Bisindole Alkaloids from Alstonia Species

Subjects: Biochemistry & Molecular Biology

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Bisindoles are structurally complex dimers and are intriguing targets for partial and total synthesis. They exhibit stronger biological activity than their corresponding monomeric units. Bisindole alkaloids are naturally occurring alkaloids containing two indole nuclei and are the products of late-stage biosynthetic processes in higher plants by combining two monomeric units. Depending on the monomeric units involved, bisindoles can be a homo- or heterodimer. As a result, bisindole alkaloids comprise much higher structural complexity than both of the monomeric units that comprise them. *Alstonia*, a major genus in the Apocynaceae family of plants, has more than 150 species and is found all over the world. Robert Brown named it in 1811 in honor of Charles Alston (1685–1760), an eminent botanist at the University of Edinburgh. The *Alstonia* genus' trees and shrubs are prevalent in the tropical and subtropical parts of Africa, Asia, and Australia. They contribute significant pharmacological activity, including anticancer, antileishmanial, antimalarial, antitussive, antiviral, antiarthritic, and antibacterial activities.

Apocynaceae | Alstonia species | Sarpagine-macroline-ajmaline type indole alkaloids | Pleiocarpamine | Bisindole synthesis | Isolation of bisindoles | Bioactivity of bisindoles

1. Bisindole Alkaloids in drug discovery

Nature has been a substantial and sustainable pool of biologically active compounds. Since ancient times natural product extracts (in crude form) have been used in traditional and folk medicines in many countries. In modern times pure (isolated) natural products and their derivatives play an important role in drug discovery, as indicated by their prevalence in approved drugs for clinical use. Out of the 1881 newly FDA-approved drugs over the last four decades (1 January 1981 to 30 September 2019), a significant portion comprising 506 (26.9%) were either natural products or derived from or inspired by natural products [1]. It is expected that the advent of modern and innovative technologies such as computational software, cheminformatics, artificial intelligence, automation, and quantum computing will further boost natural product-based drug discovery. A synergy among these technological milestones would accelerate *hit to lead to clinic* pathways of drug discovery, and natural products are expected to remain an important source [2]. Moreover, pharmacophores and their unique stereochemical interactions with natural products may stimulate more demanding targets such as protein–protein interactions in the near future and open up a new avenue in modern drug discovery [3]. The majority of biologically active natural products are produced in plants, known traditionally as medicinal plants. Alkaloids, the most important class of natural products with structural diversity and significant pharmacological effects, are mainly found in higher plants such as the Apocynaceae, Ranunculaceae, Papaveraceae, and Leguminosae families [4]. These natural products, along with flavonoids, fatty acids, etc., are the major classes of secondary metabolites that are believed to be parts of the

plants' defense mechanism. To date, many monoterpenoid indole and bisindole alkaloids have been found in the *Alstonia* genus [5]. Modern clinical application of many of these alkaloids are similar to their traditional or folklore applications; for example, cocaine and morphine were used as anesthetics while caffeine and nicotine were used as stimulants [6]. Recently, Fielding et al. illustrated that several anti-coronavirus alkaloids showed potential therapeutic value against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) in their in silico studies [7].

The indole motif is present in many naturally occurring and biologically active compounds. Some of them have been used in the clinic. In addition, there are numerous examples of synthetic compounds with useful medicinal properties that bear the indole moiety. Consequently, the indole scaffold is one of the few "privileged structures" in modern medicinal chemistry and drug discovery [8][9]. The prevalence of bioactivity of indole-containing molecules may be attributed to their similarity to the essential amino acid tryptophan, as well as important biomolecules such as tryptamine and serotonin. Many plant extracts, which likely include alkaloids, have been used from time immemorial in folk medicines for fever, general weakness, dysentery, pain, liver diseases, gastrointestinal diseases, and cancer [10]. Currently, there are many indole alkaloid-based marketed drugs such as sumatriptan for the treatment of migraine; vincristine and vinblastine for the treatment of various cancers, including leukemia and lung cancer; as well as reserpine for the treatment of hypertension and to decrease severe agitation in patients with mental disorders [11].

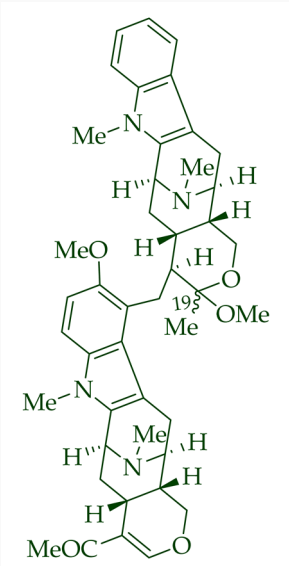
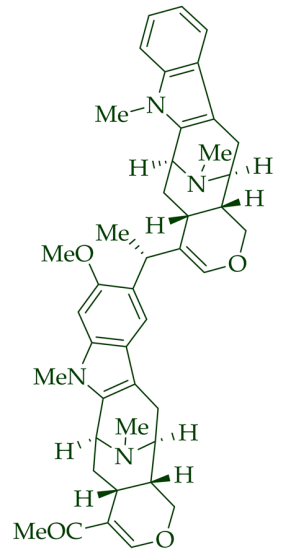
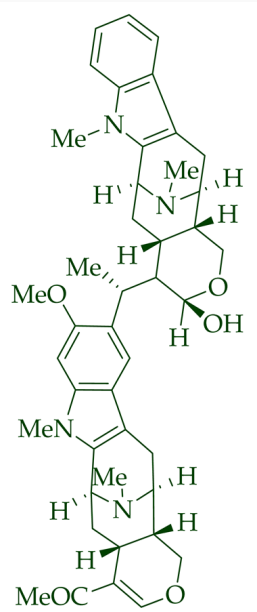
More structural diversity can be achieved via bisindole-based drug discovery by changing both monomeric units to furnish unnatural and *pseudo*-natural alkaloids. As such, bisindole alkaloids offer a large pool of natural, semi-natural, and chimeric drug candidates that have greater drug-like characteristics. As a result, due to their important biological applications and complex structural features, bisindole alkaloids have engendered the profound interest of synthetic organic and medicinal chemists, computational chemists, and chemical biologists.

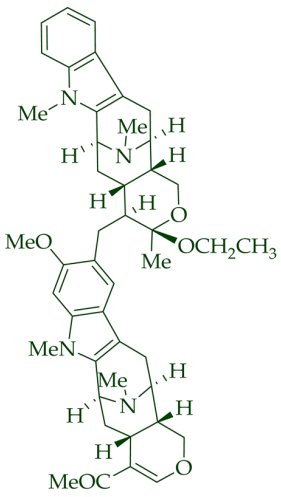
2. Isolation and Plant' Morphology of Bisindoles from *Alstonia* Species

Among various species of the *Alstonia* genus, *A. macrophylla* and *A. angustifolia* are the two major sources of bisindole alkaloids discussed herein (*vide infra*, Table 1). (+)-Alstomacroline **1**, a bisindole alkaloid consisting of a macroline and an ajmaline unit (monomeric units not shown here: please visit https://doi.org/10.3390/molecules26113459 for details), was isolated from the bark *of A. macrophylla* [12] and the leaves, stem-bark, and root-bark extracts of *A. scholaris, A. glaucescens, and A. macrophylla*[13][14]. (+)-Alstomacrophylline **2** (macroline–macroline-type) was isolated from the bark *of A. macrophylla* [12] and the leaves, stem-bark, and root-bark extracts of *A. scholaris, A. glaucescens, and A. macrophylla* [14]. (-) Alstonisidine **3**, which contains a quebrachidine and a macroline unit, was isolated from the bark of *A. muelleriana* [15][16]. The structure of (-)-alstonisidine **3** was confirmed by X-ray crystallographic data [17]. Yeap et al. recently isolated seven novel bisindoles from the methanol extract of the stem-bark of Malayan *A. penangiana* [18]. This includes (-)-angustilongine E **6**, (-)-angustilongine F **7**, (+)-angustilongine G **8**, (+)-angustilongine H **9**, (+)-angustilongine J **10**, (+)-angustilongine K **11**, and (-)-angustilongine L **12** (macroline–pleiocarpamine type). (+)-Angustilongine K **11** was

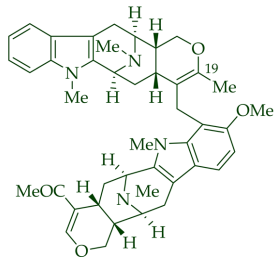
converted into (+)-*di*-*O*-acetylangustilongine K **13** by stirring it with 10 equivalents of pyridine and 15 equivalents of acetic anhydride for 6 h at room temperature in **95%** yield [18]. Among those, angustilongine G **8** and angustilongine H **9** are C-19 methyl substituted [19] bisindoles. The structures of the angustilongines were confirmed by various spectroscopic data, including ^{1}H NMR, ^{13}C NMR, 2D NMR, IR, and HRMS by Yeap et al. [18]. Angustilongine E **6**, angustilongine F **7**, angustilongine G **8**, angustilongine H **9**, angustilongine J **10**, and angustilongine K **11** are macroline–sarpagine coupled bisindoles. Angustilongine G **8** and angustilongine H **9** differ in stereochemistry only at the C-20 position.

Table 1. Structures of bisindole alkaloids from *Alstonia* species including semi-synthetic derivatives.

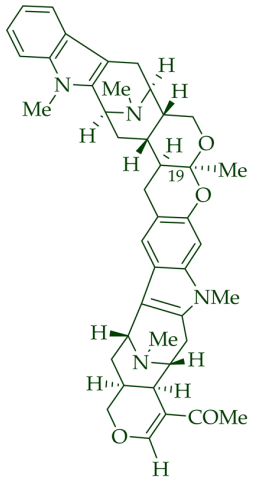
Macroline-macroline type		
 (+)-alstomacrophylline **2**	 (-)-lumusidine A **14**	 (-)-lumusidine B **15**



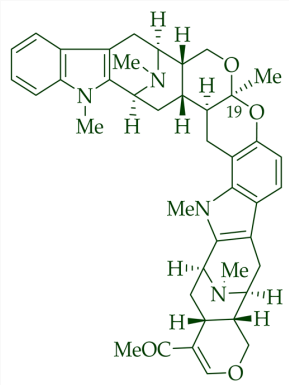
(-)-lumusidine C **16**



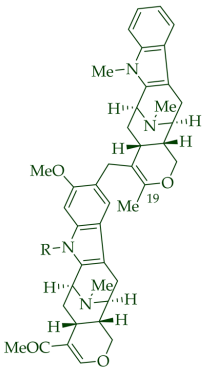
(-)-thungfaine = lumusidine D **17**



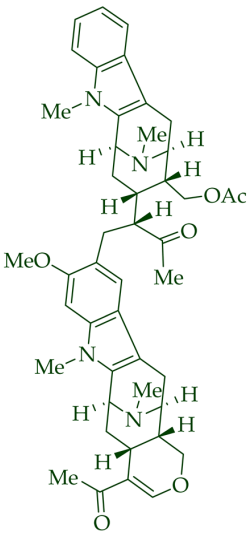
(+)- lumutinine A **18**



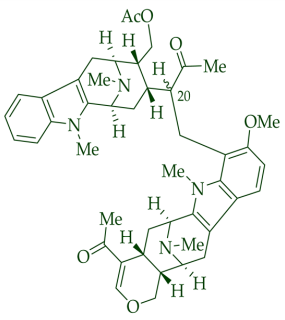
(-)-lumutinine B **19**



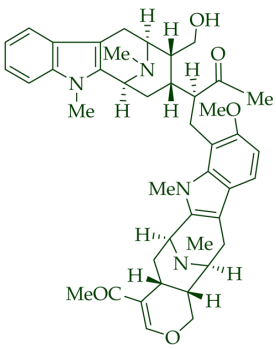
R = H, *Des-N'*$_a$-methylanhydro-macralstonine **30**
R = Me, (-)-anhydromacralstonine **27**



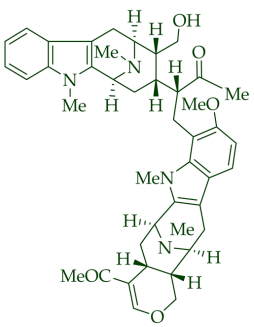
(+)-O-acetyl-*E-seco*-macralstonine **53**



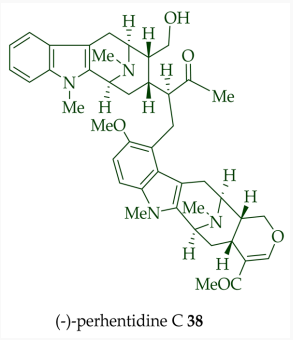
20-H, *S*, *O*-acetylperhentidine A **54**
20-H, *R*, *O*-acetylperhentidine B **55**



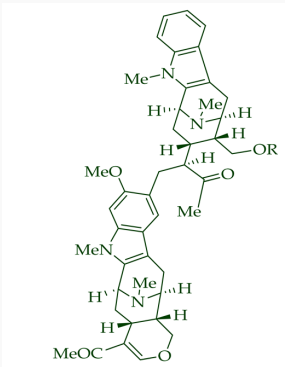
(-)-perhentidine A **36**



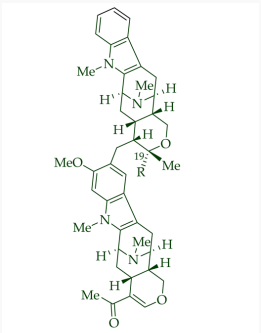
(-)-perhentidine B **37**



(-)-perhentidine C **38**

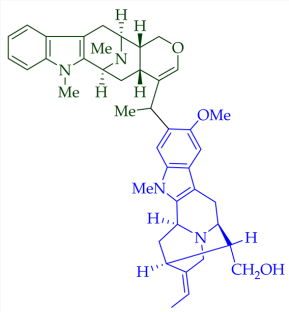


R = H, (-)-perhentinine **39**
R = Ac, *O*-acetylperhentinine **56**

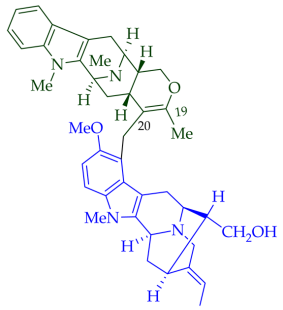


R = OH, (+)-macralstonine **24**
R = $OCOCH_3$, (+)-*O*-acetylmacralstonine **25**
R = OCH_3, (+)-*O*-methylmacralstonine **26**

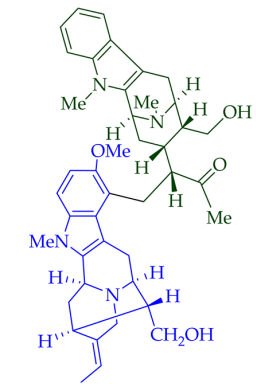
Macroline-sarpagine type



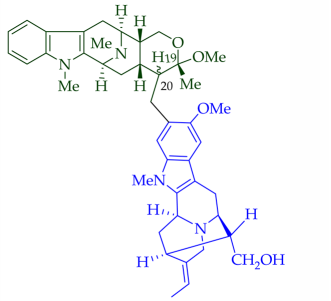
(-)-angustilongine E **6**



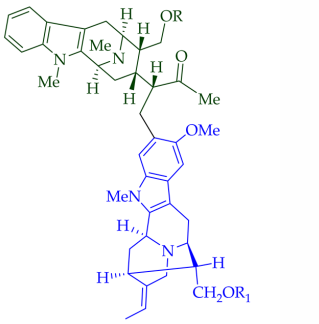
(-)-angustilongine F **7**



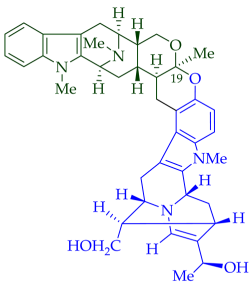
(+)-angustilongine J **10**



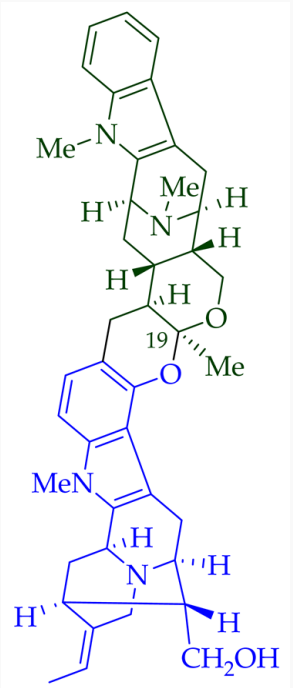
β-20- H: (+)-angustilongine G **8**
α-20- H: (+)-angustilongine H **9**



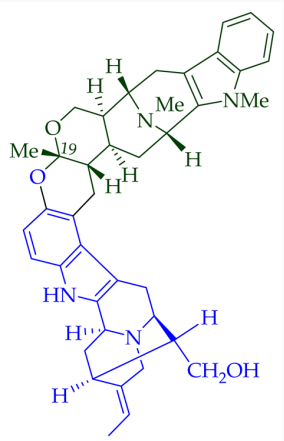
R = H, R_1 = H (+)-angustilongine K **11**
R = Ac, R_1 = Ac,
di-*O*-acetylangustilongine K **13**



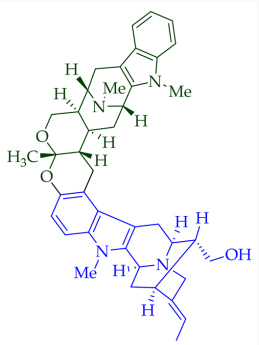
(+)-lumutinine C **20**



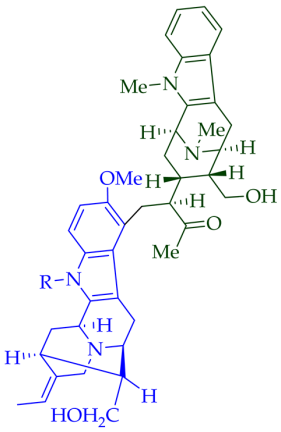
(+)- lumutinine D **21**



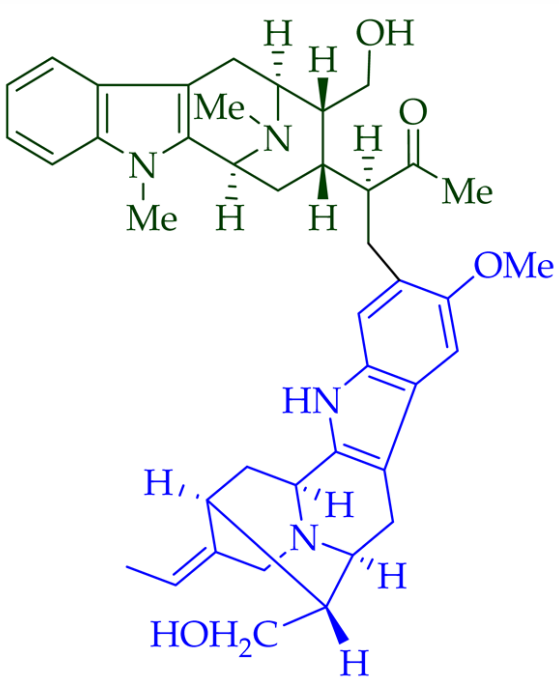
(+)-lumutinine E **22**



(+)-macralstonidine **23**

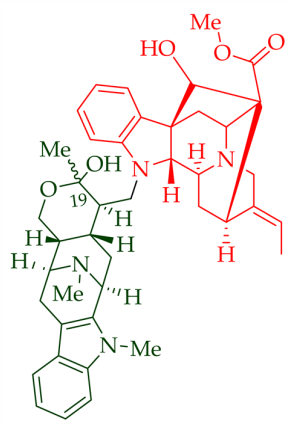


R = Me, (+)-perhentisine A **40**
R = H, (-)-perehentisine B **41**

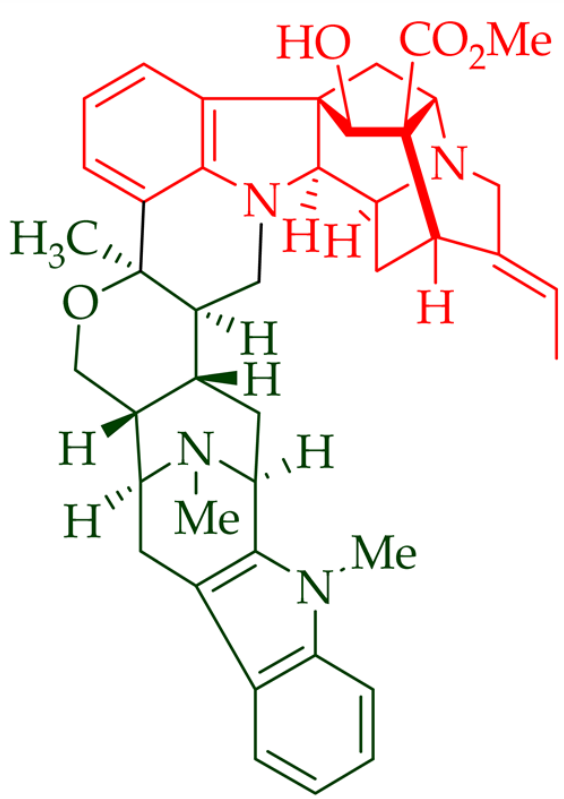


(+)-perhentisine C **42**

Macroline-ajmaline type

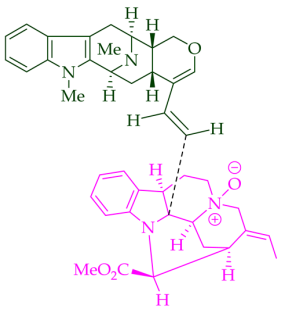


(+)-alstomacroline **1**

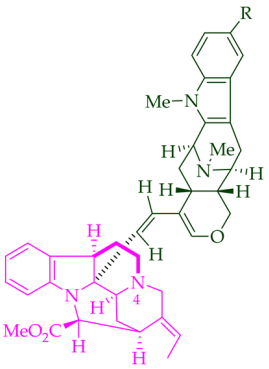


(-)-alstonisidine **3**

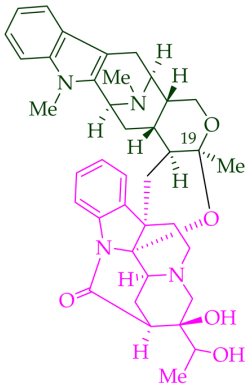
Macroline-pleiocarpamine type



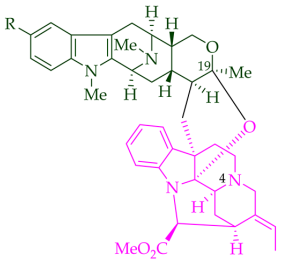
(-) angustilongine L **12**



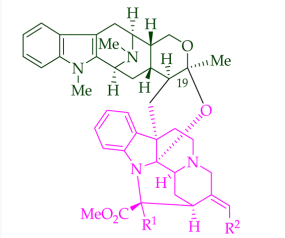
R = H, (-)-macrocarpamine **31**
R = OMe, 10-methoxymacrocarpamine **34**
R = OMe, *N*(4) → oxide (+ charge on *N*(4), 10-methoxymacrocarpamine *N*(4)-oxide **35**



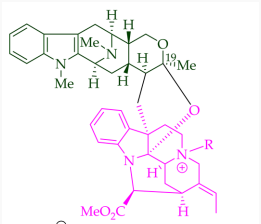
(+)-villalstonidine A **47**



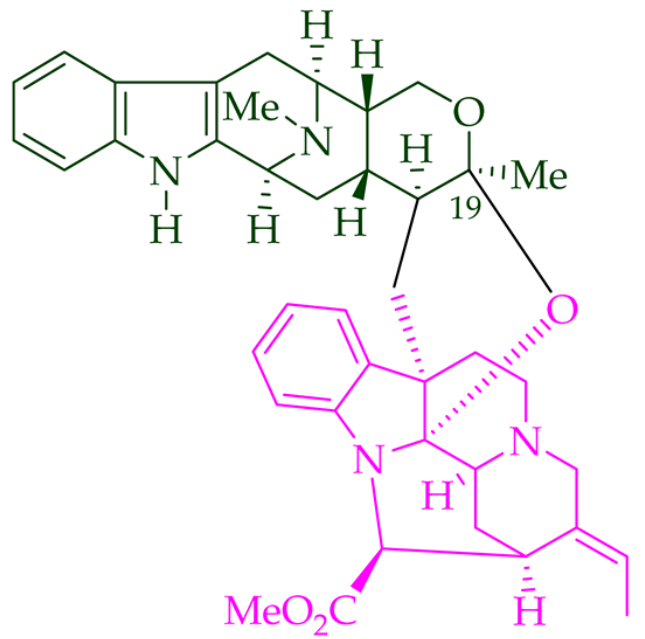
R = H, (+)-villalstonine **43**
R = OMe, 10-methoxyvillalstonine **45**
R = OMe,
N(4) ➞ Oxide (+ charge on *N*(4),
10-methoxyvillalstonine-
N(4)-oxide **46**



$R^1 = CH_2OH$, R^2 = Me, villalstonidine B **48**
R^1 = H, $R^2 = CH_2OH$, villalstonidine C **49**



R = $\ominus$O, villalstoninine *N*(4)-Oxide **44**
R = CH_3, (+)-villalstonidine D **50**
R = CH_2Cl; $Cl^{\ominus}$ (+)-villalstonidine E **51**



(+)-villalstonidine F **52**

Two macroline units are contained in (-)-lumusidine A **14**, (-)-lumusidine B **15**, (-)-lumusidine C **16**, and (-)-lumusidine D **17** bisindoles. They were isolated from the stem-bark of *A. macrophylla* and the structures were confirmed via NMR spectroscopy, mass spectrometry, UV spectroscopy, and X-ray crystallography [12]. After isolation, the group of Kam et al. converted oily (-)-lumusidine A **14**, (-)-lumusidine B **15**, and (-)-lumusidine D **17** into the corresponding crystalline dimethyl diiodide salts (structures not shown) by treatment with an excess of iodomethane for 24 h. The crystalline salts were employed to obtain X-ray crystallographic data to elucidate the exact stereochemical confirmation [12]. (-)-Lumusidine D **17** is also known as thungfaine [20]. (+)-Lumutinine A **18**, (-)-lumutinine B **19**, (+)-lumutinine C **20**, and (+)-lumutinine D **21** are linearly fused bisindoles isolated from the stem-bark of *A. macrophylla* as a light yellowish oil [21]. (+)-Lumutinine A **18** and (-)-lumutinine B **19** are macroline–macroline-type bisindoles, while (+)-lumutinine C **20**, (+)-lumutinine D **21**, and (+)-lumutinine E **22** are macroline–sarpagine-type bisindoles. The structures of the lumutinines were elucidated using spectroscopic means including

1D and 2D NMR, IR, as well as mass spectrometric analysis [21]. The structure of (+)-lumutinine D **21** was confirmed by X-ray crystallographic data [22]. (+)-Lumutinine E **22**, a macroline–sarpagine-type bisindole, was isolated from the stem-bark of *A. angustifolia* [23].

(+)-Macralstonidine **23** (macroline–sarpagine-type) was isolated from the bark of *A. macrophylla* [24][25], as well as from *A. somersentenis* [24] and *A. spectabilis* [26]. (+)-Macralstonine **24** was isolated from the leaves, stem-bark, and root-bark extracts of *A. scholaris, A. glaucescens, and A. macrophylla* extracts [14], *A. macrophylla* [24][25][27][28][29], *A. muelleriana* [30], *A. angustifolia* [31], as well as from *A. glabriflora* [26]. The structure of (+)-macralstonine **24** was confirmed by various NMR spectroscopy, mass spectrometry, and X-ray crystallography [28]. The (+)-macralstonine **24**-related bisindole, (+)-*O*-acetylmacralstonine **25,** was isolated from the leaves, stem-bark, and root-bark extracts of *A. scholaris, A. glaucescens, and A. macrophylla* [14]. Also, (+)-*O*-methylmacralstonine **26** was isolated from the leaves, stem-bark, and root-bark of *A. scholaris, A. glaucescens, and A. macrophylla* extracts [14]. (-)-Anhydromacralstonine **27** was isolated from the stem-bark of *A. angustiloba* [23] and contains (-)-alstophylline and (+)-macroline as monomeric units. Another (-)-alstophylline **28** and (+)-macroline monomeric bisindole, (+)-*Des-N'a*-methylanhydromacralstonine **30,** was isolated from the bark of *A. muelleriana* [14][30], the stem-bark of *A. angustifolia* [28], and *A. glabriflora* [26]. (+)-Macrocarpamine **31**, a hetero-dimeric bisindole containing a (+)-pleiocarpamine and (-)-anhydromacrosalhinemethine monomeric unit was isolated from the leaves, stem-bark, and root-bark of *A. scholaris, A. glaucescens, and A. macrophylla* [14], as well as the stem-bark of *A. angustifolia* [23]. 10-Methoxymacrocarpamine **34** and 10-methoxymacrocarpamine *N4'*-oxide **35** are structurally related bisindoles. These bisindoles were isolated from *A. angustifolia* leaves [31].

Lim et al. [28] reported the isolation of new macroline–macroline-type bisindoles, (-)-perhentidine A **36**, (-)-perhentidine B **37**, and (-)-perhentidine C **38** from the ethanolic extract of the stem-bark of Malayan *A. macrophylla* and *A. angustifolia.* The structurally related bisindole, (-)-perhentinine **39** (macroline–macroline-type), was isolated from the stem-bark and leaves of *A. macrophylla* and the leaves of *A. angustifolia* [12]. The exact structure of (-)-perhentinine **39** was confirmed by X-ray crystallography, converting it into the dimethyl diiodide salt by treating it with an excess of iodomethane. The X-ray crystallographic data of (-)-perhentinine **39** also helped in the structural characterizations of perhentidines A–C (**36–38**) [28]. Tan et al. isolated three macroline–sarpagine-type bisindoles: (+)-perhentisine A **40**, (-)-perhentisine B **41**, and (+)-perhentisine C **42** from the stem-bark of *A. angustifolia* as a light yellow-colored oil together with other bisindoles [23]. The structures of these bisindoles were also elucidated using various NMR and MS techniques [23]. (+)-Villastonine **43**, a macroline–pleiocarpamine-type bisindole, was isolated from the stem-bark, root-bark, and leaves of various *Alstonia* species, including *A. spectabilis* [24] and *A. muelleriana* [32] by LeQuesne et al., *A. macrophylla* [25][27][33], and *A. angustifolia* [31][34]. Schmid et al. elucidated the structure of (+)-villalstonine **43** by spectroscopic means, accompanied by degradation, and Nordman et al. confirmed the structure by X-ray crystallography [33][35].

Table 2. Isolation of bisindoles from various part' of *Alstonia* species.

Bisindoles	*Alstonia* Species	Morphology and References
(+)-Alstomacroline **1**	*A. scholaris, A. glaucescens, and A. macrophylla extracts*	Leaves, stem-bark, and root-bark [13][14]
	A. macrophylla	Bark [12]
(+)-Alstomacrophylline **2**	*A. macrophylla*	Bark [13]
	A. scholaris, A. glaucescens, and A. macrophylla extracts	Leaves, stem-bark, and root-bark [14]
(−) Alstonisidine **3**	*A. muelleriana*	Bark [15][16]
(−)-Angustilongine E **6**	*A. penangiana*	Stem-bark [18]
(−)-Angustilongine F **7**	*A. penangiana*	Stem-bark [18]
(+)-Angustilongine G **8**	*A. penangiana*	Stem-bark [18]
(+)-Angustilongine H **9**	*A. penangiana*	Stem-bark [18]
(+)-Angustilongine J **10**	*A. penangiana*	Stem-bark [18]
(+)-Angustilongine K **11**	*A. penangiana*	Stem-bark [18]
(−)-Angustilongine L **12**	*A. penangiana*	Stem-bark [18]
(−)-Anhydromacralstonine **27**	*A. angustifolia*	Stem-bark [23]
	A. muelleriana	Bark [14]
(+)-*Des-N'a*-Methylanhydromacralstonine **30**	*A. angustifolia*	Stem-bark [28]
	A. muelleriana	Leaves, stem-bark and root-bark [14][30]
(−)-Lumusidine A **14**	*A. macrophylla*	Stem-bark [12]
(−)-Lumusidine B **15**	*A. macrophylla*	Stem-bark [12]
(−)-Lumusidine C **16**	*A. macrophylla*	Stem-bark [12]
(−)-Lumusidine D **17**	*A. macrophylla*	Stem-bark [12]
(+)-Lumutinine A **18**	*A. macrophylla*	Stem-bark [21]
(−)-Lumutinine B **19**	*A. macrophylla*	Stem-bark [21]
(+)-Lumutinine C **20**	*A. macrophylla*	Stem-bark [21]

Bisindoles	*Alstonia* Species	Morphology and References
(+)-Lumutinine D **19**	*A. macrophylla*	Stem-bark [21]
(+)-Lumutinine E **21**	*A. angustifolia*	Stem-bark [23]
(+)-Macralstonidine **23**	*A. macrophylla*	Bark [24][25]
	A. somersentenis	Bark [24]
	A. spectabilis	Bark [26]
(+)-Macralstonine **24**	*A. scholaris, A. glaucescens, and A. macrophylla extracts*	Leaves, stem-bark and root-bark [13]
(+)-*O*-Acetylmacralstonine **25**	*A. macrophylla*	[24][25][27][28][29]
	A. angustifolia	[31]
	A. muelleriana	[30]
	A. glabriflora Mgf.	[26]
	A. scholaris, A. glaucescens, and A. macrophylla extracts	[14]
(+)-*O*-Methylmacralstonine **26**	*A. scholaris, A. glaucescens, and A. macrophylla extracts*	[14]
(+)-Macrocarpamine **31**	*A. scholaris, A. glaucescens, and A. macrophylla extracts*	Leaves, stem-bark, and root-bark [14]
	A. angustifolia	Stem-bark [23]
10-Methoxy macrocarpamine **34**	*A. angustifolia*	Leaves [36]
10-Methoxy macrocarpamine 4′-*N*-oxide **35**	*A. angustifolia*	Leaves [36]
(−)-Perhentidine A **36**	*A. macrophylla and A. angustifolia*	Stem-bark [23][28]
(−)-Perhentidine B **37**	*A. macrophylla and A. angustifolia*	Stem-bark [28]
(−)-Perhentidine C **38**	*A. macrophylla and A. angustifolia*	Stem-bark [23][28]
(−)-Perhentinine **39**	*A. macrophylla*	Stem-bark [12]
	A. angustifolia	Leaves [12]

Bisindoles	*Alstonia* Species	Morphology and References
	A. macrophylla	Leaves [12]
(+)-Perhentisine A **40**	*A. angustifolia*	Stem-bark [23]
(−)-Perhentisine B **41**	*A. angustifolia*	Stem-bark [23]
(+)-Perhentisine C **42**	*A. angustifolia*	Stem-bark [23]
	A. muelleriana	Leaves and stem-bark [32]
(+)-Villalstonidine A **47**	*A. angustifolia*	Stem-bark [23]
(+)-Villalstonidine B **48**	*A. angustifolia*	Stem-bark [23]
	A. macrophylla	Stem-bark [22]
(+)-Villalstonidine C **49**	*A. angustifolia*	Stem-bark [23]
(+)-Villalstonidine D **50**	*A. angustifolia*	Stem-bark [23]
(+)-Villalstonidine E **51**	*A. angustifolia*	Stem-bark [23]
(+)-Villalstonidine F **52**	*A. macrophylla*	Stem-bark [22]
(+)-Villalstonine **43**	*A. angustifolia*	Leaves and stem-bark [23][31][34][36]
	A. macrophylla	[36]
	A. villosa	[36]
	A. verticillosa	[36]
	A. somersentensis	[36]
Villalstonine *N(4)*-oxide **44**	*A. angustifolia*	Stem-bark [23]
	A. scholaris, A. glaucescens, and A. macrophylla extracts	Leaves, stem-bark, and root-bark [14][25][27][33]
(+)-10-Methoxy villalstonine **45**	*A. angustifolia*	Leaves [36]
10-Methoxy villalstonine 4′-*N*-oxide **46**	*A. angustifolia*	Leaves [36]

Villalstonine *N*(4)-oxide **44** was isolated from the stem-bark of *A. angustifolia* [23]. It was also isolated from the leaves, stem-bark, and root-bark of *A. scholaris, A. glaucescens, and A. macrophylla* extracts [14]. Moreover, two

villalstonine **44**-related bisindoles, (+)-10-methoxy villalstonine **45** and 10-methoxy villalstonine *N*(4)-oxide **46**, were isolated from the leaves of *A. angustifolia* [36]. Villalstonidine A **47**, villalstonidine B **48**, villalstonidine C **49**, villalstonidine D **50**, villalstonidine E **51**, (+)-villalstonidine F **52**, and villalstonine *N*(4)-oxide **44** are macroline–pleiocarpamine-type bisindoles and are close in structure to villalstonine **43**. Villalstonidines A–D (**47–50)** were isolated from the stem-bark of *A. angustifolia* [23]. Additionally, (+)-villalstonidine F **52** {*N*(1)-demethylderivative of villalstonine **43**} and (+)-villalstonidine B **48** were isolated from the stem-bark of *A. macrophylla* [22].

3. Bioactivity of Bisindoles from *Alstonia* Species

Studies from various groups have shown that bisindoles have anticancer activity in different cell lines, including vincristine-resistant KB/VJ300 cells. Bisindoles are also reported to have other biological activities, including antiprotozoal activity against *Plasmodium falciparum* and antileishmanial activity against promastigotes of *Entamoeba histolytica*. The reported biological activity of bisindoles from various *Alstonia* species including the semi-synthetic derivatives reviewed herein are listed in Table 3. (+)-Alstomacroline **1** and (+)-alstomacrophylline **2** bisindoles were active against the K1 (multi-drug resistant) strain of *P. falciparum* with an IC_{50} 1.12 ± 0.35 µM and IC_{50} 1.10 ± 0.30 µM, respectively [37]. Newly isolated macroline-sarpagine-type bisindoles, (−)-angustilongines E **6**, (-)-angustilongine F **7**, (+)-angustilongine G **8**, (+)-angustilongine H **9**, (+)-angustilongine J **10**, and (+)-angustilongine K **11** showed *in-vitro* growth inhibitory activity against human cancer cell lines, inclusive of KB, vincristine-resistant strains of KB, HCCT 116, PC-3, MDA-MB-231, LNCaP, MCF7, HT-29, and A549 cells with IC_{50} values ranging from 0.02 to 9.0 µM in the study from the group of Kam [18]. Kam et al. [12] also reported the anticancer activity of (−)-lumusidine A **14**, (−)-lumusidine B **15**, (−)-lumusidine C **16**, and (−)-lumusidine D **17**. These bisindoles were cytotoxic against KB/VJ300 cells ranging from IC_{50} 0.16 to 5.03 µg/mL (µM) values with 0.12 µM vincristine added [12]. Lumutinine A **18**, lumutinine B **19**, lumutinine C **20**, lumutinine D **21**, and lumutinine E **22** exhibited moderate anticancer activity against KB/VJ300 cells ranging in IC_{50} value from 0.10 to 4.61 µg/mL (µM) again with 0.12 µM vincristine added in the studies from the same group (Kam) [12].

Table 3. Bioactivity of bisindoles (including semisynthetic derivatives) from *Alstonia* species.

Bisindoles	Bioactivity	References
(+)-Alstomacroline 1	Antimalarial, with IC_{50} values of 1.12 ± 0.35 and 10.0 ± 0.4 µM against the K1 strain and T9-96 strain of *P. falciparum*, respectively.	[14]
(+)-Alstomacrophylline 2	Antimalarial, with an IC_{50} value of 1.10 ± 0.30 µM against the K1 strain of *P. falciparum.*	[14]
Angustilongines E, F, G, H, J, and K (6–11)	Anticancer, cytotoxic against various human cancer cell lines including KB, vincristine-resistant KB, HCCT 116, PC-3, MDA-MB-231, LNCaP, MCF7, HT-29, and A549 cells with IC_{50} values ranging from 0.02 to 9.0 µM.	[18]

Bisindoles	Bioactivity	References
(−)-Lumusidine A, B, and C (14–16)	Anticancer, moderately cytotoxic in KB/VJ300 cells with IC_{50} values of 0.16, 0.70, and 1.19 µg/mL (µM), respectively. The assay with 0.12 µM added vincristine did not influence KB/VJ300 cell growth.	[12]
(−)-Lumusidine D **17**	Anticancer, cytotoxic in KB/VJ300 cells with an IC_{50} value of 5.03 µg/mL (µM). The assay with 0.12 µM added vincristine did not influence KB/VJ300 cell growth.	[12]
Lumutinine A, B, C, D, and E (**18**–**22**)	Anticancer, moderately cytotoxic in KB/VJ300 cells with IC_{50} 0.21, 0.10, 4.61, 3.93, and 2.74 µg/mL (µM) values, respectively. The assay with 0.12 µM added vincristine did not influence KB/VJ300 cell growth.	[12]
(+)-Macralstonine **24**	Anticancer, strongly cytotoxic in KB/VJ300 cells with an IC_{50} 1.71 µg/mL (µM) value. The assay with 0.12 µM added vincristine did not influence KB/VJ300 cell growth.	[12]
	Antimalarial, active against the K1 strain of *P. falciparum* with an IC_{50} 8.92 ± 2.95 µM value.	[14]
(−)-Anhydromacralstonine **27**	Anticancer, moderately cytotoxic in KB/VJ300 cells with an IC_{50} value of 0.44 µg/mL (µM). The assay with 0.12 µM added vincristine did not influence KB/VJ300 cell growth.	[12]
(+)-*O*-Acetyl macralstonine **25**	Antimalarial, with IC_{50} values 0.53 ± 0.09 and 12.4 ± 1.6 (µM) against the K1 strain and T9-96 strain of *P. falciparum*, respectively.	[14]
(+)-*O*-Methyl macralstonine **26**	Antimalarial, active against the K1 strain of *P. falciparum* with an IC_{50} 0.85 ± 0.20 µM value.	[14]
O-Acetyl-*E*-*seco*-macralstonine **53**	Anticancer, strongly cytotoxic in KB/VJ300 cells with an IC_{50} value of 0.27 µg/mL (µM). The assay with 0.12 µM added vincristine did not influence KB/VJ300 cell growth.	[12]
(−)-Perhentidine A **36** and (-)-perhentidine B **37**	Anticancer, strongly cytotoxic in KB/VJ300 cells with IC_{50} values of 2.29 and 0.84 µg/mL (µM), respectively. The assay with 0.12 µM added vincristine did not influence KB/VJ300 cell growth.	[12]
(−)-*O*-Acetylperhentidine A **54** and (-)-*O*-Acetylperhentidine B **55**	Anticancer, strongly cytotoxic in KB/VJ300 cells with IC_{50} 0.36 and 0.28 µg/mL (µM) values, respectively. The assay with 0.12 µM added vincristine did not influence KB/VJ300 cell growth.	[12]
(−)-Perhentinine **39** and *O*-Acetylperhentinine **56**	Anticancer, cytotoxic in KB/VJ300 cells with IC_{50} values of 0.52 and 0.30 µg/mL (µM), respectively. The assay with 0.12 µM added vincristine did not influence KB/VJ300 cell growth.	[12]
(+)-Macralstonidine **23**	Anticancer, moderately cytotoxic in KB/VJ300 cells with an IC_{50} value of 0.13 µg/mL (µM). The assay with 0.12 µM added	[12]

Bisindoles	Bioactivity	References
	vincristine did not influence KB/VJ300 cell growth.	
(+)-Macrocarpamine **21**	Anticancer, strongly cytotoxic in KB/VJ300 cells with an IC_{50} value of 0.53 µg/mL (µM). The assay with 0.12 µM added vincristine did not influence KB/VJ300 cell growth.	[12]
	Strong antimalarial activity against the K1 strain of *P. falciparum* with an IC_{50} value of 0.36 ± 0.06 µM. Active against the T9-96 strain of *P. falciparum* with an IC_{50} >39 µM value.	[14]
	Strong antiprotozoal activity in vitro against *E. histolytica* and *P. falciparum* with ED_{50} 8.12 (95% C.I.) µM and ED_{50} 9.36 (95% C.I.) µM values, respectively.	[38]
(+)-Villalstonine **43**	Anticancer, cytotoxic in KB/VJ300 cells with an IC_{50} value of 0.42 µg/mL (µM). The assay with 0.12 µM added vincristine did not influence KB/VJ300 cell growth.	[12]
	Anticancer, cytotoxic against the HT-29 cell line with an ED_{50} 8.0 µM value (paclitaxel was used as the positive control).	[34]
	Antimalarial, with IC_{50} values of 0.27 ± 0.06 and 0.94 ± 0.07 µM against the K1 strain and T9-96 strain of *P. falciparum*, respectively.	[14]
	Antiamoebic activity against *E. histolytica* with an ED_{50} of 2.04 µM.	[38]
Villalstonine *N*(4)-oxide **44**	Antileishmanial activity against promastigotes of *L. mexicana* with an IC_{50} value of 80.3 µM (amphotericin B was used as the positive control).	[34]
	Antimalarial, active against the K1 strain of *P. falciparum* with an IC_{50} 10.7 ± 1.9 (µM) value.	[14]
(+)-Villalstonidine B **48** and (+)-villalstonidine F **52**	Anticancer, strongly cytotoxic in KB/VJ300 cells with IC_{50} values of 0.35 and 5.64 µg/mL (µM), respectively. The assay with 0.12 µM added vincristine did not influence KB/VJ300 cell growth.	[12]
(+)-Villalstodinine D **50**	Antileishmanial, active against promastigotes of *L. mexicana* with an IC_{50} value of 120.4 µM (amphotericin B was used as the positive control).	[34]
(+)-Villalstonidine E **51**	Anticancer, cytotoxic against HT-29 cell lines with an ED_{50} 6.5 µM value (paclitaxel was used as the positive control).	[34]
	Antileishmanial against promastigotes of *L. mexicana* with an IC_{50} 78 µM value (amphotericin B was used as the positive control).	[34]

(+)-Macralstonine **24** was active against the K1 strain of *P. falciparum* (IC_{50} 8.92 ± 2.95 µM). Notably, the derivatives of (+)-macralstonine **24** were more active than (+)-macralstonine **24** itself. (+)-*O*-methyl macralstonine **26** and (+)-*O*-acetyl macralstonine **25** demonstrated more potent activity against the K1 strain of *P. falciparum* with IC_{50} values of 0.85 ± 0.20 µM and IC_{50} 0.53 ± 0.09 µM, respectively [14]. Likely, the functionalization facilitated the transportation of these bisindoles through the cell membranes of parasites and red blood cells, which would have enhanced the activity as lipophilicity rose [14]. (+)-*O*-acetyl macralstonine **25** and (+)-alstomacroline **1** were also somewhat active against the T9-96 strain of *P. falciparum* with IC_{50} values of 12.4 and 10.2 µM, respectively [14]. Heterodimeric alkaloid (−)-anhydromacralstonine **27** showed moderate cytotoxicity (IC_{50} value of 0.44 µg/mL (µM) in KB/VJ300 cells with 0.12 µM of vincristine added [12]). Another semisynthetic analog of macralstonine **24**, the related *O*-acetyl-*E-seco*-macralstonine **53**, showed strong anticancer activity. It was prepared by the reaction of macralstonine **24** with acetic anhydride/pyridine in DCM [12]. It demonstrated potent activity against vincristine-resistant KB/VJ300 cells with an IC_{50} value of 0.27 µg/mL (µM), with 0.12 µM of vincristine added to the assay [12]. (+)-Macralstonidine **23** was found to exhibit moderately active anticancer activity. It was active against KB/VJ300 cells with an IC_{50} value of 0.13 µg/mL (µM) [12].

(−)-Macrocarpamine **31** exhibited antiprotozoal and anticancer activity in various studies. It showed significant antiprotozoal activity in vitro in studies from Wright et al. against *E. histolytica* and *P. falciparum* with ED_{50} values of 8.12 (95% C.I.) µM and 9.36 (95% C.I.) µM, respectively [38]. Keawpradub et al. reported significant activity of (−)-macrocarpamine **31** against the K1 strain of *P. falciparum* with an IC_{50} value of 0.36 µM [14]. Furthermore, (−)-macrocarpamine **31** showed antimalarial activity against the T9-96 strain of *P. falciparum* with an IC_{50} > 39 µM. The ancient folklore use of the extracts from *A. angustifolia* in Malaya for treatment of malaria and dysentery is supported by these in vitro studies [39]. (−)-Macrocarpamine **31** was strongly cytotoxic in KB/VJ300 cells with an IC_{50} value of 0.53 µg/mL (µM) [12]. (−)-Perhentidine A **36** and (−)-perhentidine B **37** showed strong cytotoxicity against KB/VJ300 cells with IC_{50} values of 2.29 and 0.84 µg/mL (µM), respectively [12]. *O*-Acetylperhentidine A **54** and *O*-acetylperhentidine B **55** also exhibited strong cytotoxicity against KB/VJ300 cells with IC_{50} values of 0.36 and 0.28 µg/mL (µM), respectively [12]. (−)-Perhentidine A **36** and (−)-perhentidine B **37** were treated individually by dropwise addition of acetic anhydride in a py/DCM solution, followed by stirring at room temperature for 2 h to furnish the semisynthetic (−)-*O*-acetylperhentidine A **54**, and (−)-*O*-acetylperhentidine B **55**, respectively [12]. (−)-Perhentinine **39** and *O*-acetylperhentinine **56** were cytotoxic against KB/VJ300 cells with IC_{50} values of 0.52 and 0.30 µg/mL (µM), respectively, in the studies by Kam et al. [12].

(+)-Villalstonine **43** has demonstrated various biological activities including anticancer, antimalarial, and antiamoebic activity. (+)-Villastonine **43** was 1/15th as potent as chloroquine (antimalarial drug) against malaria [40]. It exhibited potent antiplasmodial activity against the multidrug-resistant K1 strain of *P. falciparum* with an IC_{50} value of 0.27 µM [14]. Wright et al. tested this compound for antiamoebic activity against *E. histolytica* [38]. (+)-Villalstonine **43** showed six times less activity (ED_{50} 11.8 µM) than the antiamoebic drug emetine (ED_{50} 2.04 µM). These results also concur with the use of various parts of the *A. angustifolia* plant from ancient times to treat malaria and amoebic dysentery [38]. Moreover, (+)-villalstonine **43** was cytotoxic against KB cells with an ED_{50} value of 11.6 µM [38]. (+)-Villalstonine **43** was also potent against the T9-96 strain of *P. falciparum* with an IC_{50} value of 0.94 µM. It also showed anticancer activity against the HT-29 cell line with an ED_{50} value of 8.0 µM

(paclitaxel was the positive control). Also, it was cytotoxic against vincristine-resistant KB/VJ300 cells with an IC_{50} value of 0.42 µg/mL (µM) [12]. On the other hand, the derivative of (+)-villalstonine **43**, villalstonine *N(4)*-oxide **44** was less potent (IC_{50} 10.7 ± 1.9) than (+)-villalstonine **43** itself. The increase in the ionic charge (decrease in lipophilicity) might have reduced the ability of villalstonine *N(4)*-oxide **44** to cross through the cell membranes of red blood cells or the parasites, which if correct, would explain the weaker activity [14]. Villalstonine *N*(4)-oxide **44** is also an antileishmanial bisindole. It was active against promastigotes of *Leishmania mexicana* with an IC_{50} value of 80.3 µM [34]. (+)-Villalstonine **43**-related alkaloids (+)-villalstonidine B **48** and (+)-villalstonidine F **52** were found to be strongly cytotoxic against KB/VJ300 cells with IC_{50} values of 0.35 and 5.64 µg/mL (µM), respectively [12]. (+)-Villalstonidine D **50** exhibited antileishmanial activity. It was active against promastigotes of *L. mexicana* with an IC_{50} value of 120.4 µM in a study from Pan et al. [34]. Another (+)-villalstonine **43**-related alkaloid, (+)-villalstonidine E **51,** demonstrated anticancer and antimalarial activity. It was cytotoxic against the HT-29 cell lines with an ED_{50} value of 6.5 µM and was active against promastigotes of *L. mexicana* with an IC_{50} value of 78 µM in the study from the same group [34]. Many bisindoles are yet to be screened for their activity because of the paucity of material; however, their significant role in future drug discovery should be considered.

4. Conclusions

Many *Alstonia* species are rich in indole alkaloids including bisindoles. Bisindole alkaloids including semisynthetic derivatives have been found to possess significant bioactivity, including anticancer, antileishmanial, and antimalarial properties, and thus are promising leads for nature-inspired drug discovery and development. Unnatural medicinal compounds formed by combining bioactive mismatched monomeric units can furnish novel medicinal compounds. Incorporating unnatural enantiomers of monomeric alkaloids into bisindoles can provide access to novel and unnatural bioactive compounds that may have better activity and in vivo stability depending on their metabolism. Several bisindoles along with their corresponding monomeric units has been successfully synthesized (please visit https://doi.org/10.3390/molecules26113459 for details) however; many bisindoles still await their total synthesis as well as biological screening.

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