Cons	Reference
1	Soxhlet extraction	-	30 g/750 g of fresh fruits (petroleum and chloroform extracts) 20 g/750 g of fresh fruits (methanolextract)	Mechanically efficient technique, inexpensive	-	[22]
2	Conventional extraction with stirring	30 min	12.8% (acetone extract)	Good reproducibility, less time consuming	-	[51]
3	Maceration	48 h	11% (methanol extract)	Low cost	Time consuming	[43]
4	Percolation	-	11.7 mg/1 kg of fruits (n-hexane, chloroform, and methanol extracts)	-	High solvent consumption, time consuming	[48]
5	Ultrasound extraction	30 min	2.47 g (12.4%) (n-hexane extract)	Low cost, less time consuming, low solvent consumption	-	[49]
6	Accelerated solvent extraction (ASE)	-	209.7 mg/100 g of dry black pepper (ethyl acetate extract)	Good reproducibility, high yield	-	[50]

The first authors who isolated guineensine from *P. guineense* using dried, powdered fruits were Okogun et al. [22]. Soxhlet extraction with petroleum, chloroform, and methanol was used. Guineensine was isolated from petroleum and chloroform fractions and characterized by its IR and NMR spectrum [22].

In the study of Su and Horvat [51], an acetone extract was prepared by stirring from black pepper fruits. The extract was further fractionated on a column of silica gel. Thin layer chromatography followed where the yield of guineensine was 12.8%.

Fruits of *P. nigrum* were extracted using methanol for 48 h [43]. The organic solvent was evaporated under reduced pressure and the obtained extract was partitioned. Of the fractions prepared, the chloroform fraction was selected for further study. Guineensine, alongside pellitorine, pipercide, and retrofractamide A, were isolated using preparative chromatography. Guineensine yielded the highest amount, namely 2.1 mg.

In the study of Nicolussi et al. [48], the authors prepared extracts from *P. nigrum* fruits using different solvents. The chloroform extract was chosen to isolate guineensine using preparative HPLC. This fraction was purified, to finally yield 341.5 mg extract of unknown

purity. The authors obtained analytically pure guineensine (11.7 mg) by the further purification of crude guineensine (12.2 mg) using a Sephadex column.

In another study by Luca et al. [49], fruits of different *Piper* spp. were extracted with *n*-hexane in an ultrasound water bath. The organic solvent was evaporated under reduced pressure and extracts were then analyzed using liquid chromatography high-resolution tandem mass spectrometry (LC-HRMS/MS). Several piperamides were detected among which guineensine was found in most of the extracts. Its quantity varied from 11.58 ± 0.11 mg (piperine equivalents/g extract) in *P. guineense* sample to 55.52 ± 2.15 mg in *P. nigrum sample*.

Fruits from *P. nigrum* cultivated in Costa Rica were extracted with ethyl acetate by [50], using an accelerated solvent extraction (ASE) technique. Identification of the compounds presented in the extracts was performed on a UPLC QTOF-ESI-MS system and isolation of the selected alkamides was achieved using a preparative and semipreparative HPLC method. Among other piperamides, guineensine's' quantity ranged from 276.5 ± 3.6 and 421 ± 26 piperine equivalent/100 g dry sample. Interestingly, the yield obtained by the isolated compound was higher than those reported in the literature [48], reaching 209.7 mg/100 g dry black pepper.

3. Total Synthesis and Reactions

3.1. Total Synthesis

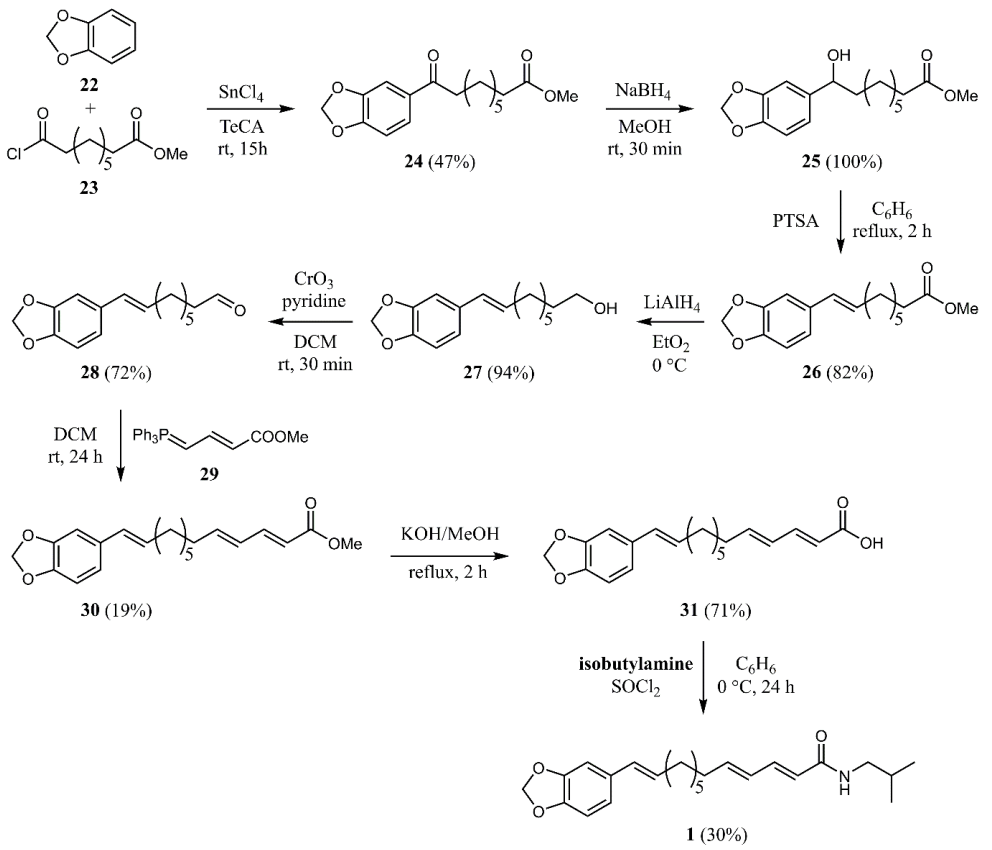
Since the isolation of guineensine by Okogun et al., four groups have presented a total synthesis of this natural product. The key steps for the multi-step preparation of **1** are the construction of the two unsaturated units: the styryl moiety and the diene moiety next to the isobutylamide terminal group. To some extent, in most cases, modified Wittig reactions [52] were employed to achieve the olefination steps.

3.1.1. The Okwute et al. Approach (1979/1984)

Okwute et al. developed a convenient route for the synthesis of guineensine (Scheme 1). The protocol was initially published in 1979 [53] and republished in 1984 in an easier-to-access literature source with more experimental details [23].

The acylation of 1,2-(methylenedioxy)benzene **22** with azelaic acid monomethyl ester chloride **23** gave the intermediate ester **24**, which subsequently reduced with $NaBH_4$ and dehydrated to afford the styryl ester **26**. Ester **26** was transformed to the corresponding aldehyde in two steps by $LiAlH_4$ reduction and then modified Collins oxidation [54] of the produced alcohol **27**. However, the authors did not provide a description of these modifications. Next, the *trans*-double bonds, along with the terminal carbonyl group were introduced by Wittig olefination. Finally, the acid **31**, which was obtained from ester **30** by base hydrolysis, reacted with excess isobutylamine in the presence of $SOCl_2$ to afford guineensine **1**. The final product was purified by preparative TLC.

This method uses mild conditions, commercially available reagents and reactants, and easy handling of the final and intermediate products. Moreover, no tedious purifications steps are needed making the protocol easy to replicate. However, it suffers by low overall yields, mainly due to low yields of the Wittig and amidation reaction steps.



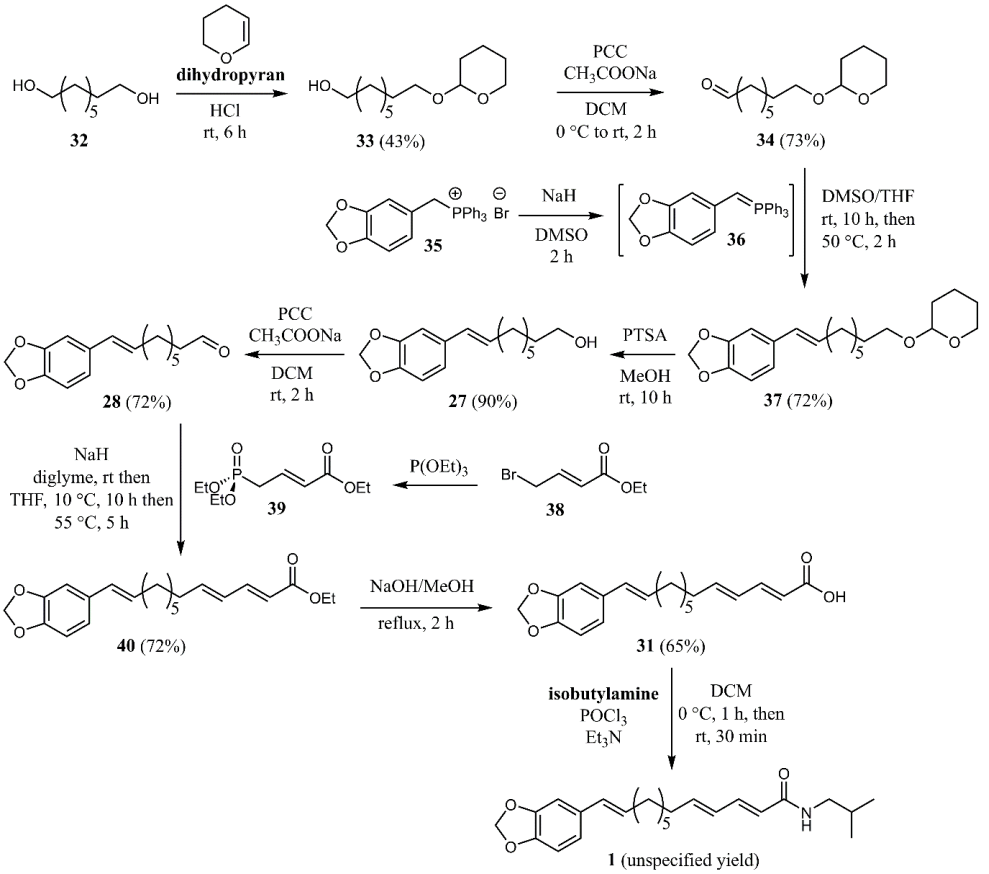
Scheme 1. Okwute et al.'s total synthesis of guineensine in 8 steps starting from 1,2-(methylenedioxy)benzene **22** (TeCA: 1,1,2,2-tetrachloroethane).

3.1.2. The Vig et al. Approach (1980)

One year later, Vig et al. reported an alternative route for the synthesis of guineensine, which is also based on Wittig reactions—two in this case—as key steps (Scheme 2) [55].

The authors utilized 1,8-octanediol **32** as the starting material. Diol **32** was partially protected with the tetrahydropyranyl (THP) group and oxidized to the corresponding aldehyde **34** to serve as one of the two Wittig components. Reaction with the triphenyl phosphorane derivative **36** resulted to the formation of the intermediate **37**, which contained the tail group of the target molecule **1**. In the next steps, the THP-protected alcohol **37** was deprotected using PTSA and oxidized to afford **28**, which gave a modified Wittig olefination with phosphonocrotonate reactant **39** to incorporate the diene moiety. The final two steps were in line with the previously reported method and included the hydrolysis of ester **40** and amidation of the carboxylic acid **31**, using $POCl_3$ this time, to finally form guineensine **1**. The final product was purified using column chromatography and crystallization.

The Vig methodology is as convenient and easy to perform as the previously reported method by Okwute et al., since both of them are performed in eight steps and require no difficult techniques or hard to obtain reagents. Typically, this protocol seems to be more efficient, although no yield for the final step is reported.



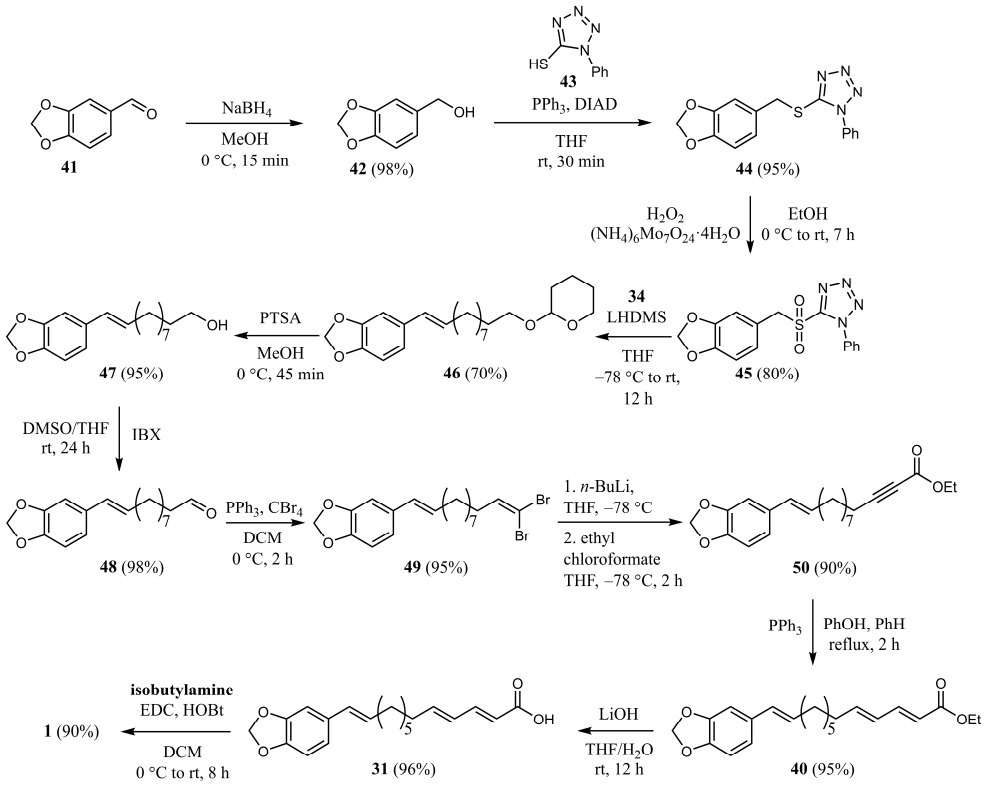
Scheme 2. Vig et al. total synthesis of guineensine in 8 steps starting from 1,2-(methylenedioxy)benzene (PCC: pyridinium chlorochromate).

3.1.3. The Shingala et al. Approach (2011)

Shingala et al. employed a Julia–Kocienski reaction for the construction of the styryl double bond. According to the authors, this was the key step to their synthetic route (Scheme 3) [56].

The intermediate sulfone **45** was prepared from the commercially available thiol **43** by a Mitsunobu reaction with the alcohol **42** followed by oxidation with ammonium heptamolybdate and H_2O_2. As with the Wittig reaction, Julia olefination allows you to select which alcohol will be converted into the sulfone. The other reactant would be used as an aldehyde. Shingala et al. abandoned the alternative option of using THP-protected decanediol-derived sulfone and piperonal early due to very low yields.

At this point, there is a controversial issue regarding the synthesis of product **46**. Bartholomäus et al., in their total synthesis publication [57], reported that they tried to prepare **46** using the Julia–Kocienski reaction, but without success due to poor *E*/*Z* selectivity (1:1.7), even though the yields were high. This inconsistency was attributed to the reaction conditions. Taking into account that the experimental protocols were not included in the initial publication, Bartholomäus et al. supposed that Shingala et al. actually followed the Barbier protocol. In this case, first the sulfone is mixed the aldehyde followed by the addition of the base, as opposed to the premetallation technique, where the sulfone is firstly mixed with the base and the addition of the aldehyde follows. This variation alters the selectivity of *E*/*Z*, often in favour of the *E*-alkene [58]. Whether these conditions ultimately lead to the product **46** with satisfactory *E*/*Z*-selectivity remains to be seen when a research team repeats the reaction under Barbier conditions.



Scheme 3. Shingala et al.'s total synthesis of guineensine in 11 steps starting from piperonal **41**. The published synthetic route starts from 3,4-dihydroxy benzaldehyde. (DIAD: diisopropyl azodicarboxylate; LHDMS: lithium bis(trimethylsilyl)amide); IBX: 2-iodobenzoic acid.)

Protected alcohol **46** was easily converted to the corresponding aldehyde **48** by acid deprotection of the hydroxyl group and oxidation using 2-iodobenzoic acid (IBX). The useful dienoate **40** was successfully accessed from **48** by an efficient approach. First, Corey–Fuchs alkynylation and carboxylation with *n*-BuLi and ethyl chloroformate of the intermediate dibromoalkene **49** provided alkynoate **50** [59]. Next, using the Rychnovsky variation in the Trost isomerization with PPh_3 and PhOH [60,61], ester **40** was obtained in excellent yield. Finally, **40** was converted to isobutylamide in two steps. The authors do not mention any technique for the purification of the final product.

Shingala and co-workers' methodology gives access to guineensine more efficiently than the previously reported methods but in more steps, employing an interesting synthetic approach.

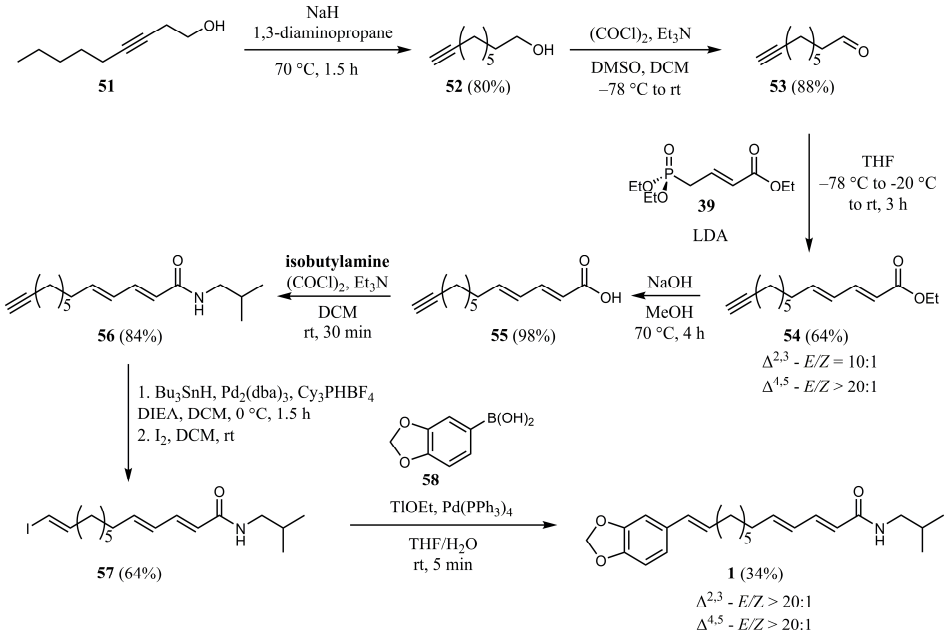
3.1.4. The Initial Bartholomäus et al. Approach (2019)

The most recent synthetic approaches to **1** were reported by Bartholomäus et al. in their impressive publication in 2019, along with the preparation of a library of guineensine analogues to perform structure–activity relationship studies on their endocannabinoid uptake inhibition properties [57].

As described earlier, their initial attempt to replicate the Julia–Kocienski olefination and base their total synthesis on this reaction was unsuccessful (see Section 3.1.3). Following this, they achieved the synthesis of **1** in seven steps, starting from the commercially available 3-nonyn-1-ol **51**. First, **51** was subjected to an alkyne zipper reaction [62] to obtain alcohol **52**, which contained the triple bond in the terminal carbons. After Swern oxidation with oxalyl chloride, the intermediate aldehyde **53** reacted with phosphonate **39** to give dienoate **54** via a Horner–Wadsworth–Emmons (HWE) olefination reaction. Dienoate

54 was easily transformed to the corresponding isobutylamide **56**, thus creating the linker and head group of the target molecule. Iodide **57** was then prepared in good yield by Pd-catalyzed hydrostannylation and treatment with iodine, and, finally, the desired molecule **1** was constructed by a Suzuki cross-coupling reaction with boronic acid **58**, using thallium (I) ethoxide as an additive (Scheme 4).

The diastereoisomeric ratio was well managed throughout the synthetic route to finally obtain the pure compound. However, in most reactions, the products had to be purified by one or more flash chromatographic runs. Moreover, the final product was provided in pure form only by preparative reverse-phase HPLC. This process was not acceptable by the authors from a total synthesis point of view, even if from a medicinal chemistry perspective it would be totally acceptable, since it ultimately leads to pure final products.



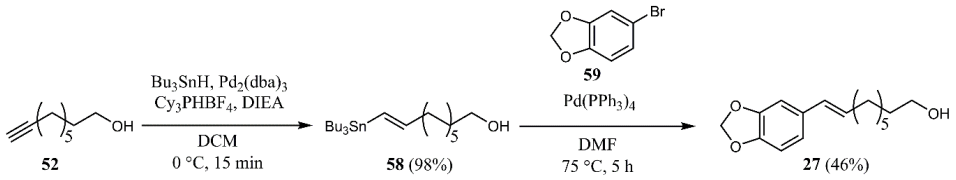
Scheme 4. Bartholomäus et al.'s first synthetic approach to guineensine. Route starts from 3-nonyn-1-ol and is accomplished in 7 steps.

3.1.5. The Revised Bartholomäus et al. Approach (2019)

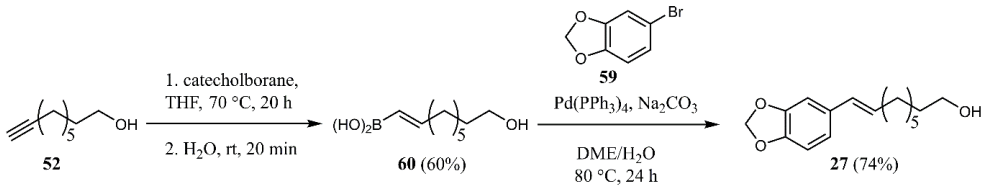
For the reasons described in the previous subsection, the same group decided to develop an alternative route, in order to access **1**, using only conventional purification techniques.

This approach involved the early incorporation of the benzodioxole moiety in contrast to the previous, in which this Suzuki coupling was the final step.

Alcohol **27** can be accessed either by a Stille coupling of the stannane intermediate **58** with the commercially available 4-bromo-1,2-(methylenedioxy)benzene **59** (Scheme 5) or by a Suzuki coupling of the boronic acid **60** with **59** (Scheme 6). Both alcohols **58** and **60** were prepared from 8-nonyn-1-ol **52**. According to the authors, the overall yields for the transformation of **52** to **27** were almost equal.



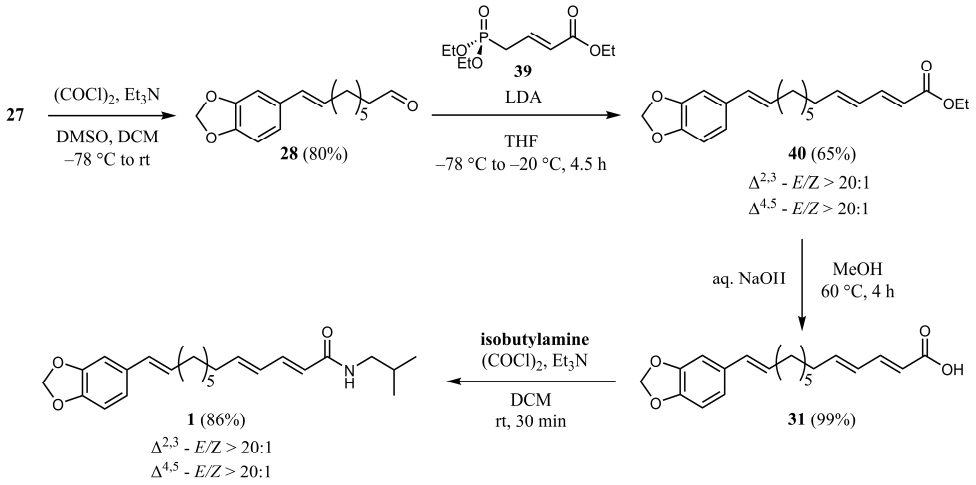
Scheme 5. Transformation of alcohol **52** to intermediate **27** via Stille coupling reaction.



Scheme 6. Transformation of alcohol **52** to intermediate **27** via Suzuki coupling reaction.

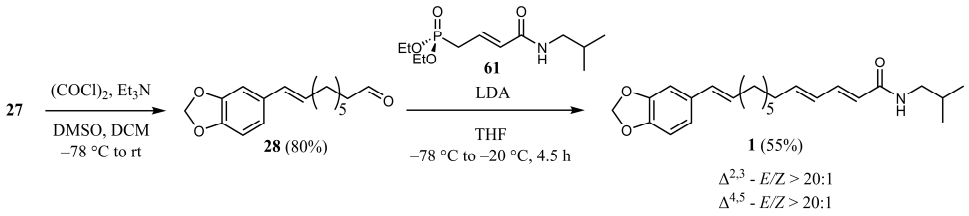
With the tail group-incorporating derivative **27** in hand, the authors proceeded to the synthesis of guineensine **1** following two equally efficient alternative routes.

Firstly, after Swern oxidation of **27** to the corresponding aldehyde, the latter would give an HWE olefination with phosphonate **39** to guineensic ethylester **40** followed by hydrolysis and amidation (Scheme 7).



Scheme 7. Synthesis of guineensine from alcohol **27** via HWE olefination of aldehyde **28** and phosphonate **39** and amidation of resulting molecule.

Alternatively, aldehyde **28** took part in an HWE olefination with amide phosphonate **61** to yield **1** in one less step (Scheme 8). This is the shortest and most efficient synthesis of this natural product reported so far. In most reactions, the products were purified by flash chromatography and sometimes with second chromatographic runs to improve the diastereoselectivity. However, no preparative HPLC was needed, thus making the methodology much more convenient. Some of these reactions were used to prepare guineensine analogues (see Section 3.2.2).



Scheme 8. Synthesis of guineensine from alcohol **27** via HWE olefination of aldehyde **28** and phosphonate amide **61**.

3.2. Reactions and Synthesis of Analogues

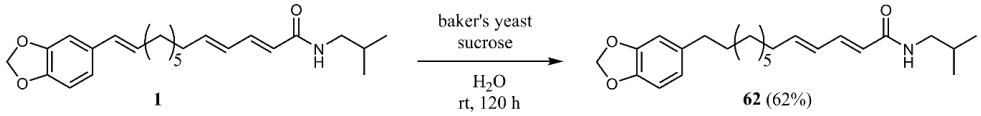
Only a few reports on the direct reactions or synthesis of guineensine analogues exist in the literature. Given that **1** belongs to a wide family of similar natural products, their synthesis could be considered as a synthesis of guineensine analogues. However, these are beyond the scope of this review. Examples include the preparations of pipercallosine [37], retrofractamide A [63,64], and dihydropipercide [64].

3.2.1. Direct Modifications

Probably due to the fact that guineensine is isolated in lower yields compared to some other piperamides, like piperine, not many known direct reactions of this natural product were reported. Even the saponification, which is a common reaction of piperine [65,66], was not used at all for the synthesis of **1** analogues, making the total synthesis of them the method of choice.

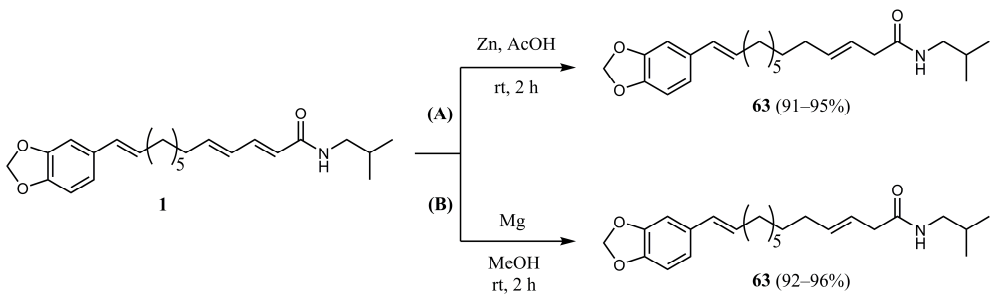
The only studied reactions are the partial saturations of the diene or the styryl moiety, with all three of them published by Das and co-workers [67–70].

They first described the reduction in the styryl double bond by baker's yeast (Scheme 9) [67]. Baker's yeast (*Saccharomyces cerevisiae*) is a useful biocatalyst for various transformations, including reduction biotransformations [68]. The reaction conditions were mild and the final product **1** was obtained in good yield (62%). Notably, brachystamide B successfully underwent the same reaction, whereas piperine and piperlonguminine did not. The reaction appears to proceed only with piperamides that have an isolated styryl double bond, rather than those with a conjugated double bond that is part of the dienamide group.



Scheme 9. Styryl double bond reduction by baker's yeast for preparation of 12,13-dihydroguineensine **62**.

Das and co-workers reported, as well, the conversion of guineensine **1** to the corresponding β, γ-unsaturated amide **63** using Zn [69] or Mg [70] (Scheme 10). The conversion yields were excellent, and the reactions took place in mild conditions especially in the case of the Mg-promoted reaction where the acidic conditions could be avoided, providing the opportunity to be applied in analogues bearing acid labile groups.



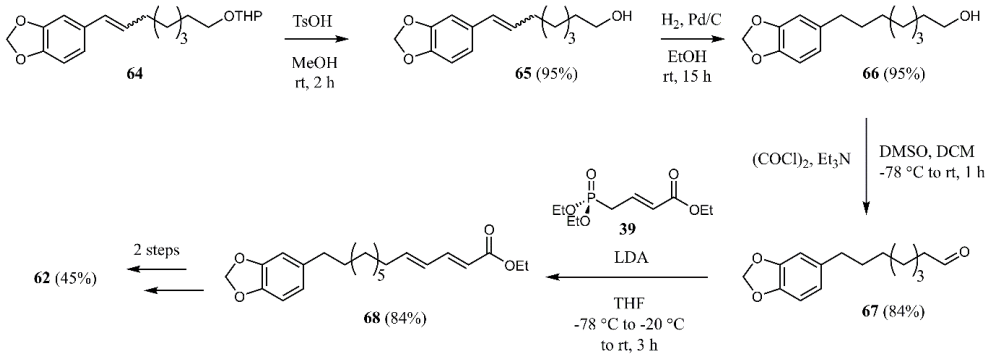
Scheme 10. Conversion of guineensine **1** to corresponding β, γ-unsaturated amide **63** using (**A**) Zn in acidic conditions and (**B**) Mg in methanol.

3.2.2. Synthesis of Analogues by Total Synthesis

Most of the limited reports that exist in the literature on structure–activity relationship studies of guineensine analogues include only nature-derived similar alkamides. Moreover, in earlier published work, for the total synthesis of **1,** no synthetic examples were included, even if the possibility of using these protocols for the preparation of analogues is mentioned.

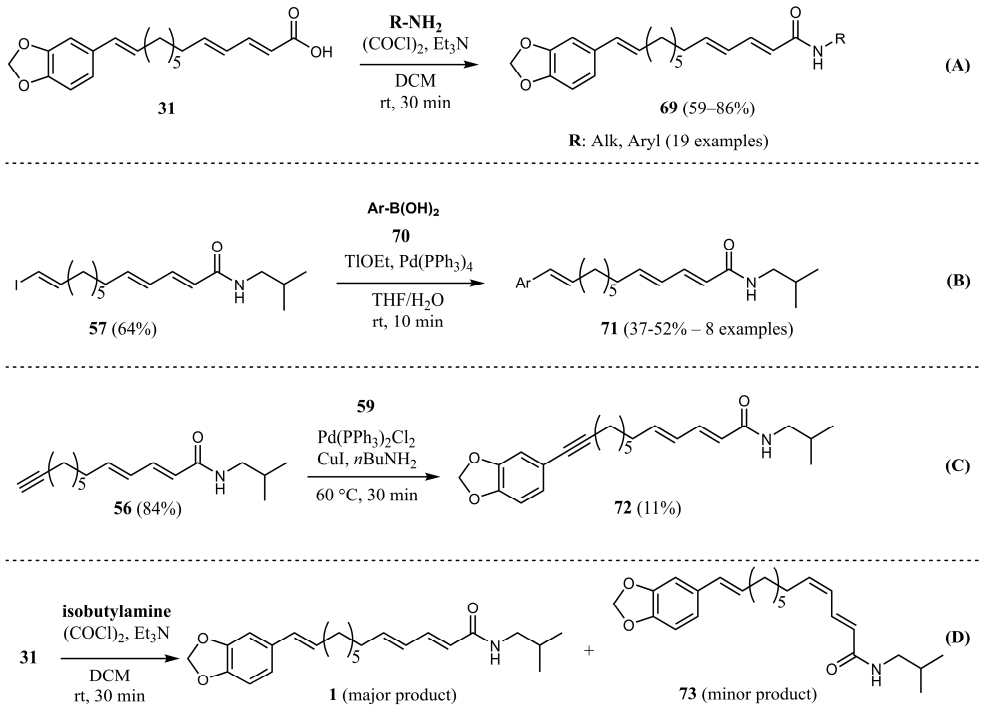
In the previously described publication by Bartholomäus et al., a library of guineensine analogues was prepared and studied, including modifications in critical parts of the molecules.

Dihydroguineensine **62** (see also Section 3.2.1—baker's yeast method) was prepared in six steps starting from the protected alcohol **64** via a six-step route using previously described reactions (Scheme 11) [57]. Moreover, **64** was used as a mixture of *cis-trans* isomers as it is the resulting product from the replication of the Julia–Kocienski reaction described in Section 3.1.4.



Scheme 11. Preparation of **62** starting from mixture of protected alcohols **64.**

Four additional types of guineensine analogues were also prepared in this work. A library of amides (**69**) was accessed by simple amidation reactions using various aliphatic or aromatic amines (Scheme 12A). Derivatives (**71**) having alternative aromatic units at the tail group of the molecule were prepared introducing these units using their boronic acids (**70**) in Suzuki cross-coupling reactions (Scheme 12B). Sonogashira coupling between alkyne **56** and bromide **59** afforded analogue **72** (Scheme 12C) occurred, while the guineensine isomer **73** was isolated as a minor side product from the amidation reaction of guineensic acid **31** with isobutylamine (Scheme 12D).



Scheme 12. Preparations toward library of guineensine analogues with modifications on (**A**) amide group, (**B**) aryl group and (**C**,**D**) linker chain.

4. Biological Activities

4.1. Inhibition of Endogenous Cannabinoids and Anti-Inflammatory Activity

The endocannabinoid system (ECS) is a cell signalling network that encompasses chemical signalling molecules called endocannabinoids (ECs), receptors and enzymes, involved in the synthesis and degradation of ECS. These include monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH). Several types of endocannabinoids receptors were described; nevertheless, the most discussed are CB1 and CB2. They are mostly expressed in the immune tissues as far as CB2 and the central nervous system concerning CB1 receptor [71]. Upon stimulation by endogenous cannabinoids, many signalling pathways are activated, giving rise to different pharmacological effects responsible for maintaining homeostasis and for regulating pathophysiology of inflammation and pain. The best discussed ECs are arachidonylethanolamide or anandamine (AEA) and arachidonylglycerol (2-AG), biosynthetic products of arachidonic acid. Both compounds are hydrophobic, and it was discussed that their penetration through the cell membrane occurs via membrane transporter proteins [72–74]. Specifically, for anandamine, a FAAH-like anandamine transporter [FLAT] was described [75]. According to this study, FLAT is expressed in brain and liver cells of rats and has low binding activity for AEA. The overexpression of the protein resulted in an accumulation of AEA to the extracellular space, without hindering other molecules entering the cell membrane. However, whether FLAT is finally a protein carrier for AEA was doubted by Leung et al. [76]. Authors found no protein expression to the peripheral nervous system of mice models, while similar results were also obtained after Western blot analysis.

ECs' activity is short due to their degradation because of the presence of hydrolytic enzymes or by their reuptake by the postsynaptic neuron. Since several disease conditions are attributed to low levels of ECs, strategies to increase their concentration in synaptic cells were examined. Some inhibitors of EC reuptake were proposed, among which natural products feature prominently on the list [48,77]. In particular, *N*-isobutylamide

guineensine was reported to induce cannabinomimetic effects related to pain [48,78]. However, central nervous system side effects, after the direct activation of cannabinoid receptors, occur often. Therefore, targeting degradation enzymes like FAAH and MAGL, inhibiting EC reuptake or alternatively, transport carriers like FLAT, are approaches under study. Such an approach was followed by Tou et al. [79] who suggested guineensine as a candidate compound to target FLAT and control neuropathic pain. Authors compared the protein bound efficacy of guineensine and retrofractamide A, an amide isolated from *Piper* spp., against FLAT. The results were compared to a control compound, namely FAAH-1 inhibitor (9*Z*)-1-(5-pyridin-2-yl-1,3,4-oxadiazol-2-yl)octadec-9-en-1-one. The length of the hydrocarbon chain, number of carbon atoms, bond distance, and electropolocial state were parameters to consider for the inhibition of FLAT. The bioactivity of guineensine was comparable to the control compound and better than that of retrofractamide A. Guineensine formed hydrophobic bonds with FLAT in contrast to retrofractamide A and the control, which formed both hydrogen and hydrophobic bonds with the protein, a result attributed to the length of their hydrocarbon skeleton. In addition, the stability of the formed complexes (ligand compound and FLAT protein) was investigated with molecular docking simulation. Binding affinity of the two tested compounds was lower than that of the control. However, guineensine caused stronger vibrations to FLAT than the control compound and this was suggested as the possible mechanism of action against FLAT inhibition.

In the study of Nicolussi et al. [48], extracts of *P. nigrum* L. and *P. longum* L. were tested for their capacity to inhibit AEA and 2-AG reuptake. Cultures of U937 and HMC-1 cells were used for the assay and results showed that the extracts prevented AEA and 2-AG from re-entering the releasing neuron at a concentration of 25 μg/mL. Both extracts were characterized as strong reuptake inhibitors in comparison to the control compounds, OMDM-2 and UCM707. Inhibition activity was also evaluated for isolated compounds such as retrofractamide A and B, brachystamide B, and guineensine. All compounds were selective against AEA inhibition with guineensine showing the greatest inhibition at a concentration of 290 nM, greater than that of the control compounds. Authors also observed no interference between guineensine and FAAH, MAGL and α/β hydrolases domain containing (ABHD) 6/12, neither with FLAT nor FABP5 proteins, possibly suggesting the presence of another target molecule for guineensine that requires further investigation (Figure 7).

Central cannabinomimetic effects by indirect activation of CB1 receptor, namely the classic "tetrad" effects, were also observed after dosing guineensine in BALB/c mice in the range of concentrations between 2.5 and 10 mg/kg. The authors suggested a possible interaction of guineensine with dopamine or serotonin receptors. The overall estimation regarding structure–activity relationship relates the number of carbon atoms of the hydrocarbon skeleton of the tested compounds. Guineensine with 13 carbons in the alkyl chain showed great activity. Retrofractamides with shorter alkyl chain were inactive while brachystamine B, bearing more carbon atoms in the alkyl chain than retrofractamides and guineensine, was less effective (Figure 8).

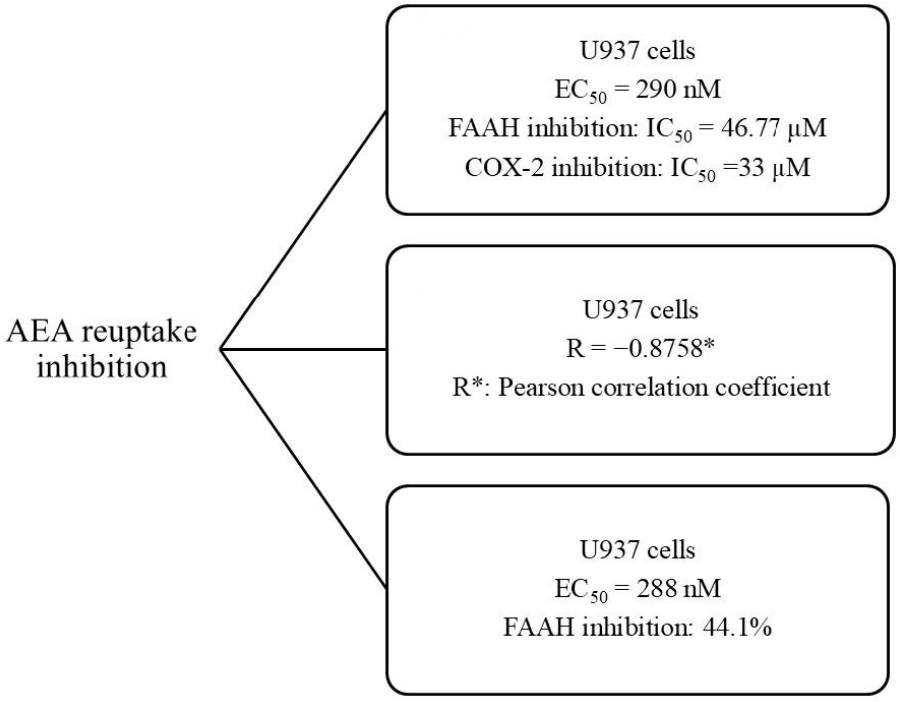


Figure 7. Activity of guineensine on AEA reuptake inhibition, as reported in studies of Nicolussi et al. [48] (top box); Luca et al. [49] (middle box); and Bartholomäus et al. [57] (bottom box).

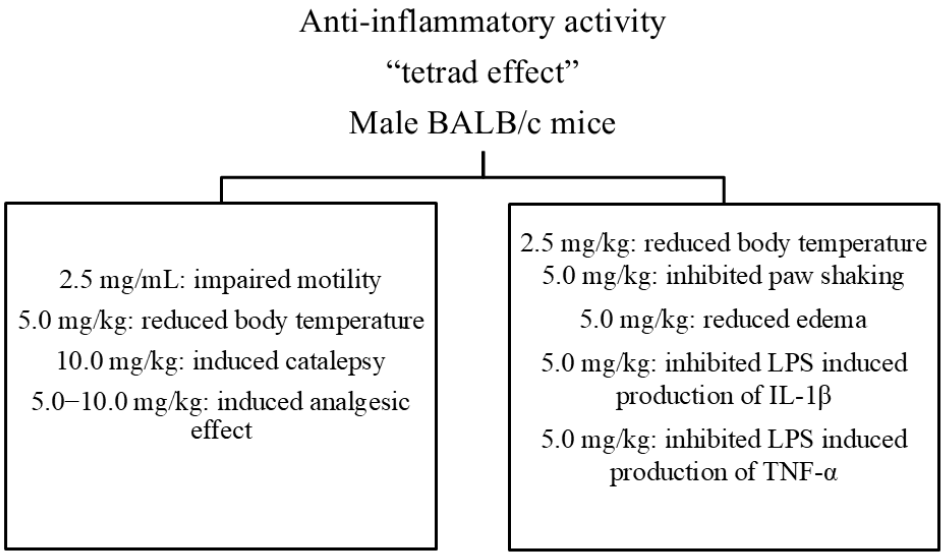


Figure 8. Anti-inflammatory activity of guineensine, as reported in studies of Nicolussi et al. [48] (left box); and Reynoso-Moreno et al. [78] (right box).

In another study by Luca et al. [49], the authors showed that extracts of black and green pepper strongly inhibited AEA reuptake on U937 cells, at a concentration of 25 μg/mL. Interestingly, the inhibition activity of the extracts was comparable to that of the reference compound WOBE437. The activity of MAGL and ABHD6/12 was not influenced; therefore, the extracts had no impact against 2-AG reuptake. The samples did not interfere with the CB1 receptor, and a moderate affinity was observed for the CB2 receptor. Considering the quantification results of the secondary metabolites presented in the

extracts, these two samples were among those that contained the highest quantity of piperine and high quantity of other piperamides, alongside a high quantity of guineensine. The authors also tested the inhibitory activity of pure compounds. According to their results, a Pearson correlation test strongly correlated guineensine as the responsible compound for such results, followed by piperolein B. Piperine showed a weak negative correlation. However, the authors supported that the total of alkamides contributes synergistic effects (Figure 7).

Endocannabinoids have immune suppressive, neuropathic, and anti-nociceptive effects and combat pain and inflammation via interaction with CB1 receptors, provided that their concentration remains high in the synaptic cleft. Reynoso-Moreno [78] observed a biphasic dose response effect of guineensine in BALB/c mice on reducing inflammatory paw shaking at 2.5 mg/kg, reducing edema, and improving pain threshold after 3 h of administration by intraperitoneal injection at a dose of 5 mg/kg (Figure 8). Furthermore, it prevented the production of IL-1β, TNFa, and partially IL-6 after LPS exposure, using the same dosage scheme. Extrapyramidal effects of guineensine were also observed, and were related to hypothermia and analgesia, mediated by the CB1 receptor, as well as catalepsy, which was the CB1 receptor's independent effect; therefore, binding guineensine to other receptors was suggested. The authors examined the ligand–receptor interactions by studying the dopamine transporter DAT and the 5HT2A and sigma receptors. They found a stronger interaction of guineensine with the last two receptors and they were suggested as potential targets for guineensine.

The progression of inflammation depends upon cell adhesion molecules. ICAM is a ligand for the integrin LFA-1 receptor and this complex triggers leucocytes to migrate toward the inflammation site, which consequently enhances excessive cell adhesion, leading to pathological conditions. The inhibition of this complex formation was studied by Lee et al. [80] with the use of metabolites isolated from *P. nigrum* and *P. longum*. The authors observed that compounds bearing a piperidine amide group, such as piperrolein B (IC_{50} = 13.4), showed a greater inhibition potential than those with isobutyl amide moiety, including guineensine.

The hydrocarbon chain linked to the benzodioxolyl moiety and the presence of the *N*-isobutyl group are the keys for guineensine's activity. Most studies relate its better (or not) activity to the length of the alkyl chain. An extensive structure–activity study (SAR) regarding guineensine's reuptake AEA inhibitory potential was performed by Bartholomäus et al. [57]. The authors synthesized various analogues by replacing either the *N*-isobutyl group or the benzodioxolyl moiety. Also, changes to the degree of unsaturation and configuration of the alkyl skeleton were examined. Generally, for analogues substituted to the *N*-isobutyl moiety, the lowest IC_{50} value was calculated at 0.096 μM for the compound with a methoxyphenylethyl group, which is close to that compound with a 3,4-dimethoxyphenylethyl moiety with an IC_{50} value of 0.097 μM. Maximum % inhibition reached 60%, while the first analogue inhibited stronger FAAH enzyme at a concentration of 1 μM. Both IC_{50} values were lower than those of guineensine (0.288 μM), which showed equal inhibitory activity and slightly better activity against FAAH (Figure 7). On the other hand, modification on the benzodioxolyl moiety led to less active analogues. The lowest IC_{50} value for these compounds was calculated at 0.849 μM for the compound bearing methoxy groups at meta position with a maximum inhibition activity of 50% and an FAAH inhibition of 30% at 10 μM. Substitution with methoxy group at para position drastically decreased the molecules' activity. Furthermore, the addition of indole moiety instead of the benzo[d][1,3]dioxolyl group induced great AEA inhibition reuptake (>60%), with an IC_{50} value at 1.66 μM. FAAH inhibition was also notable (2.4 μM expressed as IC_{50} value). Finally, the change from *E* to *Z* configuration (C4=C5) did not affect the inhibition potential of guineensine, as it occurred with the conversion of the double to triple bond

between C12 and C13, where its activity was reduced. These SAR examples are summarized in Figure 9 and depict the importance of the structural changes made to a compound, which may significantly improve or lessen its properties.

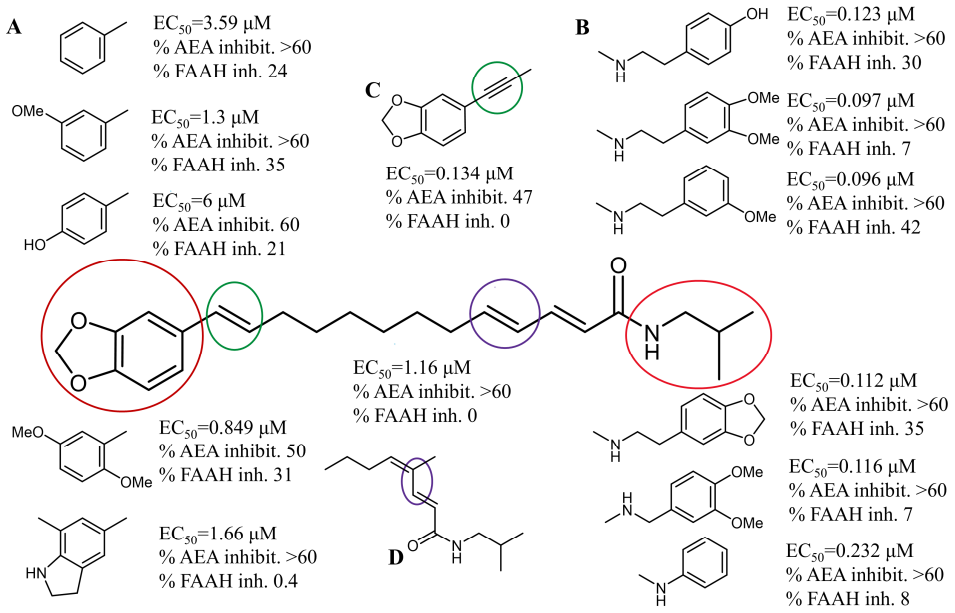


Figure 9. Synthesis of guineensine analogues and relative biological activity on AEA uptake inhibition, as reported by Bartholomäus et al. (**A**) Substitutions on benzodioxolyl moiety; (**B**) substitutions on terminal amide group. Only analogues with EC_{50} value lower than guineensine are depicted; (**C**) replacement of double bond with triple bond at position C12/C13; (**D**) changes in molecular configuration at C5/C4.

4.2. Cholinesterase Inhibition Activity

Apart from cannabinomimetic and anti-inflammatory effects, guineensine was evaluated against acetylcholinesterase (AChE), an enzyme responsible for the degradation of acetylcholine (ACh). ACh is the primary neurotransmitter of the parasympathetic nervous system responsible for many cognitive functions including memory. Alzheimer's disease is a degenerative disease related to low levels of ACh in the synaptic cleft. Generally, extracts of the *Piper* genus and their isolated aklamides, including guineensine, were tested for their anticholinesterase activity. Although the extracts showed a promising inhibition activity against AChE and BChE enzymes at 100 µg/mL, isolated compounds showed strong to moderate activity, dependent on their chemical structure. The methylenedioxyphenyl (MDP) ring seemed to enhance anticholinesterase activity, as in the case of piperine, which was the most potent compound against both AChE and BChE enzymes with IC_{50} values 63.16 ± 1.09 and 25.11 ± 0.21, respectively, whilst the length of the alkyl chain also played a critical role. For some compounds, the presence of the MDP moiety resulted in decreased activity due to long alkyl chains. On the other hand, guineensine showed comparable activity (IC_{50}= 74.37 ± 3.08 µg/mL) to piperine, without influencing BChE as piperine did [81]. In similar studies, guineensine showed only moderate binding activity for ACh, in comparison to other alkamides, such as pipercide [82]. Its IC_{50} value was calculated at 1.61 mM, while for piperine and pipercide, it was calculate at 0.32 mM and 0.61 mM, respectively. Structure–activity issues indicate that the longer hydrocarbon chain of guineensine with respect to piperine and pipercide is the reason for its lower binding affinity for AChE.

4.3. Infectious Diseases (Antiviral, Anti-Malarial Activity)

Fewer studies however have been conducted regarding other activities of guineensine. For example, only three studies have evaluated its activity against infectious diseases. In the study of Jiang et al. [83], the antiviral activity of various alkamides from *Piper longum* was evaluated against hepatitis B virus (HBV), a serious infection with chronic effects related to severe liver damage. In the study of Jiang et al. [83], a decreased secretion of HBsAg and HBeAg was discussed, which was attributed to the length of the hydrocarbon skeleton of the tested amide alkaloids and in particular for those compounds that bear more than eight carbon chains, including guineensine. The minimum inhibitory concentration was calculated for guineensine (<0.05 mM), while > 4.51 mM was the maximum IC_{50} value, evaluated for piperlonguminine. Despite the promising activity of guineensine as an antiviral agent, special attention should be given to its selectivity index (SI), as calculated at the same study. Generally, SI depicts the safety of a pharmaceutical substance. With respect to the other active compounds, the SI of guineensine was low (>3.0), indicating, possibly, toxicity related issues. Nobsathian et al. [45] examined guineensine activity against HIV-1, a disease that becomes more and more extensive. Significant antiviral activity was discussed for pellitorine and guineensine with IC_{50} values calculated at 166.1 μM and 651.9 μM, respectively. As in the study of [83], guineensine showed a low SI value, equal to 2.5.

Malaria is another infectious disease caused by a parasite. Two *Plasmodium falciparum* strains, which pose a great threat, were investigated by Yimtchui et al. [84] for their tolerance against compounds isolated from *P. guineense* seeds, including, among others, piperine, piperic acid, okalosine, and guineensine. These alkaloids showed great inhibition potential and their IC_{50} values ranged from 17.4 ± 0.7 to 23.9 ± 2.4 μg/mL regarding the Pf 3D7 strain, while for the Pf Dd2 strain, the IC_{50} values ranged from 14.6 ± 0.3 to 21.9 ±0.6 μg/mL. The best anti-malaria activity showed piperine and guineensine, with an SI higher than 50 and CC_{50} values higher than 1000 μg/mL. Their estimated IC_{50} values were close against the strain Pf 3D7, while a double fold increase showed piperine, compared to the IC_{50} value of guineensine regarding the strain Pf Dd2. For guineensine, the length of the aliphatic chain enhanced its activity.

4.4. Larvicidal–Insecticidal Activity

Natural derived products gain continuous popularity due to their effectiveness and less harmful consequences to the environment and other living organisms. Some studies refer to the insecticidal activity of guineensine. Although different organisms were tested, the results are consistent and indicate guineensine as a potential insecticidal agent [85–89]. Gbewonoyo et al. [87] made a considerable structure–activity correlation of guineensine alongside its insecticidal activity against adult female *Schistocerca gregaria*. The authors studied various amides such as pipercide, pellitorine, kalecide, and guineensine. The best activity was mentioned for kalecide both regarding knockdown and lethal activity, which was ranked after 48 h with an EC_{50} value equal to 0.08 μg/insect and 0.80 μg/insect, respectively. Guineensine presented a promising potential as authors observed a high and rapid knockdown activity, which continued for 48 h (EC_{50} = 0.50 μg/insect), with a less significant insect recovery in the first 24 h. Lethal activity was achieved at a concentration of 0.90 μg/insect (EC_{50} value) after 48 h. Insect knockdown was related to the low polarity of guineensine, in comparison to the other tested compounds, which enables its quick penetration through cell membrane. However, since this conclusion contradicts that made by Hatakoshi et al. [90], the authors agreed that apart from the lipophilicity of the molecule, other parameters should also be taken into consideration to explain its activity. Therefore, the structure of guineensine, which contains an MDP moiety, was discussed. Molecules that bear the MDP moiety seem to be more potent because this group not only

stabilizes the molecule but also interferes with mixed function oxidases, a family of enzymes that plays a critical role in insecticide detoxification. The major resistance of guineensine to insect recovery, with respect to the other compounds, was also attributed to its lower degree of unsaturation. In the studies of [85] and [43], the same mosquito species were examined for their susceptibility toward *Piper* alkamides. In the first case, adults of *C. pipiens pallens* and *A. aegypti* were used while in the second study, lavrae of these species, also including *A. togoi*, were examined. In both studies, *N*-isobutylamide alkaloids were effective; however, SAR studies indicated different behavioral effects of adults and larvae. The toxic effect of compounds bearing an isobutylamine moiety was more pronounced in adult mosquitoes than those compounds with a methylenedioxylphenyl moiety. On the contrary, larvae were sensitive to compounds with a methylenedioxyphenyl moiety. The importance of the isobutylamine moiety on the toxic potential of *Piper* compounds was also shown in the study of Park et al. [88], where the Diamondback moth lavrae, *Plutella xylostella*, was studied. *Piper nigrum* extracts and solvent fractions were first evaluated at concentrations of 2.5–5 mg/mL. Methanol, hexane, and chloroform extracts showed 100% toxicity while the water-soluble fraction was the least toxic with 3% effectiveness on the mortality of the Diamondback moth, *P. xylostella.* Pure compounds were also examined among which guineensine was the most toxic (LC_{50} equal to 0.013 mg/mL). On the other hand, piperine had no effect (LD_{50} > 50 mg/mL).

The crude extract of *P. guineense* alongside isolated compounds, for example guineensine, piperine, and propiverine, was evaluated for its insecticide, oviposition, and repellency against *Sitophilus zeamais* [89]. At 48 h post treatment, guineensine caused the highest % mortality rate (80.0 ± 7.07%) followed by piperine (60.0 ± 7.07%) and the crude extract (45.0 ± 9.48%). The mortality rate for all the tested compounds was time-dependent. At 96 h post treatment, 100% mortality was reached only by the known phosphorothionate insecticide, while guineensine also achieved a high mortality rate equal to 97.5 ± 2.5%, followed by piperine (82.5 ± 6.29%). Furthermore, the crude extract and piperine were more effective than guineensine against reduction in oviposition while the degree of repellency was the same. A synergistic activity of piperine was observed when blended with the other tested compounds. The mixture of piperine and guineensine was the most potent in terms of mortality (75 ± 10.41%) and oviposition (0.00 ± 0.00 eggs laid). Repellency activity of the mixture was also high (80%); however, the piperine/dihydrowisanine mixture (82.5%) was higher. A synergistic effect was also achieved in the study of [86] against *Callosobruchus chinensis* L. in a mixture of pipercide, dihydropipercide, and guineensine mixed at equal quantities or at a ratio of 75:5:20 (*w*/*w*), which caused the highest toxicity in comparison to the other treatments. The authors also tested mixtures of pipercide or dihydropipercide with guineensine and the pure compounds, i.e., pipercide, dihydropipercise, guineensine, pellitorine, and piperine. Piperine was the least toxic compound whereas guineensine showed a moderate activity, lower than that of the mixtures.

4.5. Other Activities

Cholesterol is a fat-like substance produced by the liver, essential for producing hormones and the correct digestion of food. Nevertheless, many lifestyle factors lead to high levels of cholesterol, increasing the risk of CVCs. Acyl-coenzyme A:cholesterol acyltransferase (ACAT) is an enzyme implicated in cholesterol homeostasis. It plays a critical role in the conversion of cholesterol to cholesteryl esters, which, in cases of accumulation, results in atherosclerosis. Several pharmacological approaches are used to combat atherosclerosis, with statins being the most prescribed medications. They are effective against high blood cholesterol levels; however, mild to severe side effects often occur. Studies have indicated natural derived products as promising alternatives to the current pharmaceutical treatment [91] and species of the genus *Piper* are among them [92]. To this end,

Lee et al. [93] isolated guineensine from *P. longum* fruits and tested its inhibitory activity against ACAT. Rat liver microsomes were used for their experiment and a natural compound named phenylpyropene A, isolated from *Penicillium griseofulvum* [94], was used as control. The results of this study showed that guineensine inhibits ACAT activity in a dose-dependent manner, with an IC_{50} value calculated at 3.12 μM, higher than that of phenylpyropene A (0.8 μM).

Gastric ulcers are common side effects of anti-inflammatory drug intake. Nevertheless, given their popularity due to their effectiveness against a variety of disorders, agents to reduce gastric ulcer disease are employed. Apart from synthetic drugs, such as proton-pump inhibitors, H2 antagonists, and others, natural products were tested for such potential [95]. One study by Soni et al. [96] examined the role of *P. attenuatum* ethanolic extracts against ulcers caused by aspirin administration. Ranitidine at 20 mg/kg was used as a control drug. Histopathological examination showed that after an induced aspirin gastric ulcer, the control medication had a mild effect against the lesion caused, while the tested extract used at 100 mg/kg and 200 mg/kg showed a moderate effect. A chemical analysis of the extracts showed the presence of different secondary metabolites and their binding affinity with specific receptors was examined via molecular docking studies. The best binding affinity score with protein 2XZB was predicted for cepharadione A (−8.7 kcal/mole), followed by δ-cadinene (−8.5 kcal/mole). In comparison to omeprazole (−7.3 kcal/mole), guineensine showed a lower binding affinity equal to −6.9 kcal/mole.

Cancer is another disease with increasing incident, and it is under continuous studies to understand its molecular mechanisms and to find new treatment approaches that are better tolerated and with fewer side effects than the existing ones. Several studies have shown that plants are a very important field for such studies [97,98] and plants of the genus *Piper* are among them [99]. In the study of Guo et al. [98], *P. longum* extracts were evaluated for their anticancer and anti-inflammatory activities. Extracts were sufficiently effective against both disorders, in contrast to isolated compounds, such as piperine, guineensine, and pipercide for which the same experiments were performed but presented variable effects. Guineensine was not effective as an anti-inflammatory agent; on the contrary, its anticancer activity was remarkable. HepG2, Hela, and SKOV-3 cells were tested; the best cytotoxic activity was exhibited by guineensine with IC_{50} values 14.90 ± 0.62; 17.13 ± 1.06 and 17.96 ± 0.39 μM, respectively. Interestingly, the calculated IC_{50} values were lower than those of the extracts and the pure compound piperine.

Monoamine oxidase (MAO) is an enzyme involved in the degradation of various amine neurotransmitters and hindering its activity is an attempt to improve mood disorders. Quineensine, piperlonguminine, and methylpiperate were evaluated for their inhibitory activity against MAO [100]. The control compounds were piperine and iproniazid, a non-selective MAO inhibitor. Among the tested compounds, methylpiperate showed the lowest IC_{50} value (3.6 μM) followed by guineensine (139.2 μM), while IC_{50} values for piperine and iproniazid were 12.3 μM and 19.7 μM, respectively. Piperlonguminine was not effective. Differences in the biological activity of the three tested compounds could be attributed to their chemical structure. For methylpiperate and piperlunguminine, both have the same length of alkyl chain, but they differ to their final end. Piperlunguminine has the same final group as guineensine and differs from ethylpiperate to the presence of a methoxy group at the final end. Guineensine showed a better bioactivity compared to piperlunguminine, a fact which aligns to previous statements that connect the length of its alkyl chain to its biological activity.

Vitiligo is a long term, not life-threatening disease that affects the production of melanocytes. The treatment goal is to diminish depigmentation of the skin, which has been achieved with various chemical preparations; nevertheless, for many of them, the results are not permanent and further development of the disease is ambiguous. Generally, the

stimulation of melanocytes is needed and *P. nigrum* extracts have shown to possess such potential [101]. In this study, extracts from *P. nigrum* and their isolated compounds showed the ability to stimulate melanocyte proliferation. More precisely, the chloroform extract was compared with piperine for its effect on melanocyte numbers. A greater activity for the extract was evaluated at 1 μM, suggesting the presence of other active compounds that act synergistically. Pure compounds, including piperine, guineensine, and pipericide, and synthetic compounds piperettine and piperlonguminine, all enhanced melanocyte proliferation with piperettine showing the greatest effect followed by piperine and guineensine. A great observation was that the methyleldioxyphenyl moiety, which is common for the above-mentioned compounds, might be the key to their activity since other compounds lacking this specific moiety were less or not active. On the other hand, protein kinase C activation was independent of the methyleldioxyphenyl moiety but strongly dependent upon the amide group, as in the case of piperine (piperidine amide) and guineensine (isobutylamide group) (Table 2).

Table 2. Biological activity of guineensine.

Biological Activity	Target	Concentration	Reference
		IC_{50}	
Cholinesterase inhibition activity	AChE and BChE	74.37 ± 3.08 μg/mL(AChE) No activity against BChE	[81]
	AChE	1.61 mM	[82]
Antiviral activity	HBV	<0.05 mM	[83]
	HIV-1	651.9 μM	[45]
Antiplasmodial activity	*Plasmodium falciparum* strain Pf 3D7	23.9 ± 2.4 μg/mL	
	Plasmodium falciparum strain Pf Dd2	14.6 ± 0.3 μg/mL	[84]
		ED_{50}	
Insecticidal activity	*Schistocerca gregaria*	0.50 μg/insect (at 48 h) knockdown activity	[88]
		0.90 μg/insect (48 h) lethal activity	
		LD_{50}	
	C. pipiens pallens (adults)	1.9 μg/♀ *	[85]
	A. aegypti (adults)	1.7 μg/♀	[85]
	C. pipiens pallens (larvae)	0.17 ppm	[43]
	A. aegypti (larvae)	0.89 ppm	[43]
	A. togoi (larvae)	0.75 ppm	[43]
Larvicidal activity	*Plutella xylostella*	0.013 mg/mL	[88]
	Sitophilus zeamais		[89]
	% Mortality at 48 h	80.0 ± 7.07	
	Oviposition	1.25 ± 0.96	
	Repellency	72.5%	
		IC_{50}	
Cholesterol homeostasis	ACAT	3.12 μM	[93]
Anticancer	HepG2	14.90 ± 0.62 μM	[99]
	Hela	17.13 ± 1.06 μM	
	SKOV-3	17.96 ± 0.39 μM	
MAO inhibition	MAO	139.2 μM	[100]

Anti-inflammatory activity	THP-1 (Inhibition of ICAM-1/LFA-1 complex)	>50	[80]
Protection against gastric ulcers	rats		[96]
	(binding affinity (kcal/mol) with protein 2XZB)	−6.9	

* ♀: *Aedes aegypti* females.

5. Discussion

Studies on the biological activity of piperamides were conducted by various researchers [26,102,103]. The most studied is piperine, in contrast to guineensine and other piperamides. However, the data presented in this review indicate that guineensine is a compound that merits further investigation. Guineensine is an alkamide found in various *Piper* species, alongside other piperamides. Its structure consists of three distinct units: a benzodioxolyl group (tail), an isobutylamide group (head), and an unsaturated carbon chain serving as the linker. It is known as an endocannabinoid uptake inhibitor but was also studied for its insecticidal and larvicidal activities. Despite its potential, guineensine remains underexplored in terms of its broader biological properties. Compared to the extensive research on piperine, guineensine can be considered as a "treasure molecule" awaiting deeper investigation. A major factor contributing to its underrepresentation is its low isolation yield. Isolation methods to obtain guineensine were developed and present several advantages and disadvantages. Conventional extraction with stirring and ultrasound extraction are the fastest methods (total time of 30 min). In relation to cost, Soxhlet extraction, maceration ,and ultrasound extraction are the most economical. Finally, accelerated solvent extraction gives higher yield than the other methods (209.7 mg/100 g of dry black pepper ethyl acetate extract) [50]. Even though the authors in this latter study do not explain the reason(s) for the high yield obtained in their experiment, this could be attributed to the sample used, which was cultivated in Costa Rica, and/or the method applied to isolate guineensine.

Because of its low isolation yield, a more active involvement of synthetic chemistry to reach Gram-scale preparations will enable researchers to perform comprehensive studies of this promising natural product. So far, three research groups have described approaches to guineensine total synthesis. The major challenges were the construction of the isolated double bonds and the purification of the final product. The first was successfully overcome by Wittig, Julia–Kocienski, or HWE olefination reactions and the more recently published studies seem to be more efficient. The final product was isolated and purified by preparative TLC or preparative HPLC, which can be considered as not acceptable from a synthetic point of view. The more recent study by Bartolomäus et al. [57] describes a method using only flash chromatography for the purification, thus being more suitable for scale up.

Generally, guineensine is a molecule with promising biological activity, with significant interactions with the endocannabinoid system, interesting antiviral and insecticidal activity, and important activity against other less studied diseases (Table 2, Figures 7–10). The substitution of methylenedioxyphenyl or isobutyl moieties or changes to the length of the alkyl chain may considerably enhance or hinder its biological activity. Each moiety has a special contribution to its pharmacological effect (Figures 9 and 10). Most attention has been given to its activity on the endocannabinoid system and focuses on its potential as an AEA and/or 2-AG reuptake inhibitor in in vitro and in vivo systems. Though the mechanism by which endogenous ECs pass through the cell membrane is still unclear, some approaches were discussed, like the presence of FAAH and MAGL enzymes and

transporters such as FLAT. Nevertheless, the results of the studies presented here demonstrate that the pharmacological effect of guineensine was FLAT independent, but authors did not propose another mechanism, except from the study of [48] and [78], where authors showed interactions of guineensine with other receptors, including DAT, 5HT2A, sigma receptors, and serotonin receptors. Therefore, more studies should be conducted to exactly understand or confirm how guineensine interferes with EC reuptake. Given its activity to increase AEA concentration at the synaptic cleft, its anti-inflammatory potential and its activity against neuropathic pain were also evaluated, inducing significant results. To evaluate whether guineensine is worth further studying, comparisons with piperine (where available) suggest that guineensine's activity depends on the biological assay. For example, in the study of Luca et al. [49], piperine was less effective than guineensine as an AEA reuptake inhibitor. In other biological studies, piperine appears as a more effective and less toxic compound than guineensine [81]. On the other hand, guineensine was more efficacious against malaria as well as its insecticidal activity was more prominent [86,88,89]. Certainly, there is yet much work to do to evaluate its therapeutic use, toxicology, pharmacokinetics, and pharmacodynamics. For instance, experiments on more cell lines should be conducted to confirm and further elucidate its potential as an AEA reuptake inhibitor and its anticancer activity. More types of microorganisms should be tested, and other damaging pests should be examined. Importantly, regarding its insecticidal activity, studies ought to examine additionally its effect not only against beneficial insects but also consider possible soil pollution. Its toxicity, discussed in the studies of [45,83] and [84], presented a great variability, possibly due to the different organisms tested. Moreover, additional SAR studies can be conducted to extend the work of Bartholomäus et al. [57] and synergism between guineensine and other compounds (natural or synthetic ones) need to be performed to lead to less toxic and more active products. Clinical trials are also required to complete its pharmacological profile. In all cases, the biological activities as presented in the above studies are a stimulus for further research.

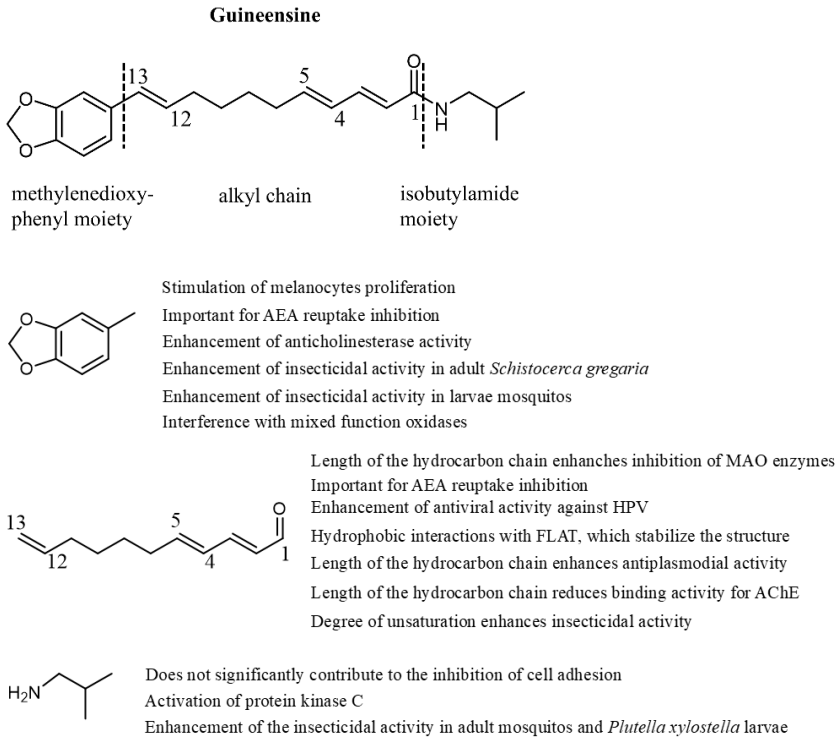


Figure 10. Relationship of chemical structure of guineensine with its biological activity.

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Abbreviations

2-AG	Arachidonylglycerol
5HT2A	Serotonin 2A receptor
ABHD	α/β hydrolase fold domain
ACAT	Acetyl-coenzyme A acetyltransferase
Ach	Acetylcholine
AChE	Acetylcholinesterase
AEA	Arachidonylethanolamide or anandamine
ASE	Accelerated solvent extraction
BChE	Butyrylcholinesterase
CC50	Cytotoxic concentration 50%
COX-2	Cycloxygenase 2
CVCs	Cardiovascular diseases
DAT	Dopamine transporter
DIAD	Diisopropyl azodicarboxylate
EC_{50}	Half maximal effective concentration
ECS	Endocannabinoid system
ECs	Endocannabinoids
E/*Z*	Entgegen/Zusammen
FAAH	Fatty acid amide hydrolase
FABP5	Fatty acid-binding protein
FLAT	FAAH-like anandamine transporter
HBeAg	Hepatitis B e-antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
Hela	Cervical cancer cells
HepG2	Human hepatocellular carcinoma cell line
HIV-1	Human Immunodeficiency Virus
HMC-1	Human mast cell line
HPLC	High-Performance Liquid Chromatography
HPLC-DAD-ESI-Q-TOF-MS	High-performance liquid chromatograph-diode array detector-electrospray ionization-quadrupole-time-of-flight-mass spectrometer
HWE	Horner–Wadsworth–Emmons
IBX	2-Iodobenzoic acid
IC_{50}	Half maximal inhibitory concentration
ICAM	Intercellular adhesion molecule-1
IL-1β	Interleukin-1 beta
IL-6	Interleukin 6
IR	Infrared Spectroscopy
LFA-1	Lymphocyte function-associated antigen 1
LPS	Lipopolysaccharide
LC-DAD	Liquid chromatograph-diode array detector
LC-HRMS/MS	Liquid chromatography high-resolution tandem mass spectrometry
LHDMS	Lithium bis(trimethylsilyl)amide)
NMR	Nuclear Magnetic Resonance
MAGL	Monoacylglycerol lipase
MAO	Monoamine oxidase
MDP	Methylenedioxyphenyl

PCC	Pyridinium chlorochromate
SAR	Structure–activity relationship
SI	Selectivity index
SKOV-3	Varian cancer cell line
THP	Tetrahydropyranyl
TeCA	1,1,2,2-Tetrachloroethane
TLC	Thin layer chromatography
TNFa	Tumour necrosis factor alpha
U-937	Human myeloid leukaemia cell line
UV	Ultraviolet radiation
UPLC-ESI-QTOF-HRMS	Electrospray ionization quadrupole time-of-flight high-resolution mass spectrometry
UPLC-MS	Ultra-performance liquid chromatography-mass spectrometry

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