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Antileishmanial Activity of new Thiophene-indole Hybrids: Design, Synthesis, Biological and Cytotoxic Evaluation, and Chemometric Studies

Mayara B. Félix [a], Edson R. de Souza [b], Maria do C. A. de Lima [b], Daiana Karla G. Frade [a], Vanessa de L. Serafim [a], Klinger Antonio da F. Rodrigues [c], Patrícia Lima do N. Néris [c], Frederico F. Ribeiro[d], Luciana Scotti [e], Marcus T. Scotti [c], Thiago M. de Aquino[f], Francisco Jaime B. Mendonça-Junior[g], Márcia R. de Oliveira[a*]

[a]*Departamento de Biologia Molecular, Centro de Ciências Exatas e da Natureza,Universidade Federal da Paraíba, João Pessoa, Paraíba 58059-900, Brazil;*

[b]*Departamento de Antibióticos, Universidade Federal de Pernambuco, Recife-PE 50670-910,Brazil;*

[c]*Programa de Pós-Graduação em Produtos Naturais e Sintéticos Bioativos. Universidade Federal da Paraíba, João Pessoa, Paraíba 58059-900, Brazil;*

[d]*Programa de Pós-Graduação em Inovação Terapêutica, Universidade Federal de Pernambuco, Campus I, Recife-PE, 50670-901, Brazil;*

[e]*Federal University of Paraíba, Campus I, João Pessoa, PB, 58051-970, Brazil;*

[f]*Instituto de Quimica e Biotecnologia, Universidade Federal de Alagoas, Maceió, AL, 57072-970, Brazil;*

[g]*Departamento de Ciências Biológicas, Universidade Estadual da Paraíba, CCBSA, João Pessoa-PB 58070-540, Brazil;*

* Corresponding author

Márcia Rosa de Oliveira

Departamento de Biologia Molecular, Centro de Ciências Exatas e da Natureza, Universidade Federal da Paraíba, Campus I, Castelo Branco, João Pessoa, Paraíba, CEP 58059-900, Brazil
Tel: +55 83 3216 7436 E-mail: mrosajp@gmail.com

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Abstract

In the present work, thirty-two hybrid compounds containing cycloalka[*b*]thiophene and indole moieties (**TN5, TN5 1-7**, **TN6, TN6 1-7**, **TN7, TN7 1-7**, **TN8, TN8 1-7**) were designed, synthesized and evaluated for their cytotoxic and antileishmanial activity against *Leishmania amazonensis* promastigotes. More than half of the compounds (18 compounds) exhibited significant antileishmanial activity (IC_{50} lower than 10.0 µg/L), showing better performance than the reference drugs (tri- and penta-valent antimonials). The most active compounds were **TN8-7**, **TN6-1** and **TN7** with respective IC_{50} values of 2.1, 2.3 and 3.2 µg/mL. Demonstrating that all of the compounds were less toxic than the reference drugs, even at the highest evaluated concentration (400 µg/mL), no compound tested presented human erythrocyte cytotoxicity. Compound **TN8-7's** effectiveness against a trivalent antimony-resistant culture was demonstrated. It was observed that **TN8-7's** antileishmanial activity is associated with DNA fragmentation of *L. amazonensis* promastigotes. Chemometric studies (CPCA, PCA, and PLS) highlight intrinsic solubility/lipophylicity, and compound size and shape as closely related to activity. Our results suggest that hybrid cycloalka[*b*]thiophene-indole derivatives may be considered as lead compounds for further development of new drugs for the treatment of leishmaniasis.

Keywords: Leishmaniasis; *Leishmania amazonensis*; cytotoxicity; 2-aminothiophene; indole, Chemometric studies.

Leishmaniasis can be defined as the array of diseases caused by protozoans of the genus *Leishmania* (Order *Kinetoplastida*, Family *Trypanosomatidae*)[1,2]. It is considered a public health concern and afflicts over two million people each year. About 350 million people live in endemic areas under risk of infection [3].

The life cycle of *Leishmania* sp. starts when the insect invertebrate host feeds on infected mammalian blood, thus imbibing amastigotes present within macrophages. In the vector-insect intestine, the amastigote form of the parasite becomes a procyclical, and later a metacyclic promastigote. When the insect bites a mammalian host again, inoculated virulent promastigotes enter the blood stream and are internalized by macrophages, where they differentiate again into amastigotes, completing the cycle [4].

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In spite of its epidemiological importance, the recommended treatment for leishmaniasis is far from satisfactory. The conventional therapy (via parenteral) uses pentavalent antimonials [5,6]. The cure rate is high, but the treatment involves difficulties such as high toxicity and possible therapeutic failure [7]. There is an urgent need to find new antileishmanial drugs that are efficient against the pathogen yet present low toxicity to the patient [8].

The thiophenes belong to a group of aromatic heterocyclic compounds [9,10], among which 2-aminothiophene compounds are well known for their therapeutic applications [11,12,13]. There are reports of thiophene derived compounds with antipromastigote activity (IC_{50} at 7.7 µg/mL) against *Leishmania amazonensis* as reported by Takahashi et al, [14]. *In vitro* activity has also been found against the intracellular amastigote form in murine peritoneal macrophages, with IC_{50} values appearing from 1.56 to >80 µM [15].

2-Aminothiophene compounds can be condensed to an indole nucleus, as described by Alves et al. [16]. Taha et al. [17], have reported the antileishmanial potential of indol-2-hydrazone compounds with IC_{50} values varying between 8.18 and 16.14 µM. Sangshetti et al. [18] also reported the antileishmanial activity of hybrid indole-coumarin compounds against *Leishmania donovani* promastigote forms, with IC_{50} values between 95.0 and 287.5 µg/mL. The most active compound ($C_{26}H_{17}Cl_2NO$ – IC_{50} 95.0 µg/mL) did not show cytotoxicity against HeLa cells.

Previous studies from our group identified promising *in vitro* antileishmanial activity in 2-aminothiophene compounds, demonstrating their potential for leishmaniasis treatment. The compounds displayed activity against promastigote forms of *L. amazonesis* with IC_{50} values reaching 3.37 µM, without cytotoxicity against blood cells or macrophages. An apoptosis associated action mechanism involving externalization of phosphatidylserine and DNA fragmentation, with cellular death by secondary necrosis was observed. The compounds were effective against infection in macrophages, and their anti-amastigote activity was associated with host immune response modulation [19]. The most effective compounds in the study presented a 2-aminothiophene hybridized indole ring, which might be involved in the increase of antileishmanial activity.

The present work reports the design and synthesis of a series of new hybrid compounds, all containing a combination of 2-aminothiophene and indole cores in a single chemical structure. These compounds were evaluated for antileishmanial activity against *L. amazonensis* promastigote forms, cytotoxicity against blood cells, and their mechanism of

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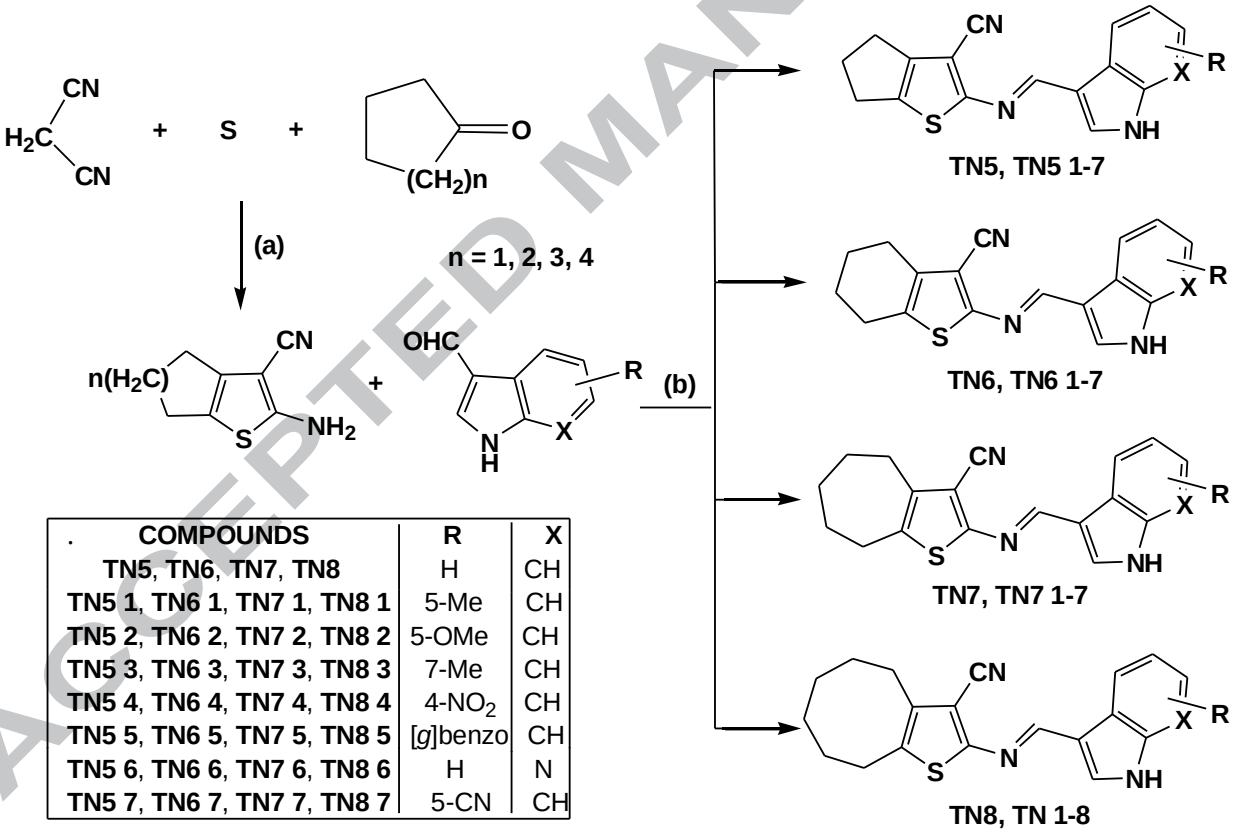
action thru death by DNA fragmentation. Finally, the compounds were subjected to computer-aided drug design (CADD) studies using Consensus PCA (CPCA), Principal Component Analysis (PCA), and Partial Least Squares (PLS) regression in order to extract structural information relating to antileishmanial activity, and to attempt a predictive model able to direct the synthesis of new derivatives with even greater antileishmanial activity.

The synthesis of the hybrid 2-[(indolyl)methyleneamino]- cycloalka[*b*]thiophene-3-carbonitrile derivatives (**TN5, TN5 1-7**, **TN6, TN6 1-7**, **TN7, TN7 1-7**, **TN8, TN8 1-7**) was performed according to procedures previously described by our group [20,21] and is depicted in **Scheme 1.**

Scheme 1. Synthesis of thiophene-indole derivatives; compounds **TN5, TN5 1-7**, **TN6, TN6 1-7**, **TN7, TN7 1-7**, **TN8, TN8 1-7**; Reagents and conditions: (a) Morpholine, EtOH, 5-10°C, 3h; (b) AcOH, EtOH, rt, 24 h.



. COMPOUNDS	R	X
TN5, **TN6**, **TN7**, **TN8**	H	CH
TN5 1, **TN6 1**, **TN7 1**, **TN8 1**	5-Me	CH
TN5 2, **TN6 2**, **TN7 2**, **TN8 2**	5-OMe	CH
TN5 3, **TN6 3**, **TN7 3**, **TN8 3**	7-Me	CH
TN5 4, **TN6 4**, **TN7 4**, **TN8 4**	4-NO_2	CH
TN5 5, **TN6 5**, **TN7 5**, **TN8 5**	[*g*]benzo	CH
TN5 6, **TN6 6**, **TN7 6**, **TN8 6**	H	N
TN5 7, **TN6 7**, **TN7 7**, **TN8 7**	5-CN	CH

All compounds were obtained in satisfactory yields (except **TN7-6** and **TN7-7**). All hybrid derivatives, with the exception of the nitro derivatives (**TN5-4**, **TN6-4**, **TN7-4** and **TN8-4**), presented yellow colors, the nitro compounds presented orange colors.

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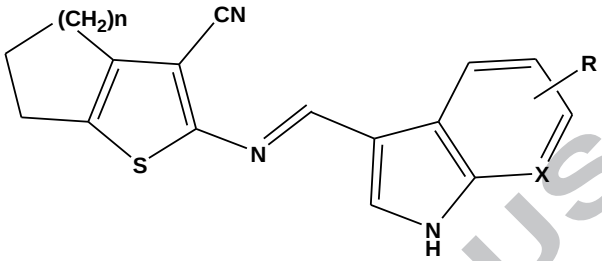
The chemical structures of all new compounds were then characterized on the basis of their physicochemical, and spectral data **(available in Supporting data)**, and were in full agreement with the proposed structures

Table 1. Chemical structures and yields of the new hybrid 2-[(indolyl)-methyleneamino]-cycloalka[*b*]thiophene-3-carbonitrile derivatives (**TN5, TN5 1-7**, **TN6, TN6 1-7**, **TN7, TN7 1-7**, **TN8, TN8 1-7**).



Compound	R	X	n	Yield (%)	Compound	R	X	n	Yield (%)
TN5	H	CH	1	57	**TN7**	H	CH	3	90
TN5-1	5-CH_3	CH	1	77	**TN7-1**	5-CH_3	CH	3	63
TN5-2	5-OCH_3	CH	1	70	**TN7-2**	5-OCH_3	CH	3	65
TN5-3	7-CH_3	CH	1	74	**TN7-3**	7-CH_3	CH	3	77
TN5-4	4-NO_2	CH	1	81	**TN7-4**	4-NO_2	CH	3	69
TN5-5	[*g*]Benzo	CH	1	59	**TN7-5**	[*g*]Benzo	CH	3	64
TN5-6	H	N	1	52	**TN7-6**	H	N	3	32
TN5-7	5-CN	CH	1	58	**TN7-7**	5-CN	CH	3	49
TN6	H	CH	2	58	**TN8**	H	CH	4	39
TN6-1	5-CH_3	CH	2	65	**TN8-1**	5-CH_3	CH	4	60
TN6-2	5-OCH_3	CH	2	59	**TN8-2**	5-OCH_3	CH	4	72
TN6-3	7-CH_3	CH	2	75	**TN8-3**	7-CH_3	CH	4	57
TN6-4	4-NO_2	CH	2	96	**TN8-4**	4-NO_2	CH	4	61
TN6-5	[*g*]Benzo	CH	2	83	**TN8-5**	[*g*]Benzo	CH	4	73
TN6-6	H	N	2	56	**TN8-6**	H	N	4	77
TN6-7	5-CN	CH	2	74	**TN8-7**	5-CN	CH	4	51

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The ^{1}H NMR and ^{13}C NMR data for all obtained compounds are given in the Supplementary Material and all proposed structures were confirmed. As observed in Souza et al. [21], the ^{1}H NMR spectra did not display signs of the precursor amino groups, but rather CH=N protons, which demonstrates successful pharmacophore (2-aminothiophene and indole) coupling.

The CH=N proton signals were observed as a singlet in the 8.14–9.02 ppm region, almost always as the second and more de-shielded signal. The most de-shielded signal was the broad singlet associated with the N-H proton of the indole ring, which appears between 11.93 and 12.95 ppm. The most shielded NMR signals were attributed to cycloalkane protons and carbons. These appeared at 1.37–2.89 ppm in ^{1}H NMR, and at 16.61–31.33 ppm in ^{13}C NMR.

The HRMS of all compounds exhibited $(M+H)^{+}$ molecular ion peaks, which is in agreement with their molecular formulas. For **TN6-3,** the molecular ion peak found was $[M-CH_3]^{+}$ which is likely due to the high incidence of α fragmentation which occurs in C-heteroatomic bonds.

The majority of the hybrid derivatives evaluated displayed antipromastigote activity, some of the compounds tested revealed higher activity than both penta- and trivalent antimony (reference drugs), as can be seen in the IC_{50} values **(Table 2) (Supporting data, Gra. S1)**.

Table 2. Data on 50% growth inhibition of the parasite population (IC_{50}), cellular viability (HC_{50}) and selectivity index (SI).

Series	Group	IC_{50} (µg/mL)	HC_{50} (µg/mL)	SI
TN5	-	48.9	>400	>8.2
TN5-1	1	7.1	>400	>56.6
TN5-2	2	124.7	>400	>3.2
TN5-3	3	91.6	n.d.	n.e.
TN5-4	4	171.2	n.d.	n.e.
TN5-5	5	7.9	>400	>50.7
TN5-6	6	289.0	>400	>1.4
TN5-7	7	7.4	>400	>53.7
TN6	-	4.5	>400	>88.8
TN6-1	1	2.3	>400	>172.4
TN6-2	2	6.0	>400	>66.8
TN6-3	3	322.0	>400	>1.2
TN6-4	4	299.0	>400	>1.3
TN6-5	5	29.7	>400	>13.5
TN6-6	6	228.0	>400	>1.7

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TN6-7	7	4.7	>400	>84.7
TN7	-	3.2	>400	>124.6
TN7-1	1	6.1	>400	>65.6
TN7-2	2	10.2	>400	>39.1
TN7-3	3	786.0	>400	>0.5
TN7-4	4	1067.0	>400	>0.3
TN7-5	5	18.7	>400	>21.4
TN7-6	6	8.7	>400	>45.8
TN7-7	7	3.4	>400	>115.6
TN8	-	5.4	>400	>73.2
TN8-1	1	6.5	>400	>61.3
TN8-2	2	6.4	>400	>62.7
TN8-3	3	4.1	>400	>96.6
TN8-4	4	9.4	>400	>42.5
TN8-5	5	9.4	>400	>42.6
TN8-6	6	651.0	>400	>0.6
TN8-7	7	2.1	>400	>193.2
Glucantime		32100.6	681.2	0.02
Amphotericin B		0.2	22.4	124.5
Trivalent Antimony		9.0	347.2	38.2

Analyzing the cyclopenta[*b*]thiophene (**TN5**) series, we observe that the eight tested compounds present antileishmanial activity with IC_{50} values between 7.07 and 171.2 µg/mL. Among the eight tested compounds, those presenting stronger antipromastigote activity were the compounds presenting 5-methyl (**TN5-1**), benzo[*g*]indol (**TN5-5**), and 5-cyano (**TN5-7**) groups. We also observed that the incorporation of such groups promoted significant increases in antileishmanial activity as compared to the non-substituted base nucleus (compound **TN5**). The incorporation of the pyrrolo[2,3-*b*]pyridin (**TN5-6**) resulted in compounds with low activity (**Supporting data, Gra. S2**).

Five compounds from the cyclohexa[*b*]thiophene (**TN6**) series showed antipromastigote activity with IC_{50} values ranging from 2.3 to 29.7 µg/mL. Three compounds displayed higher activity: **TN6-1** (5-methyl), **TN6-2** (5-methoxy) e **TN6-7** (5-cyano), presenting more pronounced antileishmanial activity when compared to the non-substituted derivative (**TN6**). Substituting the indole ring with 7-methyl (**TN6-3**), 4-nitro (**TN6-4**) and pyrrolo[2,3-*b*]pyridin (**TN6-6**) groups resulted in negative contributions to the antipromastigote activity (**Supporting data, Gra. S3**).

The cyclohepta[*b*]thiophene (**TN7**) series revealed six compounds with antipromastigote activity; IC_{50} values ranging from 3.2 to 18.7 µg/mL. However, we stress that besides the 5-cyano derivative (**TN7-7**), incorporation of substitutes to the base nucleus of this series resulted in decreased anti-parasitic activity as compared to the non-substituted

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derivative (**TN7**). The greatest difference was observed with the insertion of 7-methyl (**TN7-3**) and 4-nitro (**TN7-4**) groups; resulting in compounds with non-significant antipromastigote activity (**Supporting data, Gra. S4**).

The cycloocta[*b*]thiophene (**TN8**) series showed the best antileishmanial profile; seven hybrid compounds were active against the parasite (IC_{50} values ranging from 2.1 to 9.4 µg/mL). Analyzing the results of the non-substituted (**8CN**) compound, and comparing with the rest of the compounds in this series, one may note that incorporation of 7-methly (**TN8-3**) and 5-cyano (**TN8-7**) groups promoted significant increases in anti-*Leishmania* activity (**Supporting data, Gra. S5**).

Analyzing the influence of indole group bound substitutes; there is an emerging pattern of increasing antipromastigote activity when the methyl group is changed from position 7 to 5 on the indole ring in series **TN5**, **TN6** and **TN7**. It is important to highlight that **TN6-3** and **TN7-3** showed respectively low antileishmanial activity (IC_{50} = 322.0 and 786.0 µg/mL). With this alteration in the placement of the methyl group in **TN6-1** and **TN7-1,** a noticeable increment in the antipromastigote activity was detected (IC_{50} = 2.3 µg/mL e 6.1 µg/mL, respectively).

Concerning position C-5 substituent influence; within the same series a methyl group resulted in compounds with higher activity than substitutions with a methoxyl group, as was observed in compounds **TN5-1** e **TN5-2** (IC_{50} = 124.7 and 7.1 µg/mL, respectively), and **TN7-1** e **TN7-2** (IC_{50} = 10.2 and 6.1 µg/mL, respectively).

Substitutions resulting in indole ring size increases (compounds with benzo[*g*]indole moiety) were well tolerated, resulting in compounds with low IC_{50} values, respectively (7.9, 9.4, 18.7, and 29.7 µg/mL) for compounds **TN5-5**, **TN8-5**, **TN7-5** and **TN6-5**,.

We observed a variation in biological activity for compounds with the indole portion substituted by a 4-nitro group, with respective values for IC_{50} at **TN5-4** 171.2 µg/mL, **TN8-4** 9.4 µg/mL, **TN6-4** 299.0 µg/mL, and **TN7-4** and 1067.0 µg/mL.

The introduction of a 5-cyano group to the indole ring resulted in the most active compounds among the series studied; **TN8-7** and **TN7-7** with respective IC_{50} values of 2.1 and 3.4 µg/mL; indicating that substitutions in position 5 by electron-withdrawing groups are well tolerated, and result in compounds with more expressive biological activity.

We may infer that the quantity of methylene groups (CH_2) bound to the thiophene ring hybrid derivative exerts a progressively positive influence on antipromastigote activity. Accordingly observed in our study, the greater the size of the cycloalkane ring (more methyene groups) the more pronounced the antipromastigote activity, i.e. it was observed that

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the cycloocta[*b*]thiophene derivatives (**TN8** series) showed the best activity profile. We believe that the drug's binding site to the drug target in the parasite might be large and hydrophobic, allowing a better interaction with larger rings.

Some thiophene derivatives extracted from plants have been referred to as effective against promastigote forms of certain *Leishmania* species [22], hinting at the potential of such molecules as antileishmanial agents. Synthetic compounds derived from indole, as reported by Gupta et al. [23] demonstrated the potential of indole derivatives using [1,2,4] triazine [5,6-*b*]indol-3-ylthio-1,3,5-triazine; the antileishmanial activity of such compounds were verified against *Leishmania donovani*.

Studies with other synthetic thiophene derivatives have also reported antileishmanial potential against promastigote forms. Rodrigues et al. [19] reported utilizing 2-aminothiophene derivatives; demonstrating activity against promastigote forms with IC_{50} values ranging from 3.37 to 189.3 μM. The highest value was related to the compound containing the unsubstituted thiophene ring. When compared with our indole derivatives, one notes that an indole nucleus increases antipromastigote activity, evidenced by the low IC_{50} values registered. It is important to stress that certain drugs evaluated in the present study displayed IC_{50} values similar to those registered in our previous works [19]. For example, our two most promising compounds **TN6-1** and **TN8-7** (IC_{50}: 2.3 μg/mL (7.3 μM), and 2.1 μg/mL (5.1 μM**)**, respectively) as compared to the most promising drugs in the cited work; SB-200, SB-44, and SB-83 (9.2 μg/mL (3.6 μM), 2.3 μg/mL (7.3 μM), and 1.3 μg/mL (3.3 μM)), made evident the positive antileishmanial effect of indoles to the hybrid derivatives tested.

None of the hybrid derivatives tested showed cytotoxicity against human erythrocytes up to the highest evaluated concentration (400 μg/mL). This demonstrated that all of the evaluated compounds are less toxic to blood cells than the three reference drugs (penta- and trivalent antimonials, and Amphotericin B). It is important to highlight that these drugs, (with hemolytic activity), are the currently applied antileishmanial therapeutics, and present high cytotoxicity in humans [24]. We report that incorporation of certain groups in non-substituted compounds did not alter cytotoxicity for the new indole derivatives **(Table 2)**. The *in vitro* results suggest that these compounds might not present significant *in vivo* toxicity. Nevertheless, *in vivo* toxicity assays are necessary to confirm this hypothesis.

We also report that all of the derivatives tested were more toxic to the promastigote forms of *L. amazonensis* than to human erythrocytes, displaying selective activity for the parasite **(Table 2)**.

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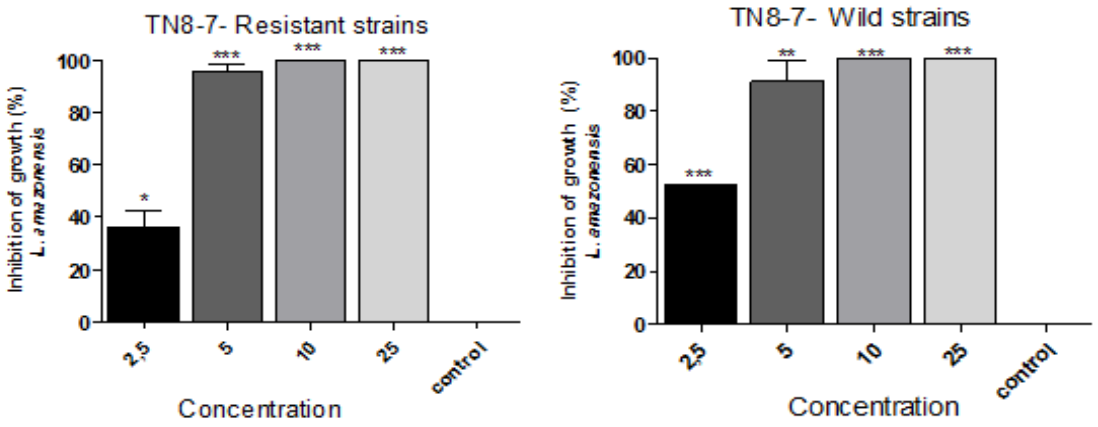
In comparison with the reference drugs, we note that based on the selectivity index most of the new thiophene-indole derivatives are significantly more selective than the penta- and trivalent antimonials. Also, compounds **TN6-1** (SI >172.4), **TN7** (SI >124.6) and **TN8-7** (SI >193.2) were more selective than Amphotericin B (SI: 124.5).

From the 32 studied molecules, 2-[(1*H*-indol-3-ilmethylene)-amino]-4,5,6,7,8,9-hexahydro-cycloocta[*b*]thiophene-3-carbonitrile **(TN8-7)** was selected for subsequent studies. This choice was made based on its lower IC_{50} value when compared to its own (and other) series. The results obtained confirm better selectivity (by index) than all three of the selected reference drugs.

We therefore performed a parasite growth inhibition test using trivalent antimony (Sb (III)) resistant strains and obtained similar values as compared to those obtained for sensitive strains, previously exposed to the same substance **(Graph 1)**. For the resistant strains and the sensitive ones we registered respective IC_{50} values of 2.8 µg/mL, and 2.1 µg/mL. These results demonstrate the efficiency of the new drug when applied against strains with resistance to the most often applied current therapeutic.

Graph 1. Antileishmanial activity of the **TN8-7** drug on promastigote forms of *Leishmania amazonensis* whether sensitive or resistant to trivalent antimony.



The promastigotes treated with **TN8-7** exhibited DNA fragmentation, which was previously reported by Rodrigues et al. [19]. As represented in **Figure 1,** the samples exposed to 1x, 2x, and 4x the IC_{50} showed DNA bands fragmented in three portions, as compared to the control (first sample). We also verified an increase of last band intensity with increasing drug concentration, suggesting higher fragmentation at four times the IC_{50}. Reports indicate

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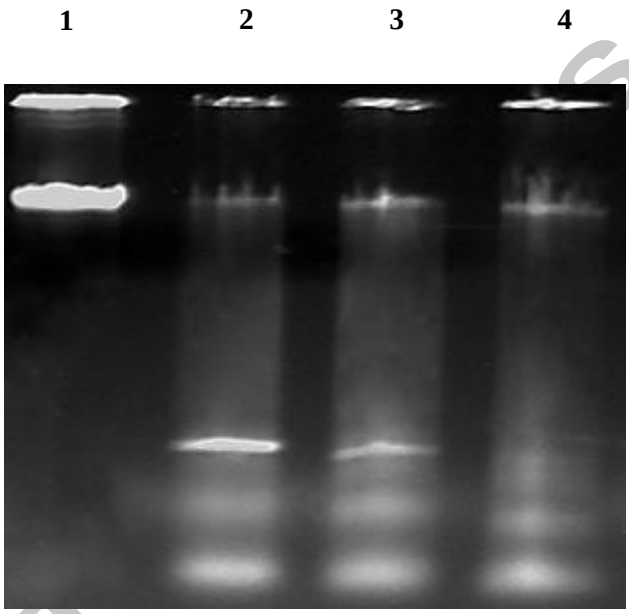
that treatment with some drugs induces endonuclease liberation, which is responsible for DNA fragmentation in *Leishmania* parasites [25]. **TN8-7** might well induce parasitic cells to enter apoptosis. Yet, more detailed studies are necessary to clarify the mechanism of action, not only for the current substance, but for the other derivatives synthesized.

Figure 1. The genomic DNA of *Leishmania amazonensis* promastigotes treated with different concentrations of **TN8-7**. Agarose gel (1%) was stained with GelRed (1:500), and analysis was performed under UV light. (1) control; (2) 1x IC_{50}; (3), 2x IC_{50} (4), and 4x IC_{50};



The CPCA (Consensus PCA) study was developed using Volsurf+ software considering 13 blocks of descriptors (independent variables), with 128 independent variables/descriptors. After auto-scaling of the data and considering PC1 and PC2, we observed that blocks H2O and DRY had greater weights in explaining the variance of the model. (Figure available in **Supporting data**) The two main principal components (PC1 and PC2) were found using a cross-validation technique, explaining 61.266% of the total variance in antileishmanial activity.

Explaining 61.266% of the total variance in antileishmanial activity, the two main principal components (PC1 and PC2) were found using a cross-validation technique, (a **t**able of the explained variance on CPCA is available in **Supporting data**).

The PCA analysis was generated considering the 40 higher weight descriptors in the highlighted CPCA. More than 60% of the total variance (60.544%) was explained by PC1 and PC2.

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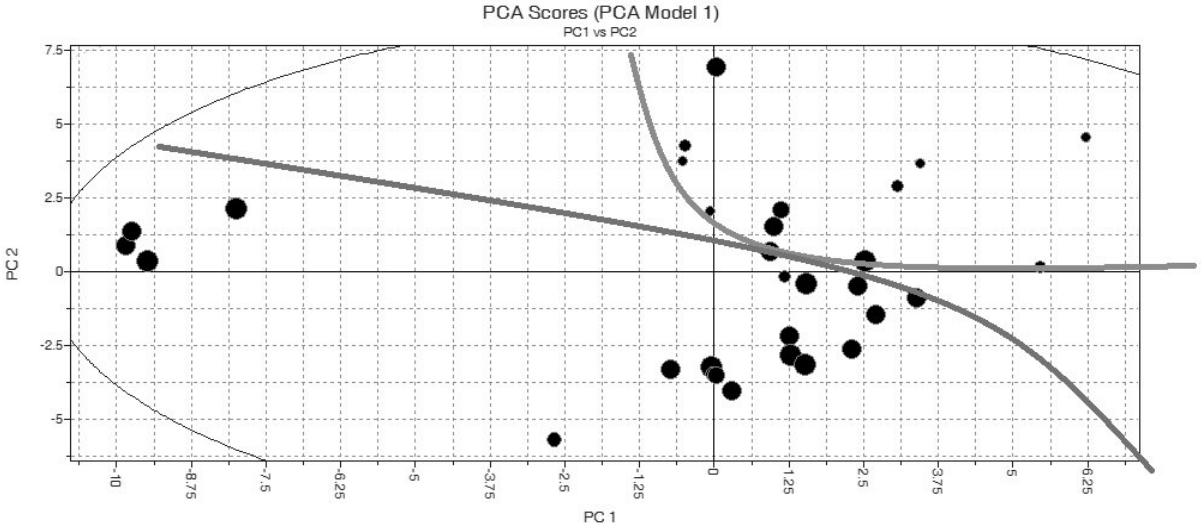
As can be observed in **Figure 2**, the plot score from the PCA (using the DRY and OH2 VolSurf+ descriptors) was able to discriminate the most active compounds (large spheres), from the less active compounds (smaller spheres).

Figure 2. Object Positions in relation to PC1 and PC2 components in the PCA. Large spheres represent more active compounds and smaller spheres represent less active compounds.



To carry out the Partial Least Squares (PLS) 33 compounds were selected to compose the training set (TN5, TN5-1, TN5-5, TN6, TN6-1, TN6-5, TN6-7, TN7, TN7-2, TN7-5, TN7-7, TN8, TN8-1, TN8-2, TN8-3, TN8-5, TN8-7, 6CN10, 6CN09, SB-80, SB-63, SB-81, SB-44 e SB-83), and 9 compounds were used for the test set (TN5-2, TN5-7, TN6-2, TN7-1, TN7-6, TN8-4, 6CN01, SB-68 e SB-200) (Compounds 6CN10, 6CN09, 6CN01, SB-80, SB-68, SB-63, SB-81, SB-200, SB-44 and SB-83 may be found in Rodrigues et al. [19].).

We applied *leave-one-out*, and the program used was Pentacle software. The best model was able to explain 78.17% of the data variance with five latent variables and showed respective r^2, q^2_{cv} and R^2 values of 0.99, 0.89 and 0.44**.** The descriptors found thru PLS were generated in interaction with the probes lipophilicity (DRY-DRY), and the molecular shape (TIP-TIP), highlighting hydrophobicity and molecular shape as important for activity to occur [21, 26, 27].

In conclusion, thirty-two hybrid compounds containing cycloalka[*b*]thiophene and indole moieties (**TN5, TN5 1-7**, **TN6, TN6 1-7**, **TN7, TN7 1-7**, **TN8, TN8 1-7**) were designed, synthesized in good yields, and well characterized by physicochemical and spectral data (^{1}H NMR, ^{13}C NMR and HRMS). These compounds were investigated for their cytotoxic and antileishmanial properties against promastigote forms of *Leishmania*

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amazonensis. It was observed that many of the substances evaluated, whose IC_{50} values were less than 10 µg/mL, demonstrated higher activity than the reference drugs (penta- and trivalent antimonials). The most active compounds were **TN8-7**, **TN6-1** and **TN7** with respective IC_{50} values of 2.1, 2.3, and 3.2 µg/mL.

To the highest concentration tested, none of the thiophene-indole derivatives evaluated revealed cytotoxicity to human erythrocytes, demonstrating that all of the evaluated compounds are less toxic to blood cells than the three reference drugs. The 2-aminothiophene derivatives were more selective for the parasite than for blood cells, presenting a high selectivity index, sometimes higher than the reference drugs. Compound **TN8-7** showed the greatest antileishmanial activity, its action associated with DNA fragmentation. The molecule demonstrated its effectiveness against antimony-resistant parasites.

For the designed compounds, the chemometric studies generated robust models with good statistical indices; especially after internal validation; highlighting that the descriptors related to hydrophobicity and molecular shape are closely related to antileishmanial activity.

Based on our data, we suggest that hybrid cycloalka[*b*]thiophene-indole compounds may be taken as lead compounds for further development as new drugs for the treatment of leishmaniasis.

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Supplementary data

Supplementary data associated with this article can be found, in the online version at;

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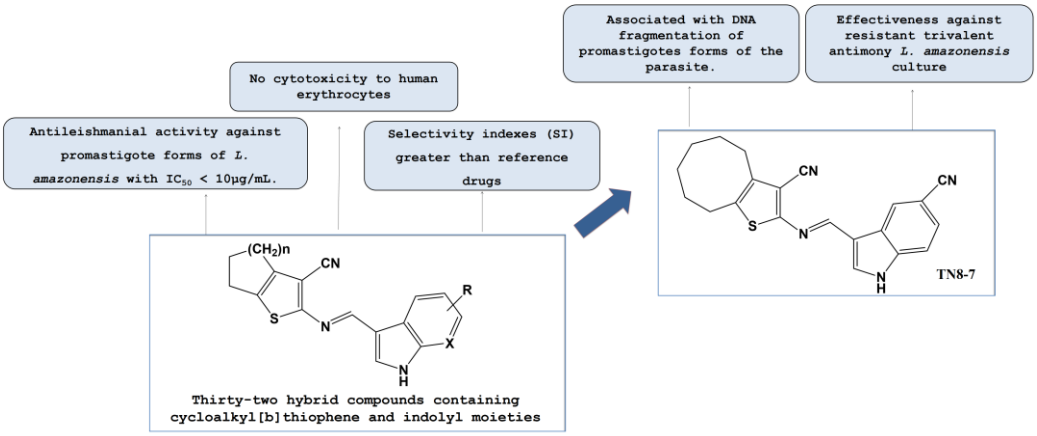
Graphical abstract



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HIGHLIGHTS

- 32 hybrid thiophene-indolyl derivatives were synthesized and characterized;
- Various compounds showed good antileishmanial activity (IC_{50} < 10µg/mL);
- Most of the compounds presenting Selectivity Indexes greater than reference drugs;
- Compound **TN8-7** showed activity associated with DNA fragmentation;
- Chemometric tools found Lipophilicity and Size/Shape as important for the activity;

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