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Synthesis, analytical behaviour and biological evaluation of new 4-substituted pyrrolo[1,2-*a*]quinoxalines as antileishmanial agents

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Abstract—An original series of 4-substituted pyrrolo[1,2-*a*]quinoxaline derivatives, new structural analogues of *Galipea* species quinoxaline alkaloids, was synthesized from various substituted 2-nitroanilines via multistep heterocyclizations and tested for in vitro anti-parasitic activity upon *Leishmania amazonensis* and *Leishmania infantum* strains. Structure–activity relationships enlighten the importance of the 4-substituted alkenyl side chain on the pyrrolo[1,2-*a*]quinoxaline moiety to modulate the antileishmanial activity. © 2006 Elsevier Ltd. All rights reserved.

1. Introduction

Protozoan parasites affect 3 billion people, with malaria and trypanosomatid parasites causing the greatest morbidity.^{1,2} Leishmaniasis is a widespread parasitic disease that is caused by protozoan parasites of the genus *Leishmania* in tropical and subtropical areas in both the old and new worlds. According to recent World Health Organization reports, 88 countries are affected, comprehending 12 million infected people worldwide, with approximately 350 million people at risk. The incidence is increasing worldwide, with 1–2 million new cases registered annually, despite all efforts being made to fight the disease.^{1–5} It occurs in two major forms; cutaneous/muco-cutaneous (CL) and visceral leishmaniasis (VL, or Kala-azar), depending upon parasite species. *Leishmania donovani* and *Leishmania infantum* are major causative agents of VL, while *Leishmania major*, *Leishmania tropica* and *Leishmania aethiopica*, *Leishmania braziliensis*, *Leishmania panamensis*, *Leishmania ama-*

zonensis and *Leishmania mexicana* cause CL.⁶ Therapy of patients with leishmaniasis is still a serious problem. Historically, leishmaniasis chemotherapy has been based on the use of toxic heavy metals, particularly antimony compounds. In fact, the drugs for leishmaniasis's treatment of all their clinical forms are meglumine antimonite (Glucantime[®]) and sodium stibogluconate (Pentostam[®]), despite the fact that they exhibit renal and cardiac toxicity (Fig. 1).^{7,8} When this kind of treatment is not effective, alternative drugs include pentamidine (Pentostam[®]) and amphotericin B (Fungizone[®], Ambisome[®]), also very toxic with serious side effects.⁹ Miltefosine, a phosphocholine analogue originally developed as an anticancer agent, has been found to be highly effective against leishmaniasis in vitro and in vivo. Now, this compound is the only oral agent against cutaneous¹⁰ and visceral¹¹ leishmaniasis, although presenting severe gastrointestinal problems.¹² As the leishmaniasis chemotherapy is still inefficient, there is an urgent need for the development of new, efficient and safe drugs.^{6,13,14} Traditional and ethnic medicine is often a good source for researchers looking for bioactive substances. Leishmaniasis is not an exception, and plants have been used for the treatment of people living far from modern medicine.^{15–17} Such compounds belong to the following groups: alkaloids, terpenes,

Keywords: Pyrrolo[1,2-*a*]quinoxaline derivatives; Antileishmanial activity; Synthesis; Lipophilicity.

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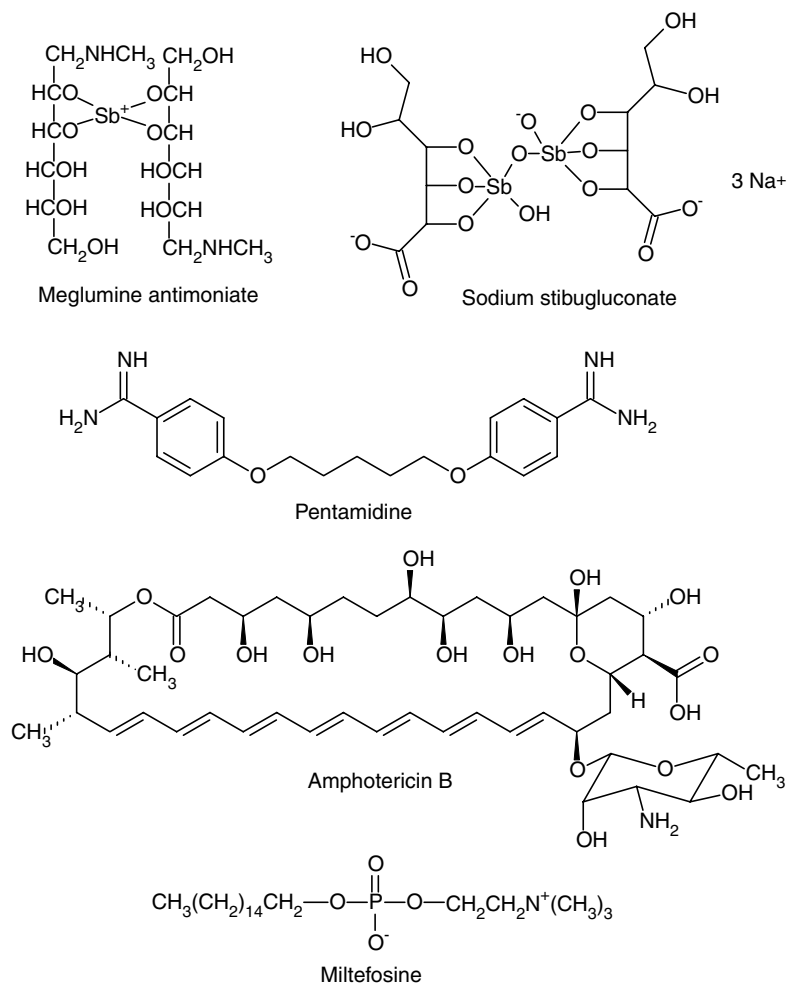


Figure 1. Structures of meglumine antimoniate (Glucantime[®]), sodium stibogluconate (Pentostam[®]), amphotericin B (Fungizone[®], Ambisome[®]), pentamidine (Pentacarinat[®]) and miltefosine.

quinines, lactones, coumarins, acetogenins of annonaceae, chalcones, tetralones, lignans and saponins.^{18–22} For example, some 2-substituted quinoline derivatives active against leishmaniasis were isolated from *Galipea longiflora*, through a bioactivity-guided fractionation process from an ethnopharmacological investigation in Bolivia.^{17,23} The 2-substituted quinoline alkaloids 2-*n*-propylquinolines, chimanines A/B/C/D, 2-*n*-pentylquinolines, 2-phenylquinolines, 2-phenylethylquinolines and 4-methoxy-2-pentenylquinoline were extracted from the bark, root and leaves of *Galipea longiflora* Krause (Rutaceae) (Fig. 2).^{23–27} *Galipea officinalis* and *Galipea bracteata* are also known to contain quinoline alkaloids such as cusparine, galipine, 2-phenylquinolines, 2-*n*-pentylquinolines and 2-pentenylquinolines (Fig. 2).^{28–30} Hence, chimanine B isolated from *Galipea* species was claimed as the lead molecule useful for further pharmacomodulations.^{31,32}

We previously described a novel synthetic approach to pyrrolo[1,2-*a*]quinoxaline derivatives designed as interesting bioactive analogues of quinoline derivatives.^{33–36} They could be developed as new isosteres of *Galipea* species quinoline alkaloids. Hence, we reported here the synthesis and in vitro antiparasitic activity study of a

series of 4-substituted pyrrolo[1,2-*a*]quinoxalines **1** upon *L. amazonensis* and *L. infantum* (Fig. 3).

As it was already noticed,³⁷ an often limiting factor for in vivo activity of new designed antiparasitic compounds is their lack of distribution to the intracellular target. As this property is often related to their molecular lipophilicity, we attempted a physico-chemical study of new pyrrolo[1,2-*a*]quinoxalines **1** achieved in order to define their analytical profile. $\log D$, the distribution coefficient between the aqueous and the lipid phase, was chosen as the parameter describing the accumulation of an ionizable drug in cells. Thus, we explored the behaviour of the 4-substituted pyrrolo[1,2-*a*]quinoxaline derivatives through $\log D$ HPLC determination achieved at physiological pH. On the other hand, pK_a values of the 4-substituted pyrrolo[1,2-*a*]quinoxaline derivatives were calculated through an in silico methodology.

Finally, all compounds were tested against *L. amazonensis* and *L. infantum* protozoa with chimanine B as the reference drug. The 4-(*E/Z*)-propenyl and 4-(*E*)-pentenyl pyrrolo[1,2-*a*]quinoxalines were found as active as the reference providing an interesting alternative to difficult extraction procedure of quinoline alkaloids from

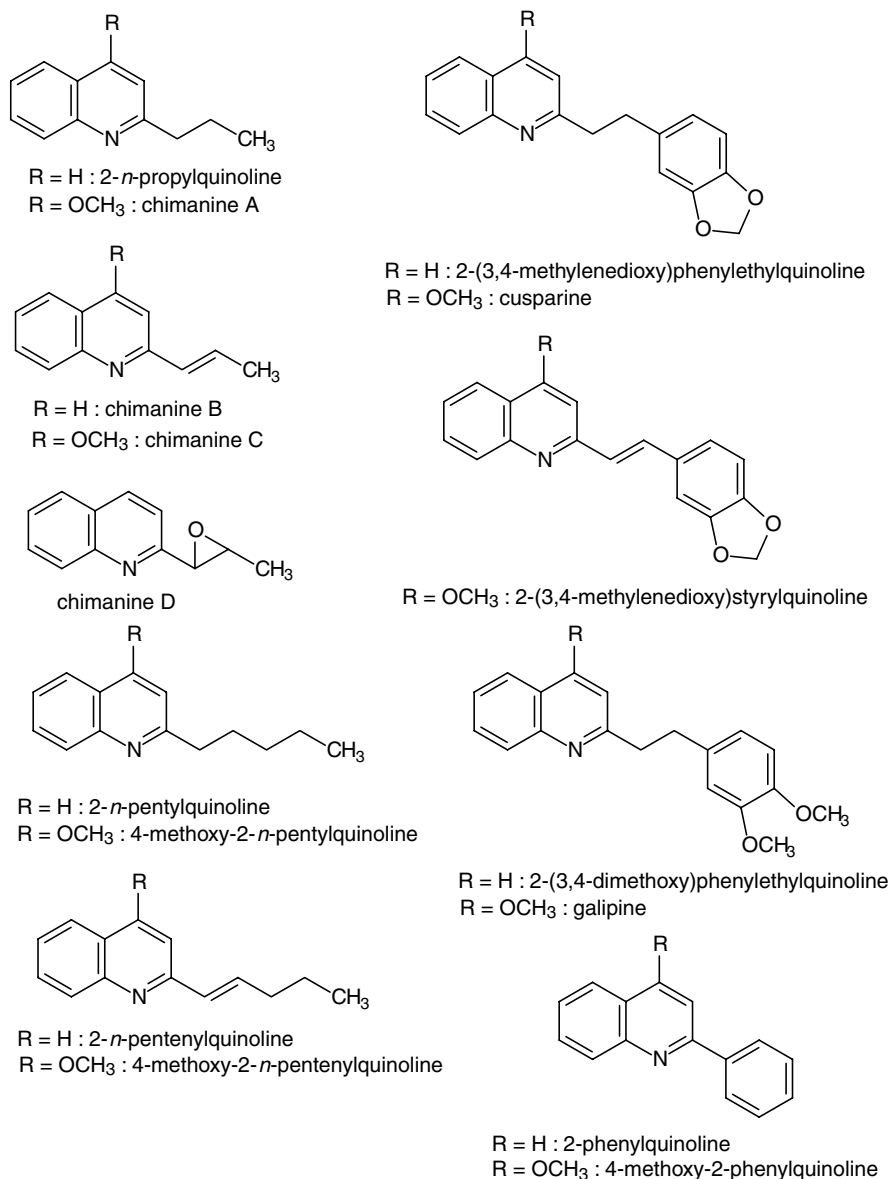


Figure 2. Structure of extracted 2-substituted quinoline alkaloids isolated from *Galipea* species.

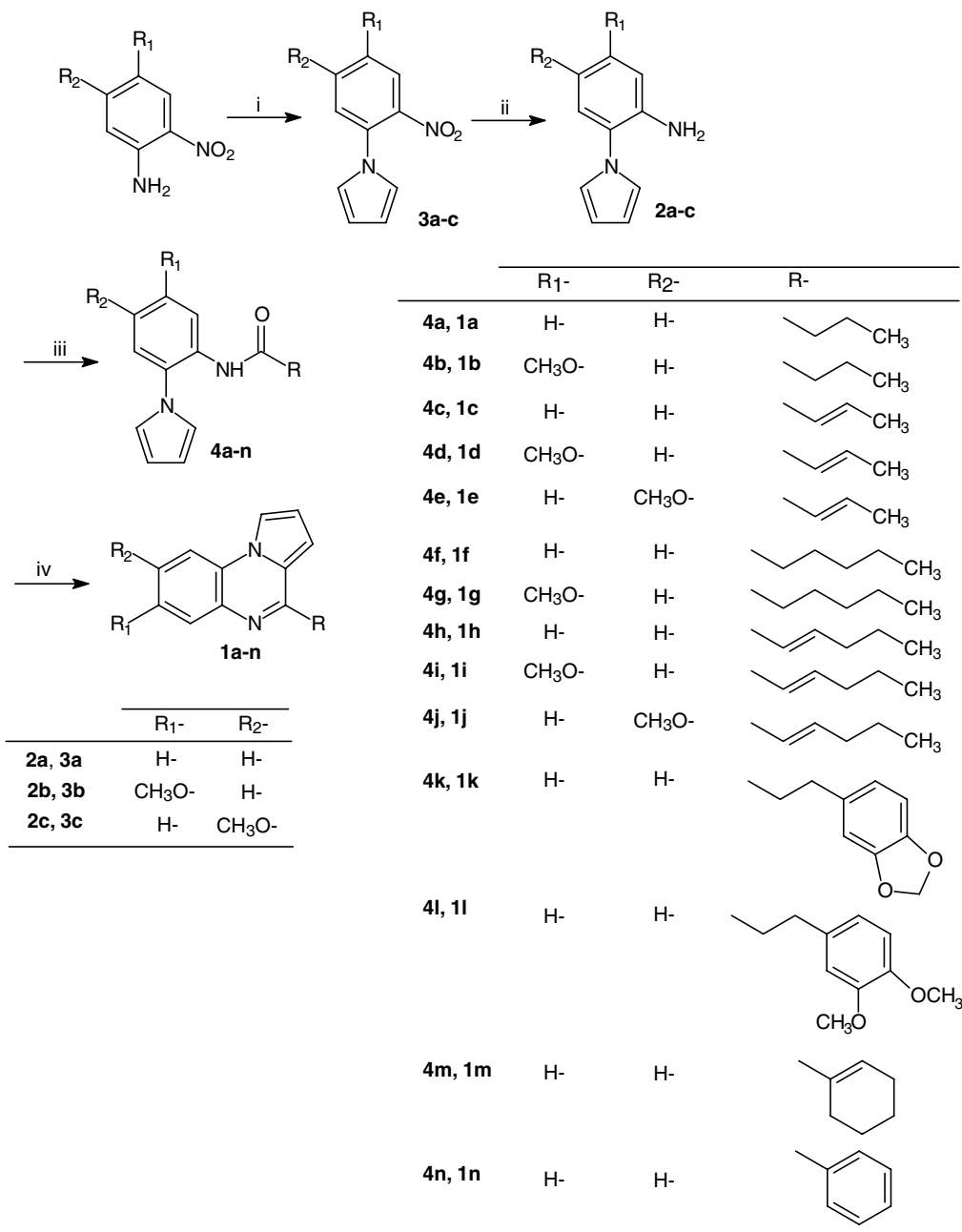
Galipea species. These results were exploited for SAR studies that enlightened the influence of the nature of the 4-substituted alkenyl side chain on the pyrrolo[1,2-*a*]quinoxaline moiety for the modulation of antiparasitic activity. These preliminary results suggest that 4-alkenylpyrrolo[1,2-*a*]quinoxalines could be further developed as potential antileishmanial drugs.

2. Results and discussion

2.1. Chemistry

All of the reported 4-substituted pyrrolo[1,2-*a*]quinoxaline derivatives **1a–n** were obtained from 1-(2-aminophenyl)pyrroles **2a–c**. Preparation of the latter was performed in acetic acid according to the Clauson–Kaas reaction starting from 2-nitroanilines and 2,5-dimethoxytetrahydrofuran (DMTHF). The resulting

1-(2-nitrophenyl)pyrroles intermediates **3a–c** were subsequently reduced into the attempted 1-(2-aminophenyl)pyrroles **2a–c** using a $BiCl_3$ – $NaBH_4$ treatment (Scheme 1). Not commercially available 5-methoxy-2-nitroaniline was prepared according to the literature.^{38,39} The reaction of various alkyl-, alkenyl-, or aryl-acid chlorides with **2a–d** led to the acetamides **4a–n**. The 4-substituted pyrrolo[1,2-*a*]quinoxalines **1a–n** were prepared by cyclisation of these amides **4a–n** in refluxing phosphorus oxychloride according to the Bischler–Napieralski reaction.⁴⁰ All the synthesized compounds were characterized by nuclear magnetic resonance (1H and ^{13}C NMR). The 4-propenyl derivatives **1c–e** were afforded as single *E* isomers. Thus, their 1H NMR spectra showed the characteristic chemical shifts of a *trans*-propenyl chain at 7.25–7.08 ppm (1H, qd, $J = 16.50$ – 15.35 and 6.80 – 6.70 Hz), 6.88–6.82 ppm (1H, dd, $J = 16.50$ – 15.35 and 1.70 – 1.25 Hz), and 2.08–2.01 ppm (3H, dd, $J = 6.80$ – 6.70 and 1.70 – 1.25 Hz). A

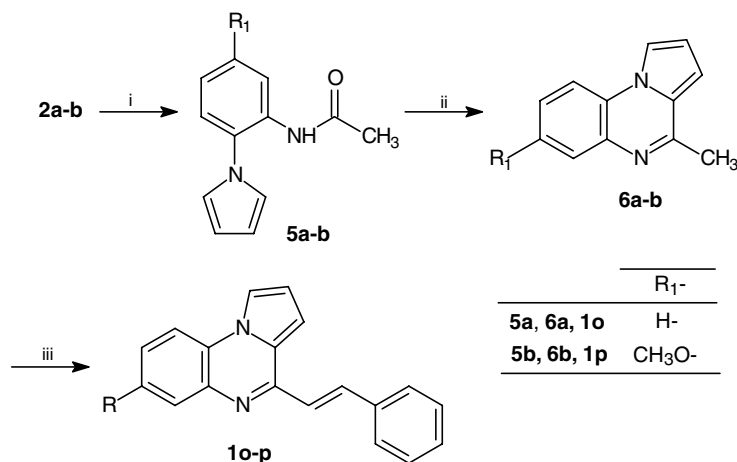


Scheme 1. Synthesis of 4-substituted pyrrolo[1,2-*a*]quinoxaline derivatives **1a–n**. Reagents and conditions: (i) DMTHF, AcOH, Δ ; (ii) BiCl₃, NaBH₄, EtOH; (iii) RCOCl, pyridine, dioxane, Δ ; (iv) 1—POCl₃, toluene, Δ ; 2—NaHCO₃, H₂O, rt.

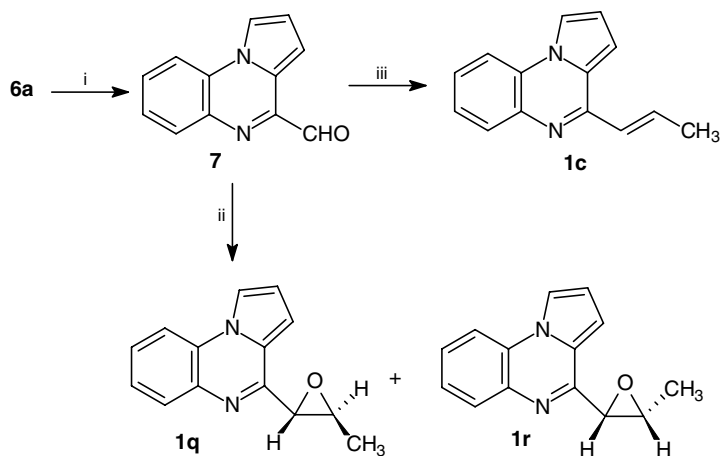
strains with chimanine B as the reference (Table 2). Amphotericin B was also used in these tests as the reference standard drug. The pyrroloquinoxalines **1c–e**, **1h–k**, **1s,t** and **1v** were found the most active compounds against the promastigote forms of *L. amazonensis* with IC₅₀ from 0.5 to 7 μ M. In particular, **1c**, designed as a chimanine B structural analogue, was found to be 10 times more active than the reference alkaloid (IC₅₀ = 0.5 μ M vs IC₅₀ = 5 μ M). The 7-methoxy and 8-methoxy-4-(*E*)-propenylpyrroloquinoxalines **1d,e** showed an IC₅₀ of 7 and 1 μ M, respectively. The introduction of a (*Z*)-propenyl or vinyl substituent on position C-4 of the pyrroloquinoxaline moiety (compounds **1s,t**) seems to slightly decrease the activity in compari-

son with compound **1c** with IC₅₀ = 4 and 3 μ M, respectively.

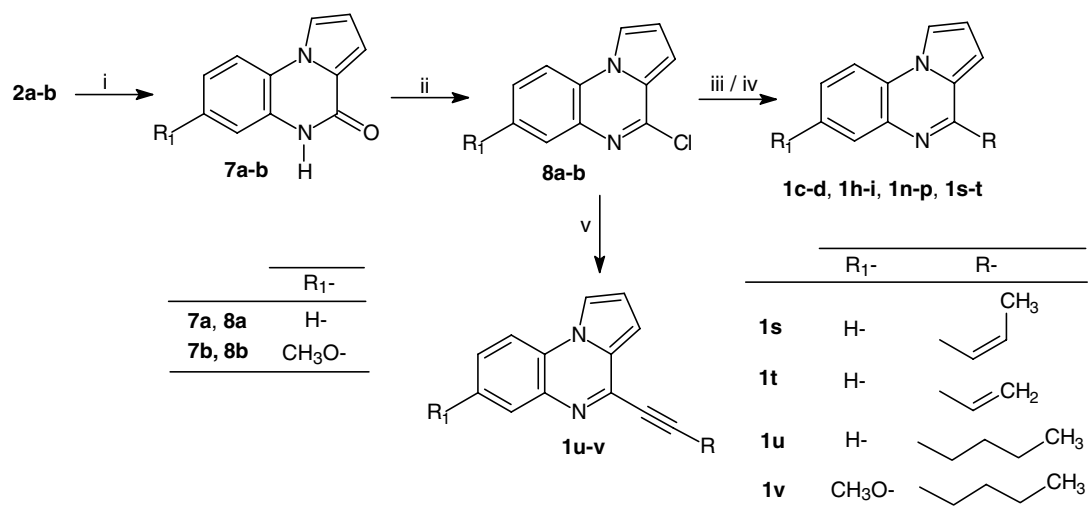
With respect to **1c**, the alkenyl chain replacement by a styryl one in compounds **1o,p** induced an activity loss, that is, IC₅₀ = 35 and 36 μ M for **1o,p**, respectively, whereas the increase of the carbon alkenyl chain length in **1h–j** gave slightly less potent compounds (IC₅₀ = 4–6 μ M). If the alkenyl function is found in a restricted configuration as observed in the cyclohexenyl ring (**1m**), this compound was found inactive until 50 μ M. On the other hand, the replacement of a pentenyl chain by an hexenyl one in compounds **1u,v** did not increase the antileishmanial activity for **1u** (IC₅₀ = 18 μ M), but is less detrimental for **1v**



Scheme 2. Synthesis of 4-substituted pyrrolo[1,2-*a*]quinoxaline derivatives **1o,p**. Reagents and conditions: (i) CH₃COCl, pyridine, dioxane, Δ; (ii) 1—POCl₃, toluene, Δ; 2—NaHCO₃, H₂O, rt; (iii) C₆H₅-CHO, Ac₂O, Δ.



Scheme 3. Synthesis of 4-substituted pyrrolo[1,2-*a*]quinoxaline derivatives **1c** and **1q,r**. Reagents and conditions: (i) SeO₂, dioxane, Δ; (ii) Et₃SBF₄, KOH, MeCN/H₂O; (iii) Et(C₆H₅)₃PBr, *t*-BuOK, toluene, 0 °C then Δ.



Scheme 4. Synthesis of 4-substituted pyrrolo[1,2-*a*]quinoxaline derivatives **1c,d, 1h,i, 1n-p, 1s,t** and **1u,v**. Reagents and conditions: (i) CO(OCCH₃)₂, toluene, Δ; (ii) POCl₃, Δ; (iii) R-B(OH)₂, Pd[P(C₆H₅)₃]₄, Na₂CO₃, C₆H₆, Δ; (iv) H₂C=CH-B(O-*n*-Bu)₂, KOH; Pd[P(C₆H₅)₃]₄, C₆H₆, Δ; (v) PdCl₂[P(C₆H₅)₃]₂, HC≡C-R, CuI, TEA, Δ.

Table 1. Physical properties of the final amines **1**

Compound	p <i>K</i> _a	log <i>D</i> _{7.4}	Salt ^a	Mp (°C) of the salts or bases	% yield
1a	9.19	3.35	HCl	132	91
1b	9.45	3.40	HCl	139	82
1c	9.59	3.44	HCl	170	89
1d	9.84	3.47	HCl	90	63
1e	9.72	3.48	HCl	156	77
1f	9.12	4.04	HCl	124	94
1g	9.37	4.38	HCl	101	68
1h	9.63	4.44	HCl	121	52
1i	9.88	4.52	HCl	155	56
1j	9.49	4.30	HCl	71	84
1k	9.25	3.84	HCl	157	92
1l	9.17	2.97	HCl	124	90
1m	9.08	4.12	HCl	88	85
1n	9.86	3.60	HCl	123	75
1o	9.51	4.84	HCl	157	70
1p	9.76	5.34	HCl	119	90
1q	10.98	2.61	— ^b	62	40
1r	10.98	2.55	— ^b	—	13
1s	9.47	3.30	— ^b	—	51
1t	9.52	3.08	HCl	>300	65
1u	9.28	4.36	HCl	91	80
1v	9.53	4.35	HCl	100	60
Chimanine B	4.67	2.23	HCl	129	89

^a The 4-substituted pyrrolo[1,2-*a*]quinoxalines **1** were dissolved in 25 mL of Et₂O and treated with hydrochloric acid gas. The hydrochloride salts which precipitated were collected by filtration and were washed with Et₂O.

^b Compound tested as a free base.

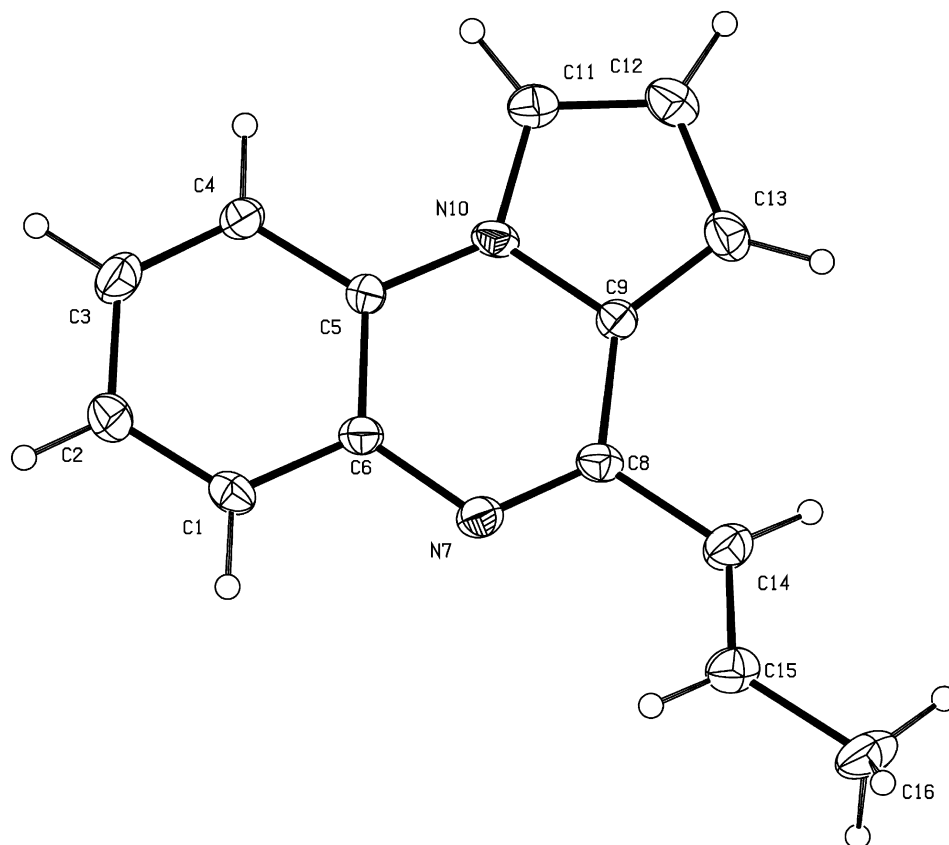
**Figure 4.** The ORTEP drawing of 4-[(*E*)-prop-1-enyl]pyrrolo[1,2-*a*]quinoxaline **1c** with thermal ellipsoids at 30% level.

Table 2. In vitro sensitivity of *Leishmania amazonensis* and *L. infantum* strains, and cytotoxicity on human peripheral blood mononuclear cells PMNC + PHA

Compound	IC ₅₀ values (μM) ^a		Index of selectivity ^b			
	<i>Leishmania</i> strains		Cytotoxicity on human peripheral blood mononuclear cells (PMNC)		<i>L. amazonensis</i>	<i>L. infantum</i>
	<i>L. amazonensis</i> promastigotes	<i>L. infantum</i> promastigotes	PMNC	PMNC + PHA		
Amphotericin B	1 ± 0.2	0.9 ± 0.1	>100	96 ± 10	96	106.6
Chimarine B	5 ± 1	7 ± 2	43 ± 3	42 ± 4	8.4	6
1a	>50	>50	>100	100 ± 6		
1b	>50	>50	>100	90 ± 10		
1c	0.5 ± 0.1	0.7 ± 0.1	6 ± 0.3	5 ± 0.4	10	7.1
1d	7 ± 1	2 ± 0.3	5 ± 1	5 ± 0.5	0.7	2.5
1e	1 ± 0.1	0.5 ± 0.1	7 ± 0.8	5 ± 0.3	5	10
1f	>50	>50	>100	30 ± 3	<1	<1
1g	>50	>50	66 ± 4	45 ± 5	<1	<1
1h	5 ± 0.2	7 ± 0.6	70 ± 12	52 ± 3	10.4	7.4
1i	6 ± 1	1 ± 0.1	60 ± 5	40 ± 4	6.7	40
1j	4 ± 0.2	2.5 ± 0.4	55 ± 5	30 ± 4	7.5	12
1k	7 ± 0.8	>50	>100	65 ± 5	9.3	
1l	30 ± 6	>50	>100	>100	>3.3	
1m	>50	>50	>100	>100		
1n	>50	>50	>100	>100		
1o	35 ± 10	>50	>100	>100	>2.9	
1p	36 ± 9	5 ± 0.6	>100	>100	>2.8	>20
1q	>50	>50	>100	>100		
1r	>50	>50	>100	>100		
1s	4 ± 0.3	0.8 ± 0.1	8 ± 0.9	6 ± 0.9	1.5	7.5
1t	3 ± 0.3	0.5 ± 0.1	3 ± 0.2	1 ± 0.2	0.3	2
1u	18 ± 6	25 ± 6	50 ± 6	25 ± 3	1.4	1
1v	3 ± 0.1	7 ± 1	6 ± 0.3	5 ± 0.3	1.7	0.7

^a IC₅₀ values were measured on the promastigotes of the *Leishmania amazonensis* (MHOM/BR/1987/BA) and *L. infantum* (clone MCAN/GR/82/LEM497) strains. The IC₅₀ (μM) values correspond to means ± SD from three independent experiments.

^b Index of selectivity (I.S.) was defined as the ratio between the IC₅₀ value on the PMNC + PHA cells and the IC₅₀ value against the *L. amazonensis* and *L. infantum* strain.

(3 μM). A total loss of activity was observed for compounds **1a,b** and **1f,g** possessing a three- or five-carbon saturated side chain (IC₅₀ > 50 μM).

With the galipine as a model we designed then compounds **1k,l** with a phenethyl-substituted C-4 chain. Whereas the substituted 4-(3,4-dimethoxyphenylethyl) compound **1l** was found with a moderate activity (IC₅₀ = 30 μM), surprisingly the 4-(3,4-methylenedioxyphenylethyl)pyrrolo[1,2-*a*]quinoxaline **1k** exhibited a potent anti-*L. amazonensis* activity with a 7 μM IC₅₀ value. Moreover, the *trans* epoxide **1q**, a structural analogue of chimanine D, was found inactive until 50 μM against *L. amazonensis*, as its *cis* epoxide homologue **1r**. Finally, compound **1n**, with a phenyl group in position 4, did not show any antileishmanial activity.

From a SAR point of view, these pharmacological results enlighten the importance of the nature of the 4-lateral chain on the pyrrolo[1,2-*a*]quinoxaline moiety, in our series. Hence, an α-unsaturated (*trans*- or *cis*-alkenyl or alkynyl) lateral chain induces a notable increase in the activity. On the other hand, the substitution at position 7 or 8 of the pyrrolo[1,2-*a*]quinoxaline heterocycle by one methoxy group is not crucial as illustrated by results for compounds **1b**, **1d,e**, **1g** and **1i,j** in comparison with those of their unsubstituted homologues (com-

pounds **1a**, **1c**, **1f** and **1h**). Only the 7-methoxysubstituted 4-alkynyl derivative **1v** was more potent than its unsubstituted analogue **1u** (IC₅₀ = 3 compared with 18 μM for **1u**).

A second evaluation was achieved against the promastigote forms of *L. infantum* in an experimental visceral leishmaniasis model, with chimanine B as the reference (Table 2). Compounds **1d**, **1h–j**, **1p** and **1v** showed almost the same level of activity with IC₅₀ ranging from 1 to 7 μM. It must be noticed that four compounds **1c**, **1e**, **1s** and **1t** showed an IC₅₀ < 1 μM, more potent than the reference (IC₅₀ = 7 μM). Hence, the introduction of a methoxy substituent in position C-7 or C-8 of the 4-alkenyl or 4-alkynylpyrrolo[1,2-*a*]quinoxaline moiety resulted in the increase of the antileishmanial activity in comparison with their respective unsubstituted 4-alkenyl or 4-alkynylpyrrolo[1,2-*a*]quinoxaline homologues (i.e., **1e** compared to **1c**, **1i,j** to **1h**, **1p** to **1o** and **1v** to **1u**).

In summary, except for **1p** and **1k**, all compounds showed almost the same level of activity on *L. amazonensis* and on *L. infantum* promastigotes. The mechanism by which these new 4-substituted pyrrolo[1,2-*a*]quinoxalines are active against *Leishmania* parasites is unknown. Further pharmacological studies should now be investigated to determine the target of action.

2.3. Cytotoxicity

All compounds **1a–v** were tested on activated (PBMC + PHA) and not activated (PBMC) human peripheral blood mononuclear cells (Table 2). Not activated cells were considered as control measurements. As expected, most of the already inactive pyrrolo[1,2-*a*]quinoxalines showed a low cytotoxicity against monocytes ($IC_{50} > 100 \mu M$), whereas cytotoxicity was noticed for most active ones. However, among these last compounds, the most active (*E*)-propenylpyrroloquinoxaline **1c** on *L. amazonensis* strain exhibited a higher level of cytotoxicity against host cells ($IC_{50} = 5 \mu M$). Such an observation was also noticed for the active (*Z*)-propenyl and vinyl compounds **1s,t** (IC_{50} of 6 and $1 \mu M$, respectively). Moreover, the (*E*)-propenylpyrrolo[1,2-*a*]quinoxalines **1d,e**, both substituted by a methoxy function in position 7 or 8, also showed high cytotoxicity ($IC_{50} = 5 \mu M$, both), while their (*E*)-pentenyl analogues **1i,j** displayed IC_{50} values of 40 and $30 \mu M$, respectively. Otherwise, the unsubstituted pentenylpyrroloquinoxaline **1h** was also found less toxic than its methoxy substituted analogues **1i,j** ($IC_{50} = 52 \mu M$). Data also indicated that the introduction of hexynyl group in **1u,v** increased the toxicity ($IC_{50} = 25$ and $5 \mu M$, respectively). The other different substitutions on the pyrrolo[1,2-*a*]quinoxaline moiety seem to have less influence on the PBMC + PHA IC_{50} values than those observed for the antileishmanial activity.

Index of selectivity (IS) was defined as the ratio of the IC_{50} value on the human mononuclear cells to the IC_{50} value on the *L. amazonensis* or *L. infantum* strains (promastigotes). This led to the identification of compound **1p** with $IS > 20$ on *L. infantum*. On the other hand, the (*E*)-pentenylpyrrolo[1,2-*a*]quinoxalines **1h–j** are remarkable with $IS =$ between 7 and 40, on both strains. These four molecules would constitute suitable pharmacophores for the design of new candidates in forthcoming pharmacological investigations.

2.4. Lipophilicity

The activity of an ionizable drug is determined by two major parameters: the partition coefficient $\log P$ between the aqueous and the lipid phases and its modification by the ionization constant pK_a of the drug. For partially ionized compounds, the partition coefficient between two phases at any fixed pH is called the distribution coefficient D , usually expressed as $\log D$.⁵³ Here, for all new synthesized compounds, $\log D$ was determined by an HPLC method at pH 7.4, considered as the biological medium pH⁵⁴ and the acidity constant pK_a calculated using Pallas® program (Table 1).⁵⁵ This allowed us to calculate the partial ionization percentage according to the Henderson–Hasselbach equation⁵⁶ at pH 7.4, always found inferior than 2% except for chimanine B (>99%).

$\log D$ values were found between 2.23 for the reference chimanine B and 5.34 for **1p**. As expected the highest lipophilic pyrroloquinoxalines **1** ($\log D > 4.0$) present a substituent in position 4 of the heterocyclic moiety that is either a 5- or 6-carbon atom chain (**1f–j** and **1u,v**) or a bulky group (**1l–p**).

A plot of antileishmanial IC_{50} versus $\log D$ values is presented in Figure 5, permitting to classify compounds in various subsets. The two more lipophilic (**1o,p**) and the three less lipophilic (**1r, 1q** and **1l**) are almost inactive while the following **1t** and **1s** are active. $\log D$ values of compounds **1a,b** substituted by a three carbon saturated linear chain and of their homologues **1c–e** substituted by a three-carbon unsaturated chain are in the same range (3.35–3.48), but not their activity. The four compounds (**1n, 1k, 1f** and **1m**) with $\log D$ between 3.60 and 4.12 are inactive. An heterogeneous behaviour was found for compounds with $\log D$ between 4.30 and 4.52 as **1j, 1v, 1u, 1h** and **1i** are active while **1g** is devoid of appreciable potency.

These results clearly indicated that the choice and the introduction of an α -unsaturation in position 4 of the pyrrolo[1,2-*a*]quinoxaline moiety correlated with a particular lipophilic behaviour seem to be fundamental

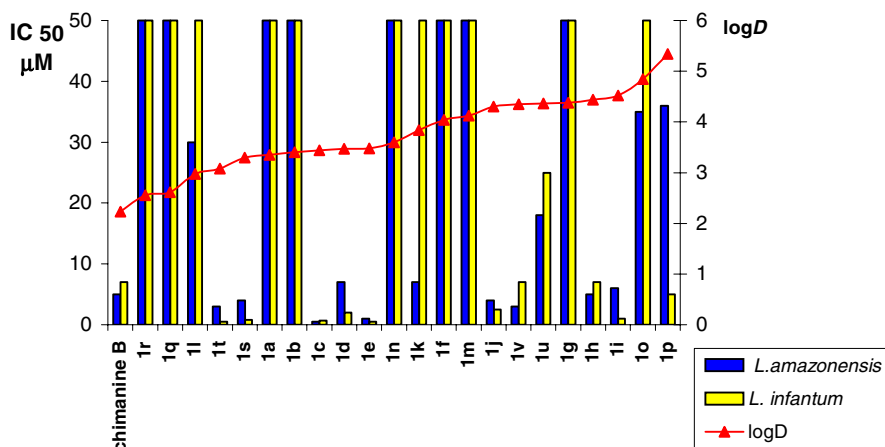


Figure 5. $\log D$ /activity relationship for pyrrolo[1,2-*a*]quinoxalines **1a–v**.

for the antileishmanial activity observed for this new series.

3. Conclusion

In conclusion, new 4-substituted pyrrolo[1,2-*a*]quinoxaline derivatives were designed as new structural analogues of *Galipea* species quinoline alkaloids. They were synthesized via Bischler-Napieralski, Perkin, Corey-Chaykovsky, Wittig, Suzuki or Sonogashira reactions, and tested for their *in vitro* antiparasitic activity on two strains: *L. amazonensis* and *L. infantum*. The use of the palladium-catalyzed Suzuki cross-coupling reactions enabled one to access easily to new substituted 4-alkenylpyrrolo[1,2-*a*]quinoxaline derivatives, more difficultly obtained through a classical 4-substituted pyrrolo[1,2-*a*]quinoxaline synthetic pathway.

The biological results obtained with chimanine B as the reference highlighted the crucial role of C-4 substitution introduced on the pyrrolo[1,2-*a*]quinoxaline nucleus, on the antileishmanial activity modulation. Hence, an α -unsaturated (*trans*- or *cis*-alkenyl or alkynyl) lateral chain induces a notable increase in the antiparasitic activity on both strains. On the other hand, the advantage of the substitution at position 7 or 8 of the pyrrolo[1,2-*a*]quinoxaline heterocycle by one methoxy group depends on the studied strain, that is, it is only beneficial for the visceral *L. infantum* strain.

The physico-chemical profile of new 4-substituted pyrrolo[1,2-*a*]quinoxaline derivatives was studied through log*D* HPLC determination achieved at physiological pH and *pK_a* calculation through an *in silico* methodology. These results have been discussed in terms of lipophilic behaviour for a preliminary SAR study. Although antiparasitic activity is generally related to the distribution of studied compounds to the intracellular target it was not possible to establish pertinent and definitive correlation. Nevertheless, it appeared that pyrrolo[1,2-*a*]quinoxalines endowed with lipophilic unsaturated carbon chain could open the way to new valuable antileishmanial chemistry scaffolding. This will be the target of further investigations.

4. Experimental

4.1. Chemistry

Commercial reagents were used as received without additional purification. Melting points were determined with an SM-LUX-POL Leitz hot-stage microscope and are uncorrected. IR spectra were recorded on a BRUKER IFS-25 spectrophotometer. NMR spectra were recorded with tetramethylsilane as an internal standard using a BRUKER AVANCE 300 spectrometer (¹H, ¹³C, 2D-COSY). Splitting patterns have been designated as follows: s, singlet; br s, broad singlet; d, doublet; dd, double doublet; qd, quadruple doublet; t, triplet; q, quartet; qt, quintuplet; sex, sextuplet; m, multiplet. Analytical TLC was carried out on 0.25 precoated silica gel

plates (POLYGRAM SIL G/UV₂₅₄) with visualisation by irradiation with a UV lamp. Silica gel 60 (70–230 mesh) was used for column chromatography. Elemental analyses (C, H, N) for new compounds were performed by CNRS (Vernaison-France) and agreed with the proposed structures within $\pm 0.3\%$ of the theoretical values. Chimanine B was synthesized in analogy to described procedure.⁴⁵

4.1.1. General procedure for 1-(2-alkanoyl- or 2-alkenoyl- or 2-benzoylaminophenyl)pyrrole (4a–n). To a solution of 1-(2-aminophenyl)pyrrole **2a–c** (0.02 mol) in dioxane (80 mL) were added pyridine (0.022 mol) then propanoyl chloride (0.02 mol). The reaction mixture was refluxed for 4 h and the solvent then removed under reduced pressure. The residue was triturated with water and extracted with diethyl ether (2 \times 80 mL). The organic layers were collected, washed with an aqueous sodium hydrogen carbonate solution (100 mL) then with water (100 mL), dried over Na₂SO₄ and evaporated to dryness. The precipitate was crystallized from petroleum ether to afford **4a–n**.

4.1.1.1. 1-(2-Propanoylaminophenyl)pyrrole (4a). White crystals (60%); mp 48 °C; IR (KBr) 3200 (NH), 1650 (C=O); ¹H NMR (CDCl₃) δ : 8.38 (d, 1H, *J* = 7.75 Hz, H-3), 7.40 (t, 1H, *J* = 7.75 Hz, H-4), 7.32 (d, 1H, *J* = 7.75 Hz, H-6), 7.16 (t, 1H, *J* = 7.75 Hz, H-5), 6.94 (br s, 1H, NH), 6.76 (dd, 2H, *J* = 1.95 and 1.95 Hz, H- α), 6.39 (dd, 2H, *J* = 1.95 and 1.95 Hz, H- β), 2.17 (t, 2H, *J* = 7.35 Hz, CH₂), 1.59 (sex, 2H, *J* = 7.35 Hz, CH₂), 0.92 (t, 3H, *J* = 7.35 Hz, CH₃); ¹³C NMR (CDCl₃) δ : 171.2 (C=O), 133.9 (C-1), 130.5 (C-2), 128.7 (C-6), 126.7 (C-3), 123.9 (C-5), 121.9 (C- α), 121.4 (C-4), 110.4 (C- β), 39.7 (CH₂), 18.7 (CH₂), 13.6 (CH₃). Anal. Calcd for C₁₄H₁₆N₂O: C, 73.65; H, 7.06; N, 12.27. Found: C, 73.69; H, 6.91; N, 12.36.

4.1.1.2. 1-(4-Methoxy-2-propanoylaminophenyl)pyrrole (4b). Pale-yellow crystals (78%); mp 51 °C; IR (KBr) 3240 (NH), 1655 (C=O); ¹H NMR (CDCl₃) δ : 8.10 (d, 1H, *J* = 2.75 Hz, H-3), 7.16 (d, 1H, *J* = 8.70 Hz, H-6), 6.89 (br s, 1H, NH), 6.71 (dd, 2H, *J* = 2.05 and 2.05 Hz, H- α), 6.65 (dd, 1H, *J* = 8.70 and 2.75 Hz, H-5), 6.36 (dd, 2H, *J* = 2.05 and 2.05 Hz, H- β), 3.84 (s, 3H, CH₃O), 2.16 (t, 2H, *J* = 7.35 Hz, CH₂), 1.60 (sex, 2H, *J* = 7.35 Hz, CH₂), 0.91 (t, 3H, *J* = 7.35 Hz, CH₃). Anal. Calcd for C₁₅H₁₈N₂O₂: C, 69.74; H, 7.02; N, 10.85. Found: C, 69.65; H, 6.85; N, 10.98.

4.1.1.3. 1-(2-*trans*-Crotonylaminophenyl)pyrrole (4c). White crystals (59%); mp 58 °C; IR (KBr) 3240 (NH), 1655 (C=O); ¹H NMR (CDCl₃) δ : 8.47 (d, 1H, *J* = 8.25 Hz, H-3), 7.36 (t, 1H, *J* = 8.25 Hz, H-4), 7.25 (d, 1H, *J* = 8.25 Hz, H-6), 7.12 (t, 1H, *J* = 8.25 Hz, H-5), 6.99 (br s, 1H, NH), 6.89 (qd, 1H, *J* = 15.15 and 6.90 Hz, =CH), 6.81 (dd, 2H, *J* = 2.10 and 2.10 Hz, H- α), 6.39 (dd, 2H, *J* = 2.10 and 2.10 Hz, H- β), 5.70 (dd, 1H, *J* = 15.15 and 1.65 Hz, HC=), 1.84 (dd, 3H, *J* = 6.90 and 1.65 Hz, CH₃); ¹³C NMR (CDCl₃) δ : 163.9 (CO), 141.8 (=CH), 134.1 (C-1), 130.7 (C-2), 128.8 (HC=), 126.9 (C-6), 125.6 (C-3), 124.1 (C-5),

122.1 (C- α), 121.6 (C-4), 110.6 (C- β), 17.9 (CH₃). Anal. Calcd for C₁₄H₁₄N₂O: C, 74.31; H, 6.23; N, 12.38. Found: C, 74.41; H, 6.28; N, 12.47.

4.1.1.4. 1-[2-(*trans*-Crotonylamino)-4-methoxyphenyl]pyrrole (4d). White crystals (71%); mp 71 °C; IR (KBr) 3240 (NH), 1665 (C=O); ¹H NMR (CDCl₃) δ : 8.18 (d, 1H, *J* = 2.75 Hz, H-3), 7.16 (d, 1H, *J* = 8.70 Hz, H-6), 6.92 (br s, 1H, NH), 6.84 (qd, 1H, *J* = 15.15 and 6.85 Hz, =CH), 6.72 (dd, 2H, *J* = 1.95 and 1.95 Hz, H- α), 6.65 (dd, 1H, *J* = 8.70 and 2.75 Hz, H-5), 6.38 (dd, 2H, *J* = 1.95 and 1.95 Hz, H- β), 5.65 (dd, 1H, *J* = 15.15 and 1.50 Hz, HC=), 3.84 (s, 3H, CH₃O), 1.83 (dd, 3H, *J* = 6.85 and 1.50 Hz, CH₃). Anal. Calcd for C₁₅H₁₆N₂O₂: C, 70.29; H, 6.29; N, 10.93. Found: C, 70.23; H, 6.20; N, 10.99.

4.1.1.5. 1-[2-(*trans*-Crotonylamino)-5-methoxyphenyl]pyrrole (4e). Pale-yellow crystals (60%); mp 127 °C; IR (KBr) 3230 (NH), 1650 (C=O); ¹H NMR (CDCl₃) δ : 8.38 (d, 1H, *J* = 8.15 Hz, H-3), 6.94–6.76 (m, 6H, H-4, H-6, NH, =CH and H- α), 6.37 (dd, 2H, *J* = 1.90 and 1.90 Hz, H- β), 5.71 (dd, 1H, *J* = 15.15 and 1.35 Hz, HC=), 3.79 (s, 3H, CH₃O), 1.84 (dd, 3H, *J* = 6.85 and 1.35 Hz, CH₃). Anal. Calcd for C₁₅H₁₆N₂O₂: C, 70.29; H, 6.29; N, 10.93. Found: C, 70.36; H, 6.45; N, 11.14.

4.1.1.6. 1-(2-Hexanoylamino-4-methoxyphenyl)pyrrole (4f). White crystals (71%); mp 40 °C; IR (KBr) 3220 (NH), 1645 (C=O); ¹H NMR (CDCl₃) δ : 8.37 (d, 1H, *J* = 7.70 Hz, H-3), 7.36 (t, 1H, *J* = 7.70 Hz, H-4), 7.25 (d, 1H, *J* = 7.70 Hz, H-6), 7.13 (t, 1H, *J* = 7.70 Hz, H-5), 7.00 (br s, 1H, NH), 6.77 (dd, 2H, *J* = 1.80 and 1.80 Hz, H- α), 6.38 (dd, 2H, *J* = 1.80 and 1.80 Hz, H- β), 2.19 (t, 2H, *J* = 7.00 Hz, CH₂), 1.57 (m, 4H, CH₂), 1.27 (m, 4H, CH₂), 0.87 (t, 3H, *J* = 7.00 Hz, CH₃); ¹³C NMR (CDCl₃) δ : 172.1 (CO), 134.4 (C-1), 131.1 (C-2), 129.3 (C-6), 127.3 (C-3), 124.6 (C-5), 122.6 (C- α), 122.0 (C-4), 110.9 (C- β), 38.4 (CH₂), 31.8 (CH₂), 25.6 (CH₂), 22.9 (CH₂), 14.5 (CH₃). Anal. Calcd for C₁₆H₂₀N₂O: C, 74.96; H, 7.86; N, 10.93. Found: C, 75.06; H, 7.95; N, 11.12.

4.1.1.7. 1-(2-Hexanoylamino-4-methoxyphenyl)pyrrole (4g). Pale-yellow crystals (74%); mp 44 °C; IR (KBr) 3220 (NH), 1645 (C=O); ¹H NMR (CDCl₃) δ : 8.09 (d, 1H, *J* = 2.70 Hz, H-3), 7.16 (d, 1H, *J* = 8.65 Hz, H-6), 6.90 (s, 1H, NH), 6.71 (dd, 2H, *J* = 1.85 and 1.85 Hz, H- α), 6.64 (dd, 1H, *J* = 8.65 and 2.70 Hz, H-5), 6.36 (dd, 2H, *J* = 1.85 and 1.85 Hz, H- β), 3.83 (s, 3H, CH₃O), 2.15 (t, 2H, *J* = 6.80 Hz, CH₂), 1.55 (m, 2H, CH₂), 1.26 (m, 4H, CH₂), 0.86 (t, 3H, *J* = 6.80 Hz, CH₃). Anal. Calcd for C₁₇H₂₂N₂O₂: C, 71.30; H, 7.74; N, 9.78. Found: C, 71.42; H, 7.96; N, 10.03.

4.1.1.8. 1-(2-*trans*-Hexenoylamino-4-methoxyphenyl)pyrrole (4h). White crystals (72%); mp 46 °C; IR (KBr) 3210 (NH), 1640 (C=O); ¹H NMR (CDCl₃) δ : 8.48 (d, 1H, *J* = 7.75 Hz, H-3), 7.37 (t, 1H, *J* = 7.75 Hz, H-4), 7.26 (d, 1H, *J* = 7.75 Hz, H-6), 7.13 (t, 1H, *J* = 7.75 Hz, H-5), 7.01 (br s, 1H, NH), 6.83 (m, 1H, =CH), 6.78 (dd,

2H, *J* = 2.15 and 2.15 Hz, H- α), 6.40 (dd, 2H, *J* = 2.15 and 2.15 Hz, H- β), 5.66 (dd, 1H, *J* = 15.45 and 1.15 Hz, HC=), 2.14 (m, 2H, CH₂), 1.45 (sex, 2H, *J* = 7.35 Hz, CH₂), 0.91 (t, 3 H, *J* = 7.35 Hz, CH₃); ¹³C NMR (CDCl₃) δ : 163.9 (CO), 146.6 (=CH), 133.9 (C-1), 130.4 (C-2), 128.7 (C-6), 126.7 (HC=), 123.9 (C-3), 123.8 (C-5), 122.0 (C- α), 121.3 (C-4), 110.4 (C- β), 34.0 (CH₂), 21.4 (CH₂), 13.6 (CH₃). Anal. Calcd for C₁₆H₁₈N₂O: C, 75.56; H, 7.13; N, 11.01. Found: C, 75.52; H, 7.21; N, 10.93.

4.1.1.9. 1-[2-(*trans*-Hexenoylamino)-4-methoxyphenyl]pyrrole (4i). Pale-yellow crystals (61%); mp 51 °C; IR (KBr) 3215 (NH), 1640 (C=O); ¹H NMR (CDCl₃) δ : 8.19 (d, 1H, *J* = 2.80 Hz, H-3), 7.16 (d, 1H, *J* = 8.65 Hz, H-6), 6.94 (br s, 1H, NH), 6.81 (m, 1H, =CH), 6.73 (dd, 2H, *J* = 1.90 and 1.90 Hz, H- α), 6.68 (dd, 1H, *J* = 8.65 and 2.80 Hz, H-5), 6.37 (dd, 2H, *J* = 1.90 and 1.90 Hz, H- β), 5.63 (d, 1H, *J* = 15.25 Hz, HC=), 3.84 (s, 3H, CH₃O), 2.15 (m, 2H, CH₂), 1.44 (sex, 2H, *J* = 7.40 Hz, CH₂), 0.90 (t, 3H, *J* = 7.40 Hz, CH₃). Anal. Calcd for C₁₇H₂₀N₂O₂: C, 71.80; H, 7.09; N, 9.85. Found: C, 71.84; H, 7.18; N, 9.77.

4.1.1.10. 1-[2-(*trans*-Hexenoylamino)-5-methoxyphenyl]pyrrole (4j). White crystals (60%); mp 82 °C; IR (KBr) 3200 (NH), 1645 (C=O); ¹H NMR (CDCl₃) δ : 8.28 (d, 1H, *J* = 8.75 Hz, H-3), 6.95 (m, 2H, H-4 and H-6), 6.87 (m, 2H, =CH and NH), 6.83 (dd, 2H, *J* = 1.95 and 1.95 Hz, H- α), 6.40 (dd, 2H, *J* = 1.95 and 1.95 Hz, H- β), 5.71 (d, 1H, *J* = 15.25 Hz, HC=), 3.82 (s, 3H, CH₃O), 2.16 (m, 2H, CH₂), 1.47 (sex, 2H, *J* = 7.35 Hz, CH₂), 0.93 (t, 3H, *J* = 7.35 Hz, CH₃). Anal. Calcd for C₁₇H₂₀N₂O₂: C, 71.80; H, 7.09; N, 9.85. Found: C, 71.95; H, 7.02; N, 9.97.

4.1.1.11. 1-[2-(3,4-Methylenedioxy)phenylethano-ylamino-phenyl]pyrrole (4k). White crystals (74%); mp 85 °C; IR (KBr) 3220 (NH), 1640 (C=O); ¹H NMR (CDCl₃) δ : 8.34 (d, 1H, *J* = 7.90 Hz, H-3), 7.38 (t, 1H, *J* = 7.90 Hz, H-4), 7.22 (d, 1H, *J* = 7.90 Hz, H-6), 7.13 (t, 1H, *J* = 7.90 Hz, H-5), 6.90 (br s, 1H, NH), 6.64 (m, 5H, H-2', H-5', H-6' and H- α), 6.35 (dd, 2H, *J* = 2.00 and 2.00 Hz, H- β), 5.89 (s, 2H, OCH₂O), 2.86 (t, 2H, *J* = 7.45 Hz, CH₂), 2.45 (t, 2H, *J* = 7.45 Hz, CH₂). Anal. Calcd for C₂₀H₁₈N₂O₃: C, 71.84; H, 5.42; N, 8.38. Found: C, 72.02; H, 5.56; N, 8.52.

4.1.1.12. 1-[2-(3,4-Dimethoxy)phenylethano-ylamino-phenyl]pyrrole (4l). White crystals (63%); mp 91 °C; IR (KBr) 3210 (NH), 1645 (C=O); ¹H NMR (CDCl₃) δ : 8.35 (d, 1H, *J* = 7.85 Hz, H-3), 7.38 (t, 1H, *J* = 7.85 Hz, H-4), 7.24 (d, 1H, *J* = 7.85 Hz, H-6), 7.14 (t, 1H, *J* = 7.85 Hz, H-5), 6.89 (br s, 1H, NH), 6.75 (m, 2H, H-5' and H-6'), 6.67 (d, 1H, *J* = 1.15 Hz, H-2'), 6.61 (dd, 2H, *J* = 1.90 and 1.90 Hz, H- α), 6.32 (dd, 2H, *J* = 1.90 and 1.90 Hz, H- β), 3.85 (s, 3H, CH₃O), 3.78 (s, 3H, CH₃O), 2.88 (t, 2H, *J* = 7.45 Hz, CH₂), 2.47 (t, 2H, *J* = 7.45 Hz, CH₂). Anal. Calcd for C₂₁H₂₂N₂O₃: C, 71.98; H, 6.33; N, 7.99. Found: C, 72.11; H, 6.27; N, 8.21.

4.1.1.13. 1-[2-(Cyclohex-1-enecarbonyl)aminophenyl]pyrrole (4m). White crystals (79%); mp 119 °C; IR (KBr) 3210 (NH), 1645 (C=O); ¹H NMR (CDCl₃) δ: 8.55 (d, 1H, *J* = 7.85 Hz, H-3), 7.42 (t, 1H, *J* = 7.85 Hz, H-4), 7.33 (br s, 1H, NH), 7.31 (d, 1H, *J* = 7.85 Hz, H-6), 7.16 (t, 1H, *J* = 7.85 Hz, H-5), 6.81 (dd, 2H, *J* = 2.10 and 2.10 Hz, H-α), 6.60 (m, 1H, =CH), 6.43 (dd, 2H, *J* = 2.10 and 2.10 Hz, H-β), 2.17 (m, 2H, CH₂), 2.09 (m, 2H, CH₂), 1.69–1.56 (m, 4 H, CH₂). Anal. Calcd for C₁₇H₁₈N₂O: C, 76.66; H, 6.81; N, 10.52. Found: C, 76.81; H, 6.82; N, 10.49.

4.1.1.14. 1-(2-Benzamidophenyl)pyrrole (4n). White crystals (71%).³²

4.1.2. General procedure for 4-alkyl- or 4-alkenyl- or 4-arylpyrrolo[1,2-*a*]quinoxaline (1a–n). A solution of acetamide **4a–n** (0.02 mol) and phosphorous oxychloride (0.1 mol) in toluene (100 mL) was heated under reflux for 4 h. After cooling, the precipitate was filtered and dissolved in water (80 mL). The solution was then made alkaline with sodium hydrogen carbonate and extracted with methylene chloride (150 mL). The organic layer was dried over Na₂SO₄ and evaporated to dryness under reduced pressure. The residue was purified by column chromatography (SiO₂, ethyl acetate/petroleum ether = 20:80) to give **1a–n**.

4.1.2.1. 4-Propylpyrrolo[1,2-*a*]quinoxaline (1a). White crystals (78%); mp 33 °C; IR (KBr) 1620 (C=N); ¹H NMR (CDCl₃) δ: 7.89 (d, 1H, *J* = 7.95 Hz, H-9), 7.85 (dd, 1H, *J* = 2.60 and 1.25 Hz, H-1), 7.77 (d, 1H, *J* = 7.95 Hz, H-6), 7.41 (m, 2H, H-7 and H-8), 6.88 (dd, 1H, *J* = 3.90 and 1.25 Hz, H-3), 6.81 (dd, 1H, *J* = 3.90 and 2.60 Hz, H-2), 2.98 (t, 2H, *J* = 7.45 Hz, CH₂), 1.91 (sex, 2H, *J* = 7.45 Hz, CH₂), 1.07 (t, 3H, *J* = 7.45 Hz, CH₃); ¹³C NMR (CDCl₃) δ: 157.9 (C-4), 136.6 (C-9a), 129.9 (C-5a), 127.8 (C-3a), 127.3 (C-8), 126.6 (C-7), 125.5 (C-6), 114.5 (C-9), 114.0 (C-1), 113.8 (C-3), 106.7 (C-2), 38.3 (CH₂), 22.4 (CH₂), 14.8 (CH₃). Anal. Calcd for C₁₄H₁₄N₂: C, 79.96; H, 6.71; N, 13.32. Found: C, 80.08; H, 6.73; N, 13.43.

4.1.2.2. 7-[(Methoxy-4-propylpyrrolo[1,2-*a*]quinoxaline (1b). Beige crystals (50%); mp 45 °C; IR (KBr) 1615 (C=N); ¹H NMR (CDCl₃) δ: 7.82 (dd, 1H, *J* = 2.55 and 1.15 Hz, H-1), 7.71 (d, 1H, *J* = 8.95 Hz, H-9), 7.38 (d, 1H, *J* = 2.75 Hz, H-6), 7.06 (dd, 1H, *J* = 8.95 and 2.75 Hz, H-8), 6.86 (dd, 1H, *J* = 3.95 and 1.15 Hz, H-3), 6.791 (dd, 1H, *J* = 3.95 and 2.55 Hz, H-2), 3.89 (s, 3H, CH₃O), 2.96 (t, 2H, *J* = 7.40 Hz, CH₂), 1.89 (sex, 2H, *J* = 7.40 Hz, CH₂), 1.06 (t, 3H, *J* = 7.40 Hz, CH₃). Anal. Calcd for C₁₅H₁₆N₂O: C, 74.97; H, 6.71; N, 11.66. Found: C, 75.06; H, 6.54; N, 11.78.

4.1.2.3. 4-[(*E*)-prop-1-enyl]pyrrolo[1,2-*a*]quinoxaline (1c). Pale-yellow crystals (23%); mp 89 °C; IR (KBr) 1615 (C=N); ¹H NMR (CDCl₃) δ: 7.98 (dd, 1H, *J* = 7.60 and 2.00 Hz, H-9), 7.94 (dd, 1H, *J* = 2.70 and 1.15 Hz, H-1), 7.85 (dd, 1H, *J* = 7.60 and 2.00 Hz, H-6), 7.51–7.41 (m, 2H, H-7 and H-8), 7.25 (qd, 1H, *J* = 15.40 and 6.80 Hz, =CH), 7.01 (dd, 1H, *J* = 3.95

and 1.15 Hz, H-3), 6.89 (dd, 1H, *J* = 3.95 and 2.70 Hz, H-2), 6.88 (dd, 1H, *J* = 15.40 and 1.70 Hz, HC=), 2.08 (dd, 3H, *J* = 6.80 and 1.70 Hz, CH₃). Anal. Calcd for C₁₄H₁₂N₂: C, 80.74; H, 5.81; N, 13.45. Found: C, 80.80; H, 5.97; N, 13.54.

4.1.2.4. 7-Methoxy-4-[(*E*)-prop-1-enyl]pyrrolo[1,2-*a*]quinoxaline (1d). Method A: yellow crystals (16%); mp 80 °C; IR (KBr) 1610 (C=N); ¹H NMR (CDCl₃) δ: 7.83 (dd, 1H, *J* = 2.55 and 1.15 Hz, H-1), 7.69 (d, 1H, *J* = 8.95 Hz, H-9), 7.38 (d, 1H, *J* = 2.75 Hz, H-6), 7.20 (qd, 1H, *J* = 15.35 and 6.75 Hz, =CH), 7.07 (dd, 1H, *J* = 8.95 and 2.75 Hz, H-8), 6.94 (dd, 1H, *J* = 3.90 and 1.15 Hz, H-3), 6.87 (dd, 1H, *J* = 15.35 and 1.55 Hz, HC=), 6.82 (dd, 1H, *J* = 3.90 and 2.55 Hz, H-2), 3.89 (s, 3H, CH₃O), 2.03 (dd, 3H, *J* = 6.75 and 1.55 Hz, CH₃). Anal. Calcd for C₁₅H₁₄N₂O: C, 75.61; H, 5.92; N, 11.76. Found: C, 75.55; H, 6.08; N, 11.64.

4.1.2.5. 8-Methoxy-4-[(*E*)-prop-1-enyl]pyrrolo[1,2-*a*]quinoxaline (1e). Yellow crystals (22%); mp 45 °C; IR (KBr) 1610 (C=N); ¹H NMR (CDCl₃) δ: 7.82 (d, 1H, *J* = 8.95 Hz, H-6), 7.78 (m, 1H, H-1), 7.19 (d, 1H, *J* = 2.40 Hz, H-9), 7.08 (qd, 1H, *J* = 16.50 and 6.70 Hz, =CH), 6.97 (dd, 1H, *J* = 8.95 and 2.40 Hz, H-7), 6.93 (m, 1H, H-3), 6.85 (m, 1H, H-2), 6.82 (dd, 1H, *J* = 16.50 and 1.25 Hz, HC=), 3.96 (s, 3H, CH₃O), 2.01 (dd, 3H, *J* = 6.70 and 1.25 Hz, CH₃). Anal. Calcd for C₁₅H₁₄N₂O: C, 75.61; H, 5.92; N, 11.76. Found: C, 75.72; H, 6.03; N, 11.89.

4.1.2.6. 4-Pentylpyrrolo[1,2-*a*]quinoxaline (1f). Colorless oil (61%); IR (KBr) 1615 (C=N); ¹H NMR (CDCl₃) δ: 7.90 (dd, 1H, *J* = 7.90 Hz, H-9), 7.81 (dd, 1H, *J* = 2.65 and 1.10 Hz, H-1), 7.72 (d, 1H, *J* = 7.90 Hz, H-6), 7.38 (m, 2H, H-7 and H-8), 6.86 (dd, 1H, *J* = 3.90 and 1.10 Hz, H-3), 6.78 (dd, 1H, *J* = 3.90 and 2.65 Hz, H-2), 2.98 (t, 2H, *J* = 7.00 Hz, CH₂), 1.89 (m, 2H, CH₂), 1.44 (m, 4H, CH₂), 0.90 (t, 3H, *J* = 7.00 Hz, CH₃); ¹³C NMR (CDCl₃) δ: 158.1 (C-4), 136.5 (C-9a), 129.9 (C-5a), 127.8 (C-3a), 127.3 (C-8), 126.5 (C-7), 125.5 (C-6), 114.6 (C-9), 114.1 (C-1), 113.9 (C-3), 106.7 (C-2), 36.5 (CH₂), 32.6 (CH₂), 28.9 (CH₂), 23.1 (CH₂), 14.6 (CH₃). Anal. Calcd for C₁₆H₁₈N₂: C, 80.63; H, 7.61; N, 11.75. Found: C, 80.57; H, 7.72; N, 11.82.

4.1.2.7. 7-Methoxy-4-pentylpyrrolo[1,2-*a*]quinoxaline (1g). Yellow oil (93%); IR (KBr) 1610 (C=N); ¹H NMR (CDCl₃) δ: 7.75 (m, 1H, H-1), 7.64 (d, 1H, *J* = 8.90 Hz, H-9), 7.36 (d, 1H, *J* = 1.70 Hz, H-6), 7.01 (dd, 1H, *J* = 8.90 and 1.70 Hz, H-8), 6.83 (m, 1H, H-3), 6.76 (m, 1H, H-2), 3.86 (s, 3H, CH₃O), 2.96 (t, 2H, *J* = 7.15 Hz, CH₂), 1.87 (m, 2H, CH₂), 1.40 (m, 4H, CH₂), 0.89 (t, 3H, *J* = 7.15 Hz, CH₃). Anal. Calcd for C₁₇H₂₀N₂O: C, 76.08; H, 7.51; N, 10.44. Found: C, 75.89; H, 7.42; N, 10.38.

4.1.2.8. 4-[(*E*)-Pent-1-enyl]pyrrolo[1,2-*a*]quinoxaline (1h). Yellow oil (54%); IR (KBr) 1615 (C=N); ¹H NMR (CDCl₃) δ: 7.92 (dd, 1H, *J* = 7.85 and 2.05 Hz, H-9), 7.84 (dd, 1H, *J* = 2.85 and 1.20 Hz, H-1), 7.73 (dd, 1H, *J* = 7.85 and 2.05 Hz, H-6), 7.37 (m, 2H, H-7 and H-8), 7.21 (m, 1H, =CH), 6.97 (dd, 1H, *J* = 4.00

and 1.20 Hz, H-3), 6.85 (dd, 1H, J = 4.00 and 2.85 Hz, H-2), 6.83 (dd, 1H, J = 15.40 and 1.25 Hz, HC=), 2.37 (m, 2H, CH₂), 1.63 (sex, 2H, J = 7.30 Hz, CH₂), 1.01 (t, 3H, J = 7.30 Hz, CH₃). Anal. Calcd for C₁₆H₁₆N₂: C, 81.32; H, 6.82; N, 11.86. Found: C, 81.29; H, 6.98; N, 11.85.

4.1.2.9. 7-Methoxy-4-[(*E*)-pent-1-enyl]pyrrolo[1,2-*a*]quinoxaline (1i). Yellow oil (37%); IR (KBr) 1610 (C=N); ¹H NMR (CDCl₃) δ : 7.84 (dd, 1H, J = 2.90 and 1.25 Hz, H-1), 7.71 (d, 1H, J = 8.95 Hz, H-9), 7.40 (d, 1H, J = 2.80 Hz, H-6), 7.15 (m, 1H, =CH), 7.06 (dd, 1H, J = 8.95 and 2.80 Hz, H-8), 6.97 (dd, 1H, J = 3.90 and 1.25 Hz, H-3), 6.83 (dd, 1H, J = 3.90 and 2.90 Hz, H-2), 6.77 (dd, 1H, J = 15.30 and 1.15 Hz, HC=), 3.90 (s, 3H, CH₃O), 2.36 (m, 2H, CH₂), 1.62 (sex, 2H, J = 7.35 Hz, CH₂), 0.97 (t, 3H, J = 7.35 Hz, CH₃). Anal. Calcd for C₁₇H₁₈N₂O: C, 76.66; H, 6.81; N, 10.52. Found: C, 76.51; H, 6.75; N, 10.56.

4.1.2.10. 8-Methoxy-4-[(*E*)-pent-1-enyl]pyrrolo[1,2-*a*]quinoxaline (1j). Yellow oil (26%); IR (KBr) 1610 (C=N); ¹H NMR (CDCl₃) δ : 7.88 (d, 1H, J = 8.95 Hz, H-6), 7.82 (dd, 1H, J = 2.75 and 1.30 Hz, H-1), 7.24 (d, 1H, J = 2.65 Hz, H-9), 7.15 (ddd, 1H, J = 15.60 and 6.95 Hz, =CH), 7.03 (dd, 1H, J = 8.95 and 2.65 Hz, H-7), 6.98 (dd, 1H, J = 4.00 and 1.30 Hz, H-3), 6.87 (dd, 1H, J = 4.00 and 2.75 Hz, H-2), 6.82 (ddd, 1H, J = 15.60 and 1.50 Hz, HC=), 3.95 (s, 3H, CH₃O), 2.38 (m, 2H, CH₂), 1.63 (sex, 2H, J = 7.40 Hz, CH₂), 1.04 (t, 3H, J = 7.40 Hz, CH₃). Anal. Calcd for C₁₇H₁₈N₂O: C, 76.66; H, 6.81; N, 10.52. Found: C, 76.44; H, 6.73; N, 10.68.

4.1.2.11. 4-(3,4-Methylenedioxyphenethyl)pyrrolo[1,2-*a*]quinoxaline (1k). White crystals (77%); mp 69 °C; IR (KBr) 1615 (C=N); ¹H NMR (CDCl₃) δ : 7.94 (d, 1H, J = 8.00 Hz, H-9), 7.88 (m, 1H, H-1), 7.82 (d, 1H, J = 8.00 Hz, H-6), 7.43 (m, 2H, H-7 and H-8), 6.87–6.74 (m, 5H, H-2, H-3, H-2', H-5' and H-6'), 5.90 (s, 2H, OCH₂O), 3.28 (m, 2H, CH₂), 3.15 (m, 2H, CH₂); ¹³C NMR (CDCl₃) δ : 156.2 (C-4), 147.8 (C-3'), 145.9 (C-4'), 135.7 (C-1'), 129.7 (C-9a), 127.4 (C-5a), 127.1 (C-3a), 126.1 (C-8), 125.2 (C-7), 121.4 (C-6'), 114.3 (C-9), 113.8 (C-6), 113.6 (C-3), 109.1 (C-1), 108.4 (C-5'), 106.2 (C-2 and C-2'), 100.9 (OCH₂O), 37.8 (CH₂), 33.9 (CH₂). Anal. Calcd for C₂₀H₁₆N₂O₂: C, 75.93; H, 5.10; N, 8.85. Found: C, 76.11; H, 5.13; N, 8.74.

4.1.2.12. 4-(3,4-Dimethoxyphenethyl)pyrrolo[1,2-*a*]quinoxaline (1l). White crystals (70%); mp 174 °C; IR (KBr) 1615 (C=N); ¹H NMR (CDCl₃) δ : 7.89 (d, 1H, J = 7.90 Hz, H-9), 7.83 (dd, 1H, J = 2.65 and 1.15 Hz, H-1), 7.78 (d, 1H, J = 7.90 Hz, H-6), 7.43 (m, 2H, H-7 and H-8), 6.88–6.81 (m, 5H, H-2, H-3, H-2', H-5' and H-6'), 3.84 (s, 3H, CH₃O), 3.82 (s, 3H, CH₃O), 3.30 (m, 2H, CH₂), 3.17 (m, 2H, CH₂); ¹³C NMR (CDCl₃) δ : 156.2 (C-4), 148.9 (C-3'), 147.4 (C-4'), 135.9 (C-1'), 134.4 (C-9a), 129.4 (C-5a), 127.2 (C-3a), 126.9 (C-8), 125.7 (C-7), 125.1 (C-6'), 120.2 (C-6), 114.2 (C-9), 113.6 (C-3), 113.5 (C-1), 112.0 (C-2'), 111.4 (C-5'), 106.1 (C-2), 55.9 (CH₃O), 55.8

(CH₃O), 37.7 (CH₂), 33.7 (CH₂). Anal. Calcd for C₂₁H₂₀N₂O₂: C, 75.88; H, 6.06; N, 8.43. Found: C, 76.07; H, 6.30; N, 8.57.

4.1.2.13. 4-(Cyclohex-1-enyl)pyrrolo[1,2-*a*]quinoxaline (1m). Yellow oil (48%); IR (KBr) 1615 (C=N); ¹H NMR (CDCl₃) δ : 7.96 (d, 1H, J = 8.00 Hz, H-9), 7.93 (dd, 1H, J = 2.75 and 1.30 Hz, H-1), 7.82 (d, 1H, J = 8.00 Hz, H-6), 7.47 (t, 1H, J = 8.00 Hz, H-8), 7.42 (t, 1H, J = 8.00 Hz, H-7), 6.98 (dd, 1H, J = 4.00 and 1.30 Hz, H-3), 6.85 (dd, 1H, J = 4.00 and 2.75 Hz, H-2), 6.59 (m, 1H, =CH), 2.69 (m, 2H, CH₂), 2.33 (m, 2H, CH₂), 1.93–1.77 (m, 4H, CH₂). Anal. Calcd for C₁₇H₁₆N₂: C, 82.22; H, 6.49; N, 11.28. Found: C, 82.10; H, 6.45; N, 11.13.

4.1.2.14. 4-Phenylpyrrolo[1,2-*a*]quinoxaline (1n). White crystals (53%).³⁴

4.1.3. General procedure for 1-(2-acetylaminophenyl)pyrrole (5a,b). To a solution of 1-(2-aminophenyl)pyrrole **2a,b** (0.02 mol) in dioxane (80 mL) were added pyridine (0.022 mol) then acetyl chloride (0.02 mol). The reaction mixture was refluxed for 4 h and the solvent then removed under reduced pressure. The residue was triturated with water and extracted with diethyl ether (2 × 80 mL). The organic layers were collected, washed with an aqueous sodium hydrogen carbonate solution (100 mL) then with water (100 mL), dried over Na₂SO₄ and evaporated to dryness. The precipitate was crystallized from petroleum ether to afford **5a,b**.

4.1.3.1. 1-(2-Acetylaminophenyl)pyrrole (5a). White crystals (67%).³⁴

4.1.3.2. 1-(2-Acetyl-amino-4-methoxyphenyl)pyrrole (5b). Pale-yellow crystals (62%); mp 73 °C; IR (KBr) 3200 (NH), 1650 (C=O); ¹H NMR (CDCl₃) δ : 8.04 (d, 1H, J = 2.50 Hz, H-3), 7.15 (d, 1H, J = 8.55 Hz, H-6), 6.90 (s, 1H, NH), 6.72 (dd, 2H, J = 1.95 and 1.95 Hz, H- α), 6.64 (dd, 1H, J = 8.55 and 2.50 Hz, H-5), 6.35 (dd, 2H, J = 1.95 and 1.95 Hz, H- β), 3.83 (s, 3H, CH₃O), 2.00 (s, 3H, CH₃). Anal. Calcd for C₁₃H₁₄N₂O₂: C, 67.81; H, 6.13; N, 12.17. Found: C, 67.75; H, 6.03; N, 12.22.

4.1.4. General procedure for 4-methylpyrrolo[1,2-*a*]quinoxaline (6a,b). A solution of 1-(2-propanoylaminophenyl)pyrrole **5a,b** (0.02 mol) and phosphorous oxychloride (0.1 mol) in toluene (100 mL) was heated under reflux for 4 h. After cooling, the precipitate was filtered and dissolved in water (80 mL). The solution was then made alkaline with sodium hydrogen carbonate and extracted with methylene chloride (150 mL). The organic layer was dried over Na₂SO₄ and evaporated to dryness under reduced pressure to give **6a,b**.

4.1.4.1. 4-Methylpyrrolo[1,2-*a*]quinoxaline (6a). White crystals (67%).³⁴

4.1.4.2. 7-Methoxy-4-methylpyrrolo[1,2-*a*]quinoxaline (6b). Pale-orange crystals (58%); mp 50 °C; IR (KBr) 1620 (C=N); ¹H NMR (CDCl₃) δ : 7.82 (dd, 1H,

$J = 2.75$ and 1.15 Hz, H-1), 7.71 (d, 1H, $J = 8.95$ Hz, H-9), 7.37 (d, 1H, $J = 2.75$ Hz, H-6), 7.07 (dd, 1H, $J = 8.95$ and 2.75 Hz, H-8), 6.85 (dd, 1H, $J = 3.90$ and 1.15 Hz, H-3), 6.80 (dd, 1H, $J = 3.90$ and 2.75 Hz, H-2), 3.89 (s, 3H, CH₃O), 2.70 (s, 3H, CH₃). Anal. Calcd for C₁₃H₁₂N₂O: C, 73.56; H, 5.70; N, 13.20. Found: C, 73.65; H, 5.78; N, 13.15.

4.1.5. General procedure for 4-styrylpyrrolo[1,2-*a*]quinoxaline (1o,p). 4-Methylpyrrolo[1,2-*a*]quinoxaline **6a,b** (3.3 mmol), benzaldehyde (3.3 mmol) and Ac₂O (5 mL) were refluxed for 5 h. The solvent was then evaporated under reduced pressure. The residue was triturated in water and extracted with dichloromethane. The organic layer was washed with an aqueous saturated sodium hydrogen carbonate solution, dried over Na₂SO₄ and evaporated and then chromatographed over Si gel. Elution with hexane/AcOEt (8:2) afforded the pure compound **1o,p**.

4.1.5.1. 4-Styrylpyrrolo[1,2-*a*]quinoxaline (1o). Yellow crystals (41%).³⁴

4.1.5.2. 7-Methoxy-4-styrylpyrrolo[1,2-*a*]quinoxaline (1p). Pale-yellow crystals (30%); mp 156 °C; IR (KBr) 1615 (C=N); ¹H NMR (CDCl₃) δ : 8.04 (d, 1H, $J = 15.95$ Hz, HC=), 7.86 (dd, 1H, $J = 2.65$ and 1.25 Hz, H-1), 7.73 (d, 1H, $J = 9.15$ Hz, H-9), 7.66 (m, 2H, Ar'-H), 7.49 (d, 1H, $J = 15.95$ Hz, =CH), 7.44 (d, 1H, $J = 2.65$ Hz, H-6), 7.39 (m, 3H, Ar'-H), 7.07 (m, 2H, H-8 and H-3), 6.87 (dd, 1H, $J = 3.90$ and 2.65 Hz, H-2), 3.92 (s, 3H, CH₃O). Anal. Calcd for C₂₀H₁₆N₂O: C, 79.97; H, 5.37; N, 9.33. Found: C, 80.13; H, 5.27; N, 9.36.

4.1.6. [Pyrrolo [1,2-*a*]quinoxaline-4-carboxaldehyde (7). 4-Methylpyrrolo[1,2-*a*]quinoxaline **6a** (2.3 mmol) and SeO₂ (3.45 mmol) were stirred in dioxane (10 mL) under reflux for 4 h. After cooling and filtration, the solution was concentrated and the residue was purified on silica gel. Elution with CH₂Cl₂ gave **7** as orange crystals (59%); mp 162 °C; IR (KBr) 1680 (C=O); ¹H NMR (CDCl₃) δ : 10.12 (s, 1H, CHO), 8.13 (d, 1H, $J = 8.05$ Hz, H-9), 8.02 (dd, 1H, $J = 2.75$ and 1.20 Hz, H-1), 7.92 (d, 1H, $J = 8.05$ Hz, H-6), 7.69 (dd, 1H, $J = 4.05$ and 1.20 Hz, H-3), 7.67 (t, 1H, $J = 8.05$ Hz, H-8), 7.54 (t, 1H, $J = 8.05$ Hz, H-7), 7.03 (dd, 1H, $J = 4.05$ and 2.75 Hz, H-2). Anal. Calcd for C₁₂H₈N₂O: C, 73.46; H, 4.11; N, 14.28. Found: C, 73.54; H, 4.31; N, 14.39.

4.1.7. 4-[(*E*)-Prop-1-enyl]pyrrolo[1,2-*a*] quinoxaline (1c). Method B: To a suspension of ethyltriphenylphosphonium bromide (2.44 mmol) in 6 mL of anhydrous toluene at 0 °C was added potassium *tert*-butoxide (2.94 mmol). After 20 min of stirring at 0 °C, a solution of aldehyde **7** (1.22 mmol) in 8 mL of anhydrous tetrahydrofuran was added to the reaction mixture. The mixture reaction was then heated at 70 °C for 1.5 h. After cooling, the mixture was hydrolysed by adding 7 mL of water. The aqueous layer was then extracted with AcOEt, the resultant organic extracts were washed

with an aqueous saturated sodium hydrogen carbonate, then with a brine solution, dried over Na₂SO₄ and evaporated to dryness. The residue was chromatographed on silica gel with dichloromethane/hexane (4:1) as eluent to give **1c** as yellow crystals (29%). IR and ¹H NMR data were in accordance with those of Method A.

4.1.8. General procedure for trans-4-(2-methyloxirane)pyrrolo[1,2-*a*]quinoxaline (1q) and cis-4-(2-methyloxirane)pyrrolo[1,2-*a*]quinoxaline (1r). A mixture of triethylsulfonium tetrafluoroborate (2.24 mmol), potassium hydroxide pellets (15.7 mmol), 8 mL of acetonitrile and 0.1 mL of water was warmed at 60 °C for 15 min in the absence of light. A solution of pyrrolo[1,2-*a*]quinoxaline-4-carboxaldehyde **7** (2.24 mmol) in 8 mL of acetonitrile was then added dropwise and the mixture was stirred at 60 °C for 2 h. The mixture was filtered and the solid was extracted with acetonitrile then with dichloromethane. The organic layers were dried over Na₂SO₄ and evaporated to dryness. The residue was chromatographed on silica gel with hexane/AcOEt (8:2) as eluent. The first eluted product ($R_f = 0.38$) was the *trans* epoxide **1q**, followed by the *cis* epoxide **1r** ($R_f = 0.20$).

4.1.8.1. Trans-4-(2-methyloxirane)pyrrolo[1,2-*a*]quinoxaline (1q). Yellow crystals (40%); mp 62 °C; IR (KBr) 1610 (C=N); ¹H NMR (CDCl₃) δ : 7.93 (d, 1H, $J = 7.85$ Hz, H-9), 7.89 (dd, 1H, $J = 2.55$ and 1.30 Hz, H-1), 7.78 (d, 1H, $J = 7.85$ Hz, H-6), 7.47 (t, 1H, $J = 7.85$ Hz, H-8), 7.39 (t, 1H, $J = 7.85$ Hz, H-7), 7.12 (dd, 1H, $J = 3.90$ and 1.30 Hz, H-3), 6.83 (dd, 1H, $J = 3.90$ and 2.55 Hz, H-2), 3.99 (d, 1H, $J = 2.00$ Hz, H-1'), 3.59 (qd, 1H, $J = 5.15$ and 2.00 Hz, H-2'), 1.54 (d, 3H, $J = 5.15$ Hz, CH₃). Anal. Calcd for C₁₄H₁₂N₂O: C, 74.98; H, 5.39; N, 12.49. Found: C, 75.15; H, 5.56; N, 12.50.

4.1.8.2. Cis-4-(2-methyloxirane)pyrrolo[1,2-*a*]quinoxaline (1r). Yellow oil (13%); IR (KBr) 1610 (C=N); ¹H NMR (CDCl₃) δ : 8.01 (d, 1H, $J = 7.80$ Hz, H-9), 7.94 (dd, 1H, $J = 2.65$ and 1.10 Hz, H-1), 7.83 (d, 1H, $J = 7.80$ Hz, H-6), 7.50 (t, 1H, $J = 7.80$ Hz, H-8), 7.41 (t, 1H, $J = 7.80$ Hz, H-7), 7.15 (dd, 1H, $J = 4.00$ and 1.10 Hz, H-3), 6.86 (dd, 1H, $J = 4.00$ and 2.65 Hz, H-2), 4.33 (d, 1H, $J = 4.35$ Hz, H-1'), 3.54 (qd, 1H, $J = 5.30$ and 4.35 Hz, H-2'), 1.25 (d, 3H, $J = 5.30$ Hz, CH₃). Anal. Calcd for C₁₄H₁₂N₂O: C, 74.98; H, 5.39; N, 12.49. Found: C, 75.06; H, 5.21; N, 12.38.

4.1.9. General procedure for 4-alkenyl- or 4-arylpyrrolo [1,2-*a*]quinoxaline (1c,d), (1h,i), (1n-p), and (1s). Method C: A mixture of the 4-chloropyrrolo[1,2-*a*]quinoxaline **8a**³⁴ or **8b**³⁶ (5 mmol), the alkenylboronic acid (5.5 mmol) and Pd(PPh₃)₄ (0.15 mmol) in benzene (21 mL), ethanol (1.6 mL) and 2 M aqueous sodium carbonate solution (5.4 mL) was stirred and heated under reflux under nitrogen for 24 h. It was then cooled, transferred to a separating funnel, and the reaction flask washed out with water (3× 50 mL) and dichloromethane (3× 90 mL), the washings being added to the separating funnel. The organic layer was separated and the aqueous

phase extracted with dichloromethane (2× 100 mL). The combined organic extracts were then washed with water (3× 130 mL), dried over Na₂SO₄, filtered and the filtrate evaporated under reduced pressure. Column chromatography of the residue on silica gel using diethyl ether/petroleum ether (1:3) as eluent gave the pure product **1c,d**, **1h,i**, **1n–p**, or **1s**.

4.1.9.1. 4-[(E)-Prop-1-enyl]pyrrolo[1,2-*a*]quinoxaline (1c). Pale-yellow crystals (77%); IR and ¹H NMR data were in accordance with those of Methods A and B.

4.1.9.2. 7-Methoxy-4-[(E)-prop-1-enyl]pyrrolo[1,2-*a*]quinoxaline (1d). Yellow crystals (65%); IR and ¹H NMR data were in accordance with those of Method A.

4.1.9.3. 4-[(E)-Pent-1-enyl]pyrrolo[1,2-*a*]quinoxaline (1h). Yellow crystals (61%); IR and ¹H NMR data were in accordance with those of Method A.

4.1.9.4. 7-Methoxy-4-[(E)-pent-1-enyl]pyrrolo[1,2-*a*]quinoxaline (1i). Yellow crystals (56%); IR and ¹H NMR data were in accordance with those of Method A.

4.1.9.5. 4-Phenylpyrrolo[1,2-*a*]quinoxaline (1n). White crystals (85%); IR and ¹H NMR data were in accordance with those of Method A.³⁴

4.1.9.6. 4-Styrylpyrrolo[1,2-*a*]quinoxaline (1o). Yellow crystals (86%); IR and ¹H NMR data were in accordance with those described above.

4.1.9.7. 7-Methoxy-4-styrylpyrrolo[1,2-*a*]quinoxaline (1p). Yellow crystals (75%); IR and ¹H NMR data were in accordance with those described above.

4.1.9.8. 4-[(Z)-Prop-1-enyl]pyrrolo[1,2-*a*]quinoxaline (1s). Yellow oil (51%); IR (KBr) 1610 (C=N); ¹H NMR (CDCl₃) δ: 7.99 (dd, 1H, *J* = 7.90 and 1.65 Hz, H-9), 7.92 (dd, 1H, *J* = 2.65 and 1.35 Hz, H-1), 7.84 (dd, 1H, *J* = 7.90 and 1.65 Hz, H-6), 7.49 (ddd, 1H, *J* = 7.90, 7.25 and 1.65 Hz, H-8), 7.43 (ddd, 1H, *J* = 7.90, 7.25 and 1.65 Hz, H-7), 6.89 (dd, 1H, *J* = 3.95 and 1.35 Hz, H-3), 6.86 (dd, 1H, *J* = 3.95 and 2.65 Hz, H-2), 6.77 (qd, 1H, *J* = 11.65 and 1.80 Hz, HC=), 6.30 (qd, 1H, *J* = 11.65 and 7.20 Hz, =CH), 2.26 (dd, 3H, *J* = 7.20 and 1.80 Hz, CH₃). Anal. Calcd for C₁₄H₁₂N₂: C, 80.74; H, 5.81; N, 13.45. Found: C, 80.60; H, 5.94; N, 13.59.

4.1.10. 4-(Eth-1-enyl)pyrrolo[1,2-*a*]quinoxaline (1t). A mixture of the 4-chloropyrrolo[1,2-*a*]quinoxaline **8a** (3.4 mmol), the vinylboronic acid di-*n*-butyl ester (3.8 mmol) and Pd(PPh₃)₄ (0.10 mmol) in benzene (15 mL), and 4 M aqueous potassium hydroxide solution (2.4 mL) was stirred and heated under reflux under nitrogen for 5 h. It was then cooled, transferred to a separating funnel, and the reaction flask washed out with water (3× 50 mL) and benzene (2× 40 mL), the washings being added to the separating funnel. The organic layer was separated, washed with an aqueous saturated sodium hydrogen carbonate, then with a brine solution, dried over Na₂SO₄, and evaporated to dryness. Column

chromatography of the residue on silica gel using diethyl ether/petroleum ether (1:3) as eluent gave the pure product **1t** as yellow crystals (30%); mp 76 °C; IR (KBr) 1610 (C=N); ¹H NMR (CDCl₃) δ: 7.99 (d, 1H, *J* = 8.05 Hz, H-9), 7.96 (dd, 1H, *J* = 2.75 and 1.20 Hz, H-1), 7.86 (d, 1H, *J* = 8.05 Hz, H-6), 7.51 (t, 1H, *J* = 8.05 Hz, H-8), 7.45 (t, 1H, *J* = 8.05 Hz, H-7), 7.18 (dd, 1H, *J* = 17.30 and 10.85 Hz, HC=), 7.04 (dd, 1H, *J* = 4.05 and 1.20 Hz, H-3), 6.91 (dd, 1H, *J* = 4.05 and 2.75 Hz, H-2), 6.65 (dd, 1H, *J* = 17.30 and 1.65 Hz, =CH₂), 5.79 (dd, 1H, *J* = 10.85 and 1.65 Hz, =CH₂). Anal. Calcd for C₁₃H₁₀N₂: C, 80.39; H, 5.19; N, 14.42. Found: C, 80.31; H, 5.34; N, 14.49.

4.1.11. General procedure for 4-(hex-1-ynyl)pyrrolo[1,2-*a*]quinoxaline (1u,v). A mixture of CuI (0.05 mmol), Pd(PPh₃)₂Cl₂ (0.05 mmol), anhydrous triethylamine (10 mL), 4-chloropyrrolo[1,2-*a*]quinoxaline **8a,b** (2.5 mmol) and 1-hexyne (3.75 mmol) was stirred and heated at 80 °C for 24 h. The reaction mixture was cooled to rt and filtered. The solid residue was washed thoroughly with hexane, combined filtrates were evaporated under reduced pressure, and the residue was purified over a column of silica gel with hexane/AcOEt (10:1) as eluent to give **1u,v**.

4.1.11.1. 4-(Hex-1-ynyl)pyrrolo[1,2-*a*]quinoxaline (1u). Yellow oil (29%); IR (KBr) 2210 (C≡C); ¹H NMR (CDCl₃) δ: 7.93 (d, 1H, *J* = 8.00 Hz, H-9), 7.89 (dd, 1H, *J* = 2.70 and 1.20 Hz, H-1), 7.80 (d, 1H, *J* = 8.00 Hz, H-6), 7.48 (t, 1H, *J* = 8.00 Hz, H-8), 7.41 (t, 1H, *J* = 8.00 Hz, H-7), 7.00 (dd, 1H, *J* = 3.95 and 1.20 Hz, H-3), 6.86 (dd, 1H, *J* = 3.95 and 2.70 Hz, H-2), 2.55 (t, 2H, *J* = 7.10 Hz, CH₂), 1.69 (qt, 2H, *J* = 7.10 Hz, CH₂), 1.52 (sex, 2H, *J* = 7.10 Hz, CH₂), 0.96 (t, 2H, *J* = 7.10 Hz, CH₃). Anal. Calcd for C₁₇H₁₆N₂: C, 82.22; H, 6.49; N, 11.28. Found: C, 82.36; H, 6.34; N, 11.35.

4.1.11.2. 4-(Hex-1-ynyl)-7-methoxypyrrrolo[1,2-*a*]quinoxaline (1v). Yellow oil (42%); IR (KBr) 2210 (C≡C); ¹H NMR (CDCl₃) δ: 7.85 (dd, 1H, *J* = 2.65 and 1.35 Hz, H-1), 7.76 (d, 1H, *J* = 9.00 Hz, H-9), 7.45 (d, 1H, *J* = 2.80 Hz, H-6), 7.13 (dd, 1H, *J* = 9.00 and 2.80 Hz, H-8), 7.02 (dd, 1H, *J* = 4.00 and 1.35 Hz, H-3), 6.87 (dd, 1H, *J* = 4.00 and 2.65 Hz, H-2), 3.91 (s, 3H, CH₃O), 2.59 (t, 2H, *J* = 7.15 Hz, CH₂), 1.73 (qt, 2H, *J* = 7.15 Hz, CH₂), 1.59 (sex, 2H, *J* = 7.15 Hz, CH₂), 1.00 (t, 3H, *J* = 7.15 Hz, CH₃). Anal. Calcd for C₁₈H₁₈N₂O: C, 77.67; H, 6.52; N, 10.06. Found: C, 77.75; H, 6.44; N, 9.86.

4.2. X-ray data

Pale-yellow single crystal (0.15 × 0.01 × 0.01 mm³) of **1c** was obtained by slow evaporation from methanol/chloroform (20:80) solution: orthorhombic, space group P₂₁₂₁₂₁, *a* = 21, *a* = 13.079(6) Å, *b* = 19.592(9) Å, *c* = 4.289(4) Å, α = 90°, β = 90°, γ = 90°, *V* = 1099.08(12) Å³, *Z* = 4, δ(calcd) = 1.259 Mg m⁻³, FW = 208.26 for C₁₄H₁₂N₂, *F*(000) = 440. The unit cell dimensions were determined using the least-squares fit from 25 reflections (25° < θ < 35°). Intensities were collected with an En-

raf-Nonius CAD-4 diffractometer using the CuK α radiation and a graphite monochromator up to $\theta = 55^\circ$. No intensity variation of two standard reflections monitored every 90 min was observed. Full crystallographic results have been deposited at the Cambridge Crystallographic Data Centre (CCDC-297422), UK, as Supplementary material.⁵⁷ The data were corrected for Lorentz and polarization effects and for empirical absorption correction.⁵⁸ The structure was solved by direct methods Shelx 86⁵⁹ and refined using Shelx 93⁶⁰ suite of programs.

4.3. Partition coefficients $\log D$ (pH 7.4)

The relative $\log D$ (pH 7.4) in this study was assessed by the micro-HPLC method.⁶¹ These determinations were performed with a chromatographic apparatus (Spectra Series, San Jose, USA) equipped with a model P1000XR pump and a model SCM 1000 vacuum membrane degasser, a model UV 150 ultraviolet detector ($203 \text{ nm} \leq \lambda_{\text{max}} \leq 253 \text{ nm}$) and a ChromJet data module integrator (ThermoFinnigan, San Jose, USA). A reversed phase column was used: a Waters SunFire™ C₁₈ ($3.0 \times 150 \text{ mm}$; $5 \mu\text{m}$ particle size) with a mobile phase consisting of acetonitrile – phosphate buffer (pH 4.5) (65:35, v/v (**1g**, **1i–m**, **1o,p** and **1u,v**); 60:40, v/v (**1f**, **1h**, **1n**, **1s**, and chimanine B); 55:45, v/v (**1e**); 50:50, v/v (**1a–d**); 40:60, v/v (**1q,r**). The compounds were partitioned between 1-octanol (HPLC grade) and phosphate buffer (pH 7.4). Octanol was presaturated with the adequate phosphate buffer (2%), and conversely. An amount of 1 mg of each compound was dissolved in an adequate volume of methanol in order to achieve 1 mg/mL stock solutions. Then an appropriate aliquot of these methanolic solutions was dissolved in buffer to obtain final concentration of 100 $\mu\text{g/mL}$. Under the above-described chromatographic conditions, 100 μL of this aqueous phase was injected into the chromatograph, leading to the determination of a peak area before partitioning (W_0). In screw-capped tubes, 2000 μL of the aqueous phase (V_{aq}) was then added to 10 μL of *n*-octanol (V_{oct}). The mixture was shaken by mechanical rotation during 30 min. Then the centrifugation was achieved at 3000 rpm in 15 min. An amount of 100 μL of the lower phase was injected into the chromatograph column. This led to the determination of a peak area after partitioning (W_1). The $\log D$ value was determined by the formula: $\log D = \log [(W_0 - W_1)V_{\text{aq}}/W_1V_{\text{oct}}]$.

4.4. Biological assays

4.4.1. In vitro *L. amazonensis* and *L. infantum* culture and drug assays.^{62,63} Promastigotes of the *L. infantum* (clone MCAN/GR/82/LEM497) and *L. amazonensis* (MHOM/BR/1987/BA) were maintained at 26 °C in RPMI-1640 medium supplemented with 10% heat-inactivated foetal calf serum (FCS), 200 U/ml penicillin, 100 $\mu\text{g/mL}$ streptomycin, sodium bicarbonate and non-essential amino acids (all from Gibco, Paisley, UK). At stationary growth phase parasites ($10^6/\text{mL}$) were harvested, washed and incubated in culture media with various molecules. The viability of promastigotes was checked using the tetrazolium-dye (MTT) colorimetric method (Promega, USA). The MTT cell proliferation assay is a colorimet-

ric assay system, which measures the reduction of a tetrazolium component (MTT) into an insoluble formazan product by the mitochondria of viable cells. After incubation of the cells with the MTT reagent, a detergent solution was added to lyse the cells and solubilize the coloured crystals. The samples were read using an ELISA plate reader at a wavelength of 570 nm. The amount of colour produced was directly proportional to the number of viable cells. In some experiments, parasites were diluted in culture medium and directly counted under microscope. Each concentration was screened in triplicate. The results are expressed as the concentrations inhibiting parasite growth by 50% (IC_{50}) after a 1–2 day incubation period.

4.4.2. Cytotoxicity test upon cells.⁶⁴ The toxicity of various molecules was evaluated on non-activated, freshly isolated normal human peripheral blood mononuclear cells (PBMC), as well as phytohaemagglutinin (PHA)-induced cells. PBMC from healthy volunteers were obtained following centrifugation on Ficoll gradient. Cells were then incubated in medium alone or induced to enter cell cycle by the addition of PHA (5 $\mu\text{g/mL}$, Murex Biotech Limited, Dartford, UK). Various molecules were added as described under results. Following cell cultures during 3–4 days, cells were harvested, washed and counted with trypan blue exclusion. In some experiments, the proliferation of PBMC was checked using the MTT colorimetric method as described previously. The 50% inhibitory concentrations (IC_{50}) were determined by linear regression analysis, expressed in $\mu\text{M} \pm \text{SD}$ and the maximum tolerated concentration expressed in μM was evaluated for each compound.

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