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Discovery of Novel Antileishmanial Agents in an Attempt to Synthesize Pentamidine−Aplysinopsin Hybrid Molecule

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In an attempt to synthesize pentamidine−aplysinopsin hybrid molecule **25**, a lead molecule **8** (containing *Z*-configured aplysinopsin moiety) was identified for antileishmanial activity. Optimization of lead **8** provided **24** (containing *E*-configured aplysinopsin) possessing 10 times more activity and 401-fold less toxicity than the drug pentamidine in cell based assays. Synthesis of **24** was possible, surprisingly, because of two innate reactivities of indole-3-carbaldehyde which provided it in diastereo- and regio-selectively pure form without recourse to the long reaction pathway.

Introduction

Leishmaniasis is a vector born parasitic disease of the tropics and subtropics which is manifested in four major clinical forms (cutaneous leishmaniasis, mucocutaneous leishmaniasis, visceral leishmaniasis, and post kala-azar dermal leishmaniasis or PKDL[a]) depending on the causative species of the protozoan *Leishmania*. In all of the above forms of leishmaniases, visceral leishmaniasis (VL) is lethal, if left untreated. There are ≈70,000 deaths, and 1.5 million new cases emerge per year due to VL. Majority of VL cases (≥90%) occur in just six countries: India, Nepal, Bangladesh, Sudan, Ethiopia, and Brazil. The situation has become complicated because of the emergence of PKDL, which appears in 0−6 months after the successful curing of VL.[1] The WHO has declared VL a neglected and emerging disease.[2]

Antimonials are the first line of treatment options for VL, which were discovered almost 70 years ago. These suffer from major side effects including cardiac arrhythmia and pancreatitis. Besides their toxicity, treatment failure with antimonials use has increased; some times, as high as 62% in some of the regions.[1] Second line treatment options for VL are pentamidine, miltefosin, and amphotericin B. However, all of these drugs suffer from moderate to severe side effects. Pentamidine, an aromatic diamidine, is not active orally and can lead to renal, pancreatic, and hepatic toxicity along with hypotension and dysglycemia.[3] Miltefosine, an alkylphosphocholine, has a long half-life (100−200 h) in humans and a low therapeutic ratio, the characteristics that could encourage the development of resistance. It is not suitable for use during pregnancy because of tetratogenecity and also cause mild to severe gastrointestinal side effects.[4] Liposomal amphotericin B is a highly effective option; however, this drug formulation is very expensive (US $2800 per treatment), limiting its use in most endemic regions. Although recent clinical trials with injectable paromomycin have shown encouraging results, an expanded catalog of new drugs for the VL causing parasite *L. donovani* is required to tackle the problem of resistance.[1]

In spite of some side effects of pentamidine, the broad range of its (dicationic class of molecules in general) biological activities,[5] relatively low propensity toward the development of resistance (resistance to pentamidine itself has never been a significant problem in the field, despite its widespread use as a prophylactic),[6] and our previous work on this class of molecules[7] prompted us to develop some novel dicationic class of molecules as potential antileishmanial agents with improved efficacy and selectivity than pentamidine. From a literature search, we found that aplysinopsins (a class of natural products possessing cyclic guanidine function)[8] act on similar biological targets (plasmepsin II and serotonin receptors) as a dicationic class of molecules.[9] Taking inspiration from a fragment based drug discovery (FBDD) approach,[10,11] we designed a hybrid molecule **25** (Figure 1), where one amidinophenoxy function of pentamidine has been replaced with aplysinopsin.

Because of their synthesis in biological settings, small molecule natural products have in built selectivity and phamacokinetic profile (necessary for a drug molecule).[12] Hence, incorporating a drug fragment with natural product may provide molecules with better activity and less toxicity.[13,14]

Herein, we describe our efforts for the synthesis of hybrid molecule **25** and its analogues and the discovery of a new class of antileishmanial agents in this endeavor.

Chemistry. To synthesize compound **25** and its analogues, we adopted the route depicted in Scheme 1. Direct synthesis of aplysinposin and its subsequent coupling with the pentamidine fragment was not feasible because of poor yield in the synthesis of aplysinopsin and a lack of chemo selectivity in its subsequent coupling with the petamidine fragment [mainly *p*-(hydroxypentyl)benzamidine]. As we proceeded according to Scheme 1, we encountered a problem in direct

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[a] Abbreviations: TBHP, *tert*-Butylhyrdroperoxide; DIPEA, diisopropylethylamine; TBAB, tetrabutylammonium bromide; VL, visceral leishmaniasis; PKDL, post kala-azar dermal leishmaniasis; FBDD, fragment based drug discovery; SE: standard error.

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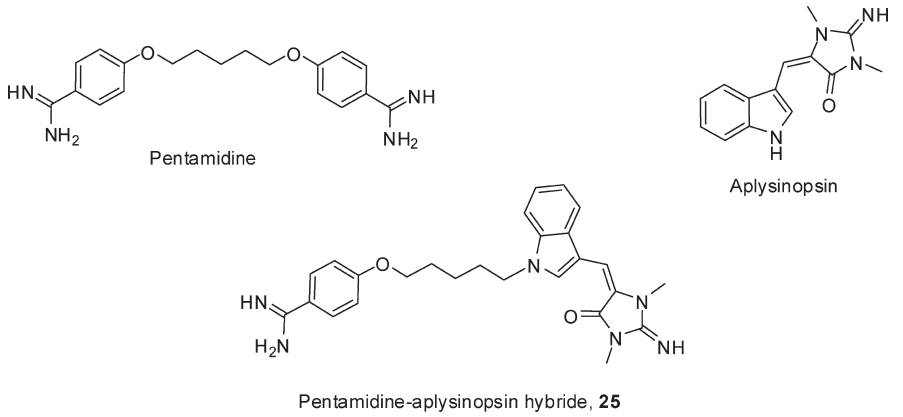
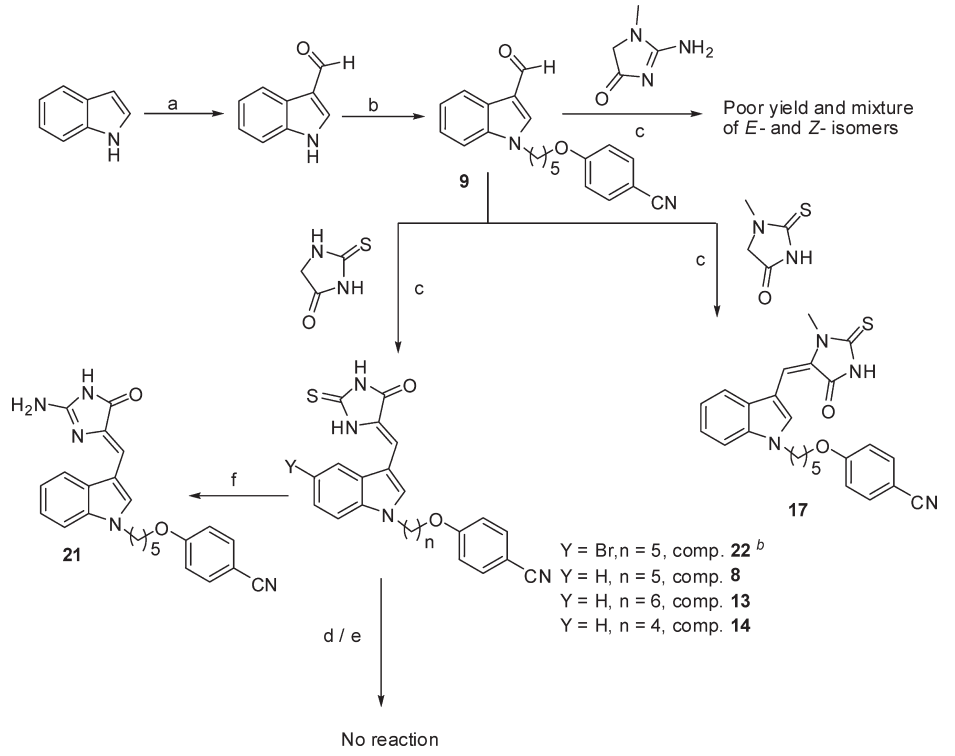


Figure 1. Hybrid of pentamidine and aplysinopsin.

Scheme 1. Compounds Formed (**1**−**9**) in an Initial Attempt to Synthesize Molecule **25**[a]



[a] Reagents and conditions: (a) DMF, $POCl_3$, 0 °C-rt. (b) *p*-cyanophenoxypentylbromide, toluene, NaOH (aq.), TBAB, rt. (c) ethanolamine (1 equiv.), ethanol (absolute), 60 °C. (d) MeOH (dry), HCl (g). (e) MeOH, MeONa. (f) TBHP/ NH_3 (aq.), MeOH, steel vessel. [b]See Supporting Information.

condensation of 2-imino-1-methyl-5*H*-imizazole-4-one (creatinine), which occurred in poor yield and provided a mixture of diastereomers (*E*- and *Z*-). Therefore, we first condensed 2-thio-4-imidazolidinon (2-thiohydantoin) with intermediate **9** to provide compounds **8**, **13**, and **14** in their *Z*- geometry. Conversion of the cyano function of compound **8** to amidine (Pinner synthesis) again proved difficult because of its high insolubility in MeOH and the presence of many NH centers (Scheme 1).

We tried a novel approach for the conversion of the cyano function of **8** to amidine by first complexing it with Zn^{2+} (using anhydrous $ZnCl_2$) and then using neat amine to form the desired amidine. But instead of getting amidine, we observed the guanylation of amine with substrate **8** to form compounds **1**−**6** (Scheme 2). Guanylation of amine was known using $HgCl_2$, Bi(III) salts, and Mukaiyama's reagent.[15] This is the first example of the use of anhydrous $ZnCl_2$ in guanylation of amines. In the absence of anhydrous $ZnCl_2$, guanylation occurred in very poor yield with many side products.

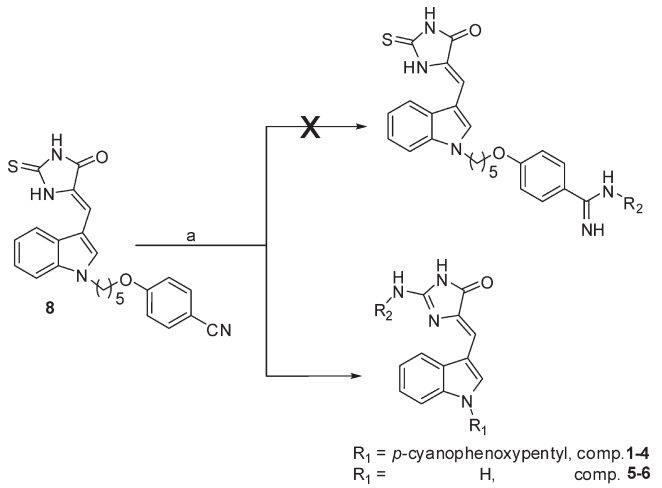
In the second phase of our endeavor, we synthesized various analogues of compound **8**, which was found active in antileishmanial screening. To synthesize intermediates **26** and **27** (Scheme 3) containing compounds other than the *p*-cyanophenoxy moiety, we have to change the sequence of coupling by first replacing one bromo group of 1,5-dibromopentane with indole-3-carbaldehyde and then replacing the other with piperazine and phenol, respectively, instead of doing the opposite, which provided only dimer. Similarly, intermediate **28** (Scheme 4) was synthesized to finally obtain compound **18**. S-methylated derivative **15** was synthesized under controlled methylation conditions (Scheme 3).

In the third phase (Schemes 4, 5, and 6), we attempted to synthesize other analogues of lead molecule **8** with a focus on

the modification of its cyano functional group, i.e., to incorporate amidine functionality in **8**. Compound **21** was obtained from compound **8** by oxidative amination of thiocarbonyl function (Scheme 1). For the synthesis of compound **19**, direct reaction of hydroxylamine hydrochloride with **8** yielded an inseparable mixture of unknown compounds, which was characterized neither by *Z*- nor *E*-isomers.

Therefore, we prepared intermediate **29** for the synthesis of **19** and **24** by coupling of *p*-amidoximophenol with 1-bromopentylindole-3-carbaldehyde. Two regio-isomers were formed in the reaction, but desired isomer **29** (phenolic OH-substituted isomer) precipitated out in pure form after initial purification by column chromatography (Scheme 4). In contrast to that described above, simple *n*-alkylation of *p*-amidoximophenol provided an inseparable mixture of two regio-isomers. Compounds **17** and **24** were formed in exclusive *E*-configuration because of the innate reactivity of indole-3-carbaldehyde (Schemes 1 and 4, respectively).[16]

Scheme 2. Guanylation of Amine in an Attempt to Convert the Cyano Function of **8** to Amidine[a]



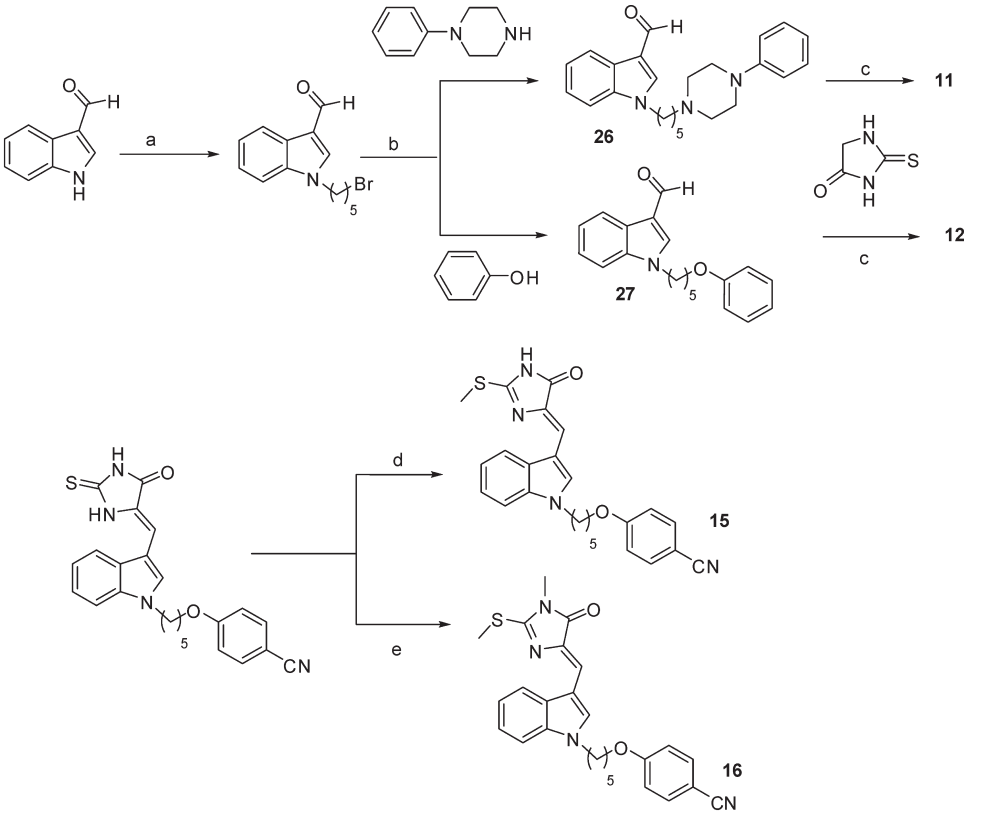
[a] Reagents and conditions: (a) $ZnCl_2$ (anhy.), R_2NH_2 (aliphatic amines or anilines), neat, 80−90 °C.

Amide analogue **20** was synthesized (Scheme 5) by first converting the cyano function of *p*-cyanophenoxypentyl bromide to amide and then proceeding as described for compound **8**.

Synthesis of compound **23** was started from alkylation of NH of azaindole to form **31** followed by Vilsmeir formylation. Reverse of the sequence for the synthesis of **23** was not feasible because of poor yield in Vilsmeir formylation of azaindole (Scheme 6).

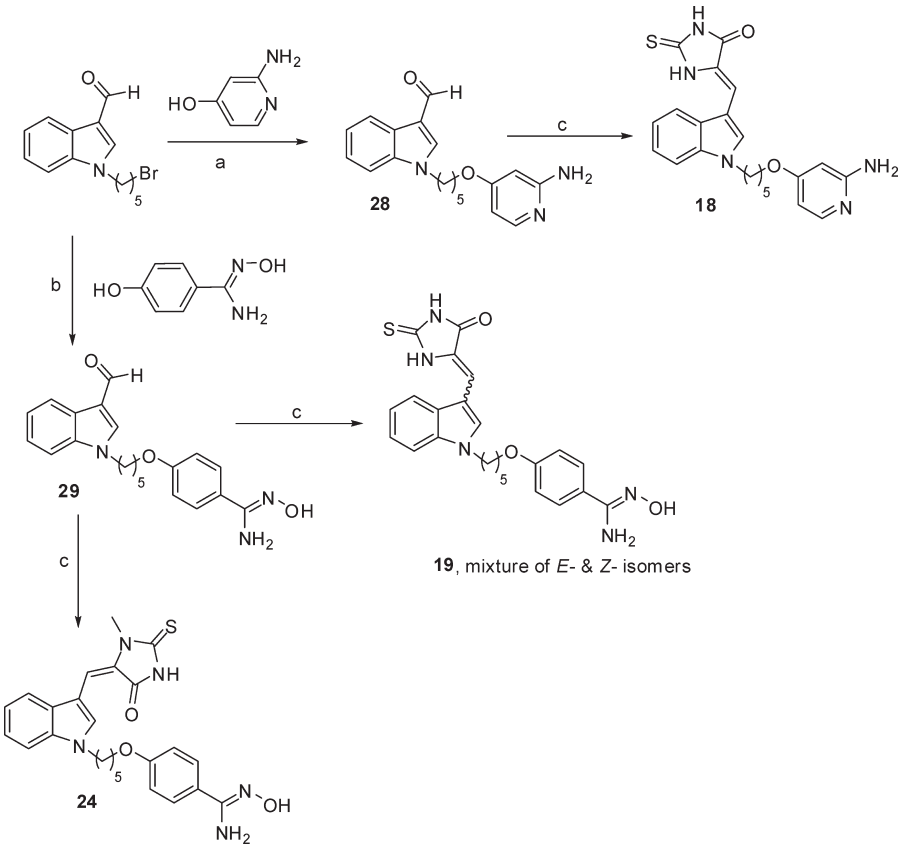
Indole-3-carbaldehyde derivatives **29** and **30** (Schemes 4 and 5, respectively) behave unusually in Knoevenagel condensation with 2-thiohydantoin. These derivatives instead of providing the usual Z- configured molecules, yielded almost 1:1 mixtures of *E*- and *Z*-isomers. The behavior of these indole-3-carbaldehydes derivatives where indole NH was substituted with *p*-amido- and *p*-amidoximo-phenoxypentyl was surprising in comparison to that of *p*-cyanophenoxypentyl substituted indole derivative **9**, which provided only the *Z*-isomer. The change of the cyano function of *p*-cyanophenoxypentylindole-3-carbaldehyde (**9**) to amidoxim or amide function may have disturbed the geometry of Knoevenagel condensation of CH acid (2-thiohydantoin) by interference in the transition state (TS) of the reaction.

Scheme 3. Synthesis of Compounds (**11**, **12**, **15**, and **16**)[a] for Optimization of **8**[b]



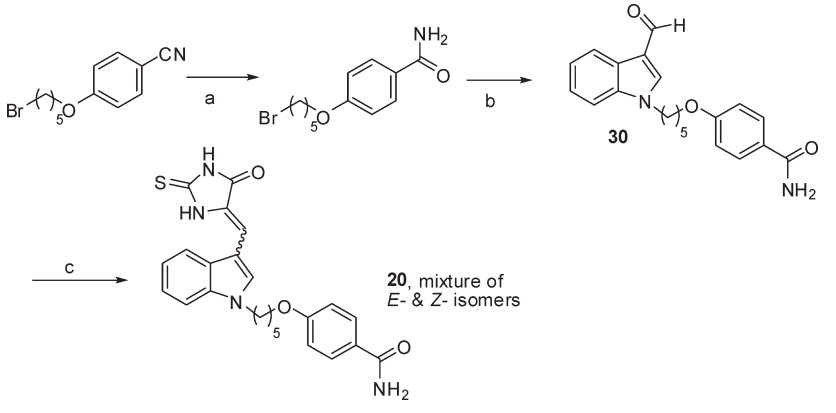
[a] See Scheme 1 for **10**, **13**, and **14**. [b] Reagents and conditions: (a) 1,5-dibromopentane (5 equiv.), K_2CO_3, acetone, 60 °C. (b) DIPEA, AcCN, 80 °C. (c) Ethanolamine (1 equiv.), ethanol (absolute), 60 °C. (d) MeI, K_2CO_3, acetone, 0 °C. (e) MeI, K_2CO_3, DMF, 70 °C.

Scheme 4. Synthesis of Compounds (**18**, **19**, **24**)[a] in a Further Attempt at Optimizing **8**[b]



[a] See Scheme 1 for **21** and **22**, Scheme 5 for **20**, and Scheme 6 for **23**. [b] Reagents and conditions: (a) K_2CO_3, acetone, 60 °C. (b) AcCN, K_2CO_3, 60 °C. (c) 2-Thiohydantoin or 1-Me-2-thiohydantoin, ethanolamine (1 equiv.), ethanol (absolute), 60 °C.

Scheme 5. Synthesis of Compound **20** in a Further Attempt at Optimizing **8**[a]

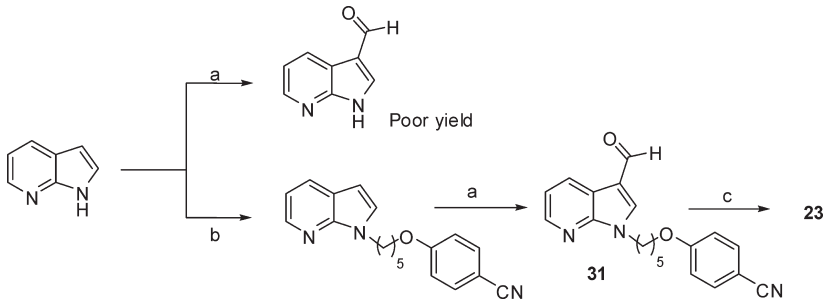


[a] Reagents and conditions: (a) NaOH (aq.), H_2O_2, MeOH. (b) indole-3-carbaldehyde, K_2CO_3, acetone, 60 °C. (c) 2-Thiohydantoin or 1-Me-2-thiohydantoin, ethanolamine (1 equiv.), ethanol (absolute), 60 °C.

Results and Discussion

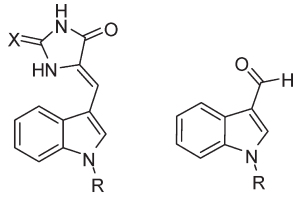
To find new antileishmanial agents, we screened initially formed guanylated derivatives **1**−**4** against the promastigote stage of *L. donovani* (using firefly luciferase gene transfected promastigotes). The structures were first screened at 10 μg/mL concentration. If more than 90% inhibition was observed, the compounds were further screened against the intracellular amastigote stage of the parasite. For the screening at the amastigote stage of parasite, mouse macrophages were infected with firefly luciferase expressing promastigotes, which transformed to amastigotes in macrophages. The inhibition of amastigote growth was determined by comparison of luciferase activity of the drug treated parasites with that of the untreated control (see Experimental Section). Using the above screening protocols, we observed that compounds **2**−**4** have shown >90% inhibition of the promastigote stage of parasites, but none has shown activity against the amastigote stage of *L. donovani*. Later, compounds **5** and **6**, in which the *p*-cyanophenoxypentyl chain has been removed, were screened. This change has even diminished the compounds' anti promastigote activity. Finally, the screening of precursor compounds **7**−**9** provided a hit molecule, **8**, which showed 62% growth inhibition of amastigotes in infected macrophages at 12.5 μg/mL without showing any toxicity (Table 1).

Scheme 6. Synthesis of Compound **23** in a Further Attempt at Optimizing **8**[a]

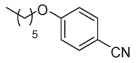
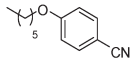
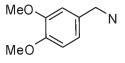
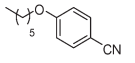
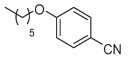
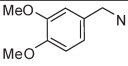
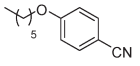
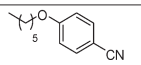


[a] Reagents and conditions: (a) DMF, $POCl_3$, 0 °C-rt. (b) NaH, DMF, rt, under N_2. (c) 2-Thiohydantoin, ethanolamine (1 equiv.), ethanol (absolute), 60 °C.

Table 1. Preliminary Antileishmanial Screening of Initially Formed Compounds **1**−**9**



1-8 **9**

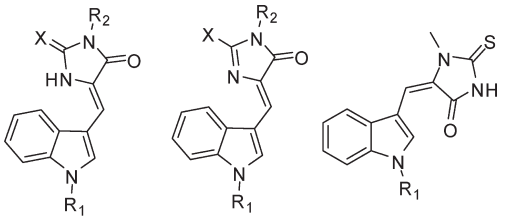
Comp.	R	X	Geometric Isomer	% Inhibition [a]	
				Prom.[b]	Amat.[c]
1			*Z*-	88.0	NI[d]
2			*Z*-	93.7	NI
3			*Z*-	92.8	NI
4			*Z*-	93.9	NI
5	H		*Z*-	NI	NI
6	H		*Z*-	NI	NI
7	H	S	*Z*-	NI	NI
8		S	*Z*-	95.1	62.0 [e, f] (IC_{50}=18.2±0.5μM)
9		-	-	NI	NI

[a] Percent inhibition measured at 10 μg/mL (for Prom.) and at 12.5 μg/mL (for Amat.). [b] Prom.: promastigote. [c] Amat.: intracellular amastigote. [d] NI: no inhibition. [e] Toxicity IC_{50} against J774 cell line > 100 μg/mL. [f] IC_{50} values are the average of two independent assays expressed as average ± standard error.

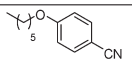
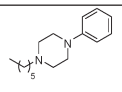
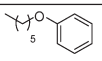
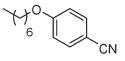
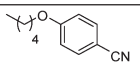
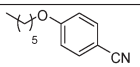
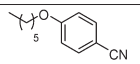
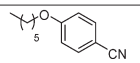
Surprisingly, the same compound **8** was also found active in vivo. Compound **8** showed on average 62.1 ± 9.8 (SE)% parasite reduction in four treated hamsters at a dosage of 4 × 50 mg/kg given through the intraperitonial route. Since in vitro parasite reduction (Table 1) and in vivo reduction (see above) are the same, it may appear that compound **8** has complete bioavailabity. Also, no adverse reactions were observed during and post-treatment in treated hamsters, which are in accordance with no in vitro toxicity of compound **8** (Table 1 footnote *e*).

From the above promising data about compound **8**, we next proceeded to optimize this lead molecule. A series of compounds **10**−**17** were synthesized with variation in chain length (**13** and **14**), thiocarbonyl function (**10**, **15**, and **16**), cyano (**12**) and phenolic moieties (**11**), and change in geometry (**17**). These were screened for antileishmanial activity. As was

Table 2. Antileishmanial Activity of Compounds **10**−**17**



10-14 **15-16** **17**

Comp.	R_1	R_2	X	Geometric Isomer	Toxicity [e] IC_{50} (μg/mL)	% Inhibition [a] Prom.[b]	% Inhibition [a] Amat.[c]
10		H	O	*Z*-	33.0	NI [d]	NI
11		H	S	*Z*-	NT [f]	88.3	NI
12		H	S	*Z*-	NT	40.7	ND [g]
13		H	S	*Z*-	2.3	71.3	ND
14		H	S	*Z*-	NT	58.8	ND
15		H	SMe	*Z*-	NT	75.4	ND
16		Me	SMe	*Z*-	NT	63.2	ND
17		-	-	*E*-	14.8	46.4	ND

[a] Percent inhibition measured at 10 μg/mL (for Prom.) and at 12.5 μg/mL (for Amat.). [b] Prom.: promastigote. [c] Amat.: intracellular amastigote. [d] NI: no inhibition. [e] Cytotoxicity for the J774 cell line. [f] NT: not toxic, i.e., $IC_{50} > 100$ μg/mL. [g] ND: not determined (because of poor activity compared to that of **8** in the Prom. assay).

evident from Table 2, none of these compounds was found to be as promising as compound **8**. Either these showed significantly less activity than **8** in the promastigote assay (< 90% at 10 μg/mL) or were toxic compared to **8** (compounds **13** and **17**). Hence, it became evident that *p*-cyanophenoxypentyl and 2-thiohydantoin moieties were essential for the optimum activity of compound **8**.

To enhance the activity of **8**, we next focused on modifying its cyano functionality by converting it to amide, amidoxim, etc. We also envisaged synthesizing the 2-imino analogue of **8** to see the effect on activity. A series of compounds **18**−**24** were synthesized in the third phase and directly screened against the amastigote internalized in macrophages (which is more relevant for drug discovery) because of the high probability of their being active.

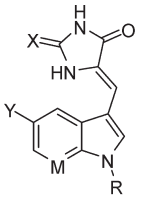
In this endeavor, we found that the introduction of 2-aminopyridine, which mimics amidine function, in place of the *p*-cyanophenoxy moiety in compound **8** proved to be almost ineffective (compound **18**, Table 3). This may be due to the one carbon shortage compared to the *p*-amidinophenoxypentyl fragment of pentamidine. Conversion of the cyano function of compound **8** to amidoxim proved to be totally ineffective (compound **19**), and conversion to amide also did not provide any significant activity (compound **20**). Reduction in the active diastereomer may be the reason for that since both compounds were formed as an ≈1:1 mixture of *E*- and *Z*-isomers. There is also a possibility that structures **19** and **20** may be inherently inactive (also see below).

Surprisingly, compound **24** with pure *E*-configuration proved to be the most promising compound of the series with $IC_{50} = 2.0$ μM and SI = 53.35 for the reduction of parasite of infected macrophages. In the intracellular amastigote assay, compound **24** was found to be 10 times more active than the drug pentamidine, while it showed 401 times less toxicity for human macrophages than the above drug. There are many examples of natural product based drug molecules which possess good bioavailability and selectivity profile (e.g., rapamycin and mevastatin).[12,19] Incorporation of the 2-thio analogue of aplysinopsin (a privileged structure, which acts on similar targets) may have provided the requisite selectivity to **24**.

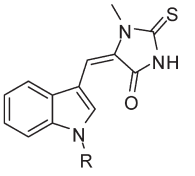
Replacing the indole ring of compound **8** with azaindole (compound **23**) has only improved the activity of compound **8**, in all synthesized compounds with *Z*- configuration. There were two other interesting findings.

First, the conversion of the 2-thio function of compound **8** to 2-imino or substituted imino (which is equivalent to cyclic guanidine function, compounds **21** and **1**−**4**), totally diminished the activity. Lipophilicity of the cationic center of

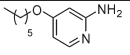
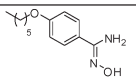
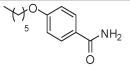
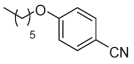
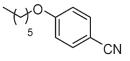
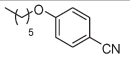
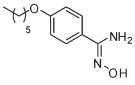
Table 3. Antileishmanial Activity of Compounds **18**−**24**



18-23



24

Comp.	R	X	M	Y	G. Iso.[g]	Inhibition Amastigote [a]		Tox. [c] IC_{50} (μM)	SI
						% [b]	IC_{50} [h] (μM)		
18		S	C	H	*Z*-	32.2	ND [d]	ND	N/A [e]
19		S	C	H	Mix.[i]	0	ND	ND	N/A
20		S	C	H	Mix.	48.9	ND	ND	N/A
21		NH	C	H	*Z*-	0	ND	ND	N/A
22		S	C	Br	Mix.	12.6	ND	ND	N/A
23		S	N	H	*Z*-	97.0	6.5±1.5	187.1±7.4	28.6
24		S	C	H	*E*-	99.3	2.0±1.8	108.5±4.1	53.4
	Control [f]	-	-	-	-	-	20.4	2.7 ±0.4	<1

[a] Intracellular amastigote. [b] Percent inhibition at 10 μg/mL. [c] Cytotoxicity for KB cell line. [d] ND: not determined. [e] N/A: not applicable. [f] Control: pentamidine isethionate salt. [g] G. Iso.: geometric isomer. [h] IC_{50} values are the average of two independent assays expressed as average ± standard error. [i] Mix.: mixture of *E*- and *Z*-isomers.

diamidines has already been reported as the enhancer of biological activity against similar species of protozoa;[17] this may be the reason for the activity enhancement of compound **8** compared to that of **21**.

Second, amidoxim containing compound **24** (possessing *E*-configuration) has shown a better activity profile than its cyano counterpart **17**, which is in the same *E*-configuration (88.2% versus 46.4% inhibition of the promastigote stage of parasite at 10 μg/mL for **24** and **17**, respectively). *p*-Amidinophenoxy functionality is already known as the recognition motif for various transporters involved in pentamidine uptake.[18] The selective enhancement of parasite cell permeability may be the reason for the better activity of **24** over **17**. Furthermore, molecule **8**, which is in *Z*-configuration, is active, while **17**, which is almost similar in functionality (except a Me group) but has opposite geometry, is inactive in the promastigote assay (Tables 1 and 2). These findings suggest that for a given geometry (*E*- or *Z*-), a particular set of functionality is essential for bioactivity. An active molecule in a given geometry may not be as active in the opposite geometry. Hence, compound **19**, which is the amidoxime counterpart of **8** but formed as almost a 50:50 mixture of *E*- and *Z*-isomers, may be inherently inactive, though further work is necessary to confirm this.

Conclusions

In conclusion, in an effort to optimize the activity of lead molecule **8** based on the aplysinopsin pentamidine hybrid scaffold, we found active molecules in both *E*- (compound **24**) and *Z* (compound **23**)-subseries of compounds. Incorporation of pentamidine substructure to the 2-thio analogue of the natural product aplysinopsin (compound **24**) increased both the selectivity and activity of the parent drug pentamidine. The concept may also be useful in improving the bioactivity profile of privileged natural product structure for other therapeutic areas. Future studies will be directed for a more detailed understanding of SAR (by finding suitable chemistry to synthesize **19** as a pure *Z*-isomer, by variation of the cationic and linker part of compound **24** in the *E*-subseries and by replacing the benzo part of the indole ring by the bioisostere of pyridine) to optimize the in vivo efficacy of this class of molecules.

Experimental Section

Promastigote Viability Assay. The *L. donovani* promastigotes (MHOM/IN/Dd_8; originally obtained from Imperial college, London) were transfected with the firefly luciferase gene, and the transfectants were maintained in medium 199 (Sigma Chemical Co., USA) supplemented with 10% fetal calf serum (GIBCO) and 1% penicillin (50 U/mL) and streptomycin (50 μg/mL) solution (Sigma) under pressure of G418 (Sigma). The in vitro effect of the compounds on the growth of promastigotes was assessed by monitoring the luciferase activity of viable cells after treatment. The transgenic promastigotes of late log phase were seeded at 6×10^4/100 μL medium 199/well in 96-well flat-bottomed microtiter (MT) plates (CELLSTAR) and incubated for 72 h in medium alone or in the presence of test dilutions of drugs in DMSO.[20] Parallel dilution of DMSO was used as controls. After incubation, an aliquot (50 μL) of promastigote suspension was aspirated from each well of a 96-well plate and mixed with an equal volume of Steady Glo reagent (Promega), and luminescence was measured by a luminometer. The values were expressed as relative luminescence unit (RLU). The inhibition of parasitic growth is determined by comparison of the luciferase activity of drug treated parasites with that of untreated controls by the following general formula:

$$\text{percentage inhibition} = (N - n \times 100)/N$$

where N is the average relative luminescence unit (RLU) of control wells, and n is the average RLU of treated wells.

Screening on Infected Macrophages. For assessing the activity of compounds against the amastigote stage of the parasite, mouse macrophage cell line (J-774A.1) infected with promastigotes expressing the luciferase firefly reporter gene was used. Cells were seeded in a 96-well plate (1.5×10^4cell/100 μL/well) in RPMI-1640 containing 10% fetal calf serum, and the plates were incubated at 37 °C in a CO_2 incubator. After 24 h, the medium was replaced with fresh medium containing stationary-phase promastigotes (4×10^3/100 μL/well). Promastigotes invade the macrophage and are transformed into amastigotes. The test material in appropriate concentrations (0.195−12.5 μg/mL) in complete medium was added after replacing the previous medium, and the plates were incubated at 37 °C in a CO_2 incubator for 72 h. After incubation, the drug containing the medium was decanted, and 50 μL of PBS was added to each well and mixed with an equal volume of Steady Glo reagent. After gentle shaking for 1−2 min, the reading was taken with a luminometer. The inhibition of parasitic growth is determined by comparison of the luciferase activity of drug treated parasites with that of untreated controls as described above.

Cytotoxicity Assay. The cell viability was determined using the MTT assay. J774.A-1 cell line or KB cell line was maintained in RPMI medium (Sigma), supplemented with 10% fetal calf serum and 40 mg/mL gentamycin. Exponentially growing cells (1×10^4cells/100 μL/well) were incubated with different drug concentrations for 72 h and were incubated at 37 °C in a humidified mixture of CO_2 and 95% air in an incubator. Stock solutions of compounds were initially dissolved in DMSO and further diluted with fresh complete medium. After incubation, 25 μL of MTT reagent (5 mg/mL) in PBS medium, followed by syringe filtration, was added to each well and incubated at 37 °C for 2 h. At the end of the incubation period, the supernatant was removed by tilting the plate completely without disturbing the cell layer, and 150 μL of pure DMSO was added to each well. After 15 min of shaking, the readings were recorded as absorbance at 544 nm on a microplate reader. The cytotoxic effect was expressed as 50% lethal dose, i.e., as the concentration of a compound which provoked a 50% reduction in cell viability compared to that of a cell in culture medium alone. IC_{50} values were estimated through the preformed template as described by Huber and Koella.[21]

In Vivo Assay. The in vivo leishmanicidal activity was determined in golden hamsters (*Mesocricetus auretus*) infected with MHOM/IN/80/Dd_8 strain of *L. donovani* obtained through the courtesy of PCC Garnham, Imperial College, London (U.K.). The method of Beveridge,[22] as modified by Bhatnagar et al.,[23] was used for in vivo screening. Golden hamsters (of either sex) weighing 40−45 g were infected intracardially with 1×10^7 amastigotes per animal. The infection was well adapted to the hamster model and establishes itself in 15−20 days. Meanwhile, hamsters gain weight (85−95 g) and can be subjected to repeated spleen biopsies. Pretreatment spleen biopsy in all of the animals was carried out to assess the degree of infection. The animals with +1 infection (5−15 amastigotes/100 spleen cell nuclei) were included in the chemotherapeutic trials. The infected animals were randomized into several groups on the basis of their parasitic burdens. Five to six animals were used for each test sample. Drug treatment by i.p. route was initiated after 2 days of biopsy and continued for 5 consecutive days. Post-treatment biopsies were done on day 7 of the last drug administration, and amastigote counts were assessed by Giemsa staining. Intensity of infection in both treated and untreated animals, and also the initial count in treated animals were compared, and the efficacy was expressed in terms of percentage inhibition (PI) using the following formula:

$$\text{PI} = 100 - [\text{ANAT} \times 100/(\text{INAT} \times \text{TIUC})]$$

where PI is percent inhibition of amastigote multiplication, ANAT is actual number of amastigotes in treated animals, INAT is initial number of amastigotes in treated animals, and TIUC is times increase of parasites in untreated control animals.

Synthetic Chemistry. Nuclear magnetic spectra were recorded on 200 MHz, 300 MHz, and 400 MHz spectrometers. In the case of multiplets, the signals are reported as intervals. Signals were abbreviated as s, singlet; d, doublet; t, triplet; m, multiplet. The electron spray mass spectra were recorded on a triple quadrupole mass spectrometer. The samples (dissolved in suitable solvents such as methanol/acetonitrile/water) were introduced into the ESI source through a syringe pump at the rate of 5 μL/min. The ESI capillary was set at 3.5 kV, and the cone voltage was 40 V. The spectra were collected in 6 s scans, and the printouts are averaged spectra of 6−8 scans. Infrared spectra were recorded using a Perkin-Elmer RX-1 spectrometer; the values were reported in cm^{-1}. The purity of all tested compounds was ascertained on the basis of their elemental analysis and was ≥95%.

Compounds Listed in Table 1. Synthesis of 1-(*p*-Cyanophenoxypentyl)indole-3-carbaldehyde (9). To a stirred solution of indole-3-carbaldehyde (1.0 g, 1.0 equiv) and, tetrabutylammonium bromide (0.1 equiv) and (*p*-cyanophenoxy)pentylbromide (1.2 equiv) in toluene (5.0 mL) was added 1.0 mL 20% w/v aqueous NaOH solution. The reaction mixture was stirred for 1.5 h until the reaction was complete. The product separated as a semisolid mass, which was decanted off. Crude solid was recrystallized from 1% MeOH in chloroform solution. Yield: 71%. M. P. >250 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.48−1.62 (m, 2H), 1.77−1.91 (m, 2H), 1.95−2.04 (m, 2H), 3.97 (t, 2H, *J* 6.2 Hz), 4.22 (t, 2H, *J* 7.0 Hz), 6.88 (d, 2H, *J* 8.8 Hz) 7.29−4.40 (m, 3H), 7.56 (d, 2H, *J* 8.8 Hz), 7.72 (s, 1H), 8.29−8.33 (m, 1H), 10.00 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6): δ 23.3 (CH_2), 26.7 (CH_2), 27.0 (CH_2), 47.3 (CH_2), 67.9 (CH_2), 110.9 (CH), 115.6 (2 CH), 122.5 (CH), 123.3 (CH), 124.6 (CH), 134.5 (CH), 138.8 (CH), 184.5 (CH), 119.6 (C), 125.8 (C), 137.5 (C), 104.5 (C), 118.6 (C), 162.3 (C). FAB Mass $[M + H]^+$ 333. IR 1656.4, 1600.1. Anal. Calcd for $C_{21}H_{20}N_2O_2$: C, 75.88; H, 6.06; N, 8.43; O, 9.63. Found: C, 75.57; H, 6.03; N, 8.47.

Representative Procedure for the Synthesis Compound 8. 1-(*p*-Cyanophenoxypentyl)indole-3-carbaldehyde (**9**) (0.245 g, 1.0 equiv) and 2-thiohydantoin (1.0 equiv) were stirred in the presence of ethanolamine (1.5 equiv) in absolute ethanol at 60 °C for 2−3 h. Compounds were precipitated out, filtered, and

crystallized from methanol or acetone. Yield: 82%. M.P. 240 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.45−1.65 (m, 2H), 1.78−1.91 (m, 2H), 1.91−2.09 (m, 2H), 4.06 (t, 2H, *J* 6.2 Hz), 4.30 (t, 2H, J 7.0 Hz), 6.84 (s, 1H), 7.07 (d, 2H, J 8.8 Hz), 7.23−738 (m, 2H), 7.63 (d, 1H, *J* 7.1 Hz), 7.74 (d, 2H, *J* 8.8 Hz), 7.87 (d, 1H, J 7.3 Hz). ^{13}C NMR (50 MHz, DMSO-d_6): δ 23.0 (CH_2), 28.5 (CH_2), 29.9 (CH_2), 46.3 (CH_2), 68.1 (CH_2), 108.6 (CH), 111.2 (CH), 118.3 (CH), 115.7 (2 CH), 120.5 (CH), 122.9 (CH), 131.7 (CH), 135.1 (2 CH), 112.9 (C), 128.2 (C), 136.5 (C), 104.6 (C), 118.6 (C), 162.8 (C), 122.4 (C), 165.2 (C), 177.3 (C). Anal. Calcd for $C_{24}H_{22}N_4O_2S$: C, 66.96; H, 5.15; N, 13.01. Found: C, 66.72; H, 5.14; N, 12.98.

Representative Procedure for the Synthesis of Compound 1. Compound **8** and anhydrous $ZnCl_2$ were stirred in dry methanol at 60 °C for 0.5 h. Methanol was removed under vacuum, and neat butyl amine (dried over anhydrous KOH pellet) was added to the remaining solid residue just to cover it (2−3 mL). The reaction mixture was stirred at 80 °C for 7−8 h until the reaction was complete. It was cooled and added to 8 mL, 1% aqueous hydrochloric acid solution. The reaction mixture was extracted by ethylacetate (3 × 4 mL) and chromatographed over 100−200 mesh silica gel eluted using methanol in chloroform solvent system with increasing concentration of methanol from 0.1% to 2.0%. Yield: 64%. Semi solid. ^{1}H NMR (200 MHz, acetone-d_6): δ 0.84 (t, 3H, 7.2 Hz), 1.31−1.84 (m, 10H), 3.42 (t, 2H, *J* 7.2 Hz), 3.89 (t, 2H, *J* 6.2 Hz), 4.20 (t, 2H, *J* 7.0 Hz), 6.82 (s, 1H), 6.82 (d, 2H, *J* 8.8 Hz), 7.06−7.14 (m, 2H), 7.39 (d, 1H, *J* 7.4 Hz), 7.53 (d, 2H, *J* 8.8 Hz), 7.84 (d, 1H, *J* 7.2 Hz), 8.20 (s, 1H). ^{13}C NMR (50 MHz, acetone-d_6): δ 14.5 (CH_3), 21.1 (CH_2), 24.4 (CH_2), 32.9 (CH_2), 47.8 (CH_2), 47.5 (CH_2), 29.1−30.6 (2 CH_2 merged with acetone-d_6 peaks), 69.3 (CH_2), 104.7 (CH), 111.3 (CH), 116.7 (2 CH), 120.2 (CH), 121.4 (CH), 123.5 (CH), 135.2 (2 CH), 136.5 (CH), 107.9 (C), 129.3 (C), 132.5 (C), 104.5 (C), 118.2 (C), 162.4 (C), 121.9 (C), 163.7 (C), 173.7 (C). FAB Mass $[M + H]^+$ 471. Anal. Calcd for $C_{28}H_{31}N_5O_2$: C, 71.62; H, 6.65; N, 14.91. Found: C, 71.37; H, 6.62; N, 14.86.

Compounds Listed in Table 2. All compounds are formed in *Z*-configuration except for those noted otherwise.

Compound 10. The title compound was synthesized following the representative procedure for compound **8**. Yield: 71%. M.P. 220 °C (decomposed). ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.53−1.69 (m, 2H), 1.80−2.12 (m, 4H), 4.02 (t, 2H, *J* 6.0 Hz), 4.30 (t, 2H, *J* 6.8 Hz), 6.86 (s, 1H), 7.20 (d, 2H, *J* 8.7 Hz), 7.25−7.41 (m, 2H), 7.71 (d, 1H, *J* 7.6 Hz), 7.85 (m, 2H), 8.34 (s, 1H), 10.22 (m, broad, 2H). ^{13}C NMR (50 MHz, DMSO-d_6): δ 23.1 (CH_2), 28.3 (CH_2), 29.5 (CH_2), 46.2 (CH_2), 68.2 (CH_2), 101.4 (CH), 110.7 (CH), 115.8 (2 CH), 119.2 (CH), 120.6 (CH), 122.7 (CH), 134.4 (2 CH), 103.0 (C), 118.7 (C), 155.9 (C), 124.1 (C), 162.4 (C), 165.2 (C), 108.2 (C), 127.7 (C), 136.1 (C). ESMS $[M + H]^+$ 415. Anal. Calcd for $C_{24}H_{22}N_4O_3$: C, 69.55; H, 5.35; N, 13.52. Found: C, 69.28; H, 5.37; 13.46.

Compounds Listed in Table 3. All of the compounds were synthesized according to the representative procedure for compound **8** given in Experimental Section and formed in Z-configuration except for those noted otherwise (also see Supporting Information).

Compound 18. Yield: 73%. M.P. >240 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.41−1.46 (m, 2H), 1.71−1.77 (m, 2H), 1.88−1.93 (m, 2H), 3.86 (t, 2H, *J* 6.0 Hz), 4.18 (t, 2H, *J* 6.8 Hz), 5.38 (s, broad, 2H), 5.92 (d, 1H, *J* 2.0 Hz), 6.03 (dd, *J* 6.0 Hz, *J*′ 2.0 Hz), 6.79 (s, 1H), 7.11−7.21 (m, 2H), 7.38 (d, 1H, J 8.0 Hz), 7.65−7.70 (m, 2H), 8.42 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6): δ 23.2 (CH_2), 28.3 (CH_2), 29.1 (CH_2), 46.8 (CH_2), 68.7 (CH_2), 92.3 (CH), 108.5 (CH), 152.1 (CH), 110.7 (CH), 112.1 (CH), 120.9 (CH), 121.7 (CH), 124.2 (CH), 131.8 (CH), 170.1 (C), 163.3 (C), 108.1 (C), 127.8 (C), 137.0 (C), 125.3 (C), 163.7 (C), 173.2 (C). ESMS $[M + H]^+$ 422. Anal. Calcd for $C_{22}H_{23}N_5O_2S$: C, 62.69; H, 5.50; N, 16.61. Found: C, 62.44; 5.48; N, 16.54.

Compound 24: *E*-24 (100%, *E*-isomer). Yield: 67%. M.P. 238 °C. ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6): δ 1.46−1.48 (m, 2H), 1.74−1.78 (m, 2H), 1.89−1.94 (m, 2H), 3.57 (s, 3H), 3.89 (t, 2H, *J* 6.3 Hz), 4.22 (t, 2H, *J* 6.9 Hz), 5.40 (s, broad, 2H), 6.77 (d, 2H, *J* 8.7 Hz), 6.85 (s, 1H), 7.17−7.22 (m, 2H), 7.42 (d, 1H, *J* 7.8 Hz), 7.52 (d, 2H, *J* 8.7 Hz), 7.84 (d, 1H, *J* 7.4 Hz), 9.03 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6): δ 23.7 (CH_2), 29.0 (CH_2), 30.1 (CH_2), 30.5 (CH_2), 47.1 (CH_2), 68.1 (CH_2), 111.4 (CH), 112.7 (CH), 114.7 (2 CH), 119.4 (CH), 121.6 (CH), 123.4 (CH), 127.5 (2 CH), 133.9 (CH), 109.0 (C), 120.6 (C), 126.3 (C), 129.3 (C), 136.6 (C), 151.8 (C), 160.1 (C), 163.9 (C), 173.8 (C). ESMS $[M + H]^+$ 478. Anal. Calcd for $C_{25}H_{27}N_5O_3S$: C, 62.87; H, 5.70; N, 14.66. Found: C, 62.62; H, 5.67; N, 14.62.

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Supporting Information Available: Synthesis procedures and analytical data of all remaining compounds and intermediates. This material is available free of charge via Internet at http://pubs.acs.org.

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