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ARTICLE

An Insight into Tetrahydro-β-carboline-Tetrazole Hybrids: Synthesis and Bioevaluation as Potent Antileishmanial Agents

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A series of 2,3,4,9 tetrahydro-β-carboline tetrazole derivatives (**14a-u**) have been synthesized utilizing Ugi multicomponent reaction and were identified as potential antileishmanial chemotype. Most of the screened derivatives exhibited significant *in vitro* activity against promastigote (IC_{50} from 0.59 ± 0.35 to 31 ± 1.27 μM) as well as intracellular amastigote (IC_{50} from 1.57 ± 0.12 to 17.6 ± 0.2 μM) of *L. donovani* and the activity is comparable with standard drug miltefosine and sodium stibogluconate. The most active compound **14t** was further studied *in vivo* against *L. donovani*/ golden hamster model at a dose of 50 mg/Kg through intraperitoneal route for 5 consecutive day and displayed 75.04 ± 7.28 % inhibition of splenic parasitic burden. Pharmacokinetics of compound **14t** was studied in golden Syrian hamster, following 50 mg/kg oral dose, the compound was detected in hamster serum up to 24 h. It exhibited a large volume of distribution (651.8 L/kg), high clearance (43.2 L/h/kg) and long mean residence time (10 h).

Introduction

Leishmaniasis is one of the most neglected tropical human disease caused by different species of genus Leishmania, a flagellate protozoa parasite transmitted by the bite of a tiny long (2-3 mm) insect vector, the phlebotomine sand fly.[1] The disease may manifest itself in three basic clinical forms i.e. cutaneous (CL), mucocutaneous (MCL), and visceral leishmaniasis (VL) depending on parasite species and response of host immune. Among them, visceral leishmaniasis (also known as kala azar) is the most severe form of leishmaniasis, mainly caused by *Leishmania donovani* and, is mainly endemic in Bangladesh, Ethiopia, India, Nepal, South Sudan and Sudan.[2] VL often affects visceral organs such as liver, spleen, bone marrow and is usually fatal in more than 90% untreated cases.[3] A recent world health organization (WHO) report indicates that 310 million people are at risk of contracting leishmaniasis, while 1.3 million new infections and 30,000 deaths takes place annually.[4] After infection in mammalian body, the protozoa multiplies within phagolysosomes of macrophages as intracellular amastigotes where it causes dysfunction and the outcome of infection depends on the production and/or secretion of immunosuppressive molecules that includes transforming growth factor (TGF)-β, interleukin (IL)-10, and prostaglandin E_2 (PGE_2).[5] These molecules suppresses host-protective microbicidal molecules, nitric oxide (NO), and reactive oxygen species (ROS) and cytokines interferon (IFN)-γ, IL-1, IL-12, and tumornecrosis factor-α (TNF-α).[6]

The present chemotherapy against leishmaniasis includes the first line drugs pentavalent antimonials (sodium stibogluconate and meglumine antimoniate) which has long period of treatment associated with severe side effects including cardiac arrhythmia and pancreatitis.[7] Pentamidine and paromomycin drugs are restricted because they demonstrate renal, hepatic and pancreatic toxicity beside with hypertension and dysglycemia.[8] On the other hand lipid formulation of Amphotericin-B is highly effective but its high cost makes this unaffordable for poor people.[9] Serendipitously discovered miltefosine is first orally active drug having long half-life (150-200 h) and due to its teratogenic effects, restricted for pregnant woman.[10] Thus, the chemotherapy against leishmaniasis is still inefficient; as a result the finding of more effective and safer drug for treating leishmaniasis remains desirable.

Nature is a rich source for production of anti-infective agents, in which alkaloids displayed potent antileishmanial activity from the ages.[11] β-carboline prototype containing natural products and synthetic molecules have been reported for antileishmanial activity for eg; harmine (1), harman (2), annomontine (3), buchtienine (4), and manzamine-A (5) demonstrated significant antileishmanial activity (Figure 1).[12-14]

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On the other hand, tetrazole scaffolds have received great attention due to their wide range of biological activities[15-17] and it is an important core of various modern drugs for eg; furofuran (**6**)[18], tetrazole PD 12 (**7**)[19] etc.

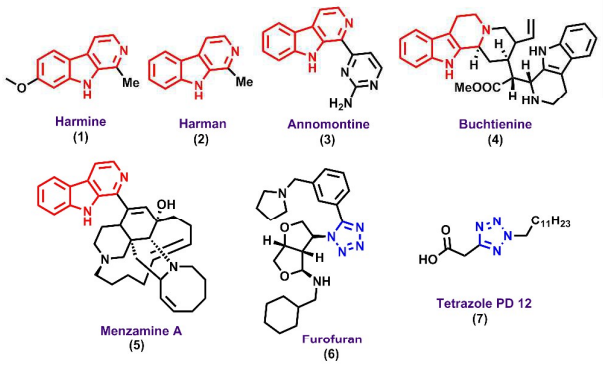


Fig. 1 Some β-carboline based natural antileishmanial agents and tetrazole containing drugs.

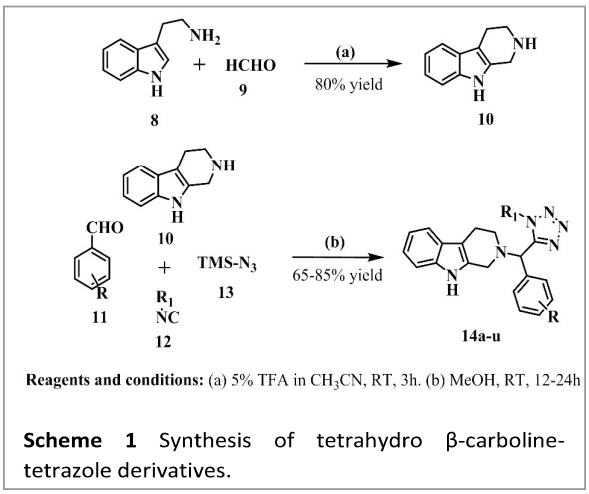
In continuation of our ongoing research on anti-parasitic diseases area, our research group had identified diverse β-carboline fused hybrid molecules as antileishmanial agents.[20-24] Other groups across the world have also reported β-carboline based organic compounds as antileishmanial agent.[25,26] Impressed by biological activities of β-carboline based hybrid molecules, we have designed a hybrid framework containing two pharmacophore units, β-carboline and tetrazole nucleus. In the present study, we synthesised tetrahydro β-carboline-tetrazole hybrids and evaluated them as antileishmanial agents. Pharmacokinetics study and *In–silico* prediction of molecular properties of compounds was also reported.

Results and discussion

Chemistry

2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole (**10**) an intermediate for synthesis of final compound, was obtained by Pictet-Spengler cyclization of commercially available tryptamine (**8**) and formaldehyde (**9**).[27] The final compounds was furnished via simple and efficient Ugi four component reaction using 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole as amine partner (**10**), aldehyde (**11**), isocyanide (**12**) and, azidotrimethylsilane (TMS-N_3) (**13**) in anhydrous methanol as solvent at room temperature for 12-24 h in good to excellent yield. The detailed synthetic route of 2,3,4,9-tetrahyrdo-β-carboline-tetrazoles (**14a-u**) is outlined in **Scheme 1**. The chemical structures of all synthesized derivatives were inferred by ^{1}H-NMR, ^{13}C-NMR, HRMS and IR spectroscopy.

Biological assay



Scheme 1 Synthesis of tetrahydro β-carboline-tetrazole derivatives.

In vitro antileishmanial activity

In an endeavor to discover novel antileishmanial β-carboline tetrazole hybrids, a series of 21 compounds have been synthesized and screened for antileishmanial activity. The *in vitro* activity results are summarized in **table 1**. All candidates were evaluated for their in *vitro* activity against WHO reference strain (MHOM/IN/80/Dd8) and murine macrophages (expressing luciferase firefly reporter gene) for extracellular promastigotes of *L. donovani* and intracellular amastigotes respectively. The *in vitro* cytotoxicity assay was achieved by using mouse macrophage cells (J-774A.1cell line), which were procured from NCCS, Pune, India. Standard antileishmanials, SSG and Miltefosine were included in the study as positive control drugs. Biological screening results indicate that the

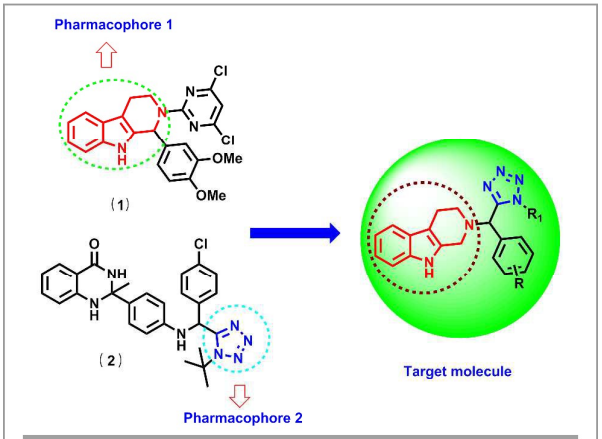


Fig. 2 Designing of target molecule based on active antileishmanial molecule.

promastigote form of parasite is more sensitive for tetrazole hybrids than amastigote form. As evident from the **table 1**, 18 derivatives showed potent to moderate activity with IC_{50} values ranges from 0.59 ± 0.35 to 31 ± 1.27 μM against promastigote. 13 compounds shows high to moderate activity with IC_{50} = 1.57 ± 0.12 to 17.6 ± 0.2 μM against amastigote

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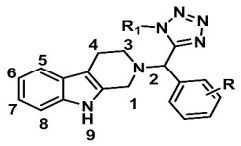
than standard. Rest of the compounds were found almost inactive with IC_{50} values ranging from ≥25 to ≥100 μM. In the present study, our main aim was to investigate the impact of substituent of phenyl ring (R) and tetrazole ring (R_1) on activity, however there is no obvious trend of activity with respect to substituent was found. Initially we have synthesized the derivative **14a** with R_1 as *tert*-butyl and R as unsubstituted phenyl ring, it showed moderate antipromastigote (IC_{50} = 4.07 ± 0.21 μM) and 1.5 times better antiamastigote (IC_{50} = 5.41 ± 0.13 μM) activity than standard drug with significant SI (27). The *para* substituted halogens on R demonstrate variable activity for eg; flouro (**14b**), chloro (**14c**), and bromo (**14d**) derivatives have shown antipromastigote (IC_{50} = 2.71 ± 1.34 μM, IC_{50} = 3.45 ± 0.42 μM, and IC_{50} = 1.58 ± 0.37 μM) and antiamastigote (IC_{50} = 17.5 ± 1.27 μM, IC_{50} = 14.15 ± 0.05 μM, and IC_{50} = 3.77 ± 0.02 μM) activity respectively. The strongly

Table 1. *In vitro* antileishmanial Actvity of tetrahydro β-carboline tetrazoles (**14a-u**) against *L. donovani* and cytotoxicity against J774A.1 cell line.



Entry	R	R_1	IC_{50} ± SEM (μM)[a]		CC_{50} (μM) on J774A.1 cell line	SI[b]
			Anti-promastigote	Anti-amastigote		
14a	4-H	*tert*. butyl	**4.07 ± 0.21**	**5.41 ± 0.13**	**146.5 ± 3.5**	**27**
14b	4-F	*tert*. butyl	2.71 ± 1.34	17.5 ± 1.27	48.8 ± 0.70	3
14c	4-Cl	*tert*. butyl	3.45 ± 0.42	14.15 ± 0.05	36.95 ± 1.5	2
14d	4-Br	*tert*. butyl	**1.58 ± 0.37**	**3.77 ± 0.02**	**144 ± 4**	**38**
14e	3-NO_2	*tert*. butyl	**4.91 ± 0.32**	**3.86 ± 0.11**	**149.5 ± 1.5**	**39**
14f	4- NO_2	*tert*. butyl	**0.59 ± 0.35**	**3.78 ± 0.20**	**139.5 ± 1.5**	**37**
14g	4-CN	*tert*. butyl	**2.82 ± 0.28**	**16.4 ± 0.1**	**149.5 ± 2.5**	**9**
14h	4-OMe	*tert*. butyl	**1.14 ± 0.28**	**4.32 ± 0.33**	**41.95 ± 1.48**	**10**
14i	3,4 di-OMe	*tert*. butyl	5.71 ± 0.03	15.95 ± 0.05	138.5 ± 3.5	8
14j	3,4,5 tri-OMe	*tert*. butyl	23.5 ± 0.96	≥50	ND[c]	ND
14k	4-isopropyl	*tert*. butyl	3.56 ± 0.08	17.6 ± 0.2	130	7
14l	4-F	cyclohexyl	14.5 ± 0.84	≥25	ND	ND
14m	4-Cl	cyclohexyl	**2.89 ± 0.13**	**2.08 ± 0.11**	**35.5 ± 0.28**	**17**
14n	2-Cl	cyclohexyl	≥100	≥50	ND	ND
14o	4-Br	cyclohexyl	16.5 ± 0.56	≥50	ND	ND
14p	4-NO_2	cyclohexyl	31 ± 1.27	≥50	ND	ND
14q	4-CN	cyclohexyl	≥100	≥50	ND	ND
14r	4-OMe	cyclohexyl	20.35 ± 2.33	≥50	ND	ND
14s	3,4 di-OMe	cyclohexyl	**1.20 ± 0.16**	**3.31 ± 0.19**	**41.9 ± 0.14**	**13**
14t	3,4,5 tri-OMe	cyclohexyl	**2.81 ± 0.08**	**1.57 ± 0.12**	**36 ± 0.98**	**23**
14u	4-OH	cyclohexyl	NA[d]	NA	ND	ND
	Miltefosine[e]		1.05 ± 0.2	8.4 ± 2.1	12.42 ± 3.2	1.48
	SSG[f]		947 ± 8.5	46.70 ± 2.8	297.38 ± 10.2	6.38

[a]IC_{50} (μM): concentration corresponding to 50 % growth inhibition of the parasite, IC_{50} and CC_{50} values are the average of three independent assays expressed as average ± standard error. [b]Selectivity index (SI): IC_{50} values of cytotoxic activity/IC_{50} values of antiamastigote antileishmanial activity. [c]ND: not determined. [d]NA: not available. [e]Miltefosine: used as standard. [f]SSG: Sodium stibogluconate: used as standard.

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electron withdrawing nitro group on R for **14f** showed excellent antipromastigote (IC_{50} = 0.59 ± 0.35 μM) and potent antiamastigote activity (IC_{50} = 3.78 ± 0.20 μM) with no toxicity for J-774A.1 cell line (CC_{50} = 139.5 ± 1.5 μM). However the isomeric meta-nitro derivative **14e** was less active in promastigote (IC_{50} = 4.91 ± 0.32 μM) and comparable activity in amastigote (IC_{50} = 3.86 ± 0.11 μM) with low toxicity (CC_{50} = 149.5 ± 1.5 μM) than compound **14f**. The cyano group containing compound **14g** displayed good antipromastigote activity with IC_{50} = 2.82 ± 0.28 μM and poor antiamastigote (IC_{50} = 16.4 ± 0.1 μM) activity than miltefosine. The compound **14h** having electron donating *para* methoxy substituent showed potent antipromastigote (IC_{50} = 1.14 ± 0.28 μM) as well as antiamastigote (IC_{50} = 4.32 ± 0.33 μM) activity, none the less increasing the methoxy substituent to dimethoxy for **14i** and trimethoxy for **14j** was less favorable resulting concomitant decrease in antileishmanial activity (see **table 1**). The *para* isopropyl group containing derivative **14k** displayed potent antipromastigote (IC_{50} = 3.56 ± 0.08 μM) activity but was found less active for amastigote form (IC_{50} = 17.6 ± 0.2 μM). Interestingly, replacement of *tert*-butyl of R_1 by cyclohexyl was found beneficial in only few cases, for eg; **14m** (R= 4-Cl), **14s** (R= 3,4 di-OMe) and **14t** (R=3,4,5 tri-OMe) which were found better active with enhanced selectivity index than respected *tert*-butyl analogs. Compounds **14l**, **14o**, **14p**, **14r** were found active against promastigote but in case of amastigote, they were almost inactive as well as toxic. Unexpectedly, the derivative **14u** containing hydroxyl substituent was found inactive. Similarly, compounds **14n** and **14q** were found almost inactive as well as toxic. Compound **14t** was found most active in the series having IC_{50} = 1.57 ± 0.12 μM. From Even after extensive biological screening, it was very tough to conclude any clear relation of biological activity with substituent on having limited number of analogues. Compounds **14a**, **14d**, **14e**, **14f**, **14g**, **14h**, **14m**, **14s**, and **14t** were found best active among all the synthesized derivatives and manifold safe in murine macrophage J-774A.1 cell line.

In continuation to SAR study of β-carboline core compounds; it was revealed in earlier synthesised compounds,[20-22,26] that activity was due to substitution at C-1 of β-carboline ring system. While in case of our study, most of the compounds (**14d**, **14e**, **14f**, **14m**, **14s**, **14t**) have shown potent *in vitro* antileishmanial activity with unsubstituted C-1 position of saturated β- carboline ring.

To optimise the antileishmanial activity of 2,3,4,9 tetrahyrdo β-carboline, the effect of substituted or unsubstituted phenyl at C-1 of 2,3,4,9 tetrahyrdo β-carboline on activity; we further synthesise C-1 substituted β-carboline and activity will be discussed in our next project.

In vivo antileishmanial activity

On the basis of *in vitro* potency results, we selected those compounds having SI = >5 for further evaluation; accordingly, *in vivo* drug response potency of compounds were performed in golden hamster model (splenic parasitic burden) at a dosage of 50 mg/kg through the intra peritoneal (IP) route for 5 consecutive days. All experiments were performed in compliance with the relevant laws and Institutional Animal Ethics committee (IAEC) guidelines approved by National Laboratory Animal Center (NLAC) of CSIR-CDRI, Lucknow. Male hamsters weighing 40-45 gm were used in the study, housed at 23 ± 2°C, with humidity at 60 to 63% and fed standard rodent pellet and fresh drinking water. Miltefosine was used as standard drug for this study. Compound **14t** showed promising inhibition (75.04 ± 7.28 %) against Leishmania parasite and rest of the compounds showed poor inhibition. Although compound **14t** in IP route shows promising activity but due to the limitation of IP administration, we further screened **14t** through oral route at a dosage of 100 mg/Kg for 5 consecutive days where it has shown moderate inhibition (50.85 ± 8.67 %) (Table 2).

Table 2. *In vivo* antileishmanial activity of some compounds against *L. donovani*/golden hamster model.

Entry	Percent Inhibition ± SD		Route of administration	Dose
	Treatment results			
	7^{th}day	28^{th}day		
14a	4.05 ± 2.12	3.79 ± 1.46	IP	50 mg/Kg
14d	33.68 ± 7.80	53.06 ± 10.42	IP	50 mg/Kg
14e	4.83 ± 3.34	2.92 ± 0.14	IP	50 mg/Kg
14f	20.41 ± 3.99	19.62 ± 8.50	IP	50 mg/Kg
14t	**75.04 ± 7.28**	**88.92 ± 8.56**	**IP**	**50 mg/Kg**
14t	**50.85 ± 8.67**	**77.45 ± 6.83**	**Oral**	**100 mg/Kg**
Miltefosine	98.1 ± 1.2		Oral	30 mg/Kg

Pharmacokinetics study

Table 3. Pharmacokinetic parameters of **14t** after 50 mg/kg oral dose in golden Syrian hamsters.[a]

Parameters		Estimate
C_{max}(ng/mL)	1	76.2 ± 5.4
	2	83.5 ± 4.0
t_{max}(h)	1	1.6 ± 0.1
	2	8.0 ± 0.0
AUC_{last}(ng/h/mL)		844.5 ± 54.5
MRT (h)		10.2 ± 2.0
Cl/F (L/h/kg)		43.2 ± 4.3
Vd/F(L/kg)		651.8 ± 59.8

Each value represents the average of five hamsters dosed orally (50 mg/kg); values are mean ± SEM.

Abbreviations: AUC_{last}= area under the serum concentration-time curve up to last sampling time, C_{max} = peak serum concentration, MRT = mean residence time, t_{max} = time to C_{max}; Cl/F, clearance; Vd/F, volume of distribution

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Encouraged by *in vitro* and *in vivo* activity of compound **14t**, we have performed the *in vivo* pharmacokinetic study for **14t**. The study animals tolerated the treatment, as no peculiarities in their behaviour were observed. Chromatographic separations and quantification of the compounds in serum was achieved by a rapid, sensitive and validated liquid chromatography-tandem mass spectrometry method using electrospray ionization. Following 50 mg/kg oral dose, the compound was monitored up to 24 h (**Fig. 3**). Due to multiple peak phenomenon (in the PK profile of any drug Enterohepatic recirculation is mostly responsible for the multiple (two) peak phenomenon. It occurs by biliary excretion and intestinal reabsorption, sometimes with hepatic conjugation and intestinal deconjugation. Enterohepatic recirculation leads to prolonged elimination half-life of the drugs. In present study, long mean residence time and two peaks in the PK profile of Compound **14t** indicates its probable enterohepatic recirculation),[28] the serum concentration-time profile was

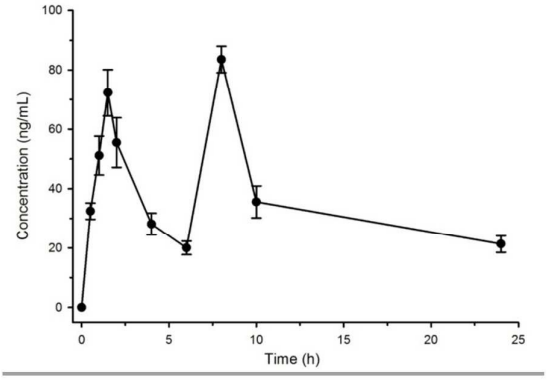

Fig. 3. Mean serum concentration-time profile of 14t after 50 mg/kg oral dose in golden Syrian hamsters (n=5). Bar represents SEM.

subjected to non-compartmental analysis using Phoenix WinNonlin (version 6.3; Certara Inc, Missouri, USA) and the calculated pharmacokinetic parameters are shown in **Table 3**. The volume of distribution (Vd/F) of **14t** (651.8 ± 59.8 L/kg) was higher than the total blood volume (0.087 L/kg) of hamster indicating extra vascular distribution. Its clearance (43.2 ± 4.3 L/h/kg) is also higher than the hepatic blood flow (0.39 L/h) of hamster indicating extra hepatic elimination.

Molecular properties

Molecular properties of any compound play crucial role in absorption, distribution, metabolism, excretion and toxicity (ADMET). *In-silico* prediction of molecular properties of tetrahydro β-carboline terazole compounds were done by moleinspiration Cheminformatics (http://www.molinspiration.com). Which compounds follow Lipinski "rule of five" parameters such as, MilogP (≤5), Molecular weight (≤500), number of hydrogen bond acceptors (≤5) and number of hydrogen bond donors (≤10) were not suffer bioavailability problem.[29] This rule is used as a filter for prediction of drug like properties of a molecule. In our synthesised compounds series, some compounds have shown violation from one of the Lipnski parameters i.e., (a) in case of **14k**, **14m**, **14n**, **14o** MilogP value were higher than 5 and, (b) for **14t** molecular weight was more than 500 (**Table 4**). So, from the *in-silico* prediction of molecular properties, it was concluded that, most of the compounds might not have any bioavailability problems.

Table 4. Predicted *In-silico* molecular properties of antileishmanial compounds.

Entry	Mol. Wt.	MilogP	HBA[a]	HBD[b]	Entry	Mol. Wt.	MilogP	HBA[a]	HBD[b]
14a	386.50	4.02	1	6	**14j**	476.58	3.56	1	9
14b	404.49	4.18	1	6	**14k**	428.58	5.53	1	6
14c	420.95	4.70	1	6	**14l**	430.53	4.90	1	6
14d	465.40	4.83	1	6	**14m**	446.99	5.42	1	6
14e	385.50	3.12	1	6	**14o**	491.44	5.55	1	6
14f	385.50	3.15	1	6	**14p**	411.53	3.87	1	6
14g	399.50	3.55	1	7	**14r**	442.57	4.80	1	7
14h	416.53	4.08	1	7	**14s**	472.59	4.39	1	8
14i	446.56	3.67	1	8	**14t**	502.62	4.37	1	9
					Miltefosine	407.58	-0.12	0	5

[a]Hydrogen bond acceptor, [b]Hydrogen bond donor

Experimental

Chemistry: general procedure

All reagents were commercial available from Sigma Aldrich and were used without further purification. Chromatography was carried on silica gel (100–200 mesh). All reactions progress was routinely monitored by TLC on pre-coated silica gel aluminium plates. Melting points were taken in open capillaries on melting point apparatus containing silicon oil and are uncorrected. Infrared spectra were recorded on a FTIR spectrometer and are recorded in terms of frequency of absorption (cm^{-1}). ^{1}H NMR and ^{13}C NMR spectra were recorded on a 400 MHz (all signals are reported in *ppm* with the internal chloroform signal at 7.28 *ppm* as standard) and 100 MHz (all signals are reported in *ppm* with the internal chloroform signal at 77.21 ppm as standard) spectrometer respectively using tetramethylsilane (TMS) as internal standard. Coupling constants (*J*) are reported in hertz (Hz). Multiplicities are reported as follows: singlet (s), doublet (d), triplet (t), multiplet (m), and broad singlet (bs). Electrospray ionization mass spectra (ESIMS) were recorded on Thermo Lcq Advantage Max-IT. High resolution mass spectra (HRMS) were recorded as ESI-HRMS on Q-TOF, LC-MS/MS mass spectrometer. Purity of final compounds was determined by analytical HPLC, which was carried out on a Water/ACN HPLC system (model pump: 515, detector PDA-2998). HPLC analysis conditions: Merck C18

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(5.0 µM), 4.6 × 250 mm column flow rate 0.8 mL/min. All biologically evaluated compounds are ≥95% pure.

General procedure (GP) for the synthesis of compounds 14a-u: In a round bottomed flask the stirred methanolic (1 M) solution of 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole (10) (1 equiv.) and corresponding benzaldehyde (1 equiv.) was added followed by addition of *tert*-butyl or cyclohexyl isocyanide (1 equiv.) and, the $TMSN_3$ (2 equiv.). This reaction mixture was allowed to run for 12-24h under N_2 atmosphere at room temperature. The progress of reaction was monitored by TLC. After completion of reaction the solvent was evaporated under reduced pressure. Then, the crude reaction mixture was diluted with DCM (20 mL) and washed with brine (10 mL). The aqueous layer was extracted with DCM (2 × 10 mL). The combined organic layer was dried over anhydrous Na_2SO_4, evaporated to dryness and the corresponding desired products (**14a-u**) were obtained by column chromatography using 100-200 mesh silica gels.

2-((1-(*tert*-butyl)-*1H*-tetrazol-5-yl)(phenyl)methyl)-2,3,4,9-tetrahydro-*1H*-pyrido[3,4-*b*]indole (14a) According to GP it was obtained by the reaction of methanolic (1 M, 3mL) solution of 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole (1 equiv., 516 mg, 3 mmol), benzaldehyde (1 equiv., 318 mg, 3 mmol), *tert*-butyl isocyanide (1 equiv., 249.4 mg, 3 mmol) and, the $TMSN_3$ (2 equiv., 691 mg, 3 mmol) in 12h with 66 % yield (0.66 mmol, 255 mg) as white solid; m.p. = 210-213°C; IR (KBr), ν_{max}: 669, 757, 1215, 1584, 3020, 3368 cm^{-1}; HPLC-PDA: tr = 21.63 min (% area = 95.33%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.71 (bs, 1H, H_{NH}), 7.50-7.44 (m, 3H, H_{Ar}), 7.40-7.37 (t, *J* = 5.00 Hz, 3H, H_{Ar}), 7.26 (s, 1H, H_{Ar}), 7.14-7.06 (m, 2H, H_{Ar}), 5.69 (s, 1H, H_{CH}), 4.15 (d, *J* = 15.10 Hz, 1H, H_{CH2}), 3.71 (d, *J* = 14.72 Hz, 1H, H_{CH2}), 3.14-3.08 (m, 1H, H_{CH2}), 3.02-2.96 (m, 1H, H_{CH2}), 2.80-2.77 (t, *J* = 5.72 Hz, 2H, H_{CH2}), 1.70 (s, 9H, $H_{3\times CH3}$) *ppm*; ^{13}C NMR (100 MHz, $CDCl_3$): 154.37 (C-5), 136.07 (C-Ar), 135.53 (C-Ar), 131.58 (C-Ar), 129.81 (C-Ar), 128.67 (C-Ar), 127.19 (C-Ar), 121.37 (C-Ar), 119.30 (C-Ar), 117.87 (C-Ar), 110.67 (C-Ar), 108.16 (C-Ar), 63.87 (C-C(Me)$_3$), 61.60 (C-CH), 48.38 (C-CH2), 46.80 (C-CH2), 30.28 (C-CH3), 21.26 (C-CH2) ppm; HRMS (EI) calcd for $[C_{23}H_{26}N_6+H]^+$ 387.2297; found 387.2290.

2-((1-(*tert*-butyl)-*1H*-tetrazol-5-yl)(4-fluorophenyl)methyl)-2,3,4,9-tetrahydro-*1H*-pyrido[3,4-*b*]indole (14b) According to GP it was obtained by reaction of methanolic (1.0 M, 3.0 mL) solution of 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole (1 equiv., 533 mg, 3.1 mmol), 4-fluorobenzaldehyde (1 equiv., 384 mg, 3.1 mmol), *tert*-butyl isocyanide (1 equiv., 257 mg, 3.1 mmol) and, the $TMSN_3$ (2 equiv., 357 mg, 3.1 mmol) in 13h with 70 % yield (0.70 mmol, 283 mg) as white solid; m.p. = 188-191°C; IR (KBr), ν_{max}: 669, 754, 1215, 1530, 3021, 3368 cm^{-1}; HPLC-PDA: tr = 15.61 min (% area = 99.40%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.69 (bs, 1H, H_{NH}), 7.51-7.45 (m, 4H, H_{Ar}), 7.15-7.10 (m, 1H, H_{Ar}), 7.09-7.08 (t, *J* = 1.44 Hz, 3H, H_{Ar}), 5.67 (s, 1H, H_{CH}), 4.11 (d, *J* = 14.88 Hz, 1H, H_{CH2}), 3.70 (d, *J* = 13.96 Hz, 1H, H_{CH2}), 3.12-3.06 (m, 1H, H_{CH2}), 3.00-2.94 (m, 1H, H_{CH2}), 280-2.77 (t, *J* = 5.64 Hz, 2H, H_{CH2}), 1.71 (s, 9H, $H_{3\times CH3}$) *ppm*; ^{13}C NMR (100 MHz, $CDCl_3$): 154.27 (C-5), 136.06 (C-Ar), 131.56 (C-Ar), 131.47 (C-Ar), 131.37 (C-Ar), 131.33 (C-Ar), 127.22 (C-Ar), 121.36 (C-Ar), 119.40 (C-Ar), 117.89 (C-Ar), 115.79 (C-Ar), 115.54 (C-Ar), 110.71 (C-Ar), 108.07 (C-Ar), 63.10 (C-C(Me)$_3$), 61.73 (C-CH), 48.15 (C-CH_2), 46.88 (C-CH_2), 30.22 (C-CH_3), 21.49 (C-CH_2) ppm; HRMS (EI) calcd for $[C_{23}H_{25}FN_6+H]^+$ 405.2203; found 405.2205.

2-((1-(*tert*-butyl)-*1H*-tetrazol-5-yl)(4-chlorophenyl)methyl)-2,3,4,9-tetrahydro-*1H*-pyrido[3,4-*b*]indole (14c)[30] According to GP it was obtained by reaction of 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole (1 equiv., 344 mg, 2 mmol), 4-chlorobenzaldehyde (1 equiv., 281 mg, 2 mmol), *tert*-butyl isocyanide (1 equiv., 166 mg, 2 mmol) and, the $TMSN_3$ (2 equiv., 461 mg, 2 mmol) in methanol (1.0 M, 2.5 mL) as solvent in 15h with 66% yield (0.66 mmol, 277 mg) as white solid; m.p. = 100-103°C; IR (KBr), ν_{max}: 670, 756, 1214, 1586, 3020, 3368 cm^{-1}; HPLC-PDA: tr = 12.80 min (% area = 97.92%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.71 (bs, 1H, H_{NH}), 7.68-7.45 (m, 2H, H_{Ar}), 7.44-7.43 (t, *J* = 2.04 Hz, 2H, H_{Ar}), 7.38-7.36 (m, 2H, H_{Ar}), 7.16-7.07 (m, 2H, H_{Ar}), 5.67 (s, 1H, H_{CH}), 4.12 (d, *J* = 14.72 Hz, 1H, H_{CH2}), 3.70 (d, *J* = 14.72 Hz, 1H, H_{CH2}), 3.12-3.06 (m, 1H, H_{CH2}), 3.00-2.94 (m, 1H, H_{CH2}), 2.80-2.77 (t, *J* = 6.56 Hz, 2H, H_{CH2}), 1.71 (s, 9H, $H_{3\times CH3}$) *ppm*; ^{13}C NMR (100 MHz, $CDCl_3$): 154.05 (C-5), 136.05 (C-Ar), 134.74 (C-Ar), 134.10 (C-Ar), 131.34 (C-Ar), 131.09 (C-Ar), 128.87 (C-Ar), 127.05 (C-Ar), 121.37 (C-Ar), 119.40 (C-Ar), 117.92 (C-Ar), 110.72 (C-Ar), 108.11 (C-Ar), 63.08 (C-C(Me)$_3$), 61.70 (C-CH), 48.18 (C-CH_2), 46.85 (C-CH_2), 30.23 (C-CH_3), 21.50 (C-CH_2) *ppm*; HRMS (EI) calcd for $[C_{23}H_{25}ClN_6+H]^+$ 421.1907; found 421.1900.

2-((4-bromophenyl)(1-(*tert*-butyl)-*1H*-tetrazol-5-yl)methyl)-2,3,4,9-tetrahydro-*1H*-pyrido[3,4-*b*]indole (14d) According to GP it was obtained by the reaction of 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole (1 equiv., 344 mg, 2 mmol), 4-bromobenzaldehyde (1 equiv., 368 mg, 2 mmol), *tert*-butyl isocyanide (1 equiv., 166 mg, 2 mmol) and, the $TMSN_3$ (2 equiv., 461 mg, 2 mmol) in methanol (1.0 M, 3 mL) as solvent in 16h with 71% yield (0.71 mmol, 329 mg) as white solid. m.p. = 193-196°C; IR (KBr), ν_{max}: 668, 754, 1214, 1580, 3015, 3369 cm^{-1}; HPLC-PDA: tr = 23.09 min (% area = 95.00%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.64 (bs, 1H, H_{NH}), 7.54-7.52 (m, 2H, H_{Ar}), 7.47 (d, *J* = 7.44 Hz, 1H, H_{Ar}), 7.39 (d, *J* = 8.52 Hz, 2H, H_{Ar}), 7.31 (d, *J* = 6.56 Hz, 1H, H_{Ar}), 7.16-7.12 (t, *J* = 7.12 Hz, 1H, H_{Ar}), 7.11-7.07 (t, *J* = 7.28 Hz, 1H, H_{Ar}), 5.66 (s, 1H, H_{CH}), 4.12 (d, *J* = 13.80 Hz, 1H, H_{CH2}), 3.70 (d, *J* = 14.88 Hz, 1H, H_{CH2}), 3.12-3.07 (m, 1H, H_{CH2}), 3.00-2.94 (m, 1H, H_{CH2}), 2.80-2.77 (t, *J* = 5.68 Hz, 2H, H_{CH2}), 1.71 (s, 9H, $H_{3\times CH3}$) *ppm*; ^{13}C NMR (100 MHz, $CDCl_3$): 153.87 (C-5), 136.02 (C-Ar), 134.64 (C-Ar), 131.83 (C-Ar), 131.38 (C-Ar), 130.89 (C-Ar), 128.87 (C-Ar), 127.09 (C-Ar), 122.90 (C-Ar), 121.42 (C-Ar), 119.40 (C-Ar), 117.92 (C-Ar), 110.77 (C-Ar), 108.15 (C-Ar), 63.22 (-C(Me)$_3$), 62.14 (CH), 48.23 (CH_2), 46.75 (CH_2), 30.33 (3 × CH3), 21.50 (CH_2) *ppm*; HRMS (EI) calcd for $[C_{23}H_{25}BrN_6+H]^+$ 465.1402; found 465.1403.

2-((1-(*tert*-butyl)-*1H*-tetrazol-5-yl)(3-nitrophenyl)methyl)-2,3,4,9-tetrahydro-*1H*-pyrido[3,4-*b*]indole (14e) According to GP it was obtained by reaction of 2,3,4,9-tetrahydro-1H-

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pyrido[3,4-b]indole (1 equiv., 361 mg, 2.1 mmol), 3-nitrobenzaldehyde (1 equiv., 317 mg, 2.1 mmol), *tert*-butyl isocyanide (1 equiv., 174 mg, 2.1 mmol) and, the $TMSN_3$ (2 equiv., 484 mg, 2.1 mmol) in methanol (1.0 M, 3 mL) as solvent in 18h with 69% yield (0.69 mmol, 298 mg) as yellow solid; m.p. = 95-98°C; IR (KBr), ν_{max}: 669, 755, 1215, 1520, 3012, 3368 cm^{-1}; HPLC-PDA: tr = 18.02 min (% area = 95.40%); ^1H NMR (400 MHz, $CDCl_3$) δ: 8.41 (t, *J* = 1.40 Hz, 1H, H_{Ar}), 8.26 (d, *J* = 8.48 Hz, 1H, H_{Ar}), 8.01 (d, *J* = 7.60 Hz, 1H, H_{Ar}), 7.71 (s, 1H, H_{NH}), 7.63-7.59 (t, *J* = 8.00 Hz, 1H, H_{Ar}), 7.47 (d, *J* = 8.00 Hz, 1H, H_{Ar}), 7.30 (d, *J* = 4.44 Hz, 1H, H_{Ar}), 7.16-7.13 (t, *J* = 7.12 Hz, 1H, H_{Ar}), 7.11-7.08 (t, *J* = 7.60 Hz, 1H, H_{Ar}), 5.79 (s, 1H, H_{CH}), 4.06 (d, *J* = 14.52 Hz, 1H, H_{CH2}), 3.75 (d, *J* = 14.36 Hz, 1H, H_{CH2}), 3.13-3.07 (m, 1H, H_{CH2}), 3.00-2.94 (m, 1H, H_{CH2}), 2.82-2.79 (m, 2H, H_{CH2}), 1.77 (s, 9H, $H_{3\times CH3}$) *ppm*; ^{13}C NMR (100 MHz, $CDCl_3$): 153.04 (C-5), 148.30 (C-Ar), 137.50 (C-Ar), 136.07 (C-Ar), 135.87 (C-Ar), 130.75 (C-Ar), 129.66 (C-Ar), 127.05 (C-Ar), 124.58 (C-Ar), 123.74 (C-Ar), 121.67 (C-Ar), 119.55 (C-Ar), 117.97 (C-Ar), 110.77 (C-Ar), 108.21 (C-Ar), 63.18 (-$C(Me)_3$), 62.09 (CH), 48.14 (CH_2), 46.75 (CH_2), 30.28 (3 × CH_3), 21.65 (CH_2) *ppm*; HRMS (EI) calcd for $[C_{23}H_{25}N_7O_2+H]^+$ 432.2148; found 432.2141.

2-((1-(*tert*-butyl)-*1H*-tetrazol-5-yl)(4-nitrophenyl)methyl)-2,3,4,9-tetrahydro-*1H*-pyrido[3,4-*b*]indole (14f) According to GP it was synthesized by reaction of solution of 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole (1 equiv., 361 mg, 2.1 mmol), 4-nitrobenzaldehyde (1 equiv., 317 mg, 2.1 mmol), *tert*-butyl isocyanide (1 equiv., 174 mg, 2.1 mmol) and, the $TMSN_3$ (2 equiv., 484 mg, 2.1 mmol) in methanol (1.0 M, 2 mL) as solvent in 20h with 72% yield (0.72 mmol, 310 mg) as yellow solid; m.p. = 178-181°C; IR (KBr), ν_{max}: 669, 758, 1215, 1584, 3019, 3369 cm^{-1}; HPLC-PDA: tr = 18.06 min (% area = 99.17%); ^1H NMR (400 MHz, $CDCl_3$) δ: 8.27 (d, *J* = 8.92 Hz, 2H, H_{Ar}), 7.75 (d, *J* = 8.92 Hz, 2H, H_{Ar}), 7.65 (bs, 1H, H_{NH}), 7.47 (d, *J* = 7.64 Hz, 1H, H_{Ar}), 7.30 (s, 1H, H_{Ar}), 7.17-7.08 (m, 2H, H_{Ar}), 5.81 (s, 1H, H_{CH}), 4.12 (d, *J* = 14.04 Hz, 1H, H_{CH2}), 3.72 (d, *J* = 14.68 Hz, 1H, H_{CH2}), 3.15-3.09 (m, 1H, H_{CH2}), 3.00-2.95 (m, 1H, H_{CH2}), 2.82-2.79 (t, *J* = 6.36 Hz, 2H, H_{CH2}), 1.75 (s, 9H, $H_{3\times CH3}$) *ppm*; ^{13}C NMR (100 MHz, $CDCl_3$): 153.09 (C-5), 148.06 (C-Ar), 142.73 (C-Ar), 136.12 (C-Ar), 130.70 (C-Ar), 126.95 (C-Ar), 123.69 (C-Ar), 121.62 (C-Ar), 119.45 (C-Ar), 117.97 (C-Ar), 110.67 (C-Ar), 108.11 (C-Ar), 63.03 (-$C(Me)_3$), 62.04 (CH), 48.23 (CH2), 46.85 (CH2), 30.31 (3 × CH3), 21.63 (CH2) ppm; HRMS (EI) calcd for $[C_{23}H_{25}N_7O_2+H]^+$ 432.2148; found 432.2149.

4-((1-(*tert*-butyl)-*1H*-tetrazol-5-yl)(1,3,4,9-tetrahydro-*2H*-pyrido[3,4-*b*]indol-2-yl)methyl)benzonitrile (14g) According to GP it was synthesized by reaction of 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole (1 equiv., 258 mg, 1.5 mmol), 4-Cyanobenzaldehyde (1 equiv., 196 mg, 1.5 mmol), *tert*-butyl isocyanide (1 equiv., 124 mg, 1.5 mmol) and, the $TMSN_3$ (2 equiv., 346 mg, 1.5 mmol) in methanol (1.0 M, 1.5 mL) as solvent in 21h with 78% yield (0.78 mmol, 321 mg) as white solid; m.p. = 75-78°C; IR (KBr), ν_{max}: 669, 758, 1215, 1580, 3022, 3369 cm^{-1}; HPLC-PDA: tr = 19.00 min (% area = 96.60%); ^1H NMR (400 MHz, $CDCl_3$) δ: 7.74 (bs, 1H, H_{NH}), 7.71-7.65 (m, 4H, H_{Ar}), 7.47 (d, *J* = 7.96 Hz, 1H, H_{Ar}), 7.29 (d, *J* = 7.80 Hz, 1H, H_{Ar}), 7.16-7.12 (t, *J* = 7.08 Hz, 1H, H_{Ar}), 7.11-7.07 (t, *J* = 7.64 Hz, 1H, H_{Ar}), 5.75 (s, 1H, H_{CH}), 4.09 (d, *J* = 14.16 Hz, 1H, H_{CH2}), 3.69 (d, *J* = 14.20 Hz, 1H, H_{CH2}), 3.12-3.07 (m, 1H, H_{CH2}), 2.98-2.92 (m, 1H, H_{CH2}), 2.80-2.77 (t, *J* = 6.04 Hz, 2H, H_{CH2}), 1.73 (s, 9H, $H_{3\times CH3}$) *ppm*; ^{13}C NMR (100 MHz, $CDCl_3$): 153.23 (C-5), 140.86 (C-Ar), 136.07 (C-Ar), 132.37 (C-Ar), 130.89 (C-Ar), 130.45 (C-Ar), 127.05 (C-Ar), 121.57 (C-Ar), 119.55 (C-Ar), 118.22 (C-Ar), 117.92 (C-Ar), 112.79 (C-Ar), 110.82 (C-Ar), 108.11 (C-Ar), 63.28 (-$C(Me)_3$), 61.99 (CH), 48.23 (CH_2), 46.71 (CH_2), 30.33 (3 × CH_3), 21.60 (CH_2) *ppm*; HRMS (EI) calcd for $[C_{24}H_{25}N_7+H]^+$ 412.2250; found 412.2253.

2-((1-(*tert*-butyl)-*1H*-tetrazol-5-yl)(4-methoxyphenyl)methyl)-2,3,4,9-tetrahydro-*1H*-pyrido[3,4-*b*]indole (14h) According to GP it was synthesized by reaction of 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole (1 equiv., 258 mg, 1.5 mmol), 4-methoxybenzaldehyde (1 equiv., 204 mg, 1.5 mmol), *tert*-butyl isocyanide (1 equiv., 124 mg, 1.5 mmol) and, the $TMSN_3$ (2 equiv., 346 mg, 1.5 mmol) in methanol (1.0 M, 3 mL) as solvent in 24h with 75% yield (0.75 mmol, 312 mg) as white solid; m.p. = 108-111°C; IR (KBr), ν_{max}: 669, 754, 1215, 2400, 3018, 3367 cm^{-1}; HPLC-PDA: tr = 12.61 min (% area = 96.34%); ^1H NMR (400 MHz, $CDCl_3$) δ: 7.70 (bs, 1H, H_{NH}), 7.47-7.44 (m, 1H, H_{Ar}), 7.41-7.36 (m, 3H, H_{Ar}), 7.14-7.12 (m, 1H, H_{Ar}), 7.11-7.06 (m, 1H, H_{Ar}), 6.91 (d, *J* = 8.48 Hz, 2H, H_{Ar}), 5.24 (s, 1H, H_{CH}), 4.11 (d, *J* = 14.68 Hz, 1H, H_{CH2}), 3.82 (s, 3H, $H_{\text{-}OCH3}$), 3.71 (d, *J* = 14.68 Hz, 1H, H_{CH2}), 3.12-3.06 (m, 1H, H_{CH2}), 3.01-2.96 (m, 1H, H_{CH2}), 2.80-2.76 (m, 2H, H_{CH2}), 1.69 (s, 9H, $H_{3\times CH3}$) *ppm*; ^{13}C NMR (100 MHz, $CDCl_3$): 154.72 (C-5), 139.82 (C-Ar), 136.09 (C-Ar), 131.69 (C-Ar), 131.01 (C-Ar), 127.49 (C-Ar), 121.23 (C-Ar), 119.23 (C-Ar), 117.84 (C-Ar), 114.01 (C-Ar), 110.75 (C-Ar), 109.13 (C-Ar), 108.05 (C-Ar), 63.20 (-$C(Me)_3$), 61.57 (CH), 55.30 (-OCH_3), 48.21 (CH_2), 46.91 (CH_2), 30.18 (3 × CH_3), 21.40 (CH_2) *ppm*; HRMS (EI) calcd for $[C_{24}H_{28}N_6O+H]^+$ 417.2403; found 417.2417.

2-((1-(*tert*-butyl)-*1H*-tetrazol-5-yl)(3,4-dimethoxyphenyl)methyl)-2,3,4,9-tetrahydro-*1H*-pyrido[3,4-*b*]indole (14i) According to GP it was obtained by reaction of 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole (1 equiv., 206 mg, 1.2 mmol), 3,4-dimethoxybenzaldehyde (1 equiv., 199 mg, 1.2 mmol), *tert*-butyl isocyanide (1 equiv., 100 mg, 1.2 mmol) and, the $TMSN_3$ (2 equiv., 276 mg, 1.2 mmol) in methanol (1.0 M, 1 mL) as solvent in 24h with 70% yield (0.70 mmol, 312) as white solid; m.p. = 107-110°C; IR (KBr), ν_{max}: 669, 758, 1216, 1578, 3021, 3368 cm^{-1}; HPLC-PDA: tr = 12.85 min (% area = 98.82%); ^1H NMR (400 MHz, $CDCl_3$) δ: 7.65 (s, 1H, H_{NH}), 7.48 (d, *J* = 7.80 Hz, 1H, H_{Ar}), 7.30 (d, *J* = 7.80 Hz, 1H, H_{Ar}), 7.20 (d, *J* = 1.60 Hz, 1H, H_{Ar}), 7.16-7.12 (t, *J* = 7.08 Hz, 1H, H_{Ar}), 7.11-7.07 (t, *J* = 7.28 Hz, 1H, H_{Ar}), 6.83 (s, 2H, H_{Ar}), 5.60 (s, 1H, H_{CH}), 4.09 (d, *J* = 15.24 Hz, 1H, H_{CH2}), 3.90 (s, 3H, $H_{\text{-}OCH3}$), 3.88 (s, 3H, $H_{\text{-}OCH3}$), 3.72 (d, *J* = 13.84 Hz, 1H, H_{CH2}), 3.13-3.07 (m, 1H, H_{CH2}), 3.03-2.98 (m, 1H, H_{CH2}), 2.81-2.78 (t, *J* = 5.32 Hz, 2H, H_{CH2}), 1.70 (s, 9H, $H_{3\times CH3}$) *ppm*; ^{13}C NMR (100 MHz, $CDCl_3$): 154.77 (C-5), 149.34 (C-Ar), 136.02 (C-Ar), 131.58 (C-Ar), 128.18 (C-Ar), 127.15 (C-Ar), 122.16 (C-Ar), 121.37 (C-Ar), 119.35 (C-Ar), 117.87 (C-Ar), 112.50 (C-Ar), 110.72 (C-Ar), 110.48 (C-Ar), 108.16 (C-Ar), 63.47 (-$C(Me)_3$), 61.60 (CH), 56.13 (-OCH_3), 55.88

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(OCH_3), 48.14 (CH_2), 47.10 (CH_2), 30.18 (3 × CH_3), 21.31 (CH_2) *ppm*; HRMS (EI) calcd for $[C_{25}H_{30}N_6O_2+H]^+$ 447.2508; found 447.2518.

2-((1-(*tert*-butyl)-*1H*-tetrazol-5-yl)(3,4,5-trimethoxyphenyl)methyl)-2,3,4,9-tetrahydro-*1H*-pyrido[3,4-*b*]indole (14j) According to GP it was obtained by reaction of 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole (1 equiv., 86 mg, 0.5 mmol), 3,4,5-trimethoxybenzaldehyde (1 equiv., 98 mg, 0.5 mmol), *tert*-butyl isocyanide (1 equiv., 41 mg, 0.5 mmol) and, the $TMSN_3$ (2 equiv., 115 mg, 0.5 mmol) in methanol (1.0 M, 1.5 mL) as solvent in 18h with 82% yield (0.82 mmol, 390 mg) as white solid; m.p. = 143-146°C; IR (KBr), ν_{max}: 669, 754, 1214, 1520, 3020, 3369 cm^{-1}; HPLC-PDA: tr = 9.52 min (% area = 98.43%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.80 (bs, 1H, H_{NH}), 7.48 (d, *J* = 7.24 Hz, 1H, H_{Ar}), 7.29 (d, *J* = 7.24 Hz, 1H, H_{Ar}), 7.13-7.07 (m, 2H, H_{Ar}), 6.71 (s, 2H, H_{Ar}), 5.57 (s, 1H, H_{CH}), 4.08 (d, *J* = 13.40 Hz, 1H, H_{CH2}), 3.87 (s, 3H, H_{-OCH3}), 3.83 (s, 6H, $H_{2\times -OCH3}$), 3.73 (d, *J* = 14.68 Hz, 1H, H_{CH2}), 3.13-3.07 (m, 1H, H_{CH2}), 3.04-2.98 (m, 1H, H_{CH2}), 2.82-2.79 (t, *J* = 10.80 Hz, 2H, H_{CH2}), 1.72 (s, 9H, $H_{3\times CH3}$) *ppm*; ^{13}C NMR (100 MHz, $CDCl_3$): 154.60 (C-5), 153.30 (C-Ar), 138.17 (C-Ar), 136.07 (C-Ar), 131.49 (C-Ar), 131.29 (C-Ar), 127.17 (C-Ar), 121.34 (C-Ar), 119.32 (C-Ar), 117.86 (C-Ar), 110.77 (C-Ar), 108.02 (C-Ar), 106.94 (C-Ar), 106.79 (C-Ar), 63.72 (-$C(Me)_3$), 61.60 (CH), 60.86 (-OCH_3), 56.32 (2 × -OCH_3), 48.23 (CH_2), 47.15 (CH_2), 30.28 (3 × CH_3), 21.26 (CH_2) *ppm*; HRMS (EI) calcd for $[C_{26}H_{32}N_6O_3+H]^+$ 477.2614; found 477.2612.

2-((1-(*tert*-butyl)-*1H*-tetrazol-5-yl)(4-isopropylphenyl)methyl)-2,3,4,9-tetrahydro-*1H*-pyrido[3,4-*b*]indole (14k) According to GP it was obtained by reaction of 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole (1 equiv., 120 mg, 0.7 mmol), 4-isopropylbenzaldehyde (1 equiv., 104 mg, 0.7 mmol), *tert*-butyl isocyanide (1 equiv., 58 mg, 0.7 mmol) and, the $TMSN_3$ (2 equiv., 161 mg, 0.7 mmol) in methanol (1.0 M, 1 mL) as solvent in 12h with 72% yield (0.72 mmol, 308 mg) as white solid; m.p. = 105-108°C; IR (KBr), ν_{max}: 668, 756, 1216, 1589, 3022, 3368 cm^{-1}; HPLC-PDA: tr = 12.78 min (% area = 96.60%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.67 (s, 1H, H_{NH}), 7.46 (d, *J* = 7.88 Hz, 1H, H_{Ar}), 7.42 (d, *J* = 7.84 Hz, 2H, H_{Ar}), 7.27-7.26 (m, 1H, H_{Ar}), 7.24-7.22 (m, 2H, H_{Ar}), 7.14-7.10 (t, *J* = 7.36 Hz, 1H, H_{Ar}), 7.102-7.06 (t, *J* = 7.76 Hz, 1H, H_{Ar}), 5.64 (s, 1H, H_{CH}), 4.12 (d, *J* = 13.84 Hz, 1H, H_{CH2}), 3.72 (d, *J* = 14.04 Hz, 1H, H_{CH2}), 3.12-3.07 (m, 1H, H_{CH2}), 3.02-2.97 (m, 1H, H_{CH2}), 2.94-2.89 (m, 1H, $H_{-C(Me)2}$), 2.81-2.77 (m, 2H, H_{CH2}), 1.70 (s, 9H, $H_{3\times CH3}$), 1.33 (s, 6H, $H_{2\times CH3}$) *ppm*; ^{13}C NMR (100 MHz, $CDCl_3$): 154.58 (C-5), 149.38 (C-Ar), 136.06 (C-Ar), 135.62 (C-Ar), 132.61 (C-Ar), 131.68 (C-Ar), 130.32 (C-Ar), 129.76 (C-Ar), 127.22 (C-Ar), 126.67 (C-Ar), 121.25 (C-Ar), 119.27 (C-Ar), 117.86 (C-Ar), 110.69 (C-Ar), 108.12 (C-Ar), 63.57 (-$C(Me)_3$), 61.56 (CH), 48.34 (CH_2), 46.82 (CH_2), 33.77 (-$C(Me)_2$), 30.22 (3 × CH_3), 23.86 (2 × CH_3), 21.46 (CH_2) *ppm*; HRMS (EI) calcd for $[C_{26}H_{32}N_6+H]^+$ 429.2767; found 429.2768.

2-((1-cyclohexyl-*1H*-tetrazol-5-yl)(4-fluorophenyl)methyl)-2,3,4,9-tetrahydro-*1H*-pyrido[3,4-*b*]indole (14l) According to GP it was obtained by reaction of 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole (1 equiv., 86 mg, 0.5 mmol), 4-fluorobenzaldehyde (1 equiv., 62 mg, 0.5 mmol), cyclohexyl isocyanide (1 equiv., 55 mg, 0.5 mmol) and, the $TMSN_3$ (2 equiv., 115 mg, 0.5 mmol) in methanol (1.0 M, 1.2 mL) as solvent in 22h with 68% yield (0.68 mmol, 293 mg) as yellow solid; m.p. = 123-126°C; IR (KBr), ν_{max}: 668, 753, 1215, 2400, 3019, 3369 cm^{-1}; HPLC-PDA: tr = 28.47 min (% area = 96.42%); ^{1}H NMR (400 MHZ, $CDCl_3$) δ: 7.77 (bs, 1H, H_{NH}), 7.54-7.50 (m, 3H, H_{Ar}), 7.32 (d, *J* = 7.88 Hz, 1H, H_{Ar}), 7.19 – 7.15 (t, *J* = 7.64 Hz, 1H, H_{Ar}), 7.12-7.08 (t, *J* = 8.28 Hz, 3H, H_{Ar}), 5.38 (s, 1H, H_{CH}), 4.57-4.50 (m, 1H, H_{cy}), 3.95 (d, *J* = 14.68 Hz, 1H, H_{CH2}), 3.65 (d, *J* = 14.68 Hz, 1H, H_{CH2}), 3.08-3.01 (m, 1H, H_{CH2}), 2.92-2.88 (m, 1H, H_{CH2}), 2.86-2.82 (m, 2H, H_{CH2}), 2.02-1.98 (m, 1H, H_{cy}), 1.92-1.89 (t, *J* = 8.92 Hz, 4H, H_{cy}) 1.87- 1.77 (m, 1H, H_{cy}), 1.59 (d, *J* = 12.76 Hz, 1H, H_{cy}), 1.34-1.30 (m, 3H, H_{cy}) *ppm*; ^{13}C NMR (100 MHz, $CDCl_3$): 153.40 (C-5), 136.11 (C-Ar), 131.58 (C-Ar), 130.84 (C-Ar), 130.33 (C-Ar), 130.25 (C-Ar), 126.97 (C-Ar), 121.59 (C-Ar), 119.50 (C-Ar), 117.96 (C-Ar), 116.04 (C-Ar), 115.83 (C-Ar), 110.89 (C-Ar), 108.02 (C-Ar), 62.84 (CH), 58.25 (C-Cy), 48.98 (CH_2), 48.21 (CH_2), 32.88 (C-Cy), 32.84 (C-Cy), 25.39 (C-Cy), 25.23 (C-Cy), 24.73 (C-Cy), 21.04 (CH_2) ppm; HRMS (EI) calcd for $[C_{25}H_{27}FN_6+H]^+$ 431.2359; found 431.2351.

2-((4-chlorophenyl)(1-cyclohexyl-*1H*-tetrazol-5-yl)methyl)-2,3,4,9-tetrahydro-*1H*-pyrido[3,4-*b*]indole (14m) According to GP it was obtained by reaction of 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole (1 equiv., 206 mg, 1.2 mmol), 4-chlorobenzaldehyde (1 equiv., 125 mg, 1.2 mmol), cyclohexyl isocyanide (1 equiv., 131 mg, 1.2 mmol) and, the $TMSN_3$ (2 equiv., 276 mg, 1.2 mmol) in methanol (1.0 M, 1.5 mL) as solvent in 22h with 74% yield (0.74 mmol, 330 mg) as white solid; m.p. = 187-190°C; IR (KBr), ν_{max}: 669, 757, 1215, 1584, 3019, 3368 cm^{-1}; HPLC-PDA: tr = 23.41 min (% area = 97.36%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.80 (bs, 1H, H_{NH}), 7.50 -7.47(m, 3H, H_{Ar}), 7.39 (d, *J* = 8.52 Hz, 2H, H_{Ar}), 7.32 (d, *J* = 8.00 Hz, 1H, H_{Ar}), 7.18-7.15 (t, *J* = 7.44 Hz, 1H, H_{Ar}), 7.14-7.10 (t, *J* = 7.44 Hz, 1H, HAr), 5.38 (s, 1H, H_{CH}), 4.57-4.49 (m, 1H, H_{cy}), 3.95 (d, *J* = 14.56 Hz, 1H, H_{CH2}), 3.65 (d, *J* = 14.36 Hz, 1H, H_{CH2}), 3.07-3.01 (m, 1H, H_{CH2}), 2.92-2.88 (m, 1H, H_{CH2}), 2.86-2.81 (m, 2H, H_{CH2}), 2.01-1.95 (m, 1H, H_{cy}), 1.92-1.87 (m, 3H, H_{cy}) 1.84- 1.75 (t, *J* = 23.60 Hz, 2H, H_{cy}), 1.61 (d, *J* = 11.88 Hz, 1H, H_{cy}), 1.34-1.25 (m, 3H, H_{cy}) *ppm*; ^{13}C NMR (100 MHz, $CDCl_3$): 153.16 (C-5), 136.12 (C-Ar), 134.72 (C-Ar), 134.24 (C-Ar), 130.73 (C-Ar), 129.92 (C-Ar), 129.17 (C-Ar), 126.95 (C-Ar), 121.63 (C-Ar), 119.51 (C-Ar), 117.96 (C-Ar), 110.92 (C-Ar), 107.96 (C-Ar), 62.89 (CH), 58.30 (C-Cy), 48.99 (CH_2), 48.19 (CH_2), 32.92 (C-Cy), 32.86 (C-Cy), 25.38 (C-Cy), 25.22 (C-Cy), 24.73 (C-Cy), 21.02 (CH2) ppm; HRMS (EI) calcd for $[C_{25}H_{27}ClN_6+H]+$ 447.2064; found 447.2076.

2-((2-chlorophenyl)(1-cyclohexyl-*1H*-tetrazol-5-yl)methyl)-2,3,4,9-tetrahydro-*1H*-pyrido[3,4-*b*]indole (14n) According to GP it was obtained by reaction of 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole (1 equiv., 124 mg, 1.3 mmol), 2-chlorobenzaldehyde (1 equiv., 182 mg, 1.3 mmol), cyclohexyl isocyanide (1 equiv., 142 mg, 1.3 mmol) and, the $TMSN_3$ (2

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equiv., 300 mg, 1.3 mmol) in methanol (1.0 M, 1.5 mL) as solvent in 15h with 82% yield (0.82 mmol, 366 mg) as white solid; m.p. = 188-191°C; IR (KBr), ν_{max}: 669, 759, 1213, 2413, 3020, 3369 cm^{-1}; HPLC-PDA: tr = 30.29 min (% area = 96.54%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.91 (d, *J* = 7.72 Hz, 1H, H_{Ar}), 7.81 (bs, 1H, H_{NH}), 7.49 (d, *J* = 7.44 Hz, 1H, H_{Ar}), 7.45 (d, *J* = 7.64 Hz, 1H, H_{Ar}), 7.38-7.33 (m, 1H, H_{Ar}), 7.31-7.29 (m, 2H, H_{Ar}), 7.17-7.13 (t, *J* = 7.60 Hz, 1H, H_{Ar}), 7.12-7.08 (t, *J* = 7.60 Hz, 1H, H_{Ar}), 5.84 (s, 1H, H_{CH}), 4.43-4.36 (m, 1H, H_{cy}), 3.86-3.77 (m, 2H, H_{CH2}), 3.12-3.06 (m, 1H, H_{CH2}), 2.98-2.92 (m, 1H, H_{CH2}), 2.88-2.80 (m, 2H, H_{CH2}), 2.02-1.95 (m, 1H, H_{cy}), 1.90-1.80 (m, 4H, H_{cy}) 1.72- 1.69 (t, *J* = 6.72 Hz, 1H, H_{cy}), 1.61 (d, *J* = 4.44 Hz, 1H, H_{cy}), 1.33-1.28 (m, 3H, H_{cy}) *ppm*; ^{13}C NMR (100 MHz, $CDCl_3$): 153.26 (C-5), 136.11 (C-Ar), 134.02 (C-Ar), 133.54 (C-Ar), 131.14 (C-Ar), 130.95 (C-Ar), 130.00 (C-Ar), 129.93 (C-Ar), 127.59 (C-Ar), 127.06 (C-Ar), 121.48 (C-Ar), 119.39 (C-Ar), 117.95 (C-Ar), 110.84 (C-Ar), 108.16 (C-Ar), 58.56 (CH), 58.24 (C-Cy), 48.77 (CH_2), 48.17 (CH_2), 33.00 (C-Cy), 32.88 (C-Cy), 25.31 (C-Cy), 24.75 (C-Cy), 21.14 (CH_2) *ppm*; HRMS (EI) calcd for $[C_{25}H_{27}ClN_6+H]^+$ 447.2064; found 447.2097.

2-((4-bromophenyl)(1-cyclohexyl-*1H*-tetrazol-5-yl)methyl)-2,3,4,9-tetrahydro-*1H*-pyrido[3,4-*b*]indole (14o) According to GP it was obtained by reaction of 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole (1 equiv., 344 mg, 2 mmol), 4-bromobenzaldehyde (1 equiv., 368 mg, 2 mmol), cyclohexyl isocyanide (1 equiv., 218 mg, 2 mmol) and, the TMSN3 (2 equiv., 461 mg, 2 mmol) in methanol (1.0 M, 2 mL) as solvent in 16h with 80% yield (0.80 mmol, 392 mg) as yellow solid; m.p. = 99-102°C; IR (KBr), ν_{max}: 669, 757, 1215, 2400, 3019, 3366 cm^{-1}; HPLC-PDA: tr = 24.23 min (% area = 98.49%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.73 (bs, 1H, H_{NH}), 7.55 (d, *J* = 8.40 Hz, 2H, H_{Ar}), 7.50 (d, *J* = 7.56 Hz, 1H, H_{Ar}), 7.44-7.40 (m, 2H, H_{Ar}), 7.32 (d, *J* = 7.68 Hz, 1H, H_{Ar}), 7.19-7.10 (m, 2H, H_{Ar}), 5.36 (s, 1H, H_{CH}), 4.58-4.50 (m, 1H, H_{cy}), 3.95 (d, *J* = 14.88 Hz, 1H, H_{CH2}), 3.64 (d, *J* = 14.48 Hz, 1H, H_{CH2}), 3.08-3.00 (m, 1H, H_{CH2}), 2.92-2.88 (m, 1H, H_{CH2}), 2.87-2.82 (m, 2H, H_{CH2}), 2.03-1.99 (m, 1H, H_{cy}), 1.97-1.87 (m, 3H, H_{cy}) 1.81 (d, *J* = 15.32 Hz, 1H, H_{cy}), 1.72 (bs, 1H, H_{cy}), 1.32-1.29 (m, 4H, H_{cy}) *ppm*; ^{13}C NMR (100 MHz, $CDCl_3$): 153.07 (C-5), 137.46 (C-Ar), 136.11 (C-Ar), 134.85 (C-Ar), 134.72 (C-Ar), 132.12 (C-Ar), 130.22 (C-Ar), 126.97 (C-Ar), 122.81 (C-Ar), 121.64 (C-Ar), 119.54 (C-Ar), 117.97 (C-Ar), 110.89 (C-Ar), 109.18 (C-Ar), 108.03 (C-Ar), 62.98 (CH), 58.30 (C-Cy), 48.99 (CH_2), 48.20 (CH_2), 32.88 (C-Cy), 25.24 (C-Cy), 24.73 (C-Cy), 21.05 (CH_2) ppm; HRMS (EI) calcd for $[C_{25}H_{27}BrN_6+H]^+$ 491.1559; found 491.1592.

2-((1-cyclohexyl-*1H*-tetrazol-5-yl)(4-nitrophenyl)methyl)-2,3,4,9-tetrahydro-*1H*-pyrido[3,4-*b*]indole (14p) According to GP it was obtained by the reaction of 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole (1 equiv., 138 mg, 0.8 mmol), 4-nitrobenzaldehyde (1 equiv., 121 mg, 0.8 mmol), cyclohexyl isocyanide (1 equiv., 87 mg, 0.8 mmol) and, the $TMSN_3$ (2 equiv., 184 mg, 0.8 mmol) in methanol (1.0 M, 1.5 mL) as solvent in 18h with 83% yield (0.83 mmol, 379 mg) as white solid; m.p. = 106-109°C; IR (KBr), ν_{max}: 669, 754, 1215, 1584, 3022, 3369 cm^{-1}; HPLC-PDA: tr = 21.65 min (% area = 95.07%); 1H NMR (400 MHz, $CDCl_3$) δ: 8.29 (d, *J* = 8.92 Hz, 2H, H_{Ar}), 7.79 (d, *J* = 9.12 Hz, 3H, H_{Ar}, H_{NH}), 7.51 (d, *J* = 7.44 Hz, 1H, H_{Ar}), 7.32 (d, *J* = 8.08 Hz, 1H, H_{Ar}), 7.18-7.11 (m, 1H, H_{Ar}), 5.24 (s, 1H, H_{CH}), 4.59-4.51 (m, 1H, H_{cy}), 3.93 (d, *J* = 14.48 Hz, 1H, H_{CH2}), 3.68 (d, *J* = 14.24 Hz, 1H, H_{CH2}), 3.09-3.00 (m, 1H, H_{CH2}), 2.95-2.91 (m, 1H, H_{CH2}), 2.89-2.84 (m, 2H, H_{CH2}), 2.05-1.99 (m, 1H, H_{cy}), 1.95-1.89 (m, 2H, H_{cy}), 1.87-1.85 (t, *J* = 2.96 Hz, 3H, H_{cy}), 1.75 (d, *J* = 6.80 Hz, 1H, H_{cy}), 1.37-1.29 (m, 3H, H_{cy}) *ppm*; ^{13}C NMR (100 MHz, $CDCl_3$): 152.26 (C-5), 148.01 (C-Ar), 142.76 (C-Ar), 137.50 (C-Ar), 136.12 (C-Ar), 129.64 (C-Ar), 126.87 (C-Ar), 124.04 (C-Ar), 121.80 (C-Ar), 120.13 (C-Ar), 118.00 (C-Ar), 110.91 (C-Ar), 109.12 (C-Ar), 107.99 (C-Ar), 62.72 (CH), 58.50 (C-Cy), 48.97 (CH_2), 48.01 (CH_2), 32.92 (C-Cy), 25.24 (C-Cy), 24.67 (C-Cy), 21.11 (CH_2) ppm; HRMS (EI) calcd for $[C_{25}H_{27}N_7O_2+H]^+$ 458.2304; found 458.2309.

4-((1-cyclohexyl-*1H*-tetrazol-5-yl)(1,3,4,9-tetrahydro-*2H*-pyrido[3,4-*b*]indol-2-yl)methyl)benzonitrile(14q) According to GP it was obtained by reaction of 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole (1 equiv., 215 mg, 1.25 mmol), 4-cyanobenzaldehyde (1 equiv., 164 mg, 1.25 mmol), cyclohexyl isocyanide (1 equiv., 136 mg, 1.25 mmol) and, the $TMSN_3$ (2 equiv., 288 mg, 1.25 mmol) in methanol (1.0 M, 2 mL) as solvent in 18h with 85% yield (0.85 mmol, 372 mg) as white solid; m.p. = 90-93°C; IR (KBr), ν_{max}: 668, 758, 1216, 1580, 3022, 3368 cm^{-1}; HPLC-PDA: tr = 23.52 min (% area = 96.76%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.76 (bs, 1H, H_{NH}), 7.71 (s, 4H, H_{Ar}), 7.50 (d, *J* = 7.44 Hz, 1H, H_{Ar}), 7.32 (d, *J* = 7.80 Hz, 1H, H_{Ar}), 7.19-7.11 (m, 2H, H_{Ar}), 5.24 (s, 1H, H_{CH}), 4.55-4.50 (m, 1H, H_{cy}), 3.92 (d, *J* = 14.52 Hz, 1H, H_{CH2}), 3.66 (d, *J* = 14.88 Hz, 1H, H_{CH2}), 3.05-2.99 (m, 1H, H_{CH2}), 2.93-2.89 (m, 1H, H_{CH2}), 2.87-2.83 (m, 2H, H_{CH2}), 2.30 (d, *J* = 12.76 Hz, 1H, H_{cy}), 2.03-1.99 (m, 1H, H_{cy}), 1.96 (d, *J* = 5.32 Hz, 1H, H_{cy}), 1.85-1.82 (t, *J* = 3.16 Hz, 2H, H_{cy}), 1.74 (d, *J* = 9.24 Hz, 2H, H_{cy}) 1.34-1.29 (m, 3H, H_{cy}) *ppm*; ^{13}C NMR (100 MHz, $CDCl_3$): 152.39 (C-5), 141.06 (C-Ar), 137.48 (C-Ar), 136.12 (C-Ar), 132.66 (C-Ar), 130.41 (C-Ar), 129.41 (C-Ar), 126.87 (C-Ar), 121.74 (C-Ar), 119.59 (C-Ar), 117.98 (C-Ar), 112.73 (C-Ar), 110.93 (C-Ar), 109.14 (C-Ar), 107.92 (C-Ar), 62.93 (CH), 58.45 (C-Cy), 48.98 (CH_2), 48.06 (CH_2), 32.89 (C-Cy), 31.58 (C-Cy), 25.21 (C-Cy), 24.68 (C-Cy), 22.65 (CH_2) *ppm*; HRMS (EI) calcd for $[C_{26}H_{27}N_7+H]^+$ 438.2406; found 438.2409.

2-((1-cyclohexyl-*1H*-tetrazol-5-yl)(4-methoxyphenyl)methyl)-2,3,4,9-tetrahydro-*1H*-pyrido[3,4-*b*]indole (14r) According to GP it was obtained by reaction of 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole (1 equiv., 430 mg, 2.5 mmol), 4-methoxybenzaldehyde (1 equiv., 340 mg, 2.5 mmol), cyclohexyl isocyanide (1 equiv., 273 mg, 2.5 mmol) and, the $TMSN_3$ (2 equiv., 576 mg, 2.5 mmol) in methanol (1.0 M, 3 mL) as solvent with 74% yield (0.74 mmol, 327 mg) as white solid; m.p. = 94-97°C; IR (KBr), ν_{max}: 669, 752, 1284, 1584, 3021, 3369 cm^{-1}; HPLC-PDA: tr = 12.41 min (% area = 95.49%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.74 (s, 1H, H_{NH}), 7.50 (d, *J* = 7.88 Hz, 1H, H_{Ar}), 7.44-7.40 (m, 2H, H_{Ar}), 7.32 (d, *J* = 8.08 Hz, 1H, H_{Ar}), 7.18-7.10 (m, 1H, H_{Ar}), 6.93 (d, *J* = 8.28 Hz, 2H, H_{Ar}), 5.24 (s, 1H, H_{CH}), 4.58-4.51 (m, 1H, H_{cy}), 3.97 (d, *J* = 14.92 Hz, 1H, H_{CH2}), 3.82 (s, 3H, H_{-OCH3}), 3.65 (d, *J* = 14.88 Hz, 1H, H_{CH2}), 3.09-3.03 (m, 1H,

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H_{CH2}), 2.92-2.88 (m, 1H, H_{CH2}), 2.85-2.82 (m, 2H, H_{CH2}), 2.30 (d, *J* = 12.12 Hz, 1H, H_{cy}), 1.99-1.95 (m, 1H, H_{cy}), 1.88-1.84 (m, 3H, H_{cy}), 1.74-1.70 (m, 2H, H_{cy}), 1.31-1.28 (m, 3H, H_{cy}) *ppm*; ^{13}C NMR (100 MHz, $CDCl_3$): 153.88 (C-5), 140.58 (C-Ar), 137.42 (C-Ar), 136.11 (C-Ar), 132.47 (C-Ar), 131.15 (C-Ar), 129.75 (C-Ar), 127.72 (C-Ar), 127.06 (C-Ar), 121.48 (C-Ar), 119.43 (C-Ar), 117.93 (C-Ar), 114.29 (C-Ar), 110.87 (C-Ar), 109.20 (C-Ar), 108.07 (C-Ar), 63.13 (CH), 58.11 (C-Cy), 49.01 (CH_2), 48.32 (CH_2), 32.83 (C-Cy), 25.41 (C-Cy), 24.78 (C-Cy), 21.04 (CH_2) *ppm*; HRMS (EI) calcd for $[C_{26}H_{30}N_6O+H]^+$ 443.2559; found 443.2545.

2-((1-cyclohexyl-*1H*-tetrazol-5-yl)(3,4-dimethoxyphenyl)methyl)-2,3,4,9-tetrahydro-*1H*-pyrido[3,4-*b*]indole (14s) According to GP it was obtained by reaction of of 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole (1 equiv., 34 mg, 0.2 mmol), 3,4-dimethoxybenzaldehyde (1 equiv., 33 mg, 0.2 mmol), cyclohexyl isocyanide (1 equiv., 22 mg, 0.2 mmol) and, the $TMSN_3$ (2 equiv., 46 mg, 0.2 mmol) in methanol (1.0 M, 0.5 mL) as solvent in 20h with 82% yield (0.82 mmol, 387 mg) as white solid; m.p. = 219-222°C; IR (KBr), ν_{max}: 669, 757, 1215, 1584, 3020, 3368 cm^{-1}; HPLC-PDA: tr = 12.67 min (% area = 98.40%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.77 (s, 1H, H_{NH}), 7.51 (d, *J* = 7.44 Hz, 1H, H_{Ar}), 7.32 (d, *J* = 7.44 Hz, 1H, H_{Ar}), 7.18-7.15 (t, *J* = 7.08 Hz, 1H, H_{Ar}), 7.14-7.10 (t, *J* = 7.60 Hz, 1H, H_{Ar}), 7.07 (d, *J* = 1.92 Hz, 1H, H_{Ar}), 7.01 (d, *J* = 7.96 Hz, 1H, H_{Ar}), 6.86 (d, *J* = 8.12 Hz, 1H, H_{Ar}), 5.28 (s, 1H, H_{CH}), 4.58-4.52 (t, *J* = 11.56 Hz, 1H, H_{cy}), 3.96 (d, *J* = 14.92 Hz, 1H, H_{CH2}), 3.89 (s, 3H, H_{-OCH3}), 3.86 (s, 3H, H_{-OCH3}), 3.68 (d, *J* = 14.92 Hz, 1H, H_{CH2}), 3.10-3.04 (m, 1H, H_{CH2}), 2.95-2.90 (m, 1H, H_{CH2}), 2.85-2.82 (t, *J* = 5.64 Hz, 2H, H_{CH2}), 1.98-1.86 (m, 4H, H_{cy}), 1.72-1.65 (m, 3H, H_{cy}), 1.32-1.27 (t, *J* = 10.12 Hz, 3H, H_{cy}) *ppm*; ^{13}C NMR (100 MHz, $CDCl_3$): 153.86 (C-5), 149.51 (C-Ar), 149.29 (C-Ar), 136.09 (C-Ar), 131.06 (C-Ar), 128.44 (C-Ar), 127.03 (C-Ar), 121.54 (C-Ar), 120.79 (C-Ar), 119.47 (C-Ar), 117.94 (C-Ar), 111.27 (C-Ar), 111.05 (C-Ar), 110.89 (C-Ar), 108.04 (C-Ar), 63.26 (CH), 58.13 (C-Cy), 49.01 (CH_2), 48.42 (CH_2), 32.83 (C-Cy), 25.42 (C-Cy), 25.32 (C-Cy), 24.76 (C-Cy), 20.85 (CH_2) *ppm*; HRMS (EI) calcd for $[C_{27}H_{32}N_6O_2+H]^+$ 473.2665; found 473.2661.

2-((1-cyclohexyl-*1H*-tetrazol-5-yl)(3,4,5-trimethoxyphenyl)methyl)-2,3,4,9-tetrahydro-*1H*-pyrido[3,4-*b*]indole (14t) According to GP it was obtained by reaction of 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole (1 equiv., 103 mg, 0.6 mmol), 3,4,5-trimethoxybenzaldehyde (1 equiv., 118 mg, 0.6 mmol), cyclohexyl isocyanide (1 equiv., 65 mg, 0.6 mmol) and, the $TMSN_3$ (2 equiv., 138 mg, 0.6 mmol) in methanol (1.0 M, 1.5 mL) as solvent in 8h with 85% yield (0.85 mmol, 427 mg) as white solid; m.p. = 193-196°C; IR (KBr), ν_{max}: 669, 757, 1214, 1585, 3020, 3368 cm^{-1}; HPLC-PDA: tr = 13.05 min (% area = 97.52%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.80 (bs, 1H, H_{NH}), 7.52 (d, *J* = 7.60 Hz, 1H, H_{Ar}), 7.33 (d, *J* = 7.64 Hz, 1H, H_{Ar}), 7.19-7.16 (t, *J* = 7.08 Hz, 1H, H_{Ar}), 7.15-7.11 (t, *J* = 7.44 Hz, 1H, H_{Ar}), 6.74 (s, 2H, H_{Ar}), 5.30 (s, 1H, H_{CH}), 4.66-4.58 (m, 1H, H_{cy}), 3.98 (d, *J* = 14.72 Hz, 1H, H_{CH2}), 3.85 (s, 3H, H_{-OCH3}), 3.84 (s, 6H, $H_{2\times -OCH3}$), 3.66-3.50 (t, *J* = 14.72 Hz, 1H, H_{CH2}), 3.11-3.06 (m, 1H, H_{CH2}), 2.96-2.91 (m, 1H, H_{CH2}), 2.87-2.84 (m, 2H, H_{CH2}), 2.03-1.97 (m, 1H, H_{cy}), 1.92-1.85 (m, 3H, H_{cy}) 1.78-1.65 (m, 3H, H_{cy}), 1.33-1.25 (m, 3H, H_{cy}) *ppm*; ^{13}C NMR (100 MHz, $CDCl_3$): 153.64 (C-5), 138.12 (C-Ar), 136.11 (C-Ar), 131.75 (C-Ar), 130.97 (C-Ar), 126.97 (C-Ar), 121.60 (C-Ar), 119.50 (C-Ar), 117.93 (C-Ar), 110.93 (C-Ar), 107.94 (C-Ar), 105.32 (C-Ar), 63.74 (CH), 60.90 (-OMe), 58.26 (C-Cy), 56.28 (2 × -OMe), 49.22 (CH_2), 48.62 (CH_2), 32.90 (C-Cy), 32.83 (C-Cy), 25.48 (C-Cy), 25.38 (C-Cy), 24.75 (C-Cy), 20.86 (CH_2) *ppm*; HRMS (EI) calcd for $[C_{28}H_{34}N_6O_3+H]^+$ 503.2771; found 503.2771.

4-((1-cyclohexyl-*1H*-tetrazol-5-yl)(1,3,4,9-tetrahydro-*2H*-pyrido[3,4-*b*]indol-2-yl)methyl)phenol (14u) According to GP it was obtained by reaction of 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole (1 equiv., 86 mg, 0.5 mmol), 4-hydroxybenzaldehyde (1 equiv., 61 mg, 0.5 mmol), cyclohexyl isocyanide (1 equiv., 54 mg, 0.5 mmol) and, the $TMSN_3$ (2 equiv., 115 mg, 0.5 mmol) in methanol (1.0 M, 1 mL) as solvent in 12h with 65% yield (0.65 mmol, 278 mg) as white solid; m.p. = 102-105°C; IR (KBr), ν_{max}: 669, 756, 1215, 1524, 3020, 3370 cm^{-1}; HPLC-PDA: tr = 12.72 min (% area = 99.50%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.81 (bs, 1H, H_{NH}), 7.49 (d, *J* = 7.64 Hz, 1H, H_{Ar}), 7.34-7.29 (m, 3H, H_{Ar}), 7.17-7.139 (t, *J* = 6.56 Hz, 1H, H_{Ar}), 7.13-7.09 (t, *J* = 7.48 Hz, 1H, H_{Ar}), 6.86-6.83 (m, 2H, H_{Ar}), 5.26 (s, 1H, H_{CH}), 4.58-4.51 (m, 1H, H_{cy}), 3.93 (d, *J* = 15.32 Hz, 1H, H_{CH2}), 3.61 (d, *J* = 15.12 Hz, 1H, H_{CH2}), 3.01-2.96 (m, 1H, H_{CH2}), 2.92-2.87 (m, 1H, H_{CH2}), 2.84-2.80 (m, 2H, H_{CH2}), 1.96-1.90 (m, 2H, H_{cy}), 1.87-1.83 (m, 3H, H_{cy}), 1.73-1.64 (m, 2H, H_{cy}), 1.30-1.26 (m, 3H, H_{cy}) *ppm*; ^{13}C NMR (100 MHz, $CDCl_3$): 154.31 (C-5), 136.14 (C-Ar), 131.11 (C-Ar), 129.88 (C-Ar), 126.97 (C-Ar), 126.66 (C-Ar), 121.44 (C-Ar), 119.35 (C-Ar), 117.89 (C-Ar), 116.19 (C-Ar), 111.02 (C-Ar), 109.21 (C-Ar), 107.75 (C-Ar), 63.24 (CH), 58.32 (C-Cy), 49.16 (CH_2), 48.12 (CH_2), 32.80 (C-Cy), 25.32 (C-Cy), 24.74 (C-Cy), 20.87 (CH_2) *ppm*; HRMS (EI) calcd for $[C_{25}H_{28}N_6O+H]^+$ 429.2403; found 429.2496.

Conclusions

In conclusion, a series of tetrahydro β-carboline tetrazole hybrid were synthesised in good yield by efficient, easy and one step Ugi multicomponent reaction and identified as potent antileishmanial agents. The biological data revealed that most of the synthesized derivatives of tetrahydro β-carboline tetrazoles have exhibited moderate to potent *in vitro* antileishmanial activity with good selectivity index compared to standard drug miltefosine and SSG. Compound **14t** showed highest activity against intracellular amastigotes *in vitro*. This compound has also displayed promising *in vivo* potency in *L. donovani*/golden hamster model. In pharmacokinetic study, it indicates high volume of distribution and systemic clearance. Therefore, these β-carboline analogues are found to be good candidates for a new lead optimization in the field of antileishmanial chemotherapy.

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Conflict of Interest

The authors declare no competing interests.

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An Insight into Tetrahydro-*β*-carboline-Tetrazole Hybrids: Synthesis and Bioevaluation as Potent Antileishmanial Agents

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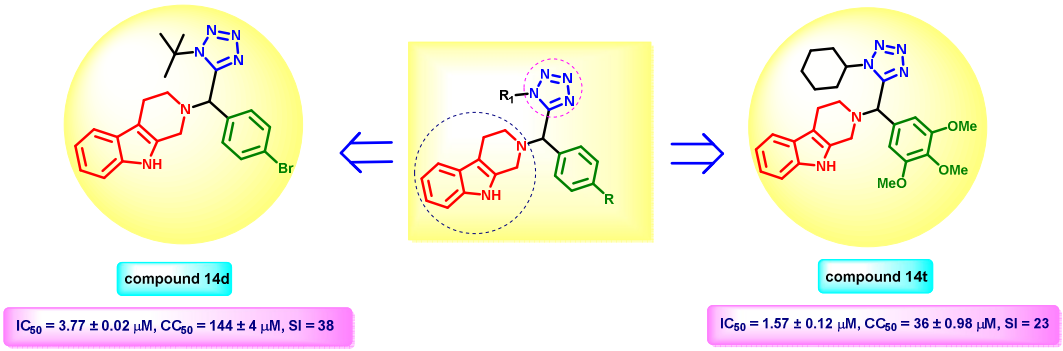

A series of 2,3,4,9 tetrahydro-*β*-carboline tetrazole derivatives have been synthesized utilizing Ugi-tetrazole multicomponent reaction and were identified as potential antileishmanial chemotype.