A new dimeric imidazole alkaloid plasmid conjugation inhibitor from *Lepidium sativum*

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Abstract

Phytochemical investigation of the methanolic extract of *Lepidium sativum* seeds led to the isolation of a new compound, named 2-(3-(3-((1*H*-imidazol-2-yl)methyl)-5-methoxyphenoxy)benzyl)-1*H*-imidazole and given the trivial name Lepidine AK (**1**), along with three known compounds; Lepidine E (**2**), Lepidine B (**3**) and 2-(3-(2-((1*H*-imidazol-2-yl)methyl)-6-methoxyphenoxy)benzyl)-1*H*-imidazole (**4**). The structures were elucidated based on NMR spectroscopy, UV, IR and high-resolution electrospray ionization mass spectrometry. The isolated compounds were tested for bacterial conjugation inhibition. Lepidine AK (**1**, 100 µg/mL) reduced the conjugal transfer of the IncI$_2$ plasmid TP114 to 44.7 ± 3.5% but interestingly promoted the conjugation of the IncN plasmid pKM101 to greater than 120%.

Keywords: Dimeric; Imidazole; Lepidine; Plasmids; Conjugation

Introduction

Lepidium sativum (garden cress) is an annual herb of the family Brassicaceae. It is a native of Egypt and West Asia but is widely farmed in temperate countries globally for its culinary and medicinal uses.[1,2] In China, garden cress seeds are used for the treatment of abdominal colic, asthma, pleurisy and dropsy.[3] In Africa, Ethiopians use the seeds primarily for treatment of throat diseases, asthma, bronchitis, headache and for baking and as a condiment.[4,5] The Mauritians use the seeds for the treatment of hiccough and stomachache.[5] Both seeds and leaves of *L. sativum* are used for the treatment of inflammation, bronchitis, rheumatism and muscular pain in the Unani system of medicine.[3,6] It is also reported to be useful in the treatment of diabetes, hypertension, cough and bleeding piles.[7–9] Phytochemical studies on *L. sativum* have shown the presence of essential oil, glucosinolates, alkaloids, sterols, cyanogenic glycosides (traces), flavonoids, tannins, saponins and triterpenes.[3,6] Bahroun and Damak first reported dimeric imidazole alkaloids for *L. sativum* seeds after which Meinhart Zenk's group isolated five additional analogues.[10]

In this paper, we report the isolation and structural characterization by spectroscopic methods of a new dimeric imidazole alkaloid, 2-(3-(3-((1*H*-imidazol-2-yl)methyl)-5-methoxyphenoxy)benzyl)-1*H*-imidazole (**1**) (Fig. 1) along with three known compounds. The antibacterial ~~and antifungal~~ activit~~ies~~y and inhibition of bacterial conjugal transfer of plasmids of the isolated compounds are reported herein for the first time.
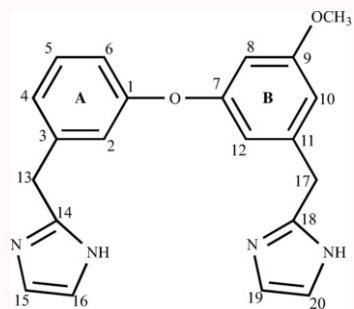
Fig. 1 Structure and numbering of compound **1**.

Results and discussion

L. sativum seeds (245 g) were obtained from Seed Parade, UK, and defatted with petroleum ether and subsequently extracted with methanol. The MeOH extract was mixed with water, acidified with concentrated HCl and it was then liquid-liquid extracted with EtOAc. The EtOAc fraction was chromatographed over silica gel 60 (gravity column, 70–230 mesh, column size: 50 × 4 cm). Compound **1** (3.6 mg, *Rf* value: 0.39) was obtained by purification of the column fractions using preparative TLC (silica gel, CHCl₃-MeOH-NH₃, 90:9:1, $^V/_V$).

Compound **1** was isolated as a pale yellow solid and HRTOFESIMS gave an *m/z* at 361.1659 [M+H]⁺, which indicated that its molecular formula was $C_{21}H_{20}N_4O_2$. The ¹H and COSY NMR spectra revealed two aromatic rings; di-substituted (A) and tri-substituted (B) aromatic rings. The ¹H data was similar to the known compound **4** (2-(3-(2-((1*H*-imidazol-2-yl)methyl)-6-methoxyphenoxy)benzyl)-1*H*-imidazole, only varying in the positioning of the methoxyl and imidazolyl methyl group on the B-ring. The di-substituted aromatic ring was identified by proton resonances at δ_H 6.70 (dd, *J* = 2.0, 1.5 Hz, H-2), 6.86 (dd, *J* = 8.0, 0.5 Hz, H-4), 7.15 (t, *J* = 8.0 Hz, H-5), 6.64 (dd, *J* = 8.0, 2.0 Hz, H-6). The couplings between the aromatic hydrogens, and the meta-coupling of H-2 by H-6 ($^4J_{HH}$ = 2.0 Hz) confirmed their arrangement on the di-substituted ring. On the B-ring a meta-coupling of a hydrogen signal at δ_H 7.01 (d, *J* = 1.5 Hz, H-10) to δ_H 6.85 (s, *J* = 1.5 Hz, H-12) in the COSY spectrum also indicated the presence of an aromatic spin system, which was 1,3,5-tri-substituted (ring B). Both spin systems were confirmed by the HMQC and HMBC correlations (Table 1, Fig. 2). The aromatic rings were linked by oxygen, which was confirmed by a strong IR absorption band at 1053 cm⁻¹ (the ether functional group). A $^3J_{H-C}$ correlation of the methoxyl hydrogens (3H, s, δ_H 3.70) to the aromatic quaternary carbon (δ_C 151.9, C-9) revealed the position of the methoxyl group on the aromatic ring B. It was meta-positioned to the imidazolylmethyl moiety and this was supported by the splitting pattern of the aromatic hydrogens on the ring B. The NOESY spectrum revealed a cross peak between the methoxyl hydrogens (3H, s, δ_H 3.70) and the aromatic hydrogens H-8 and H-10 (δ_H 7.01), which confirmed this structural arrangement. The hydrogen signal at δ_H 6.91 (4H, s, H-15, H-16, H-19 and H-20) and the carbon signals at δ_C 123.3 (2C, C-15 and C-16), 123.0 (2C, C-19 and C-20), 148.1 (C-14) and 148.4 (C-18) accounted for the two-imidazole rings. The signals at δ_H 3.97 (2H, s, H₂-13), δ_c 34.3 (C-13) and δ_H 3.95 (2H, s, H₂-17), δ_c 35.1 (C-17) were identified as the two methylene groups connecting the imidazole rings to the aromatic rings. The IR spectrum confirmed this with the presence of secondary amine functional group absorbance at 2920 cm⁻¹. The combined NMR, IR and HRTOFESIMS spectra data led to an unambiguous assignment of the full structure of compound **1**, as 2-(3-(3-((1*H*-imidazol-2-yl)methyl)-5-methoxyphenoxy)benzyl)-1*H*-imidazole. The known compounds were identified by comparison of their spectroscopic data with reported data as Lepidine E (**2**), Lepidine B (**3**) and 2-(3-(2-((1*H*-imidazol-2-yl)methyl)-6-methoxyphenoxy)benzyl)-1*H*-imidazole (**4**).[10]

Table 1 ¹H (500 MHz) and ¹³C NMR (125 MHz) NMR data and HMBC correlations of **1** recorded in methanol-*d₄*.

Position	δ_C, type	δ_H, (*J* in Hz)	HMBC
1	159.9, C		
2	117.6, CH	6.70, dd (2.0, 1.5)	
3	141.0, C		
4	123.3, CH	6.86, dd (8.0, 0.5)	C-5
5	130.6, CH	7.15, t	C-1, C-3
6	115.6, CH	6.64, dd (8.0, 2.0)	
7	145.6, C		
8	114.5, CH	7.01, s	C-9
9	151.9, C		
10	126.4, CH	7.01, d (1.5)	C-11
11	132.5, C		
12	123.0, CH	6.85, d (1.5)	
13	34.3, CH₂	3.97, s	C-2, C-3, C-4, C-14
14	148.1, C		
15	123.3, CH	6.91, s	C-14
16	123.3, CH	6.91, s	C-14

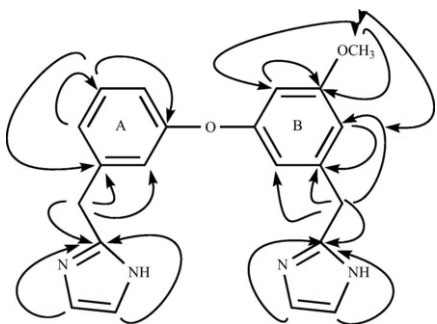|			
17	35.1, CH$_2$	3.95, s	C-10, C-11, C-12, C-18
18	148.4, C		
19	123.0, CH	6.91, s	C-18
20	123.0, CH	6.91, s	C-18
O-Me	56.5, CH$_3$	3.70, s	C-9

Fig. 2 Key HMBC (single-headed arrows) and NOESY (double-headed arrows) correlations of compound **1**.

The compounds were tested for their ability to inhibit bacterial plasmid conjugation[11] (Table 2). The essence of this assay is to identify compounds that can inhibit the spread of antibiotic-resistance genes via the bacterial type-IV secretion systems (conjugation machinery). The inhibition of this process is important because of the clinical significance of conjugation machinery in the transfer of toxins and effector proteins directly into eukaryotic target cells, and its involvement in biofilm formation as well as the aforementioned transfer of antibiotic-resistance genes among microorganisms.[12,13]

Table 2 The effect of the isolated dimeric imidazoles on conjugal transfer of plasmids pKM101 and TP114.

Conjugation pair		Compounds (100 µg/mL)				Controls	
Donor	Recipient	1	2	3	4	Novobiocin (10 µg/mL)	No drug
pKM101[a]	ER1793[c]	120 ± 0.0	31.0 ± 7.0	120 ± 0.0	28.0 ± 5.0	—	100.0 ± 0.0
TP114[b]		44.0 ± 3.5	26.0 ± 4.0	120 ± 0.0	23.5 ± 4.0	17.0 ± 4.2	100.0 ± 0.0

The values represent the mean transfer frequency (%) ± standard deviation of at least three independent experiments.

[a] *E. coli strain* WP2 bearing plasmid pKM101 (IncN; ampicillin-resistant).

[b] *E. coli strain* K12 J53-2 bearing plasmid TP114 (IncI$_2$; kanamycin-resistant).

[c] *E. coli strain* ER1793, streptomycin resistant.

It was interesting to note that some of the dimeric imidazoles (compounds **2** and **4**) at sub-inhibitory concentration (100 µg/mL) exhibited anti-conjugal activity in *Escherichia coli* while compound **3** at the same concentration enhanced conjugation activity (conjugal transfer frequency, greater than 120%). With compound **1**, the activity varied.

Compound **2** reduced the conjugal transfer frequency of the IncN plasmid pKM101 and IncI$_2$ plasmid TP114 to 31.0 ± 7.0% and 26.0 ± 4.0%, respectively. In comparison to compound **2**, **4** had a slightly better reduction in conjugal transfer frequency, a 28.0 ± 5.0% for IncN plasmid pKM101 and 23.5 ± 4.9% for IncI$_2$ plasmid TP114. Although the difference in anti-conjugal activity for compounds **2** and **4** is marginal, we suspect that the structural differences of substituent groups on the aromatic ring B of the compounds may have contributed to this. At position 8 of the aromatic ring B, compound **2** has a hydroxyl group attached while compound **4** has a methoxyl group. The imidazolylmethyl moiety is attached to position 11 for compound **2**, while for compound **4** it is at the position 12. A general observation made was that, the isolated compounds were similar to each other but only varied in the positioning of the substituent groups on the aromatic ring B (either an hydroxyl or a methoxyl group and an imidazolylmethyl moiety), and this may have influenced the varied outcome of activity against the conjugal transfer of plasmids in *E. coli*.

The only compound, which showed specificity in activity against the tested plasmids strains, was the new imidazole (**1**). It exhibited moderate anti-conjugal activity against the IncI$_2$ plasmid TP114 (transfer frequency 44.0 ± 3.5%) and enhanced the conjugation activity of the IncN plasmid pKM101 to greater than 120%. We found this interesting, as specificity would limit its potential use but promotion may have utility in promoting plasmid transfer, which would be useful in many areas of molecular biology. In conclusion, this finding could serve as a good start point for structural modification for improved anti-conjugal activity with specificity. The development of an anti-conjugal molecule has potential druggability in reducing transfer and spread of resistance and reducing virulence.

Structure elucidation

2-(3-(3-((1*H*-imidazol-2-yl)methyl)-5-methoxyphenoxy)benzyl)-1*H*-imidazole (compound **1**).

Pale yellow solid; λ_{max} (log ε) 218 (3.97), 228 (4.18) nm; IR (film) V$_{max}$: 2919.76, 1045.02, 971.15, 799.93, 610.64 cm^{-1}; ^{1}H NMR (500 MHz, methanol-d_4) and ^{13}C NMR (125 MHz, methanol-d_4): see Table 1; positive HRTOFESIMS *m/z* 361.1659 [M+H]$^+$ (calcd for C$_{21}$H$_{20}$N$_4$O$_2$, 361.1664).

Acknowledgments

A Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.tetlet.2018.04.028.

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A Supplementary data

Multimedia Component 1

Supplementary data 1

Graphical abstract
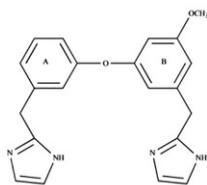
Highlights

- A new dimeric imidazole alkaloid was isolated from the seeds of *Lepidium sativum.*

- Structure elucidation was achieved by analysis of the NMR spectroscopic and mass spectral data.

- The new compound, named lepidine AK (**1**), reduced the conjugal transfer of the Incl$_2$ plasmid TP114 to 44.7%.

- Interestingly, compound **1** also promoted the conjugation of the IncN plasmid pKM101 to greater than 120%.

- Plasmid transfer inhibitors may have utility in reducing the spread of antibiotic resistance genes and bacterial virulence.

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