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Further investigation of harmicines as novel antiplasmodial agents: Synthesis, structure-activity relationship and insight into the mechanism of action

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ABSTRACT

The rise of the resistance of the malaria parasite to the currently approved therapy urges the discovery and development of new efficient agents. Previously we have demonstrated that harmicines, hybrid compounds composed from β-carboline alkaloid harmine and cinnamic acid derivatives, linked *via* either triazole or amide bond, exert significant antiplasmodial activity. In this paper, we report synthesis, antiplasmodial activity and cytotoxicity of expanded series of novel triazole- and amide-type harmicines. Structure-activity relationship analysis revealed that amide-type harmicines **27**, prepared at N-9 of the β-carboline core, exhibit superior potency against both erythrocytic stage of *P. falciparum* and hepatic stages of *P. berghei*. Notably, harmicine **27a**, *m*-(trifluoromethyl)cinnamic acid derivative, exhibited the most favourable selectivity index (SI = 1105). Molecular dynamics simulations revealed the ATP binding site of *P. falciparum* heat shock protein 90 as a druggable binding location, confirmed the usefulness of the harmine's N-9 substitution and identified favourable N–H … π interactions involving Lys45 and the aromatic phenyl unit in the attached cinnamic acid fragment as crucial for the enhanced biological activity. Thus, those compounds were identified as promising and valuable leads for further derivatization in the search of novel, more efficient antiplasmodial agents.

1. Introduction

Malaria is the deadliest human protozoan infectious disease widely spread in the tropical and subtropical areas of the world. Five identified species of the parasite responsible for causing human malaria are *Plasmodium falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*. Of these, *P. falciparum* and *P. vivax* are responsible for more than 95% of malaria cases in the world [1–3]. Despite years of continuous efforts, it is still a major cause of morbidity and mortality (229 million cases and 409 000 deaths in 2019), especially among young children and pregnant women [3]. As existing antimalarial drugs are becoming less effective due to the emergence of resistant strains of *P. falciparum*, there is an urgent need for novel and effective agents to combat malaria [2].

One of the widely employed approaches to find novel antimalarial chemotherapeutic agents is the design of hybrid molecules [4–8]. Hybrids represent chemical entities in which at least two pharmacological agents, acting on the same disease, are covalently linked in a single molecule. Ideally, the novel molecule should have greater activity than the sum of its parts and reduce the risk of drug resistance, drug-drug interactions and adverse effects. In the antimalarial field, hybrids comprised of agents belonging to the most of the known antimalarial drug classes have shown enhanced activity *in vitro* and *in vivo* [5]. Among those compounds, conjugates with cinnamic acid and its derivatives (CADs) have been extensively explored by us and others [9–13] and recently reviewed by Gomes and co-workers [14].

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In our previous work, we presented harmicines, hybrid compounds derived from harmine, a β-carboline alkaloid with antiplasmodial properties, and CADs [10,15]. The reported harmicines differ in 1) the type of the linker between two moieties, which is either a triazole ring or an amide bond, 2) the position of the substitution on the harmine's β-carboline core, which is either O-7 or N-9, and 3) type of CAD. Harmicines exerted remarkable antiplasmodial activity against erythrocytic and hepatic stages of the *Plasmodium* infection, while molecular dynamics (MD) simulations confirmed binding of the most active compounds within the ATP binding site of *P. falciparum* heat shock protein 90 (*Pf*Hsp90), crucial for the intraerythrocytic development of *Plasmodium* [16,17].

To further extend our knowledge of the harmicines structure-activity relationship, we decided to derivatize the harmine scaffold at C-1, C-3, O-6 and N-9 of the β-carboline core and combine it with different CADs (Fig. 1). Both triazole-type (TT) and amide-type (AT) harmicines were prepared. As a continuation of our research, we investigated their antiplasmodial activity against both erythrocytic and hepatic stages of the *Plasmodium* parasite, as well as cytotoxicity against human liver hepatocellular carcinoma cell line (HepG2). We also report computational analysis of their binding to *Pf*Hsp90 using MD simulations.

2. Results and discussion

2.1. Chemistry

The synthetic part of this work could be divided into two parts: 1) synthesis of TT harmicines and 2) synthesis of AT harmicines, with a general synthetic scheme shown in Fig. 2, while the detailed reactions and conditions are given in Schemes 1–4.

TT harmicines (**5a-e**, **14a-e**, **15a-e**, **20a-e**) were prepared by Cu(I) catalysed azide-alkyne cycloaddition (CuAAC). Different reagents and reaction conditions were used depending on the position of the β-carboline ring functionalization. Harmine-based azides **3** or **10** and CAD-based alkynes **4a-e** served as building blocks for the synthesis of TT harmicines at C-1 (**5a-e**) or C-3 (**15a-e**), respectively. CuAAC was performed with $CuSO_4 \times 5H_2O$ in the presence of sodium ascorbate, as a source of Cu(I) ions, in *t*-BuOH/H_2O 1:1 mixture. On the contrary, the starting compounds for the synthesis of TT harmicines at O-6 (**20a-e**) and N-9 (**14a-e**) were harmine-based alkynes **12** or **19** and cinnamyl azides **13a-e**. In this case, the reaction was carried out in MeOH, with Cu(II) acetate (MeOH acts as a reducing agent to generate Cu(I)).

To obtain the required starting compounds for CuAAC, synthetic routes to alkynes and azides were developed. Harmine-based azides **3** and **10** were generated in several steps: 1) synthesis of substituted β-carbolines **1** and **8** at positions 1 and 3 [18,19], 2) synthesis of the corresponding alcohols **2** and **9** [20], and 3) conversion of alcohols to azides **3** and **10** *via* 2-azido-1,3-dimethylimidazolinium hexafluorophosphate (ADMP) and 1,8-diazabicyclo(5.4.0)undec-7-ene (DBU) [21]. Alcohol **9** was also alkylated at N-9 with propargyl bromide in the presence of Cs_2CO_3, to obtain alkyne **12**. Next, we have prepared β-carboline **17**, substituted with a methoxy group at C-6, a starting compound for harmicines **20a-e** and **23a-h**. Subsequent demethylation yielded phenol **18**, which was further propargylated to yield alkyne **19**. CADs were efficiently transformed to CAD-based alkynes **4a-e** and cinnamyl azides **13a-e**. Alkynes **4a-e** were successfully prepared by the reaction of CAD with propargyl bromide, in the presence of K_2CO_3, while the synthesis of cinnamyl azides **13a-e** involved several steps: esterification of CADs, reduction to the corresponding alcohols, and conversion to azides *via* ADMP/DBU, as described previously [10].

On the other hand, synthesis of AT harmicines (**7a-h**, **16a-h**, **23a-h**, **27a**,**b**) was straightforward and involved two steps: 1) synthesis of harmine-based primary amines **6**, **11**, **22** and **26** and 2) coupling reactions with different CADs. The above listed primary amines were obtained by either the reduction of azides **3** and **10** by catalytic hydrogenation or the O-6 alkylation of phenol **18**/N-9 alkylation of harmine with 2-(Boc-amino)ethyl bromide, followed by the removal of the Boc-protecting group under acidic conditions [15]. Coupling reactions were efficiently performed by activating CADs with 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo

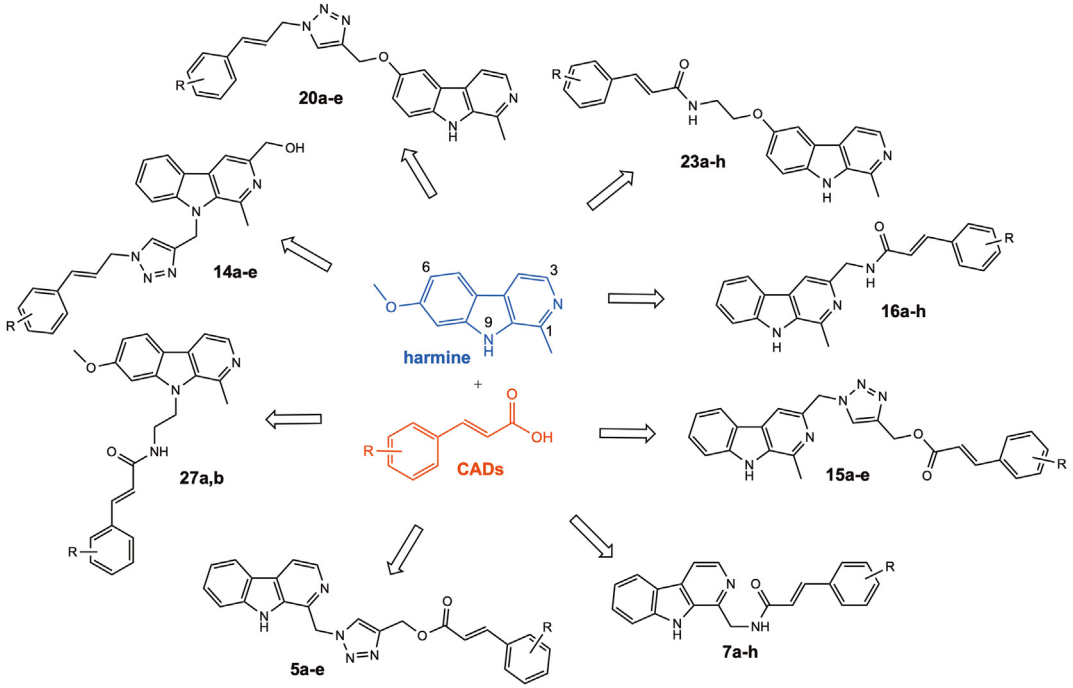


Fig. 1. Novel harmicines.

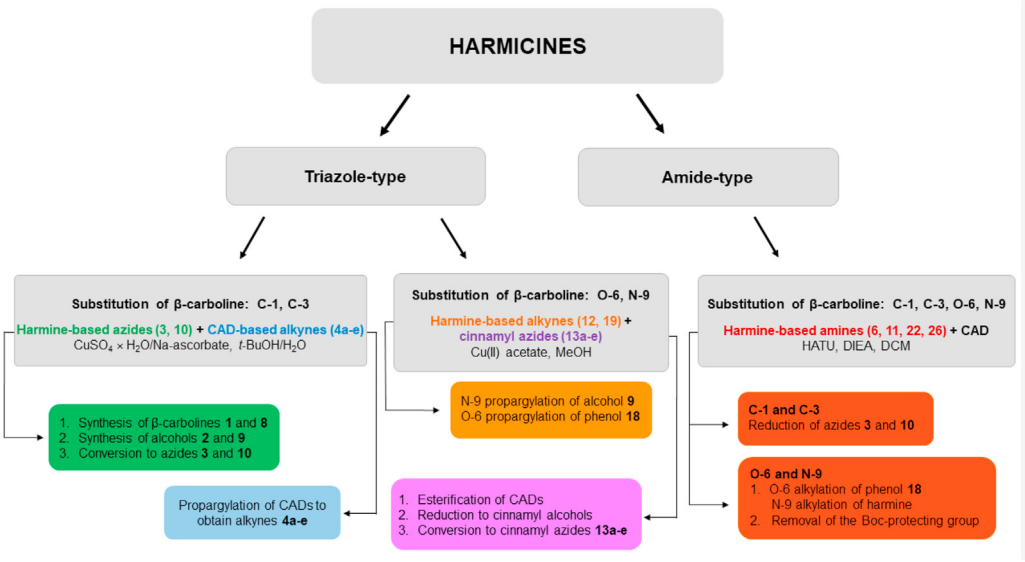
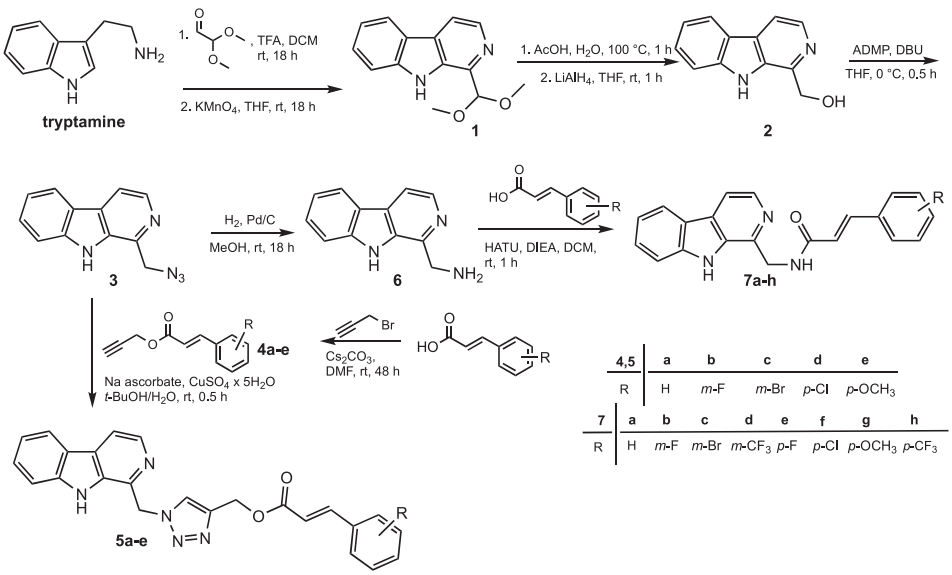


Fig. 2. Synthesis of triazole-type (TT) and amide-type (AT) harmicines.



Scheme 1. Synthesis of TT harmicines **5a**-**e** and AT harmicines **7a**-**h**.

[4,5-*b*]pyridinium-3-oxide hexafluorophosphate (HATU), in the presence of *N,N*-diisopropylethylamine (DIEA), followed by the addition of an appropriate harmine-based amine. The reactions proceeded smoothly, in dichloromethane at room temperature for 1 h.

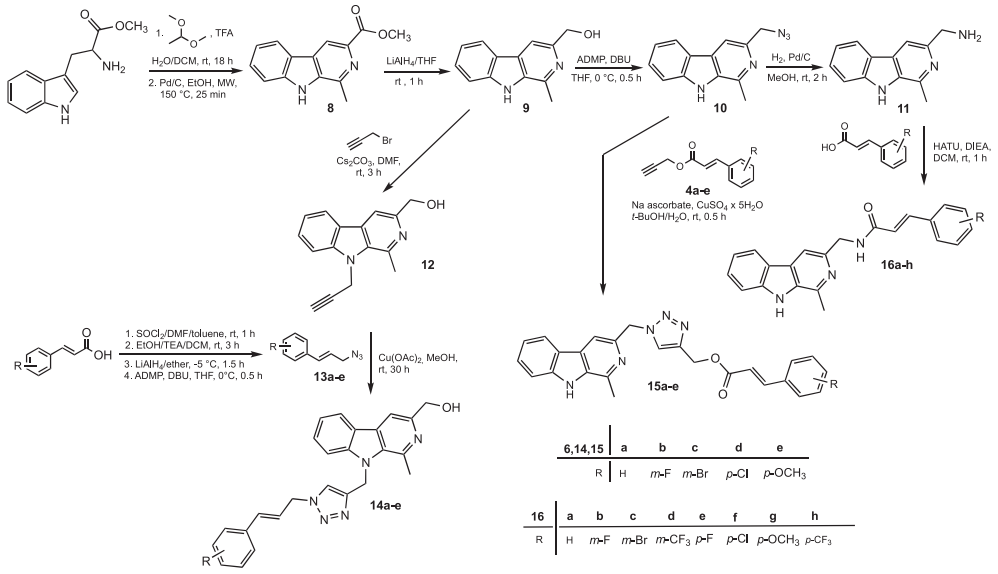
Altogether, we have obtained 45 new compounds in high purity, as determined by HPLC-ESI/MS analysis (>95%) or CHN analysis (values for C, H, and N within 0.4% of the calculated values for the proposed formula). Spectroscopic and spectrometric methods (^{1}H and ^{13}C NMR, IR, MS) were used to confirm the proposed structures. The data obtained are provided in short in the Materials and Methods section, and in detail in the Supplementary Material. The evaluation of the drug-like properties, *i.e.* relevant physicochemical parameters included in the Lipinski's rule and Gelovani's rules, using Chemicalize.org software [22] disclosed that both TT and AT harmicines are in complete agreement with both sets of rules, respectively (Table S15).

2.2. Biological evaluations

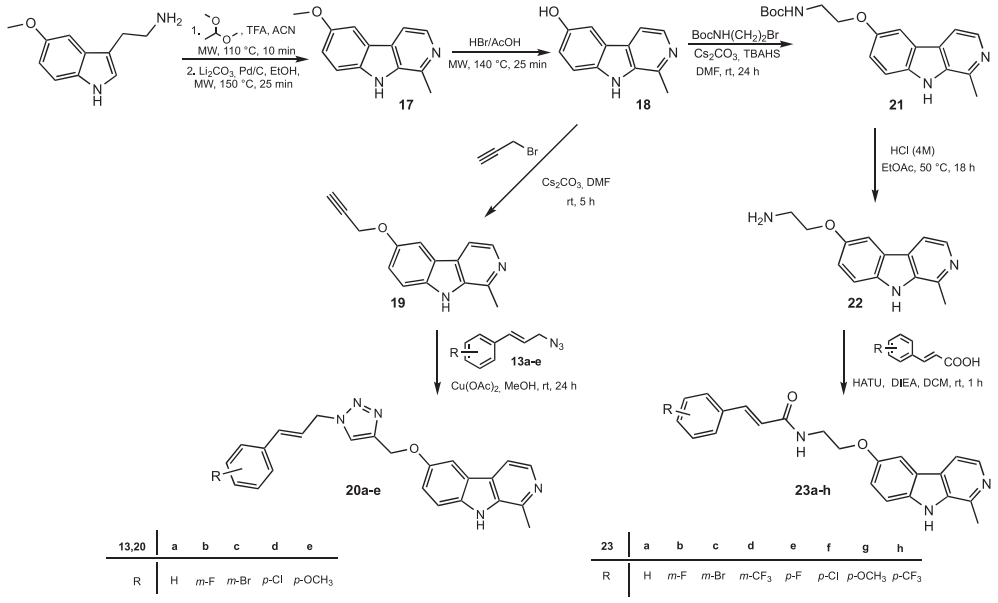
2.2.1. In vitro antiplasmodial activity and SAR analysis

The ability of novel harmicines to inhibit the erythrocytic stage of *P. falciparum* infection (chloroquine-sensitive (*Pf*3D7) and chloroquine-resistant (*Pf*Dd2) strains), as well as infection of the human hepatoma cell line (Huh7) by *P. berghei* parasites, was evaluated as described previously [23–27]. The diversity of the harmicines allowed us to explore the significance of certain structural features for the activity: 1) position of the substitution at the β-carboline ring, 2) linker between β-carboline and CAD, and 3) position and the type of substituent on the phenyl ring in CAD.

The results of the *in vitro* screening of blood schizonticidal activity indicated significant differences in antiplasmodial activities between harmicines prepared at the different positions of the β-carboline ring (Table 1). In general, the activity decreased according to the pattern: N-9 > O-6 > C-3 > C-1, except for TT harmicines **14**



Scheme 2. Synthesis of TT harmicines **14a**-**e**, **15a**-**e** and AT harmicines **16a**-**h**.



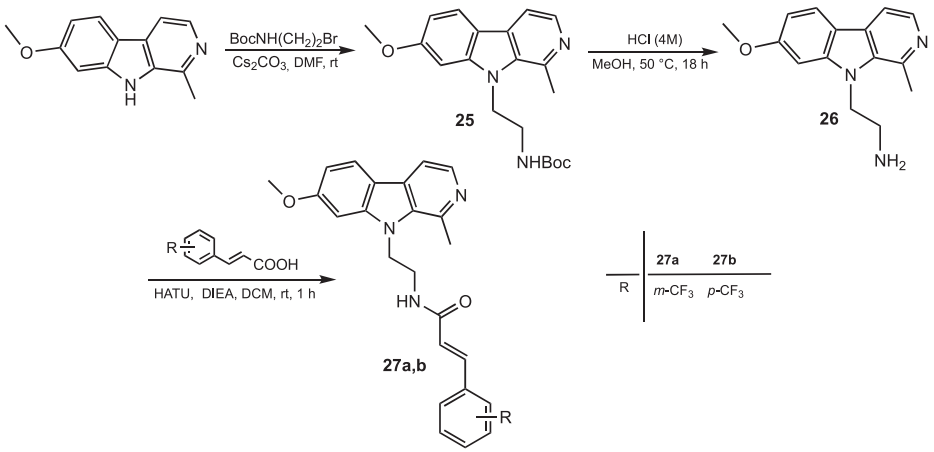
Scheme 3. Synthesis of TT harmicines **20a**-**e** and AT harmicines **23a**-**h**.

(substitution at N-9), which were among the least active compounds.

The most active compounds, AT harmicines **27a**,**b**, hybrids comprised of harmine-based amine at N-9 (compound **26**) and *m*- or *p*-(trifluoromethyl)cinnamic acid, displayed at least two orders of magnitude stronger activities than the parent compound, harmine, in nanomolar (*Pf*3D7) and submicromolar concentrations (*Pf*Dd2). These results are in agreement with IC_{50} values obtained previously for the series of AT harmicines, in which the derivative with *p*-chloro substituent (trifluoromethyl isostere) in the cinnamic scaffold exerted the highest activity [15]. Interestingly, harmicines **14**, also hybrids at N-9, with triazole linker and an additional hydroxymethyl substituent at C-3, exerted lower activity than harmine, with the exception of compound **14c**, *m*-bromocinnamic acid derivative. It is noteworthy that the analogues TT harmicines reported by Perković et al., without C-3 hydroxymethyl substituent, exhibited activities in submicromolar concentrations, suggesting that such substitution results in the loss of activity [10].

Harmicines **20** and **23**, both bearing substituents at O-6, showed similar activities in submicromolar and micromolar concentrations, regardless of the type of the linker. Comparison of the antiplasmodial activities within the series of TT harmicines **20** showed that the substituent R^1 on cinnamic moiety had a minor effect on the activity. Compound **20c**, a *m*-bromocinnamic acid derivative, exerted on average 2.2-fold higher activity than the other compounds. Among the AT harmicines, compounds **23d** and **23h**, *m*- and *p*-(trifluoromethyl)cinnamic acid derivatives, exhibited the highest antiplasmodial activities, which were still 2 to 3-fold lower than the activities of analogous compounds **27a** and **27b**.

On the other hand, considering the antiplasmodial activities of the AT and TT harmicines **16** and **15**, hybrids at C-3, two conclusions could be drawn. First, the IC_{50} values were in the micromolar range.



Scheme 4. Synthesis of harmicines **27**.

Table 1
In vitro antiplasmodial activity of harmicines **5**, **7**, **14**–**16**, **20**, **23** and **27** against the erythrocytic stage of *P. falciparum* (*Pf*3D7 and *Pf*Dd2 strains).

TT harmicines	β-carboline substitution	IC_{50}[a] (μM)		AT harmicines	IC_{50} (μM)	
		*Pf*3D7	*Pf*Dd2		*Pf*3D7	*Pf*Dd2
5a	C-1	>27.78[b]	>20.25	**7a**	>27.99	>55.56
5b		>17.32	>20.07	**7b**	>27.78	>27.78
5c		>13.24	>19.07	**7c**	>27.78	>27.78
				7d	>27.78	>27.78
				7e	>13.89	>13.89
5d		>55.56	>55.56	**7f**	>27.78	>27.78
5e		>55.56	39.20 ± 0.25	**7g**	>27.78	>27.78
				7h	>55.56	>55.56
15a	C-3	>20.04	>27.78	**16a**	5.78 ± 1.05	10.18 ± 1.68
15b		>27.78	>27.78	**16b**	5.74 ± 0.33	12.71 ± 1.76
15c		16.41 ± 3.35	11.14 ± 2.76	**16c**	3.23 ± 0.32	1.48 ± 035
				16d	2.01 ± 0.09	7.40 ± 0.74
				16e	4.13 ± 0.61	13.30 ± 7.41
15d		21.44 ± 2.27	>27.78	**16f**	2.92 ± 0.03	8.38 ± 3.16
15e		>27.78	>27.78	**16g**	6.50 ± 0.29	10.72 ± 3.18
				16h	2.30 ± 0.41	5.97 ± 1.33
20a	O-6	0.61 ± 0.09	2.12 ± 0.40	**23a**	1.55 ± 0.45	2.47 ± 0.25
20b		0.55 ± 0.08	2.28 ± 0.33	**23b**	1.11 ± 0.14	1.30 ± 0.09
20c		0.26 ± 0.17	0.73 ± 0.40	**23c**	0.19 ± 0.02	0.63 ± 0.22
				23d	0.12 ± 0.02	0.32 ± 0.08
				23e	0.95 ± 0.06	1.67 ± 0.06
20d		0.57 ± 0.06	0.95 ± 0.16	**23f**	0.36 ± 0.02	0.92 ± 0.06
20e		0.60 ± 0.03	1.81 ± 0.04	**23g**	1.17 ± 0.03	2.86 ± 0.52
				23h	0.12 ± 0.02	0.35 ± 0.06
14a	N-9	>27.78	>19.78			
14c		6.28 ± 1.04	18.41 ± 2.42			
14d		>19.92	>23.60			
14e		18.19	>16.93			
				27a	0.06 ± 0.02	0.33 ± 0.06
				27b	0.04 ± 0.003	0.17 ± 0.01
CQ[d]		0.003 ± 0.002	0.20 ± 0.10	HAR[e]	8.25 ± 2.83	>27.7

[c] Results represent mean ± SD, $n \geq 2$.
[a] IC_{50}, the concentration of the tested compound necessary for 50% growth inhibition.
[b] The exact IC_{50} could not be obtained, as activity could only be detected at the highest tested concentration.
[d] CQ, chloroquine;[e] HAR, harmine.

This result, together with those obtained for harmicines **14**, indicate that the position 3 of the β-carboline ring is not optimal for substitution. Secondly, there is a marked difference in the antiplasmodial activities of harmicines **15** and **16**. AT harmicines **16** were significantly more potent than their triazole-linker counterparts, compounds **15**. Comparison of the activities of the homologous compounds from the series **16** and **15** revealed at least a 3.5-fold (**16a** *vs* **15a**) increase in the activity. The most pronounced effect was observed for harmicines **16g** and **15d** (a 7.3-fold increase in the activity). Remarkably, the comparison of IC_{50} values of AT and TT harmicines, hybrids at N-9 of the β-carboline, reported earlier, revealed the same tendency [10,15].

Harmicines **5** and **7**, prepared at C-1of the β-carboline core, were inactive against the erythrocytic stage of *P. falciparum*, at the highest concentration tested, regardless of the type of the linker and CAD used for conjugation.

As a continuation of our research, screening of *in vitro* activities of harmicines **5**, **7**, **14**, **15**, **16**, **20**, **23** and **27** against the hepatic stages

of *Plasmodium* parasite was performed. Both AT and TT harmicines were initially tested at two concentrations, 1 and 10 μM. The results obtained have shown that the potency of harmicines against the hepatic stages followed a similar pattern as against the erythrocytic stage of *Plasmodium* infection (Fig. 3). The most pronounced activity was observed for harmicines **27** and **14**, prepared at N-9 of the β-carboline ring, followed by **20** and **23** (O-6), **15** and **16** (C-3) and finally harmicines **5** and **7** (C-1), which were mostly inactive. In general, AT harmicines exhibited better activity, *i.e.* **27** > **14**, **23** > **20**, **16** > **15**. In view of those results, compound **27a**, *m*-(trifluoromethyl)cinnamic acid derivative, was selected as the most promising for IC_{50} determination (Fig. S1). The obtained IC_{50} value was 4.3-fold lower than IC_{50} of the reference drug primaquine (1.94 ± 0.68 μM *vs* 8.4 ± 3.4 μM) [28].

2.2.2. *In vitro cytotoxicity screening and selectivity*

Cytotoxicity of the harmicines active against the erythrocytic stage of *P. falciparum* was evaluated against HepG2, as described previously [29] (Table 2). In addition, the selectivity index (SI) for each harmicine was calculated as the fractional ratio between the IC_{50}s for HepG2 and *P. falciparum Pf*3D7 strain (Table 2).

To our delight, the most active compounds against *Pf*3D7, **27a**,**b**, exhibited favourable SIs. Remarkably, the most selective compound of all prepared harmicines was **27a** (SI = 1105). A comparison of the IC_{50} values obtained for harmicines prepared at O-6 revealed that, in general, TT harmicines **20** were more cytotoxic to HepG2 cells than AT harmicines **23** ($IC_{50} \leq 10$ μM, with the exception of the compound **20e**). It is worth mentioning that harmicines **23e**-**g** were not cytotoxic at all at the highest concentration tested.

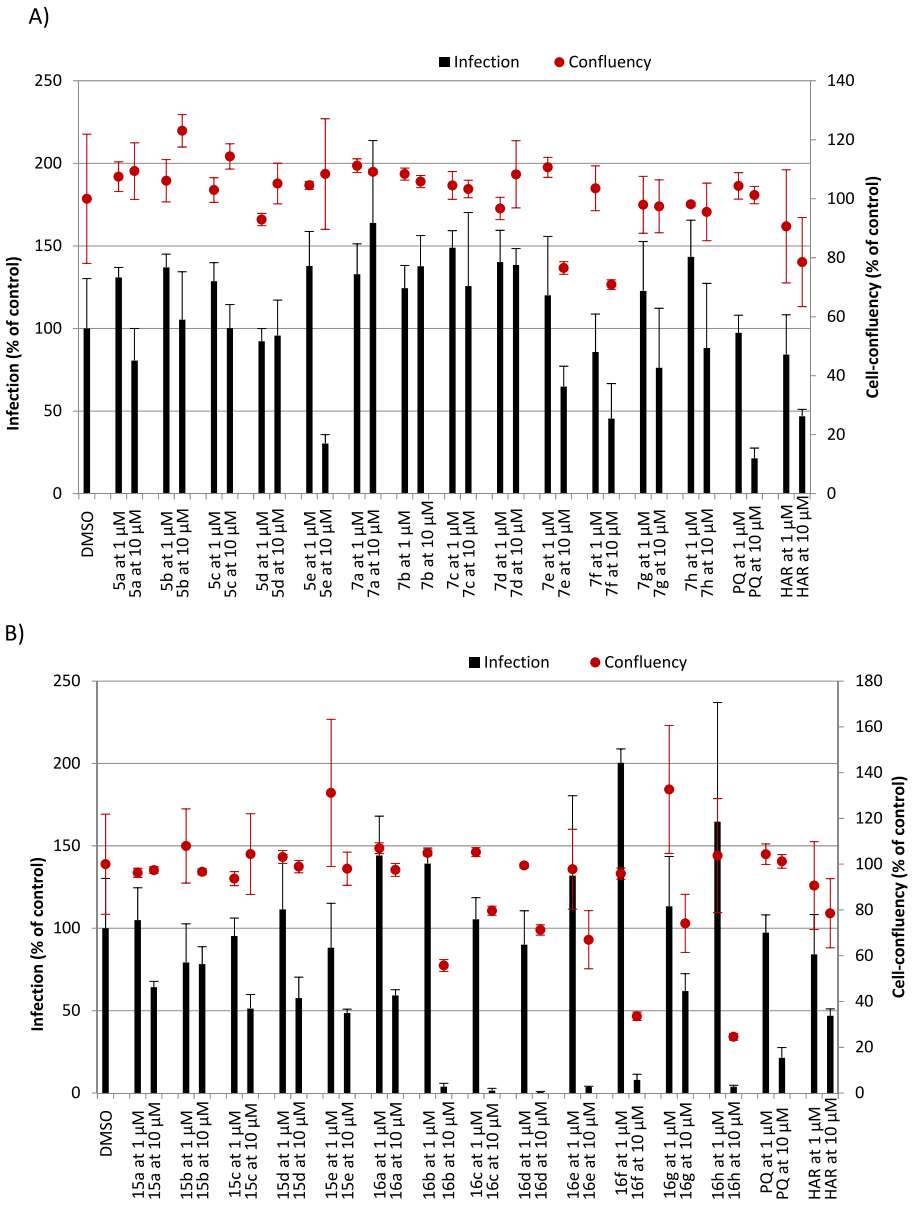


Fig. 3. *In vitro* activity of harmicines against *P. berghei* liver stages: a) **5a**-**e** and **7a**-**h**, b) **15a**-**e** and **16a**-**h**, c) **20a**-**e** and **23a**-**h** and d) **14a**,**c**-**d** and **27a**,**b** at 1 and 10 μM concentrations. Total parasite load (infection scale, bars) and cell viability (cell confluency scale, dots) are shown. Results were normalized to the negative control, DMSO, and are represented as mean ± SD, n = 1 (PQ, primaquine; HAR, harmine).

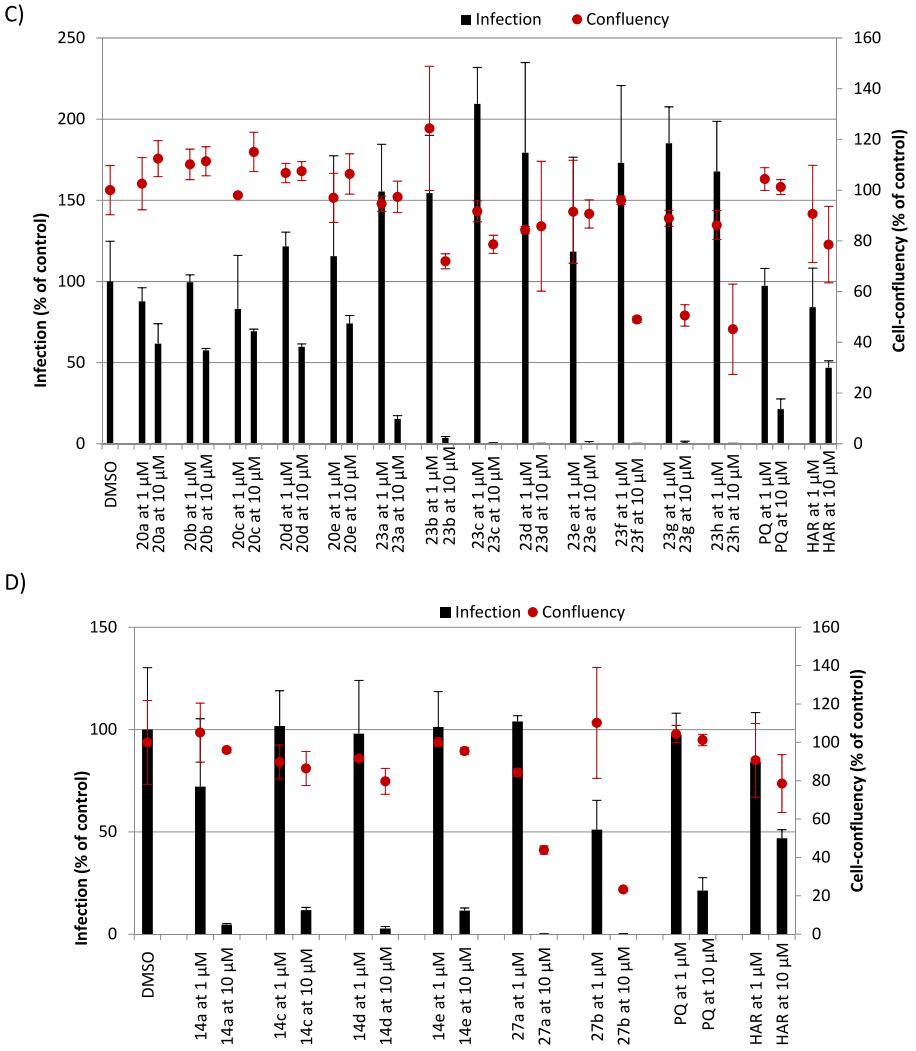


Fig. 3. (*continued*).

Cytotoxicity of other tested harmicines did not differ much from their antiplasmodial activities against erythrocytic stages (in most cases their SI was below 7). Therefore, those compounds can't be considered for future development as antimalarial agents. However, those results open the window for their repurposing as anticancer agents, which will be explored in our future investigations.

2.3. Molecular dynamics simulations

Given a very large number of compounds evaluated in this work, we utilized MD simulations on a representative set of systems, selected to feature the most potent derivative **27b**, together with **7b**, **16a** and **23h**, which should provide enough structural and electronic information to discriminate among various substitution patterns and their biological activities, while offering guidelines how to focus subsequent synthetic efforts towards even more effective analogues based on the employed organic framework.

The calculated binding free energies (ΔG_{BIND}) for the studied systems are given in Table 3, together with their decomposition into contributions from individual residues. The residues considered for the analysis include those identified as responsible for the

Table 2
In vitro cytotoxicity screening of harmicines against HepG2 and calculated selectivity indices.

Compd.	IC_{50}[a] (μM)	SI[b]	Compd.	IC_{50}[a] (μM)	SI[b]
14c	>125[c]	19.90	**20d**	3.95 ± 0.83	6.93
15c	110.19 ± 56.92	6.71	**20e**	88.01 ± 50.81	146.68
15d	70.50 ± 25.64	3.29	**23a**	12.02 ± 4.95	7.75
16a	7.05 ± 0.11	1.29	**23b**	37.57 ± 3.10	33.85
16b	15.83 ± 3.23	2.76	**23c**	7.57 ± 2.56	39.84
16c	9.64 ± 4.71	2.98	**23d**	6.14 ± 0.11	51.17
16d	9.37 ± 4.90	4.66	**23e**	>250	263.16
16e	12.46 ± 5.26	2.99	**23f**	>250	694.44
16f	11.33 ± 0.65	3.88	**23g**	>250	213.68
16g	10.38 ± 4.15	1.60	**23h**	3.59 ± 1.26	29.92
16h	33.26 ± 1.66	14.46	**27a**	66.32 ± 3.79	1105.33
20a	10.13 ± 3.50	16.61	**27b**	5.84 ± 1.67	146
20b	10.51 ± 5.39	19.11	HAR[d]	>250	30
20c	6.13 ± 2.71	23.58			

[a] IC_{50}, the concentration of the tested compound necessary for 50% growth inhibition.
[b] SI, selectivity index, the ratio between IC_{50} (HepG2) and IC_{50} (*Pf*3D7).
[c] The exact IC_{50} could not be obtained, as activity could only be detected at the highest tested concentration.
[d] HAR, harmine.

Table 3
Total binding free energies (ΔG_{BIND}) and their decomposition on a per-residue basis following the MM-GBSA analysis of the obtained molecular dynamics trajectories.[a].

System	7b	16a	23h	27b	HAR[b]
Total ΔG_{BIND}	−21.3	−25.8	−22.9	−30.0	−7.5
Lys44	−0.13	−1.41	−0.51	−1.89	0.00
Arg98	−1.63	0.04	−1.71	−1.67	0.00
Asn37	−2.25	−2.17	−1.80	−1.40	0.00
Met84	−0.89	−1.63	−2.06	−1.26	0.00
Ala41	−0.58	−1.51	−1.59	−1.02	0.00
Tyr47	0.01	−0.02	0.01	−0.68	0.00
Phe124	−0.78	−0.73	−0.74	−0.68	0.00
Ile82	−0.10	−0.98	−1.30	−0.64	0.00
Asp40	0.14	−0.79	−0.48	−0.63	0.00
Leu93	−0.61	−0.61	−0.58	−0.47	0.00
Ile173	−0.21	−0.66	−0.56	−0.43	−0.01
Thr171	−0.37	−1.03	−0.75	−0.37	0.00
Leu34	−0.13	−0.48	−0.19	−0.31	0.00
Ala38	−0.15	−0.82	−0.69	−0.23	0.00
Lys102	0.01	0.01	0.01	0.03	−0.34
Asp52	0.01	0.01	0.01	0.04	0.00
Thr95	0.02	0.03	0.03	0.04	0.00
Arg167	0.03	0.04	0.02	0.05	0.00
Glu48	0.03	0.06	0.08	0.13	0.00
Asp43	0.05	0.04	0.04	0.20	0.00
Asp79	0.23	1.51	1.62	0.31	0.00

[a] Residues are selected to list those belonging to the ATP binding pocket (Asn37, Asp79, Arg98, Phe124, in bold) and all of those with contributions higher than −0.20 and lower than 0.03 kcal mol^{-1} for the most potent **27b**. All values are in kcal mol^{-1}.
[b] HAR, harmine.

binding of ATP within the ATP binding pocket of *Pf*Hsp90 (Asn37, Asp79, Arg98, Phe124) [30,31] and all those with contributions higher than −0.20 and lower than 0.03 kcal mol^{-1} for the most potent **27b**.

It turns out that all compounds are associated with negative ΔG_{BIND} values, implying that their binding is exergonic and favourable. All four systems are clearly positioned within the ATP binding site of *Pf*Hsp90 (Fig. 4), as they form significant interactions with residues that define that site, which underlines the importance of this structural element as a druggable target. Still, their orientations and specific ligand-protein interactions are largely different, thus resulting in diverse affinities.

The binding of **27b** is the most exergonic, ΔG_{BIND}(**27b**) = −30.0 kcal mol^{-1}, thus confirming its highest biological activity measured here. Its harmine fragment enters deeper within the binding site, where it uses its aromatic framework to interact with Asn37 (N−H … π interactions) and Met84 (C−H … π interactions), evidenced in their significant individual contributions

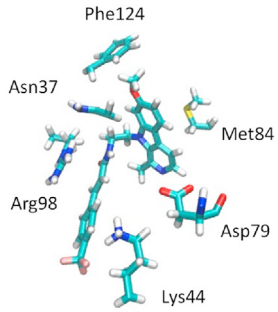


Fig. 5. Specific interactions governing the binding of the most potent **27b** within the ATP binding site of the *Pf*Hsp90 protein.

of −1.40 and −1.26 kcal mol^{-1}, respectively (Fig. 5), or its attached −OMe group to form C−H … π interactions with Phe124 (−0.68 kcal mol^{-1}). The mentioned three residues already contribute around 11% of the total binding energy, which is significant. In addition, the introduced N-9 amide functionality further promotes the binding, first by allowing Arg98 to stack above the amide linker (−1.67 kcal mol^{-1}), and second, even more significantly, to facilitate positive N−H … π contacts between the cationic Lys44 residue and the terminal *p*-CF_3-phenyl unit, being persistent during simulations (Fig. S2). The latter promotes Lys44 as the most dominant *Pf*Hsp90 residue in binding **27b**, related with the individual contribution of −1.89 kcal mol^{-1}. Taken together, indicated five residues already account for 23% of the total binding energy, suggesting that **27b** is rather well positioned within the binding site. All of this clearly confirms the suitability of the N-9 substitution as a useful strategy in designing effective compounds and justifies placing an aromatic unit at an appropriate distance from the harmine N-9 site to benefit the binding, where the amide linker seems to be optimal and well-tuned for the purpose. In addition, knowing that the *p*-CF_3 group exerts an electron-withdrawing effect on the attached phenyl ring, which likely diminishes the mentioned N−H … π interactions with Lys44, it would be useful to consider replacing *p*-CF_3 with different electron-donating groups, which will be tackled in the subsequent synthetic work. Having said that, among others, *p*-OMe does not appear suitable as such a derivative possesses reduced activity [15], albeit lower toxicity, so other substituents would have to be evaluated.

The effect of the anionic Asp79 is also interesting and worth discussing. This is a significant residue, since it is also identified as part of the ATP binding motif, yet its interactions with **27b** are strongly

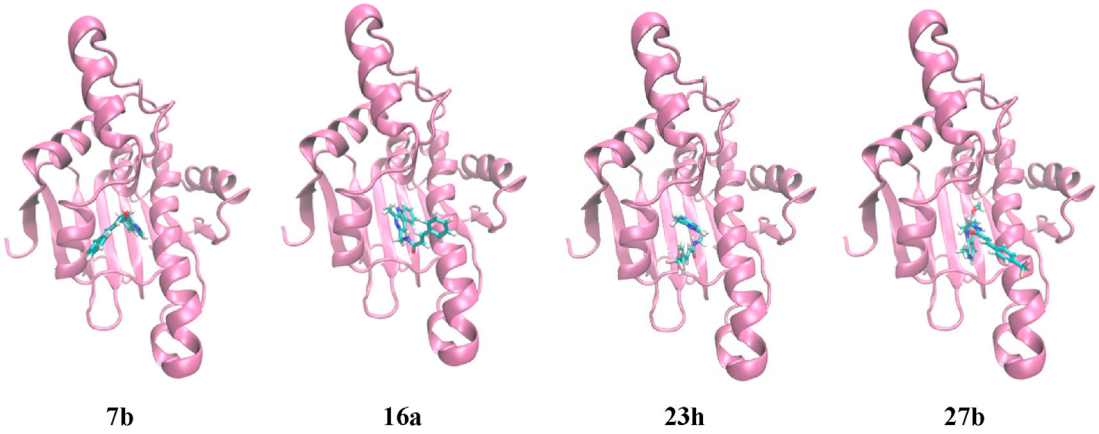


Fig. 4. Position of the investigated ligands within the ATP binding site of the *Pf*Hsp90 protein.

disfavouring the binding, as Asp79 is associated with a positive contribution of +0.31 kcal mol^{-1}, being the highest endergonic among all residues. The underlying reason is that Asp79 is located in the vicinity of the harmine's pyridine unit without any notable favourable contacts that could counterbalance a negative steric and electronic interference from the anionic carboxylate. This suggests that despite its mostly optimal design, **27b** could further benefit from modifying its pyridine unit with something that could revert this fragment into a favourable pairing with Asp79. Yet, this is not achieved in **16a**, as the binding affinity of this C-3 derivative is reduced by 4.2 kcal mol^{-1} to ΔG_{BIND}(**16a**) = −25.8 kcal mol^{-1}, suggesting that the introduced amide-linked cinnamic acid moiety is likely too large for this purpose. There, the unfavourable interaction with Asp79 is even enhanced (Fig. S3), as its individual contribution rises from +0.31 in **27b** to +1.51 kcal mol^{-1}. Such structural modification even changes the binding orientation so that, for example, the entire positive contribution from Arg98, observed in **27b**, is lost here, which leads to a reduced affinity, being in line with experiments. An even less favourable outcome is seen in **7b**, where an analogous structural modification occurs on the other part of the pyridine ring, the C-1 atom, where the resulting binding affinity is further reduced to ΔG_{BIND}(**7b**) = −21.3 kcal mol^{-1}, being the lowest among considered systems, thus confirming its low activity observed experimentally. Even though **7b** seems to be able to compensate the unfavourable contribution from Asp79 down to +0.23 kcal mol^{-1}, its position within the ATP binding site disfavours favourable contributions from several significant residues, including Lys44, which is diminished to only −0.13 kcal mol^{-1}, despite, for example, improving the contribution of Asn37 up to −2.25 kcal mol^{-1} due to positive N−H … π interactions.

In general, all considered systems, selected to correspond to different substitution patterns, show much more exergonic binding affinities than the parent harmine, which justifies the utilized synthetic strategy. The calculated value of ΔG_{BIND}(harmine) = −7.5 kcal mol^{-1}, demonstrates its poor binding features, being consistent with its elevated IC_{50} value of 8.25 μM (Table 1). In addition, although the relationship between IC_{50} and ΔG_{BIND} values is not so straightforward in absolute terms, their relative ratio is connected through the Cheng-Prusoff equation [32]. In this context, harmine's IC_{50} value roughly translates to the binding energy of −6.9 kcal mol^{-1}, thus placing our calculations in close agreement with experiments, and confirming the validity of the employed computational setup. Harmine seems like a good reference, since it was identified as a selective inhibitor of *Pf*Hsp90, which is essential for the erythrocytic development of *Plasmodium* [16,33] and our calculations show it predominantly binds outside the ATP binding site (Table 3). Hence, the approach to structurally modify harmine is likely to lead to more efficient compounds, although, in some cases evaluated here, the introduced amide/triazole-linked cinnamic acid unit, despite being placed at right positions on the harmine's scaffold, appears as too bulky or electronically less optimized to maximize the effect. Nevertheless, computations confirm the substitution at the N-9 position as the most promising route in this respect, which likely opens the door towards further improvements in the desired biological activities.

3. Conclusions

In conclusion, we have successfully prepared and characterized 45 novel harmicines of the triazole- and amide-type in the positions 1, 3, 6 and 9 of the β-carboline ring. Further, we evaluated their antiplasmodial activities against erythrocytic and hepatic stages of the *Plasmodium* infection and cytotoxicity against HepG2. The results clearly showed remarkable blood shizontocidal activity in nanomolar concentrations, as well as selectivity of amide-type harmicines **27**, in comparison to other compounds. Harmicines **27** were also the most active against hepatic stages of *P. berghei* (4.3-fold more active than the reference drug primaquine). Molecular dynamics simulations revealed that all investigated compounds bind within the ATP binding site and confirmed **27b** as the most potent derivative. In addition, Lys44 was identified as crucial for the enhanced activity, evident in favourable N−H … π contacts with the phenyl ring within the attached cinnamic acid unit, particularly when the latter is introduced at the harmine's N-9 position, being in line with the experiments. Lastly, computations underlined the unfavourable contribution from the ATP binding site residue Asp79, seen in the steric and electronic interference with the bound ligands, which defines a promising route to advance the developed synthetic methodology towards even more efficient compounds based on the employed organic framework. Taken together with previously obtained results, this study revealed amide-type harmicines at N-9 of the β-carboline ring as the best performers against *Plasmodium*. Our ongoing work focuses on establishing a reliable quantitative structure-activity relationship model, with the final goal of identifying lead compounds for further development.

4. Materials and Methods

4.1. Chemistry

4.1.1. General information

Melting points were determined on a Stuart Melting Point Apparatus (Barloworld Scientific, UK) in open capillaries and were uncorrected. FTIR-ATR spectra were recorded using a Fourier-Transform Infrared Attenuated Total Reflection UATR Two spectrometer (PerkinElmer, Waltham, MA, USA) in the range from 450 to 4000 cm^{-1}. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III HD operating at 300, 400 or 600 MHz for the ^{1}H and 75, 101 or 151 MHz for the ^{13}C nuclei (Bruker, Billerica, MA, USA). Samples were measured in DMSO-d_6 solutions at 20 °C in 5 mm NMR tubes. Chemical shifts (δ) are reported in parts per million (ppm) using tetramethylsilane (TMS) as a reference in the ^{1}H and DMSO residual peak as a reference in the ^{13}C spectra (39.52 ppm). Coupling constants (J) are reported in hertz (Hz). Mass spectra were recorded on Agilent 1200 Series HPLC coupled with Agilent 6410 Triple Quad (Agilent Technologies, St. Clara, CA, USA). The mobile phase consisted of Milli Q water as component A and MeOH (HPLC grade, J. T. Baker) as component B, and as the stationary phase, Zorbax XDC C18 column (4.6 × 75 mm, 3.5 μm) was used. Gradient elution was used at a flow rate of 0.5 mL/min, and 5 μL of analyte solution was injected per analysis. The starting conditions and gradient steepness were adjusted according to the analyte polarity. Diode array detector was utilized, while the data were presented as a total wavelength chromatogram (TWC). Mass spectrometry conditions were as follows: electrospray ionization (ESI) in positive and negative mode was used. Capillary voltage and current were set to 4.0 kV and 20 nA, respectively. Nebulizer pressure was set to 15 psi, while the drying gas (nitrogen) temperature and flow were 300 °C and 11 L/min. For the MS data analysis, Agilent MassHunter software (Agilent Technologies, St. Clara, CA, USA) was used. Elemental analyses were performed on a CHNS LECO analyser (LECO Corporation, St. Joseph, MI, USA). Analyses indicated by the symbols of the elements were within ±0.4% of their theoretical values. Microwave-assisted reactions were performed in a microwave reactor CEM Discover (CEM, USA) in a glass reaction vessel. All compounds were routinely checked by TLC with silica gel 60F-254 glass plates (Merck, Germany) using DCM/MeOH 8:1, 85:15, 95:5, 97:3, cyclohexane/EtOAc/MeOH 1:1:0.5 and cyclohexane/EtOAc 2:1, 9:1 as the solvent systems. Spots were visualized by UV light (λ = 254 nm; 365 nm) and iodine vapour. Column chromatography was performed on silica gel 0.063−0.200 mm (Sigma-Aldrich, USA)

with the same eluents used for TLC, with an additional Al_2O_3 layer to remove Cu-salts (compounds **5a**-**e**, **14a**,**c**-**d**, **15a**-**e**, **20a**-**e**). All chemicals and solvents were of analytical grade and purchased from commercial sources. CADs were purchased as predominantly *trans* stereoisomers (≥99%).

Harmine, acetic acid, hydrochloric acid, cinnamic acid, *m*-fluorocinnamic acid, *p*-fluorocinnamic acid, *p*-methoxycinnamic acid, *p*-chlorocinnamic acid, *m*-(trifluoromethyl)cinnamic acid, *p*-(trifluoromethyl)cinnamic acid, *t*-BuOH, acetaldehyde dimethyl acetal, 2,2-dimethoxyacetaldehyde, DBU, lithium aluminium hydride, toluene, lithium carbonate, 10% palladium on activated charcoal were purchased from Sigma-Aldrich (USA). Caesium carbonate, 2-(*tert*-butoxycarbonylamino)ethyl bromide, tryptamine, 5-methoxytryptamine, L-tryptophan methyl ester hydrochloride, tetrabutylammonium hydrogensulfate, ADMP, HATU, *m*-bromocinnamic acid, propargyl bromide and DMF were purchased from TCI Chemicals (Japan). Hydrobromic acid (47%), dry tetrahydrofuran and aluminium oxide were purchased from Merck (Germany), DIEA, copper(II) acetate and TEA from Alfa Aesar (USA), 1 M hydrochloric acid in EtOAc, trifluoroacetic acid, DCM and dry diethyl ether from Thermo Fischer Scientific (USA), diethyl ether from ITW Reagents (Germany), cyclohexane, EtOAc, MeOH, absolute EtOH and petroleum ether from Honeywell (USA), acetonitrile from CARLO ERBA Reagents (France), DMF, sodium hydroxide, sodium bicarbonate and potassium carbonate from Kemika (Croatia), anhydrous sodium sulphate, potassium permanganate, ammonium chloride and sodium chloride from Gram-Mol (Croatia), thionyl chloride from Fluka (Switzerland), sodium ascorbate from Acros Organics (Belgium), copper(II) sulphate pentahydrate from Zorka Šabac (Serbia).

β-carbolines **1**, **8**, **17** and alcohol **9** were prepared according to the literature procedures [18–20,34]. Alcohol **2** is a known compound, but prepared by alternative methods [35,36]. Phenol **18** was prepared according to the modified literature procedure from β-carboline **17** using HBr/glacial acetic acid mixture, under the MW irradiation [37]. Among alkynes **4**, only **4c** is a new compound. Nevertheless, the established synthetic method for their preparation differs from the one described in the literature [38]. Although amine **6** is commercially available, we have included its synthesis and characterization, since those data are not available. Cinnamyl azides **13a**-**e**, as well as compounds **25** and **26** were prepared according to our published methods [10,15].

*4.1.2. Synthesis of (9H-pyrido [3,4]-b]indol-1-yl)methanol (**2**)*

A solution of β-carboline **1** (0.700 g, 2.889 mmol), AcOH (3.63 mL) and H_2O (5.45 mL) was refluxed for 1 h at 100 °C. After cooling to rt, pH was adjusted to 9 with 5% NaOH and the reaction mixture extracted with EtOAc (3 × 50 mL). The collected organic layers were filtered through phase separator and solvent evaporated under the reduced pressure. Obtained yellow crude (0.453 g, 2.309 mmol) was suspended in dry THF (6 mL). Afterwards $LiAlH_4$ (0.175 g, 4.618 mmol) was added in small portions, and the reaction mixture was stirred under argon atmosphere for 1 h at rt. The reaction was quenched with H_2O (20 mL), pH adjusted to 8 with 1% HCl, and the resulting solution extracted with EtOAc (4 × 50 mL). The collected organic layers were filtered through phase separator and solvent evaporated under the reduced pressure. The crude product was triturated with diethyl ether, to obtain alcohol **2**. Yield: 0.417 g (91%).

*4.1.3. General procedure for the synthesis of azides **3** and **10***

To a solution of **2** or **9** (1.447 mmol) in dry THF (5 mL), ADMP (1.032 g, 3.618 mmol) and DBU (0.584 mL, 3.907 mmol) were added at 0 °C. The reaction was stirred at 0 °C for 0.5 h, diluted with saturated NH_4Cl solution (40 mL) and extracted with DCM (2 × 30 mL). Organic layers were collected and extracted with brine (2 × 30 mL) and H_2O (1 × 20 mL), filtered through phase separator and solvent evaporated under the reduced pressure. The crude product (brownish oil) was purified by column chromatography (cyclohexane/EtOAc/MeOH 1:1:0.5).

*4.1.3.1. 1-(azidomethyl)-9H-pyrido[3,4-b]indole (**3**).* Alcohol **2**: 0.287 g. Yield: 0.236 g (73%); oil; IR (ATR, ν/cm^{-1}) 3663, 3451, 3288, 3059, 2937, 2855, 2105, 1730, 1631, 1566, 1493, 1428, 1371, 1321, 1248, 1118, 1052, 971, 938, 873, 824, 751, 718, 620, 579, 432; ^{1}H NMR (DMSO-d_6) δ 11.78 (s, 1H), 8.36 (d, 1H, J = 5.2 Hz), 8.29-8.23 (m, 1H), 8.13 (d, 1H, J = 5.2 Hz), 7.64 (dt, 1H, J = 8.3, 0.9 Hz), 7.60-7.57 (m, 1H), 7.29-7.26 (m, 1H), 4.89 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 140.58, 139.19, 137.75, 133.86, 128.50, 128.44, 121.87, 120.74, 119.57, 114.77, 112.02, 51.59.

*4.1.3.2. 3-(azidomethyl)-1-methyl-9H-pyrido[3,4-b]indole (**10**).* Alcohol **9**: 0.307 g. Yield: 0.261 g (76%); oil; IR (ATR, ν/cm^{-1}) 3671, 3451, 3239, 3051, 2937, 2863, 2447, 2325, 2104, 1689, 1631, 1566, 1509, 1452, 1403, 1354, 1264, 1085, 1036, 971, 848, 759, 718, 644, 579, 431; ^{1}H NMR (DMSO-d_6) δ 11.66 (s, 1H), 8.20 (d, 1H, J = 7.9 Hz), 8.00 (s, 1H), 7.62-7.60 (m, 1H), 7.57-7.53 (m, 1H), 7.26-7.23 (m, 1H), 4.57 (s, 2H), 2.78 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 143.23, 142.12, 140.74, 133.94, 128.02, 127.56, 121.74, 121.01, 119.39, 112.07, 111.72, 55.30, 20.41.

*4.1.4. General procedure for the synthesis of alkynes **4a**-**e***

An appropriate CAD (3.375 mmol) and K_2CO_3 (0.653 g, 4.725 mmol) were suspended in dry DMF (5 mL). Under the argon atmosphere, propargyl bromide (0.601 mL, 5.4 mmol, 80% solution in toluene) was added dropwise, and the reaction was stirred at rt for 48 h. Upon completion, the reaction mixture was diluted with H_2O (50 mL) and extracted with EtOAc (3 × 40 mL). The collected organic layers were washed with H_2O (2 × 100 mL), dried over anhydrous sodium sulphate and solvent evaporated under the reduced pressure. The crude product (oil) was purified by column chromatography (cyclohexane/EtOAc/MeOH 1:1:0.5).

*4.1.4.1. Prop-2-yn-1-yl cinnamate (**4a**).* CAD: 0.500 g of *trans*-cinnamic acid; yield: 0.584 g (93%).

*4.1.4.2. Prop-2-yn-1-yl (E)-3-(3-fluorophenyl)acrylate (**4b**).* CAD: 0.561 g of *m*-fluorocinnamic acid; yield: 0.648 g (94%).

*4.1.4.3. Prop-2-yn-1-yl (E)-3-(3-bromophenyl)acrylate (**4c**).* CAD: 0.766 g of *m*-bromocinnamic acid; yield: 0.779 g (87%); mp 79.0–81.0 °C; IR (ATR, ν/cm^{-1}) 3416, 3279, 3060, 3042, 2940, 2132, 1716, 1637, 1561, 1479, 1419, 1360, 1295, 1201, 1169, 1073, 991, 965, 851, 780, 754, 696, 665, 632, 554; ^{1}H NMR (DMSO-d_6) δ 8.02 (t, 1H, J = 1.8 Hz), 7.78 (dt, 1H, J = 7.7, 1.4 Hz), 7.69 (d, 1H, J = 16.1 Hz), 7.64-7.62 (m, 1H), 7.39 (t, 1H, J = 7.9 Hz), 6.79 (d, 1H, J = 16.1 Hz), 4.84 (d, 2H, J = 2.4 Hz), 3.59 (t, 1H, J = 2.5 Hz); ^{13}C NMR (DMSO-d_6) δ 165.21, 143.81, 136.38, 133.17, 130.99, 130.97, 127.45, 122.32, 118.90, 77.80, 51.93; ESI-MS: decomposition.

*4.1.4.4. Prop-2-yn-1-yl (E)-3-(4-chlorophenyl)acrylate (**4d**).* CAD: 0.616 g of *p*-chlorocinnamic acid; yield: 0.7 g, (94%).

*4.1.4.5. Prop-2-yn-1-yl (E)-3-(4-methoxyphenyl)acrylate (**4e**).* CAD: 0.601 g of *p*-methoxycinnamic acid; yield: 0.540 g, (74%).

*4.1.5. General procedure for the synthesis of harmicines **5a**-**e***

Azide **3** (0.04 g, 0.179 mmol) and a corresponding alkyne **4a-e** (0.197 mmol) were suspended in 5 mL of a 1:1 H_2O/*t*-BuOH mixture. Sodium ascorbate (0.2 mmol, 200 μL of freshly prepared 1 M

solution in H_2O) was added, followed by $CuSO_4 \times 5H_2O$ (0.02 mmol, 20 μL of 1 M solution in H_2O). The mixture was stirred at rt for 0.5 h, diluted with 5 mL of ice-cold H_2O and filtered. A yellow precipitate was washed with H_2O (3 × 2 mL), dried under vacuum, purified by column chromatography (mobile phase DCM/MeOH 95:5). The crude product was triturated with diethyl ether/petroleum ether mixture/recrystallized from EtOH.

*4.1.5.1. (1-((9H-pyrido[3,4-b]indol-1-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl cinnamate (**5a**).* Alkyne **4a**: 0.037 g; purification: trituration with diethyl ether/petroleum ether mixture; yield: 0.036 g (49%); mp 181.5–183.5 °C; IR (ATR, ν/cm^{-1}) 3220, 3168, 3093, 3059, 3029, 2994, 2961, 2898, 2877, 2798, 2776, 1713, 1637, 1567, 1505, 1452, 1432, 1402, 1370, 1346, 1325, 1307, 1281, 1243, 1203, 1167, 1066, 1038, 1003, 972, 859, 820, 804, 764, 739, 707, 682, 641, 605, 593, 573, 547, 525, 483; ^{1}H NMR (DMSO-d_6) δ 11.98 (s, 1H), 8.32 (s, 1H), 8.30 (d, 1H, J = 5.2 Hz), 8.27 (d, 1H, J = 7.9 Hz), 8.13 (d, 1H, J = 5.2 Hz), 7.73-7.66 (m, 4H), 7.62-7.58 (m, 1H), 7.44-7.38 (m, 3H), 7.31-7.26 (m, 1H), 6.66 (d, 1H, J = 16.0 Hz), 6.11 (s, 2H), 5.28 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 165.93, 145.03, 141.79, 140.70, 137.98, 137.88, 133.92, 133.84, 130.57, 128.91, 128.74, 128.57, 128.42, 125.73, 121.92, 120.74, 119.66, 117.65, 114.87, 112.07, 57.29, 51.21; ESI-MS: *m/z* 410.1 (M+1)$^+$; Anal. Calcd. for $C_{24}H_{19}N_5O_2$: C, 70.40; H, 4.68; N, 17.10; found: C, 70.27; H, 4.47; N, 17.35.

*4.1.5.2. (1-((9H-pyrido[3,4-b]indol-1-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl (E)-3-(3-fluorophenyl)acrylate (**5b**).* Alkyne **4b**: 0.040 g; purification: trituration with diethyl ether/petroleum ether mixture; yield: 0.043 g (56%); mp 185.5–187.5 °C; IR (ATR, ν/cm^{-1}) 3311, 3230, 3198, 3147, 3105, 3063, 3019, 2998, 2973, 1715, 1639, 1585, 1569, 1502, 1485, 1472, 1449, 1429, 1403, 1390, 1349, 1323, 1272, 1234, 1190, 1168, 1147, 1122, 1075, 1060, 1035, 983, 954, 939, 874, 858, 837, 819, 780, 728, 671, 643, 605, 582, 562, 534, 521; ^{1}H NMR (DMSO-d_6) δ 11.98 (s, 1H), 8.32 (s, 1H), 8.30 (d, 1H, J = 5.2 Hz), 8.27 (d, 1H, J = 7.9 Hz), 8.13 (d, 1H, J = 5.2 Hz), 7.69-7.63 (m, 3H), 7.61-7.59 (m, 1H), 7.56 (dt, 1H, J = 7.8, 1.2 Hz), 7.47-7.43 (m, 1H), 7.30-7.24 (m, 2H), 6.75 (d, 1H, J = 16.1 Hz), 6.11 (s, 2H), 5.28 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 165.75, 162.40 (d, J = 243.8 Hz), 143.66, 141.71, 140.71, 137.99, 137.89, 136.49 (d, J = 8.1 Hz), 133.85, 130.87 (d, J = 8.4 Hz), 128.75, 128.59, 125.78, 124.94 (d, J = 2.5 Hz), 121.94, 120.75, 119.68, 119.30, 117.26 (d, J = 21.4 Hz), 114.89, 114.58 (d, J = 22.1 Hz), 112.08, 57.40, 51.22; ESI-MS: *m/z* 428.1 (M+1)$^+$; Anal. Calcd. for $C_{24}H_{18}FN_5O_2$: C, 67.44; H, 4.24; N, 16.38; found: C, 67.40; H, 4.49; N, 16.21.

*4.1.5.3. (1-((9H-pyrido[3,4-b]indol-1-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl (E)-3-(3-bromophenyl)acrylate (**5c**).* Alkyne **4c**: 0.052 g; purification: recrystallisation from EtOH; yield: 0.032 g (37%); mp 184.0–186.0 °C; IR (ATR, ν/cm^{-1}) 3311, 3223, 3194, 3147, 3102, 3060, 2997, 2971, 1715, 1640, 1607, 1564, 1501, 1472, 1457, 1428, 1404, 1389, 1347, 1308, 1276, 1234, 1189, 1168, 1122, 1090, 1060, 1036, 984, 860, 830, 812, 777, 735, 670, 643, 605, 578, 542; ^{1}H NMR (DMSO-d_6) δ 11.97 (s, 1H), 8.32 (s, 1H), 8.30 (d, 1H, J = 5.2 Hz), 8.26 (d, 1H, J = 7.8 Hz), 8.13 (d, 1H, J = 5.2 Hz), 7.98 (t, 1H, J = 1.8 Hz), 7.73 (dt, 1H, J = 7.8, 1.3 Hz), 7.69-7.57 (m, 4H), 7.36 (t, 1H, J = 7.9 Hz), 7.31-7.27 (m, 1H), 6.75 (d, 1H, J = 16.0 Hz), 6.11 (s, 2H), 5.28 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 165.68, 143.33, 141.71, 140.69, 137.97, 137.87, 136.45, 133.83, 133.03, 130.93, 128.74, 128.57, 127.32, 125.74, 122.28, 121.92, 120.73, 119.66, 119.37, 114.87, 112.07, 57.40, 51.21; ESI-MS: *m/z* 488.0 (M+1)$^+$, 489.9 (M+1)$^+$; Anal. Calcd. for $C_{24}H_{18}BrN_5O_2$: C, 59.03; H, 3.72; N, 14.34; found: C, 59.19; H, 3.94; N, 14.52.

*4.1.5.4. (1-((9H-pyrido[3,4-b]indol-1-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl (E)-3-(4-chlorophenyl)acrylate (**5d**).* Alkyne **4d**: 0.043 g; purification: trituration with diethyl ether/petroleum ether mixture; yield: 0.02 g (25%); mp 201.0–203.5 °C; IR (ATR, ν/cm^{-1}) 3227, 3192, 3096, 3061, 2993, 2965, 2926, 2894, 2872, 1713, 1642, 1594, 1568, 1503, 1490, 1468, 1453, 1431, 1404, 1370, 1346, 1308, 1271, 1242, 1201, 1171, 1148, 1087, 1068, 1038, 1012, 972, 868, 818, 775, 737, 695, 683, 669, 637, 593, 560, 547, 529, 491, 455; ^{1}H NMR (DMSO-d_6) δ 11.97 (s, 1H), 8.31 (s, 1H), 8.30 (d, 1H, J = 5.3 Hz), 8.27 (d, 1H, J = 7.9 Hz), 8.13 (d, 1H, J = 5.2 Hz), 7.77-7.74 (m, 2H), 7.70-7.64 (m, 2H), 7.62-7.58 (m, 1H), 7.50-7.45 (m, 2H), 7.31-7.26 (m, 1H), 6.69 (d, 1H, J = 16.1 Hz), 6.11 (s, 2H), 5.28 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 165.79, 143.60, 141.74, 140.69, 137.97, 137.87, 135.08, 133.83, 132.91, 130.15, 128.94, 128.74, 128.57, 125.73, 121.92, 120.73, 119.66, 118.49, 114.87, 112.07, 57.35, 51.21; ESI-MS: *m/z* 444.0 (M+1)$^+$; Anal. Calcd. for $C_{24}H_{18}ClN_5O_2$: C, 64.94; H, 4.09; N, 15.78; found: C, 64.75; H, 4.27; N, 15.93.

*4.1.5.5. (1-((9H-pyrido[3,4-b]indol-1-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl (E)-3-(4-methoxyphenyl)acrylate (**5e**).* Alkyne **4e**: 0.043 g; purification: trituration with diethyl ether/petroleum ether mixture; yield: 0.032 g (41%); mp 155.0–157.5 °C; IR (ATR, ν/cm^{-1}) 3223, 3170, 3131, 3094, 3082, 2996, 2965, 2934, 2900, 2837, 1712, 1636, 1607, 1573, 1513, 1455, 1429, 1402, 1375, 1322, 1308, 1287, 1258, 1240, 1202, 1163, 1065, 1037, 1002, 971, 867, 852, 824, 774, 734, 592, 547, 527, 513; ^{1}H NMR (DMSO-d_6) δ 11.98 (s, 1H), 8.32-8.29 (m, 2H), 8.27 (d, 1H, J = 7.9 Hz), 8.12 (d, 1H, J = 5.2 Hz), 7.69-7.59 (m, 5H), 7.28 (t, 1H, J = 7.5 Hz), 6.96 (d, 2H, J = 8.4 Hz), 6.50 (d, 1H, J = 16.0 Hz), 6.11 (s, 2H), 5.25 (s, 2H), 3.79 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 166.22, 161.24, 144.85, 141.92, 140.71, 138.01, 137.88, 133.85, 130.26, 128.74, 128.58, 126.56, 125.70, 121.94, 120.75, 119.67, 114.88, 114.38, 112.09, 57.11, 55.34, 51.21; ESI-MS: *m/z* 440.1 (M+1)$^+$; Anal. Calcd. for $C_{25}H_{21}N_5O_3$: C, 68.33; H, 4.82; N, 15.94; found: C, 68.27; H, 4.99; N, 15.68.

*4.1.6. General procedure for the synthesis of amines **6** and **11***

A suspension of **3** or **10** (0.856 mmol) and 10% Pd/C (0.041 g) in MeOH (5 mL) was stirred at rt under hydrogen atmosphere for 18 h (compound **6**) or 2 h (compound **11**). Upon completion, the reaction mixture was filtered through celite, and the solvent was removed under the reduced pressure. The crude product was triturated with diethyl ether/petroleum ether mixture.

*4.1.6.1. (9H-pyrido[3,4-b]indol-1-yl)methanamine (**6**).* Azide **3**: 0.191 g; yield: 0.120 g (71%); IR (ATR, ν/cm^{-1}) 3347, 3282, 3120, 3051, 2955, 2856, 2776, 1625, 1600, 1562, 1501, 1477, 1460, 1430, 1355, 1327, 1316, 1240, 1212, 1163, 1127, 1108, 1071, 960, 896, 848, 829, 772, 747, 671, 595, 564, 516; ^{1}H NMR (DMSO-d_6) δ 8.26 (d, 1H, J = 5.2 Hz), 8.21 (dt, 1H, J = 7.8, 1.0 Hz), 7.97 (d, 1H, J = 5.2 Hz), 7.63-7.62 (m, 1H), 7.55-7.52 (m, 1H), 7.24-7.22 (m, 1H), 4.19 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 146.51, 140.35, 137.18, 133.23, 127.82, 127.42, 121.60, 120.88, 119.15, 113.19, 112.01, 44.59; ESI-MS: *m/z* 196.0 (M − 1)$^-$.

*4.1.6.2. (1-methyl-9H-pyrido[3,4-b]indol-3-yl)methanamine (**11**).* Azide **10**: 0.203 g; yield: 0.110 g (61%); IR (ATR, ν/cm^{-1}) 3338, 3239, 3130, 3057, 2978, 2940, 2914, 2882, 2850, 2783, 2737, 2690, 2637, 1626, 1606, 1566, 1503, 1453, 1401, 1374, 1344, 1317, 1284, 1250, 1176, 1147, 1103, 1083, 1009, 970, 945, 891, 838, 813, 775, 734, 643, 588, 545; ^{1}H NMR (DMSO-d_6) δ 11.42 (s, 1H), 8.14 (d, 1H, J = 7.6 Hz), 7.91 (s, 1H), 7.57-7.47 (m, 2H), 7.20 (t, 1H, J = 6.9 Hz), 3.89 (s, 2H), 2.73 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 151.13, 140.89, 140.74, 133.29, 127.76, 127.62, 121.53, 121.19, 118.98, 111.89, 109.01, 47.59, 20.38; ESI-MS: *m/z* 210.0 (M − 1)$^-$.

*4.1.7. General procedure for the synthesis of harmicines **7a-h***

A solution of a corresponding CAD (0.212 mmol), DIEA (0.073 mL, 0.424 mmol) and HATU (0.081 g, 0.212 mmol) in DCM

(4 mL) was stirred at rt for 20 min, followed by the addition of amine **6** (0.038 g, 0.193 mmol). The reaction mixture was stirred at rt for 1 h. The formed precipitate was filtered off. After purification by column chromatography (DCM/MeOH 8:1) and trituration with diethyl ether, compounds **7a-h** were obtained.

*4.1.7.1. N-((9H-pyrido[3,4-b]indol-1-yl)methyl)cinnamamide (**7a**).* CAD: 0.031 g of *trans*-cinnamic acid; yield: 0.033 g (52%); mp 225.0–227.0 °C; IR (ATR, ν/cm^{-1}) 3375, 3328, 3226, 3195, 3101, 3060, 2901, 1661, 1623, 1576, 1514, 1435, 1404, 1325, 1243, 1205, 994, 914, 811, 767, 731, 718, 706, 620, 565, 490, 478; ^{1}H NMR (DMSO-d_6) δ 11.64 (s, 1H), 8.79 (t, 1H, J = 5.4 Hz), 8.33 (d, 1H, J = 5.2 Hz), 8.25 (d, 1H, J = 7.9 Hz), 8.08 (d, 1H, J = 5.3 Hz), 7.67-7.64 (m, 1H), 7.61-7.50 (m, 4H), 7.46-7.35 (m, 3H), 7.29-7.24 (m, 1H), 6.88 (d, 1H, J = 15.8 Hz), 4.92 (d, 2H, J = 5.3 Hz); ^{13}C NMR (DMSO-d_6) δ 165.43, 141.41, 140.45, 139.04, 137.34, 134.92, 133.52, 129.49, 128.94, 128.17, 127.82, 127.58, 122.10, 121.76, 120.87, 119.45, 113.96, 112.08, 41.65; ESI-MS: m/z 328.1 (M+1)$^+$; HPLC purity > 99.5%.

*4.1.7.2. (E)-N-((9H-pyrido[3,4-b]indol-1-yl)methyl)-3-(3-fluorophenyl)acrylamide (**7b**).* CAD: 0.035 g of *m*-fluorocinnamic acid; yield: 0.034 g (51%); mp 224.5–225.5 °C; IR (ATR, ν/cm^{-1}) 3350, 3217, 3149, 3080, 2995, 2905, 2814, 1672, 1627, 1586, 1516, 1448, 1409, 1325, 1273, 1239, 1225, 1146, 1011, 968, 878, 851, 807, 784, 728, 611, 564, 521; ^{1}H NMR (DMSO-d_6) δ 11.63 (s, 1H), 8.78 (t, 1H, J = 5.4 Hz), 8.32 (d, 1H, J = 5.2 Hz), 8.24 (d, 1H, J = 7.8 Hz), 8.07 (d, 1H, J = 5.2 Hz), 7.64 (d, 1H, J = 8.2 Hz), 7.57 (t, 1H, J = 7.6 Hz), 7.52 (d, 1H, J = 15.8 Hz), 7.49-7.43 (m, 3H), 7.26 (t, 1H, J = 7.4 Hz), 7.23-7.20 (m, 1H), 6.94 (d, 1H, J = 15.8 Hz), 4.92 (d, 2H, J = 5.3 Hz); ^{13}C NMR (DMSO-d_6) δ 165.08, 162.46 (d, J = 244.0 Hz), 141.26, 140.45, 137.71 (d, J = 2.4 Hz), 137.58 (d, J = 7.8 Hz), 137.37, 133.48, 130.90 (d, J = 8.4 Hz), 128.16, 127.80, 123.75 (d, J = 2.5 Hz), 123.70, 121.76, 120.87, 119.44, 116.14 (d, J = 21.2 Hz), 113.96 (d, J = 21.8 Hz), 112.07, 41.65; ESI-MS: m/z 346.1 (M+1)$^+$; HPLC purity 98.2%.

*4.1.7.3. (E)-N-((9H-pyrido[3,4-b]indol-1-yl)methyl)-3-(3-bromophenyl)acrylamide (**7c**).* CAD: 0.048 g of *m*-bromocinnamic acid; yield: 0.056 g (72%); mp 236.5–239.0 °C; IR (ATR, ν/cm^{-1}) 3191, 3016, 1671, 1626, 1564, 1497, 1471, 1437, 1392, 1328, 1222, 1128, 1078, 971, 929, 820, 778, 722, 667, 584; ^{1}H NMR (DMSO-d_6) δ 11.62 (s, 1H), 8.74 (t, 1H, J = 5.4 Hz), 8.31 (d, 1H, J = 5.2 Hz), 8.23 (d, 1H, J = 7.8 Hz), 8.06 (d, 1H, J = 5.2 Hz), 7.80 (t, 1H, J = 1.9 Hz), 7.63 (d, 1H, J = 8.2 Hz), 7.59 (d, 1H, J = 7.8 Hz), 7.57-7.54 (m, 2H), 7.47 (d, 1H, J = 15.8 Hz), 7.37 (t, 1H, J = 7.9 Hz), 7.25 (t, 1H, J = 7.4 Hz), 6.94 (d, 1H, J = 15.8 Hz), 4.91 (d, 2H, J = 5.3 Hz); ^{13}C NMR (DMSO-d_6) δ 165.01, 141.20, 140.46, 137.53, 137.34, 137.30, 133.46, 131.98, 131.02, 130.06, 128.17, 127.82, 126.49, 123.82, 122.26, 121.76, 120.85, 119.44, 113.96, 112.06, 41.62; ESI-MS: m/z 405.9 (M+1)$^+$, 407.9 (M+1)$^+$; HPLC purity 99.4%.

*4.1.7.4. (E)-N-((9H-pyrido[3,4-b]indol-1-yl)methyl)-3-(3-(trifluoromethyl)phenyl)acrylamide (**7d**).* CAD: 0.046 g of *m*-(trifluoromethyl)cinnamic acid; yield: 0.040 g (52%); mp 225.5–226.5 °C; IR (ATR, ν/cm^{-1}) 3197, 3014, 1669, 1628, 1560, 1498, 1438, 1329, 1226, 1177, 1167, 1109, 1077, 972, 885, 804, 742, 721, 691, 581; ^{1}H NMR (DMSO-d_6) δ 11.65 (s, 1H), 8.78 (t, 1H, J = 5.3 Hz), 8.33 (d, 1H, J = 5.2 Hz), 8.24 (dt, 1H, J = 7.9, 1.0 Hz), 8.07 (d, 1H, J = 5.2 Hz), 7.96 (t, 1H, J = 2.0 Hz), 7.90 (d, 1H, J = 7.8 Hz), 7.73 (d, 1H, J = 7.8 Hz), 7.67-7.63 (m, 2H), 7.60 (d, 1H, J = 15.8 Hz), 7.58-7.55 (m, 1H), 7.28-7.25 (m, 1H), 7.06 (d, 1H, J = 15.9 Hz), 4.93 (d, 2H, J = 5.3 Hz); ^{13}C NMR (DMSO-d_6) δ 164.96, 141.19, 140.47, 137.35, 137.26, 136.19, 133.47, 131.37, 130.05, 129.74 (q, J = 31.7 Hz), 128.15, 127.80, 125.72 (q, J = 2.7 Hz), 124.33, 124.06 (q, J = 273.3 Hz), 123.92 (q, J = 3.6 Hz), 121.75, 120.87, 119.44, 113.96, 112.07, 41.67; ESI-MS: m/z 396.1 (M+1)$^+$; HPLC purity 97.3%.

*4.1.7.5. (E)-N-((9H-pyrido[3,4-b]indol-1-yl)methyl)-3-(4-fluorophenyl)acrylamide (**7e**).* CAD: 0.035 g of *p*-fluorocinnamic acid; yield: 0.045 g (67%); mp 231.5–233.0 °C; IR (ATR, ν/cm^{-1}) 3380, 3244, 3147, 3075, 2989, 2900, 1655, 1616, 1599, 1556, 1505, 1430, 1426, 1413, 1354, 1325, 1239, 1220, 1161, 1128, 1098, 1010, 977, 828, 755, 734, 628, 508; ^{1}H NMR (DMSO-d_6) δ 11.62 (s, 1H), 8.75 (t, 1H, J = 5.4 Hz), 8.32 (d, 1H, J = 5.2 Hz), 8.24 (d, 1H, J = 7.8 Hz), 8.06 (d, 1H, J = 5.2 Hz), 7.66-7.63 (m, 3H), 7.56 (t, 1H, J = 7.6 Hz), 7.51 (d, 1H, J = 15.8 Hz), 7.27-7.24 (m, 3H), 6.83 (d, 1H, J = 15.8 Hz), 4.91 (d, 2H, J = 5.3 Hz); ^{13}C NMR (DMSO-d_6) δ 165.31, 162.69 (d, J = 247.0 Hz), 141.39, 140.42, 137.80, 137.35, 133.49, 131.56 (d, J = 3.0 Hz), 129.71 (d, J = 8.4 Hz), 128.12, 127.77, 122.00 (d, J = 1.7 Hz), 121.73, 120.86, 119.41, 115.89 (d, J = 21.8 Hz), 113.92, 112.05, 41.64; ESI-MS: m/z 346.1 (M+1)$^+$; Anal. Calcd. for $C_{21}H_{16}FN_3O$: C, 73.03; H, 4.67; N, 12.17; found: C, 73.15; H, 4.81; N, 12.29.

*4.1.7.6. (E)-N-((9H-pyrido[3,4-b]indol-1-yl)methyl)-3-(4-chlorophenyl)acrylamide (**7f**).* CAD: 0.039 g of *p*-chlorocinnamic acid; yield: 0.036 g (52%); mp 241.0–243.5 °C (decomp.); IR (ATR, ν/cm^{-1}) 3384, 3275, 3053, 1671, 1627, 1532, 1505, 1494, 1457, 1435, 1405, 1323, 1237, 1219, 1094, 1028, 1013, 972, 819, 732, 609, 589, 573, 495; ^{1}H NMR (DMSO-d_6) δ 11.62 (s, 1H), 8.79 (t, 1H, J = 5.4 Hz), 8.32 (d, 1H, J = 5.2 Hz), 8.24 (d, 1H, J = 7.8 Hz), 8.06 (d, 1H, J = 5.2 Hz), 7.65-7.61 (m, 3H), 7.58-7.55 (m, 1H), 7.52-7.48 (m, 3H), 7.26 (t, 1H, J = 7.4 Hz), 6.89 (d, 1H, J = 15.8 Hz), 4.91 (d, 2H, J = 5.4 Hz); ^{13}C NMR (DMSO-d_6) δ 165.20, 141.35, 140.45, 137.67, 137.38, 133.92, 133.51, 129.29, 128.99, 128.16, 127.80, 122.93, 121.77, 120.88, 119.45, 113.96, 112.08, 41.66; ESI-MS: m/z 362.1 (M+1)$^+$; Anal. Calcd. for $C_{21}H_{16}ClN_3O$: C, 69.71; H, 4.46; N, 11.61; found: C, 69.95; H, 4.31; N, 11.99.

*4.1.7.7. (E)-N-((9H-pyrido[3,4-b]indol-1-yl)methyl)-3-(4-methoxyphenyl)acrylamide (**7g**).* CAD: 0.038 g of *p*-methoxycinnamic acid; yield: 0.034 g (49%); mp 230.5–232.0 °C; IR (ATR, ν/cm^{-1}) 3350, 3173, 3097, 3006, 1662, 1624, 1603, 1511, 1432, 1366, 1325, 1254, 1240, 1174, 1029, 981, 914, 818, 791, 733, 596, 528, 506; ^{1}H NMR (DMSO-d_6) δ 11.58 (s, 1H), 8.66 (t, 1H, J = 5.5 Hz), 8.29 (d, 1H, J = 5.3 Hz), 8.22 (d, 1H, J = 7.8 Hz), 8.04 (d, 1H, J = 4.7 Hz), 7.62 (d, 1H, J = 8.2 Hz), 7.56-7.51 (m, 3H), 7.44 (d, 1H, J = 15.7 Hz), 7.24 (t, 1H, J = 7.5 Hz), 6.96 (d, 2H, J = 8.2 Hz), 6.69 (d, 1H, J = 15.7 Hz), 4.88 (d, 2H, J = 5.5 Hz), 3.77 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 165.82, 160.39, 141.57, 140.46, 138.83, 137.34, 133.54, 129.20, 128.20, 127.85, 127.49, 121.78, 120.90, 119.57, 119.48, 114.42, 113.98, 112.10, 55.28, 41.67; ESI-MS: m/z 358.1 (M+1)$^+$; HPLC purity 99.0%.

*4.1.7.8. (E)-N-((9H-pyrido[3,4-b]indol-1-yl)methyl)-3-(4-(trifluoromethyl)phenyl)acrylamide (**7h**).* CAD: 0.046 g of *p*-(trifluoromethyl)cinnamic acid; yield: 0.050 g (65%); mp 262.5–265.0 °C; IR (ATR, ν/cm^{-1}) 3341, 3236, 1664, 1622, 1504, 1436, 1416, 1323, 1239, 1217, 1155, 1116, 1068, 1013, 988, 826, 755, 740, 572, 532, 494; ^{1}H NMR (DMSO-d_6) δ 11.65 (s, 1H), 8.87 (t, 1H, J = 5.4 Hz), 8.33 (d, 1H, J = 5.3 Hz), 8.25 (d, 1H, J = 7.9 Hz), 8.08 (d, 1H, J = 5.3 Hz), 7.83-7.76 (m, 4H), 7.66-7.54 (m, 3H), 7.29-7.24 (m, 1H), 7.03 (d, 1H, J = 15.8 Hz), 4.94 (d, 2H, J = 5.3 Hz); ^{13}C NMR (DMSO-d_6) δ 164.89, 141.23, 140.46, 139.04, 137.38, 137.32, 133.49, 129.21 (q, J = 32.3 Hz), 128.20, 128.16, 127.81, 125.81 (q, J = 3.0 Hz), 124.95, 124.12 (q, J = 272.7 Hz), 121.76, 120.87, 119.44, 113.96, 112.07, 41.66; ESI-MS: m/z 396.1 (M+1)$^+$; HPLC purity 99.4%.

*4.1.8. Synthesis of (1-methyl-9-(prop-2-yn-1-yl)-9H-pyrido[3,4-b]indol-3-yl)methanol (**12**)*

Alcohol **9** (0.200 g, 0.942 mmol) was dissolved in dry DMF (5 mL). Under argon atmosphere caesium carbonate (0.430 g, 1.319 mmol) was added, followed by dropwise addition of

propargyl bromide (0.126 mL, 1.130 mmol, 80% solution in toluene). The reaction mixture was stirred at rt for 5 h. Upon completion, the reaction mixture was diluted with H_2O (50 mL) and extracted with EtOAc (3 × 40 mL). The collected organic layers were washed with H_2O (2 × 100 mL), dried over anhydrous sodium sulphate and the solvent evaporated under the reduced pressure. After purification by column chromatography (DCM/MeOH 95:5) and trituration with diethyl ether, alkyne **12** was obtained as a white solid.

Yield: 0.170 g (72%); mp 225.0–230.0 °C (decomp.); IR (ATR, ν/cm^{-1}) 3154, 2929, 2823, 2109, 1621, 1561, 1475, 1453, 1360, 1333, 1283, 1206, 1139, 1063, 1040, 965, 933, 877, 744, 724, 641, 578; ^{1}H NMR (DMSO-d_6) δ 8.25 (d, 1H, J = 7.8 Hz), 8.03 (s, 1H), 7.78 (d, 1H, J = 8.3 Hz), 7.63-7.58 (m, 1H), 7.29 (t, 1H, J = 7.4 Hz), 5.45 (d, 2H, J = 2.4 Hz), 5.35 (t, 1H, J = 5.8 Hz), 4.68 (d, 2H, J = 5.8 Hz), 3.35 (t, 1H, J = 2.4 Hz), 3.04 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 150.83, 141.27, 140.52, 133.22, 129.53, 128.23, 121.52, 121.05, 120.04, 110.37, 109.11, 75.50, 64.34, 34.19, 22.45; ESI-MS: m/z 251.1 (M+1)$^+$.

*4.1.9. General procedure for the synthesis of harmicines **14a-e***

To a solution of alkyne **12** (0.040 g, 0.160 mmol) and the corresponding cinnamyl azide **13a-e** (0.176 mmol) in MeOH (5 mL), a catalytic amount of $Cu(OAc)_2$ was added. The reaction mixture was stirred at rt for 24 h. Upon completion of the reaction, the solvent was removed under the reduced pressure. The residue was purified by column chromatography with DCM/MeOH, cyclohexane/EtOAc/MeOH or EtOAc/MeOH as a mobile phase. The crude product was triturated with diethyl ether/petroleum ether to obtain harmicines **14a-e**. Compound **14b** was obtained, but decomposed quickly at −20 °C.

*4.1.9.1. (9-((1-cinnamyl-1H-1,2,3-triazol-4-yl)methyl)-1-methyl-9H-pyrido[3,4-b]indol-3-yl)methanol (**14a**).* **13a**: 0.028 g; mobile phase: cyclohexane/EtOAc/MeOH 3:1:0.5; yield: 0.032 g (49%); mp 197.0–200.0 °C; IR (ATR, ν/cm^{-1}) 3113, 3060, 2966, 2917, 2859, 1657, 1619, 1560, 1512, 1445, 1402, 1362, 1337, 1290, 1252, 1220, 1190, 1156, 1136, 1122, 1062, 1043, 962, 934, 860, 842, 813, 780, 748, 691, 638, 620, 577, 530, 511, 477, 463; ^{1}H NMR (DMSO-d_6) δ 8.23 (d, 1H, J = 7.7 Hz), 8.02 (s, 1H), 7.98 (s, 1H), 7.82 (d, 1H, J = 8.3 Hz), 7.59-7.55 (m, 1H), 7.40-7.24 (m, 6H), 6.51 (d, 1H, J = 15.9 Hz), 6.47-6.35 (m, 1H), 5.90 (s, 2H), 5.33 (t, 1H, J = 5.7 Hz), 5.07 (d, 2H, J = 6.0 Hz), 4.67 (d, 2H, J = 5.7 Hz), 3.05 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 150.27, 144.17, 141.35, 140.55, 135.63, 133.45, 133.41, 129.10, 128.66, 128.10, 128.06, 126.51, 123.66, 122.72, 121.41, 120.99, 119.71, 110.61, 109.07, 64.35, 51.27, 39.73, 23.15; ESI-MS: m/z 410.3 (M+1)$^+$; Anal. Calcd. for $C_{25}H_{23}N_5O$: C, 73.33; H, 5.66; N, 17.10; found: C, 73.19; H, 5.39; N, 17.34.

*4.1.9.2. (E)-(9-((1-(3-(3-bromophenyl)allyl)-1H-1,2,3-triazol-4-yl)methyl)-1-methyl-9H-pyrido[3,4-b]indol-3-yl)methanol (**14c**).* **13c**: 0.042 g; mobile phase: DCM/MeOH 95:5; yield: 0.031 g, (39%); mp 164.5–167.5 °C; IR (ATR, ν/cm^{-1}) 3117, 3060, 2959, 2922, 2861, 1619, 1592, 1560, 1471, 1460, 1445, 1380, 1358, 1336, 1290, 1252, 1222, 1199, 1154, 1120, 1095, 1062, 1044, 995, 965, 934, 875, 861, 829, 786, 747, 719, 685, 668, 636, 621, 577, 534, 492, 463; ^{1}H NMR (DMSO-d_6) δ 8.23 (d, 1H, J = 7.7 Hz), 8.02 (s, 1H), 7.97 (s, 1H), 7.82 (d, 1H, J = 8.3 Hz), 7.70 (t, 1H, J = 2.2 Hz), 7.57 (t, 1H, J = 8.0 Hz), 7.47-7.42 (m, 2H), 7.30-7.24 (m, 2H), 6.67-6.60 (m, 2H), 5.93 (s, 2H), 5.32 (t, 1H, J = 5.7 Hz), 5.07 (d, 2H, J = 5.1 Hz), 4.67 (d, 2H, J = 5.6 Hz), 3.08 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 150.26, 144.16, 141.34, 140.54, 138.20, 133.45, 131.73, 130.74, 130.69, 129.09, 129.00, 128.04, 125.64, 125.56, 122.78, 122.12, 121.40, 120.99, 119.71, 110.60, 109.06, 64.34, 51.11, 39.72, 23.13; ESI-MS: m/z 488.1 (M+1)$^+$, 490.1 (M+1)$^+$; Anal. Calcd. for $C_{25}H_{22}BrN_5O$: C, 61.48; H, 4.54; N, 14.34; found: C, 61.78; H, 4.59; N, 14.62.

*4.1.9.3. (E)-(9-((1-(3-(4-chlorophenyl)allyl)-1H-1,2,3-triazol-4-yl)methyl)-1-methyl-9H-pyrido[3,4-b]indol-3-yl)methanol (**14d**).* **13d**: 0.034 g; mobile phase: EtOAc/MeOH 10:0.5; yield: 0.029 g, (41%); mp 183.0–186.5 °C; IR (ATR, ν/cm^{-1}) 3111, 3064, 2971, 2918, 2858, 2822, 1727, 1658, 1619, 1592, 1559, 1489, 1471, 1447, 1405, 1361, 1336, 1292, 1254, 1221, 1192, 1158, 1121, 1085, 1041, 1014, 964, 938, 861, 837, 819, 799, 779, 748, 719, 685, 637, 577, 537, 502, 462; ^{1}H NMR (DMSO-d_6) δ 8.23 (d, 1H, J = 7.8 Hz), 8.02 (s, 1H), 7.97 (s, 1H), 7.82 (d, 1H, J = 8.3 Hz), 7.57 (t, 1H, J = 7.5 Hz), 7.43-7.35 (m, 4H), 7.27 (t, 1H, J = 7.4 Hz), 6.52-6.41 (m, 2H), 5.90 (s, 2H), 5.32 (t, 1H, J = 5.7 Hz), 5.07 (d, 2H, J = 5.1 Hz), 4.67 (d, 2H, J = 5.7 Hz), 3.05 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 150.27, 144.18, 141.35, 140.55, 134.61, 133.44, 132.47, 132.05, 129.10, 128.64, 128.24, 128.06, 124.71, 122.76, 121.41, 120.99, 119.72, 110.60, 109.06, 64.35, 51.17, 39.73, 23.15; ESI-MS: m/z 444.2 (M+1)$^+$; Anal. Calcd. for $C_{25}H_{22}ClN_5O$: C, 67.64; H, 5.00; N, 15.78; found: C, 67.34; H, 5.16; N, 15.63.

*4.1.9.4. (E)-(9-((1-(3-(4-methoxyphenyl)allyl)-1H-1,2,3-triazol-4-yl)methyl)-1-methyl-9H-pyrido[3,4-b]indol-3-yl)methanol (**14e**).* **13e**: 0.033 g; mobile phase: DCM/MeOH 95:5; yield: 0.030 g, (43%); mp 202.0–205.0 °C; IR (ATR, ν/cm^{-1}) 3180, 3119, 3067, 3024, 3002, 2945, 2923, 2869, 1622, 1563, 1488, 1462, 1441, 1379, 1364, 1333, 1298, 1254, 1222, 1203, 1160, 1135, 1122, 1067, 1037, 1003, 965, 915, 857, 771, 745, 696, 636, 582, 533, 509, 471, 454; ^{1}H NMR (DMSO-d_6) δ 8.23 (d, 1H, J = 7.8 Hz), 8.02 (s, 1H), 7.96 (s, 1H), 7.82 (d, 1H, J = 8.3 Hz), 7.57 (t, 1H, J = 7.5 Hz), 7.34-7.30 (m, 2H), 7.26 (t, 1H, J = 7.5 Hz), 6.89-6.85 (m, 2H), 6.47 (d, 1H, J = 15.8 Hz), 6.25 (dt, 1H, J = 15.8, 6.5 Hz), 5.90 (s, 2H), 5.33 (t, 1H, J = 5.7 Hz), 5.03 (d, 2H, J = 7.2 Hz), 4.67 (d, 2H, J = 5.7 Hz), 3.74 (s, 3H), 3.05 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 159.20, 150.26, 144.14, 141.35, 140.56, 133.45, 133.19, 129.09, 128.24, 128.05, 127.86, 122.61, 121.40, 121.05, 120.98, 119.71, 114.06, 110.60, 109.07, 64.35, 55.11, 51.41, 39.73, 23.15; ESI-MS: m/z 440.3 (M+1)$^+$; Anal. Calcd. for $C_{26}H_{25}N_5O_2$: C, 71.05; H, 5.73; N, 15.93; found: C, 71.27; H, 5.79; N, 15.72.

*4.1.10. General procedure for the synthesis of harmicines **15a-e***

To a suspension of azide **10** (0.04 g, 0.169 mmol) and a corresponding alkyne **4a-e** (0.186 mmol) in 5 mL of a 1:1H_2O/*t*-BuOH mixture, sodium ascorbate (0.2 mmol, 200 μL of freshly prepared 1 M solution in H_2O) and $CuSO_4 \times 5H_2O$ (0.02 mmol, 20 μL of 1 M solution in H_2O) were added. The mixture was stirred at rt for 0.5 h, diluted with 5 mL of ice-cold H_2O and filtered. A yellow precipitate was washed with H_2O (3 × 2 mL) and dried under vacuum. After purification by column chromatography (mobile phase DCM/MeOH 95:5) and trituration with diethyl ether/petroleum ether mixture, compounds **15a-e** were obtained as white solids.

*4.1.10.1. (1-((1-methyl-9H-pyrido[3,4-b]indol-3-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl cinnamate (**15a**).* Alkyne **4a**: 0.035 g; yield: 0.026 g (36%); mp 194.5–195.5 °C; IR (ATR, ν/cm^{-1}) 3220, 3194, 3175, 3145, 3101, 3060, 3030, 2989, 2945, 1712, 1694, 1644, 1626, 1605, 1565, 1503, 1451, 1392, 1376, 1356, 1329, 1312, 1271, 1254, 1223, 1202, 1170, 1116, 1059, 1037, 1002, 972, 934, 898, 859, 843, 827, 804, 765, 752, 738, 708, 681, 642, 611, 587, 524, 480; ^{1}H NMR (DMSO-d_6) δ 11.67 (s, 1H), 8.26 (s, 1H), 8.17 (d, 1H, J = 7.9 Hz), 7.99 (s, 1H), 7.72-7.70 (m, 2H), 7.67 (d, 1H, J = 16.0 Hz), 7.60 (dt, 1H, J = 8.2, 1.0 Hz), 7.55-7.53 (m, 1H), 7.44-7.39 (m, 3H), 7.24-7.21 (m, 1H), 6.65 (d, 1H, J = 16.1 Hz), 5.77 (s, 2H), 5.27 (s, 2H), 2.75 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 165.94, 145.02, 142.54, 142.16, 141.92, 140.78, 134.00, 133.93, 130.58, 128.91, 128.42, 128.10, 127.60, 125.13, 121.72, 120.91, 119.46, 117.66, 112.10, 112.05, 57.34, 55.25, 20.40; ESI-MS: m/z 424.0 (M+1)$^+$; Anal. Calcd. for $C_{25}H_{21}N_5O_2$: C, 70.91; H, 5.00; N, 16.54; found: C, 70.83; H, 5.17; N, 16.69.

*4.1.10.2. (1-((1-methyl-9H-pyrido[3,4-b]indol-3-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl (E)-3-(3-fluorophenyl)acrylate (**15b**).* Alkyne **4b**: 0.038 g, yield: 0.045 g (60%); mp 197.0–198.5 °C; IR (ATR, ν/cm^{-1}) 3223, 3198, 3166, 3104, 3067, 3043, 3012, 2970, 2895, 1707, 1627, 1608, 1583, 1568, 1506, 1483, 1449, 1387, 1355, 1320, 1271, 1252, 1213, 1151, 1115, 1084, 1057, 1036, 1008, 980, 942, 911, 898, 864, 832, 788, 766, 738, 693, 675, 649, 609, 586, 555, 537, 520, 480; ^{1}H NMR (DMSO-d_6) δ 11.67 (s, 1H), 8.26 (s, 1H), 8.18 (d, 1H, J = 7.9 Hz), 8.00 (s, 1H), 7.67 (d, 1H, J = 16.1 Hz), 7.65-7.62 (m, 1H), 7.61-7.59 (m, 1H), 7.56-7.53 (m, 2H), 7.46-7.43 (m, 1H), 7.27-7.22 (m, 2H), 6.74 (d, 1H, J = 16.0 Hz), 5.77 (s, 2H), 5.28 (s, 2H), 2.75 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 165.73, 162.39 (d, J = 243.8 Hz), 143.61, 142.52, 142.16, 141.83, 140.77, 136.47 (d, J = 8.1 Hz), 134.00, 130.84 (d, J = 8.4 Hz), 128.09, 127.60, 125.14, 124.91 (d, J = 2.5 Hz), 121.71, 120.90, 119.44, 119.29, 117.23 (d, J = 21.3 Hz), 114.56 (d, J = 22.1 Hz), 112.09, 112.06, 57.43, 55.25, 20.40; ESI-MS: m/z 442.1 (M+1)$^+$; HPLC purity 97.6%.

*4.1.10.3. (1-((1-methyl-9H-pyrido[3,4-b]indol-3-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl (E)-3-(3-bromophenyl)acrylate (**15c**).* Alkyne **4c**: 0.049 g; yield: 0.045 g (53%); mp 204.5–205.5 °C; IR (ATR, ν/cm^{-1}) 3347, 3281, 3170, 3080, 3054, 3023, 2970, 2950, 1708, 1684, 1638, 1592, 1569, 1498, 1474, 1455, 1423, 1383, 1354, 1341, 1313, 1290, 1272, 1247, 1231, 1188, 1146, 1110, 1073, 1053, 1032, 1009, 980, 928, 899, 872, 829, 791, 766, 731, 697, 666, 649, 623, 586, 563, 549, 527; ^{1}H NMR (DMSO-d_6) δ 11.67 (s, 1H), 8.26 (s, 1H), 8.17 (d, 1H, J = 7.9 Hz), 7.99 (s, 1H), 7.97 (t, 1H, J = 1.8 Hz), 7.75-7.71 (m, 1H), 7.64 (d, 1H, J = 16.0 Hz), 7.62-7.58 (m, 2H), 7.56-7.52 (m, 1H), 7.36 (t, 1H, J = 7.9 Hz), 7.25-7.21 (m, 1H), 6.74 (d, 1H, J = 16.1 Hz), 5.77 (s, 2H), 5.28 (s, 2H), 2.75 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 165.67, 143.31, 142.52, 142.14, 141.84, 140.77, 136.44, 133.99, 133.01, 130.91, 128.08, 127.59, 127.29, 125.11, 122.27, 121.70, 120.90, 119.43, 119.38, 112.08, 112.03, 57.44, 55.24, 20.39; ESI-MS: m/z 501.9 (M+1)$^+$, 503.9 (M+1)$^+$; HPLC purity 97.4%.

*4.1.10.4. (1-((1-methyl-9H-pyrido[3,4-b]indol-3-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl (E)-3-(4-chlorophenyl)acrylate (**15d**).* Alkyne **4d**: 0.041 g; yield: 0.044 g (57%); mp 209.0–211.0 °C; IR (ATR, ν/cm^{-1}) 3250, 3157, 3103, 3086, 3067, 3052, 1703, 1633, 1592, 1566, 1493, 1475, 1456, 1428, 1408, 1388, 1356, 1305, 1278, 1251, 1222, 1204, 1182, 1160, 1117, 1089, 1058, 1036, 1004, 964, 899, 865, 840, 823, 805, 753, 739, 719, 702, 646, 605, 586, 550, 524, 501, 456; ^{1}H NMR (DMSO-d_6) δ 11.67 (s, 1H), 8.26 (s, 1H), 8.18 (d, 1H, J = 7.9 Hz), 8.00 (s, 1H), 7.76-7.73 (m, 2H), 7.66 (d, 1H, J = 16.0 Hz), 7.60 (dt, 1H, J = 8.3, 1.0 Hz), 7.56-7.53 (m, 1H), 7.48-7.45 (m, 2H), 7.24-7.22 (m, 1H), 6.68 (d, 1H, J = 16.1 Hz), 5.77 (s, 2H), 5.28 (s, 2H), 2.75 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 165.80, 143.60, 142.53, 142.17, 141.88, 140.78, 135.09, 134.01, 132.92, 130.15, 128.94, 128.11, 127.61, 125.13, 121.72, 120.91, 119.46, 118.51, 112.10, 112.07, 57.40, 55.26, 20.41; ESI-MS: m/z 458.0 (M+1)$^+$; HPLC purity 98.3%.

*4.1.10.5. (1-((1-methyl-9H-pyrido[3,4-b]indol-3-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl (E)-3-(4-methoxyphenyl)acrylate (**15e**).* Alkyne **4e**: 0.040 g; yield: 0.048 g (62%); mp 240.5–243.0 °C; IR (ATR, ν/cm^{-1}) 3236, 3201, 3165, 3103, 3068, 3036, 3007, 2965, 2940, 2911, 2839, 1699, 1626, 1601, 1574, 1514, 1456, 1426, 1389, 1357, 1307, 1290, 1252, 1223, 1207, 1179, 1155, 1115, 1056, 1023, 1009, 992, 971, 937, 898, 868, 830, 810, 776, 751, 735, 710, 693, 663, 652, 637, 608, 586, 555, 521; ^{1}H NMR (DMSO-d_6) δ 11.67 (s, 1H), 8.25 (s, 1H), 8.18 (d, 1H, J = 7.9 Hz), 7.99 (s, 1H), 7.68-7.64 (m, 2H), 7.64-7.59 (m, 2H), 7.55-7.53 (m, 1H), 7.24-7.22 (m, 1H), 6.97-6.94 (m, 2H), 6.49 (d, 1H, J = 16.0 Hz), 5.77 (s, 2H), 5.25 (s, 2H), 3.79 (s, 3H), 2.75 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 166.19, 161.20, 144.80, 142.53, 142.14, 142.03, 140.76, 133.99, 130.22, 128.08, 127.59, 126.54, 125.05, 121.70, 120.90, 119.44, 114.88, 114.35, 112.08, 112.04, 57.14, 55.32, 55.23, 20.39; ESI-MS: m/z 454.1 (M+1)$^+$; HPLC purity 98.6%.

*4.1.11. General procedure for the synthesis of harmicines **16a-h***

A solution of a corresponding CAD (0.162 mmol), DIEA (0.056 mL, 0.324 mmol) and HATU (0.062 g, 0.162 mmol) in DCM (4 mL) was stirred at rt for 20 min, followed by the addition of amine **11** (0.031 g, 0.147 mmol). The resulting solution was stirred at rt for 1 h. Purification was performed by either Method A or Method B.

Method A: The resulting precipitate was filtered off, purified by column chromatography (DCM/MeOH 8:1) and triturated with diethyl ether/petroleum ether mixture.

Method B: After completion of the reaction, the solvent was evaporated and EtOAc (10 mL) was added. The formed precipitate was filtered off, purified by column chromatography (DCM/MeOH 8:1) and triturated with diethyl ether/petroleum ether mixture.

*4.1.11.1. N-((1-methyl-9H-pyrido[3,4-b]indol-3-yl)methyl)cinnamamide (**16a**).* CAD: 0.024 g of *trans*-cinnamic acid; purification: Method A; yield: 0.032 g (64%); mp 207.0–208.0; IR (ATR, ν/cm^{-1}) 3352, 3284, 3089, 3048, 2896, 2851, 1659, 1621, 1568, 1510, 1451, 1388, 1354, 1319, 1284, 1246, 1206, 1176, 1075, 1015, 973, 899, 871, 741, 714, 643, 612, 587, 557, 487; ^{1}H NMR (DMSO-d_6) δ 11.54 (s, 1H), 8.69 (t, 1H, J = 5.7 Hz), 8.19 (d, 1H, J = 7.8 Hz), 7.88 (s, 1H), 7.60-7.58 (m, 3H), 7.53-7.49 (m, 2H), 7.44-7.41 (m, 2H), 7.39-7.37 (m, 1H), 7.21 (t, 1H, J = 7.4 Hz), 6.81 (d, 1H, J = 15.8 Hz), 4.62 (d, 2H, J = 5.8 Hz), 2.78 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 164.89, 146.07, 141.27, 140.79, 138.77, 134.98, 133.55, 129.41, 128.92, 127.86, 127.79, 127.52, 122.38, 121.70, 120.99, 119.17, 111.93, 110.08, 44.64, 20.26; ESI-MS: m/z 342.4 (M+1)$^+$; HPLC purity 97.1%.

*4.1.11.2. (E)-3-(3-fluorophenyl)-N-((1-methyl-9H-pyrido[3,4-b]indol-3-yl)methyl)acrylamide (**16b**).* CAD: 0.027 g of *m*-fluorocinnamic acid; purification: Method A; yield: 0.022 g (41%); mp 118.0–119.0 °C; IR (ATR, ν/cm^{-1}) 3242, 3080, 2972, 2907, 2783, 1658, 1620, 1571, 1501, 1451, 1420, 1341, 1247, 1144, 1032, 983, 899, 859, 779, 740, 632, 561, 517; ^{1}H NMR (DMSO-d_6) δ 11.52 (s, 1H), 8.69 (t, 1H, J = 5.5 Hz), 8.18 (d, 1H, J = 7.4 Hz), 7.87 (s, 1H), 7.59-7.42 (m, 6H), 7.24-7.19 (m, 2H), 6.85 (d, 1H, J = 15.7 Hz), 4.61 (d, 2H, J = 4.3 Hz), 2.77 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 164.59, 162.46 (d, J = 243.9 Hz), 146.03, 141.35, 140.75, 137.63 (d, J = 7.9 Hz), 137.47, 133.57, 130.89 (d, J = 8.3 Hz), 127.83, 127.73, 123.97, 123.67, 121.68, 121.00, 119.15, 116.07 (d, J = 21.4 Hz), 113.92 (d, J = 21.8 Hz), 111.94, 110.07, 44.73, 20.34; ESI-MS: m/z 360.3 (M+1)$^+$; Anal. Calcd. for $C_{22}H_{18}FN_3O$: C, 73.52; H, 5.05; N, 11.69; found: C, 73.35; H, 5.27; N, 11.83.

*4.1.11.3. (E)-3-(3-bromophenyl)-N-((1-methyl-9H-pyrido[3,4-b]indol-3-yl)methyl)acrylamide (**16c**).* CAD: 0.037 g of *m*-bromocinnamic acid; purification: Method A; yield: 0.018 g (28%); mp 121.5–123.5 °C; IR (ATR, ν/cm^{-1}) 3243, 3082, 1743, 1725, 1655, 1617, 1562, 1501, 1452, 1419, 1470, 1396, 1340, 1248, 1234, 1150, 1069, 1029, 984, 899, 863, 779, 741, 697, 666, 638, 609, 561; ^{1}H NMR (DMSO-d_6) δ 11.54 (s, 1H), 8.68 (t, 1H, J = 5.8 Hz), 8.18 (d, 1H, J = 7.9 Hz), 7.87 (s, 1H), 7.80 (t, 1H, J = 1.8 Hz), 7.61-7.56 (m, 3H), 7.52 (t, 1H, J = 7.4 Hz), 7.47 (d, 1H, J = 15.8 Hz), 7.39 (t, 1H, J = 7.9 Hz), 7.21 (t, 1H, J = 7.4 Hz), 6.87 (d, 1H, J = 15.8 Hz), 4.62 (d, 2H, J = 5.8 Hz), 2.78 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 164.53, 146.00, 141.36, 140.76, 137.60, 137.12, 133.58, 131.93, 131.03, 130.03, 127.83, 127.73, 126.42, 124.09, 122.26, 121.68, 121.00, 119.15, 111.94, 110.06, 44.73, 20.34; ESI-MS: m/z 420.0 (M+1)$^+$, 422.0 (M+1)$^+$; HPLC purity 98.3%.

*4.1.11.4. (E)-N-((1-methyl-9H-pyrido[3,4-b]indol-3-yl)methyl)-3-(3-(trifluoromethyl)phenyl)acrylamide (**16d**).* CAD: 0.035 g of *m*-(trifluoromethyl)cinnamic acid; purification: Method B; yield: 0.023 g

(39%); mp 235.5–237.0 °C (decomp.); IR (ATR, ν/cm^{-1}) 3339, 3251, 3084, 2992, 2950, 2893, 2855, 2786, 1664, 1622, 1570, 1517, 1470, 1451, 1396, 1331, 1284, 1249, 1229, 1165, 1122, 1095, 1072, 1020, 969, 937, 899, 864, 803, 777, 738, 695, 657, 596, 559, 524, 507; ^{1}H NMR (DMSO-d_6) δ 11.53 (s, 1H), 8.70 (t, 1H, J = 5.8 Hz), 8.18 (d, 1H, J = 7.9 Hz), 7.95 (t, 1H, J = 2.0 Hz), 7.90 (d, 1H, J = 7.8 Hz), 7.88 (s, 1H), 7.74 (d, 1H, J = 7.8 Hz), 7.67 (t, 1H, J = 7.8 Hz), 7.61-7.58 (m, 2H), 7.54-7.51 (m, 1H), 7.22-7.20 (m, 1H), 6.96 (d, 1H, J = 15.9 Hz), 4.63 (d, 2H, J = 5.8 Hz), 2.78 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 164.49, 145.97, 141.37, 140.77, 137.06, 136.23, 133.58, 131.31, 130.07, 129.73 (q, J = 31.7 Hz), 127.85, 127.75, 125.69 (q, J = 3.5 Hz), 124.06 (q, J = 272.8 Hz), 124.56, 123.88 (q, J = 3.5 Hz), 121.69, 121.01, 119.16, 111.95, 110.07, 44.74, 20.34; ESI-MS: m/z 410.1 (M+1)$^+$; HPLC purity 98.4%.

*4.1.11.5. E)-3-(4-fluorophenyl)-N-((1-methyl-9H-pyrido[3,4-b]indol-3-yl)methyl)acrylamide (**16e**).* CAD: 0.027 g of *p*-fluorocinnamic acid; purification: Method A; yield: 0.018 g (34%); mp 217.5–219.5 °C (decomp.); IR (ATR, ν/cm^{-1}) 3243, 3077, 2993, 2950, 2913, 2890, 2851, 2785, 1655, 1620, 1597, 1571, 1553, 1507, 1451, 1416, 1394, 1341, 1318, 1282, 1249, 1226, 1156, 1124, 1094, 1033, 1011, 984, 933, 899, 859, 830, 788, 739, 693, 643, 608, 591, 565, 506, 462; ^{1}H NMR (DMSO-d_6) δ 11.52 (s, 1H), 8.67 (t, 1H, J = 5.1 Hz), 8.18 (d, 1H, J = 7.8 Hz), 7.87 (s, 1H), 7.67-7.63 (m, 2H), 7.59-7.48 (m, 3H), 7.26 (t, 2H, J = 8.7 Hz), 7.20 (t, 1H, J = 7.4 Hz), 6.75 (d, 1H, J = 15.8 Hz), 4.61 (d, 2H, J = 5.5 Hz), 2.77 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 164.81, 162.66 (d, J = 246.9 Hz), 146.14, 141.32, 140.75, 137.57, 133.56, 131.62 (d, J = 3.2 Hz), 129.66 (d, J = 8.4 Hz), 127.82, 127.72, 122.28, 121.68, 121.00, 119.14, 115.90 (d, J = 21.7 Hz), 111.93, 110.04, 44.70, 20.33; ESI-MS: m/z 360.1 (M+1)$^+$; HPLC purity 98.8%.

*4.1.11.6. (E)-3-(4-chlorophenyl)-N-((1-methyl-9H-pyrido[3,4-b]indol-3-yl)methyl)acrylamide (**16f**).* CAD: 0.030 g of *p*-chlorocinnamic acid; purification: Method A; yield: 0.012 g (21%); mp 255.0–257.5 °C (decomp.); IR (ATR, ν/cm^{-1}) 3649, 3239, 3055, 2918, 2874, 2798, 1982, 1925, 1897, 1662, 1624, 1551, 1502, 1454, 1404, 1318, 1283, 1251, 1220, 1176, 1088, 1044, 1011, 976, 902, 867, 815, 735, 646, 590, 550, 497; ^{1}H NMR (DMSO-d_6) δ 11.53 (s, 1H), 8.70 (t, 1H, J = 5.8 Hz), 8.18 (d, 1H, J = 7.9 Hz), 7.87 (s, 1H), 7.63-7.56 (m, 3H), 7.54-7.46 (m, 4H), 7.23-7.18 (m, 1H), 6.81 (d, 1H, J = 15.8 Hz), 4.61 (d, 2H, J = 5.7 Hz), 2.77 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 164.67, 146.08, 141.33, 140.75, 137.41, 133.96, 133.82, 133.56, 129.22, 128.97, 127.82, 127.72, 123.19, 121.68, 121.00, 119.14, 111.93, 110.06, 44.72, 20.33; ESI-MS: m/z 374.0 (M − 1)$^-$; Anal. Calcd. for $C_{22}H_{18}ClN_3O$: C, 70.30; H, 4.83; N, 11.18; found: C, 70.39; H, 4.65; N, 11.37.

*4.1.11.7. (E)-3-(4-methoxyphenyl)-N-((1-methyl-9H-pyrido[3,4-b]indol-3-yl)methyl)acrylamide (**16g**).* CAD: 0.029 g of *p*-methoxycinnamic acid; purification: Method A; yield: 0.028 g (51%); mp 263.0–264.0 °C (decomp.); IR (ATR, ν/cm^{-1}) 3249, 3162, 3079, 2964, 2910, 2835, 2787, 2684, 1652, 1603, 1555, 1512, 1454, 1419, 1345, 1287, 1251, 1175, 1109, 1026, 987, 932, 900, 873, 827, 778, 744, 592, 549, 520; ^{1}H NMR (DMSO-d_6) δ 11.54 (s, 1H), 8.59 (t, 1H, J = 5.6 Hz), 8.18 (d, 1H, J = 7.9 Hz), 7.87 (s, 1H), 7.59-7.50 (m, 4H), 7.45 (d, 1H, J = 15.8 Hz), 7.20 (t, 1H, J = 7.5 Hz), 6.98 (d, 2H, J = 8.2 Hz), 6.66 (d, 1H, J = 15.8 Hz), 4.61 (d, 2H, J = 5.9 Hz), 3.79 (s, 3H), 2.78 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 165.20, 160.29, 146.31, 141.29, 140.75, 138.47, 133.54, 129.09, 127.81, 127.73, 127.55, 121.68, 121.01, 119.89, 119.14, 114.38, 111.93, 109.98, 55.25, 44.66, 20.33; ESI-MS: m/z 372.3 (M+1)$^+$; Anal. Calcd. for $C_{23}H_{21}N_3O_2$: C, 74.37; H, 5.70; N, 11.31; found: C, 74.19; H, 5.93; N, 11.54.

*4.1.11.8. (E)-N-((1-methyl-9H-pyrido[3,4-b]indol-3-yl)methyl)-3-(4-(trifluoromethyl)phenyl)acrylamide (**16h**).* CAD: 0.035 g of *p*-(trifluoromethyl)cinnamic acid; purification: Method A; yield: 0.026 g (43%); mp 264.5–266.5 °C; IR (ATR, ν/cm^{-1}) 3339, 3263, 3058, 2920, 1666, 1626, 1575, 1496, 1452, 1413, 1324, 1248, 1164, 1120, 1065, 1012, 972, 900, 858, 822, 778, 737, 702, 586, 522, 491; ^{1}H NMR (DMSO-d_6) δ 11.54 (s, 1H), 8.79 (t, 1H, J = 5.8 Hz), 8.18 (d, 1H, J = 7.9 Hz), 7.88 (s, 1H), 7.82-7.76 (m, 4H), 7.59-7.49 (m, 3H), 7.23-7.18 (m, 1H), 6.94 (d, 1H, J = 15.8 Hz), 4.63 (d, 2H, J = 5.7 Hz), 2.77 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 164.43, 145.97, 141.38, 140.77, 139.09, 137.12, 133.59, 129.17 (q, J = 31.8 Hz), 128.16, 127.84, 127.74, 125.81 (q, J = 3.9 Hz), 125.20, 124.13 (q, J = 272.0 Hz), 121.70, 121.01, 119.16, 111.95, 110.14, 44.78, 20.34; ESI-MS: m/z 410.1 (M+1)$^+$; HPLC purity 97.3%.

*4.1.12. Synthesis of 1-methyl-6-(prop-2-yn-1-yloxy)-9H-pyrido[3,4-b]indole (**19**)*

To a solution of compound **18** (0.200 g, 1.009 mmol) in dry DMF (5 mL), under argon atmosphere caesium carbonate (0.460 g, 1.413 mmol) was added, followed by the dropwise addition of propargyl bromide (0.135 mL, 1.211 mmol, 80% solution in toluene). The reaction mixture was stirred at rt for 6 h. Upon completion, reaction mixture was diluted with H_2O (50 mL) and extracted with EtOAc (3 × 40 mL). The collected organic layers were washed with H_2O (2 × 100 mL), dried over anhydrous sodium sulphate and the solvent was evaporated under the reduced pressure. After purification by column chromatography (DCM/MeOH = 97:3 → 95:5) and trituration with diethyl ether, **19** was obtained as a white solid. Yield: 0.122 g (51%); mp 175.5–177.5 °C; IR (ATR, ν/cm^{-1}) 3292, 3122, 3054, 2950, 2860, 2766, 2716, 2366, 2330, 2128, 2058, 1866, 1764, 1736, 1606, 1570, 1506, 1476, 1448, 1412, 1380, 1334, 1292, 1260, 1200, 1120, 1068, 1030, 986, 944, 914, 886, 818, 758, 646, 524; ^{1}H NMR (DMSO-d_6) δ 11.42 (s, 1H), 8.17 (d, 1H, J = 5.4 Hz), 7.88 (d, 1H, J = 5.3 Hz), 7.81 (d, 1H, J = 2.5 Hz), 7.53 (d, 1H, J = 8.9 Hz), 7.23 (dd, 1H, J = 8.8, 2.5 Hz), 4.88 (d, 2H, J = 2.4 Hz), 3.56 (t, 1H, J = 2.4 Hz), 2.75 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 151.09, 142.26, 137.03, 135.67, 135.12, 126.63, 121.27, 118.34, 112.72, 112.62, 105.62, 78.04, 56.26, 20.40; ESI-MS: m/z 237.1 (M+1)$^+$.

*4.1.13. General procedure for the synthesis of harmicines **20a-e***

To a solution of alkyne **19** (0.050 g, 0.212 mmol) and the corresponding cinnamyl azide **13a-e** (0.254 mmol) in MeOH (5 mL), catalytic amount of $Cu(OAc)_2$ was added. The reaction mixture was stirred at rt for 24 h. Upon completion of the reaction, the solvent was removed under the reduced pressure. The residue was purified by column chromatography with DCM/MeOH or cyclohexane/EtOAc/MeOH as a mobile phase. The crude product was triturated with diethyl ether/petroleum ether mixture to obtain harmicines **20a-e**.

*4.1.13.1. 6-((1-Cinnamyl-1H-1,2,3-triazol-4-yl)methoxy)-1-methyl-9H-pyrido[3,4-b]indole (**20a**).* **13a**:0.040 g; mobile phase: DCM/MeOH 8:1; yield: 0.059 g (70%); mp 205.0–206.5 °C; IR (ATR, ν/cm^{-1}) 3136, 3120, 3030, 2945, 2856, 2760, 2693, 2676, 1632, 1604, 1579, 1565, 1504, 1481, 1460, 1412, 1389, 1361, 1335, 1289, 1237, 1202, 1123, 1054, 1034, 1002, 967, 888, 858, 817, 780, 755, 725, 705, 694, 625, 584, 520, 502; ^{1}H NMR (DMSO-d_6) δ 11.39 (s, 1H), 8.30 (s, 1H), 8.16 (d, 1H, J = 5.3 Hz), 7.91-7.89 (m, 2H), 7.51 (d, 1H, J = 8.8 Hz), 7.45-7.22 (m, 6H), 6.65-6.49 (m, 2H), 5.26 (s, 2H), 5.21 (d, 2H, J = 6.3 Hz), 2.74 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 151.94, 143.26, 142.22, 136.98, 135.70, 135.42, 135.10, 133.62, 128.68, 128.14, 126.70, 126.56, 124.43, 123.72, 121.36, 118.35, 112.76, 112.69, 105.12, 61.89, 51.34, 20.42; ESI-MS: m/z 396.3 (M+1)$^+$; Anal. Calcd. for $C_{24}H_{21}N_5O$: C, 72.89; H, 5.35; N, 17.71; found: C, 72.74; H, 5.52; N, 17.56.

*4.1.13.2. (E)-6-((1-(3-(3-fluorophenyl)allyl)-1H-1,2,3-triazol-4-yl)methoxy)-1-methyl-9H-pyrido[3,4-b]indole (**20b**).* **13b**: 0.045 g; mobile phase: cyclohexane/EtOAc/MeOH 1:1:0.5 and DCM/MeOH 9:1; yield: 0.039 g (44%); mp 145.0–147.0 °C; IR (ATR, ν/cm^{-1}) 3357,

3293, 3166, 3071, 3052, 2975, 2937, 2900, 2866, 1601, 1583, 1568, 1493, 1473, 1459, 1404, 1379, 1365, 1330, 1286, 1262, 1207, 1149, 1120, 1067, 1054, 1039, 976, 938, 884, 847, 812, 792, 776, 752, 736, 715, 675, 641, 620, 593, 561, 528, 493, 456; ^{1}H NMR (DMSO-d_6) δ 11.41 (s, 1H), 8.31 (s, 1H), 8.17 (d, 1H, J = 5.4 Hz), 7.91-7.89 (m, 2H), 7.52 (d, 1H, J = 8.8 Hz), 7.39-7.32 (m, 2H), 7.27-7.23 (m, 2H), 7.13-7.08 (m, 1H), 6.64-6.62 (m, 2H), 5.26 (s, 2H), 5.23-5.21 (m, 2H), 2.75 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 162.49 (d, J = 243.3 Hz), 151.97, 143.26, 142.16, 138.36 (d, J = 7.9 Hz), 136.80, 135.48, 135.07, 132.34, 130.58 (d, J = 8.5 Hz), 126.78, 125.50, 124.47, 130.00 (d, J = 1.7 Hz), 121.34, 118.42, 114.81 (d, J = 21.3 Hz), 112.80 (d, J = 17.4 Hz), 112.77, 112.67, 105.11, 61.89, 51.16, 20.32; ESI-MS: m/z 414.2 $(M+1)^+$; Anal. Calcd. for $C_{24}H_{20}FN_5O$: C, 69.72; H, 4.88; N, 16.94; found: C, 69.47; H, 4.61; N, 17.15.

*4.1.13.3. (E)-6-((1-(3-(3-bromophenyl)allyl)-1H-1,2,3-triazol-4-yl) methoxy)-1-methyl-9H-pyrido[3,4-b]indole (**20c**).* **13c**: 0.060 g; mobile phase: cyclohexane/EtOAc/MeOH 3:1:0.75; yield: 0.051 g (50%); mp 181.5–183.0 °C; IR (ATR, ν/cm^{-1}) 3252, 3198, 3151, 3089, 3064, 3039, 3015, 2965, 2945, 2916, 2892, 1601, 1584, 1566, 1495, 1475, 1422, 1400, 1373, 1350, 1337, 1289, 1256, 1233, 1217, 1199, 1129, 1095, 1066, 1052, 1033, 1008, 977, 944, 882, 857, 807, 782, 738, 707, 674, 620, 589, 563, 542, 479; ^{1}H NMR (DMSO-d_6) δ 11.40 (s, 1H), 8.31 (s, 1H), 8.16 (d, 1H, J = 5.4 Hz), 7.92-7.89 (m, 2H), 7.71 (t, 1H, J = 2.0 Hz), 7.51 (d, 1H, J = 8.8 Hz), 7.48-7.43 (m, 2H), 7.30 (t, 1H, J = 7.8 Hz), 7.24 (dd, 1H, J = 8.8, 2.5 Hz), 6.68-6.58 (m, 2H), 5.26 (s, 2H), 5.21 (d, 2H, J = 4.6 Hz), 2.74 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 151.98, 143.28, 142.20, 138.30, 136.89, 135.46, 135.09, 132.03, 130.77, 129.05, 126.75, 125.67, 124.47, 122.17, 121.36, 118.40, 112.78, 112.71, 105.10, 61.91, 51.20, 20.37; ESI-MS: m/z 474.2 $(M+1)^+$, 476.2 $(M+1)^+$. Anal. Calcd. for $C_{24}H_{20}BrN_5O$: C, 60.77; H, 4.25; N, 14.76; C, 60.92; H, 4.43; N, 14.56.

*4.1.13.4. (E)-6-((1-(3-(4-chlorophenyl)allyl)-1H-1,2,3-triazol-4-yl) methoxy)-1-methyl-9H-pyrido[3,4-b]indole (**20d**).* **13d**: 0.049 g; mobile phase: cyclohexane/EtOAc/MeOH 1:1:0.5; yield: 0.046 g (51%); mp 195.0–196.5 °C; IR (ATR, ν/cm^{-1}) 3208, 3136, 3089, 3069, 2969, 2923, 2893, 2870, 2802, 1602, 1582, 1567, 1494, 1480, 1459, 1405, 1385, 1340, 1289, 1257, 1237, 1207, 1163, 1127, 1094, 1054, 1033, 1015, 987, 971, 950, 884, 857, 843, 818, 742, 702, 687, 662, 623, 583, 563, 533, 487; ^{1}H NMR (DMSO-d_6) δ 11.39 (s, 1H), 8.30 (s, 1H), 8.16 (d, 1H, J = 5.4 Hz), 7.91-7.89 (m, 2H), 7.51 (d, 1H, J = 8.8 Hz), 7.47-7.44 (m, 2H), 7.39-7.36 (m, 2H), 7.23 (dd, 1H, J = 8.9, 2.5 Hz), 6.62-6.51 (m, 2H), 5.26 (s, 2H), 5.21 (d, 2H, J = 5.1 Hz), 2.74 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 151.93, 143.27, 142.22, 136.98, 135.44, 135.10, 134.68, 132.51, 132.23, 128.65, 128.28, 126.71, 124.77, 124.48, 121.36, 118.38, 112.76, 112.69, 105.15, 61.89, 51.23, 20.41; ESI-MS: m/z 430.4 $(M+1)^+$; Anal. Calcd. for $C_{24}H_{20}ClN_5O$: C, 67.05; H, 4.69; N, 16.29; found: C, 67.22; H, 4.78; N, 16.31.

*4.1.13.5. (E)-6-((1-(3-(4-methoxyphenyl)allyl)-1H-1,2,3-triazol-4-yl) methoxy)-1-methyl-9H-pyrido[3,4-b]indole (**20e**).* **13e**: 0.048 g; mobile phase: cyclohexane/EtOAc/MeOH 1:1:0.5; yield: 0.069 g (77%); mp 200.0–203.0 °C; IR (ATR, ν/cm^{-1}) 3208, 3138, 3069, 2953, 2929, 2894, 2873, 2836, 2802, 1743, 1607, 1582, 1567, 1513, 1498, 1480, 1460, 1441, 1405, 1386, 1337, 1289, 1257, 1238, 1207, 1175, 1127, 1109, 1054, 1032, 1015, 987, 968, 884, 856, 819, 760, 741, 703, 666, 624, 582, 563, 529, 498, 477; ^{1}H NMR (DMSO- d_6) δ 11.39 (s, 1H), 8.28 (s, 1H), 8.16 (d, 1H, J = 5.4 Hz), 7.92-7.89 (m, 2H), 7.51 (d, 1H, J = 8.8 Hz), 7.37 (d, 2H, J = 8.2 Hz), 7.23 (d, 1H, J = 8.9 Hz), 6.88 (d, 2H, J = 8.2 Hz), 6.58 (d, 1H, J = 15.8 Hz), 6.39-6.32 (m, 1H), 5.25 (s, 2H), 5.16 (d, 2H, J = 6.5 Hz), 3.75 (s, 3H), 2.74 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 159.22, 151.94, 143.23, 142.21, 136.98, 135.42, 135.09, 133.36, 128.30, 127.89, 126.70, 124.31, 121.36, 121.11, 118.34, 114.06, 112.75, 112.68, 105.12, 61.89, 55.11, 51.46, 20.41; ESI-MS: m/z 426.3 $(M+1)^+$; Anal. Calcd. for $C_{25}H_{23}N_5O_2$: C, 70.57; H, 5.45; N, 16.46; found: C, 70.72; H, 5.63; N, 16.65.

*4.1.14. Tert-butyl (2-((1-methyl-9H-pyrido[3,4-b]indol-6-yl)oxy) ethyl)carbamate (**21**)*

To a stirred solution of **18** (0.647 g, 3.264 mmol) in dry DMF (6 mL), under argon atmosphere, caesium carbonate (2.978 g, 9.139 mmol) and tetrabutylammonium hydrogensulphate (0.887 g, 2.611 mmol) was added. The resulting suspension was stirred at rt for 20 min, followed by the addition of 2-(*tert*-butoxycarbonylamino)ethyl bromide (2.926 g, 13.056 mmol). The reaction mixture was stirred at rt for 24 h, poured into H_2O (50 mL) and extracted with EtOAc (3 × 50 mL). The collected organic layers were washed with H_2O, filtered through phase separator and evaporated under the reduced pressure. After purification by column chromatography (DCM/MeOH 8:1) and trituration with diethyl ether/petroleum ether mixture, compound **21** was obtained. Yield: 0.624 g (56%); ^{1}H NMR (DMSO-d_6) δ 11.42 (s, 1H), 8.16 (d, 1H, J = 5.3 Hz), 7.92 (d, 1H, J = 5.3 Hz), 7.77 (d, 1H, J = 1.7 Hz), 7.51 (d, 1H, J = 8.8 Hz), 7.18 (dd, 1H, J = 8.8, 2.3 Hz), 7.06 (t, 1H, J = 5.0 Hz), 4.05 (t, 2H, J = 5.8 Hz), 3.39-3.34 (m, 2H), 2.75 (s, 3H), 1.40 (s, 9H); ^{13}C NMR (DMSO-d_6) δ 155.73, 152.39, 142.04, 136.57, 135.41, 135.03, 126.88, 121.35, 118.42, 112.78, 112.75, 104.68, 77.76, 67.19, 39.61, 28.24, 20.23; ESI-MS: m/z 342.4 $(M+1)^+$.

*4.1.15. 2-((1-Methyl-9H-pyrido[3,4-b]indol-6-yl)oxy)ethan-1-amine (**22**)*

A solution of the **21** (0.642 g, 1.880 mmol) and 4.7 mL 4 M HCl (18.80 mmol) in EtOAc (6 mL) was stirred at 50 °C for 18 h. Upon completion, solvent was removed under the reduced pressure. The residue was dissolved in H_2O (20 mL), basified to pH 11 with 5% NaOH. The resulting precipitate was filtered off. After trituration with diethyl ether, 0.313 g, (69%) of **22** was obtained; mp 170.5–172.0 °C; IR (ATR, ν/cm^{-1}) 3645, 3359, 3241, 3065, 2925, 2869, 1605, 1581, 1566, 1500, 1478, 1458, 1401, 1288, 1234, 1211, 1126, 1071, 1059, 992, 905, 884, 847, 825, 816, 741, 703, 632; ^{1}H NMR (DMSO-d_6) δ 11.36 (s, 1H), 8.15 (d, 1H, J = 5.3 Hz), 7.90 (d, 1H, J = 5.3 Hz), 7.74 (d, 1H, J = 2.3 Hz), 7.50 (d, 1H, J = 8.8 Hz), 7.19 (dd, 1H, J = 8.8, 2.5 Hz), 4.02 (t, 2H, J = 5.8 Hz), 2.94 (t, 2H, J = 5.7 Hz), 2.74 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 152.47, 141.87, 136.70, 135.19, 134.93, 126.54, 121.29, 117.98, 112.34, 112.18, 104.86, 71.06, 40.87, 19.97; ESI-MS: m/z 242.2 $(M+1)^+$.

*4.1.16. General procedure for the synthesis of harmicines **23a-h***

A solution of a corresponding CAD (0.249 mmol), DIEA (0.086 mL, 0.498 mmol) and HATU (0.095 g, 0.249 mmol) in DCM (4 mL) was stirred at rt for 20 min, followed by the addition of amine **22** (0.050 g, 0.208 mmol). The resulting solution was stirred at rt for 1 h. The formed precipitate was filtered off. After purification by column chromatography (DCM/MeOH 8:1) and trituration with diethyl ether/petroleum ether mixture compounds **23a-h** were obtained.

*4.1.16.1. N-(2-((1-methyl-9H-pyrido[3,4-b]indol-6-yl)oxy)ethyl)cinnamamide (**23a**).* CAD: 0.037 g of *trans*-cinnamic acid; yield: 0.045 g (58%); mp 128.0–129.5 °C; IR (ATR, ν/cm^{-1}) 3640, 3220, 3062, 2986, 2938, 2811, 2609, 1871, 1665, 1622, 1545, 1502, 1458, 1422, 1343, 1286, 1209, 1121, 1074, 970, 897, 808, 766, 714, 659, 630, 574, 512, 483; ^{1}H NMR (DMSO-d_6) δ 11.39 (s, 1H), 8.45 (t, 1H, J = 5.6 Hz), 8.16 (d, 1H, J = 5.3 Hz), 7.91 (d, 1H, J = 5.3 Hz), 7.81 (d, 1H, J = 2.5 Hz), 7.57 (d, 2H, J = 7.3 Hz), 7.53-7.46 (m, 2H), 7.44-7.36 (m, 3H), 7.22 (dd, 1H, J = 8.9, 2.6 Hz), 6.73 (d, 1H, J = 15.9 Hz), 4.17 (t, 2H, J = 5.6 Hz), 3.64 (q, 2H, J = 5.6 Hz), 2.74 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 165.27, 152.37, 142.04, 138.83, 136.54, 135.46, 135.03, 134.89, 129.45, 128.93, 127.52, 126.90, 122.07, 121.37, 118.43, 112.79, 112.77,

104.73, 67.12, 38.67, 20.20; ESI-MS: *m/z* 372.0 $(M+1)^+$; HPLC purity > 99.5%.

*4.1.16.2. (E)-3-(3-fluorophenyl)-N-(2-((1-methyl-9H-pyrido[3,4-b]indol-6-yl)oxy)ethyl)acrylamide (**23b**).* CAD: 0.041 g of *m*-fluorocinnamic acid; yield: 0.062 g (76%); mp 225.0–226.0 °C; IR (ATR, ν/cm^{-1}) 3658, 3236, 3068, 2974, 2936, 2882, 2822, 2608, 1871, 1666, 1625, 1585, 1551, 1501, 1448, 1344, 1286, 1248, 1207, 1146, 1075, 1037, 974, 902, 856, 807, 738, 666, 630, 573, 519, 467; ^{1}H NMR (DMSO-d_6) δ 11.38 (s, 1H), 8.46 (t, 1H, J = 5.5 Hz), 8.16 (d, 1H J = 5.4 Hz), 7.91 (d, 1H, J = 5.3 Hz), 7.80 (d, 1H, J = 2.4 Hz), 7.51 (d, 1H, J = 8.9 Hz), 7.50-7.40 (m, 4H), 7.24-7.19 (m, 2H), 6.77 (d, 1H, J = 15.8 Hz), 4.17 (t, 2H, J = 5.5 Hz), 3.64 (q, 2H, J = 5.5 Hz), 2.74 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 164.99, 162.45 (d, J = 243.8 Hz), 152.32, 142.15, 137.54, 137.49, 136.83, 135.37, 135.07, 130.89 (d, J = 8.4 Hz), 126.76, 123.65, 121.40, 118.29, 116.11 (d, J = 21.2 Hz), 113.93 (d, J = 21.8 Hz), 112.74, 112.72, 104.73, 67.07, 38.71, 20.34; ESI-MS: *m/z* 390.4 $(M+1)^+$; HPLC purity > 99.5%.

*4.1.16.3. (E)-3-(3-bromophenyl)-N-(2-((1-methyl-9H-pyrido[3,4-b]indol-6-yl)oxy)ethyl)acrylamide (**23c**).* CAD: 0.057 g of *m*-bromocinnamic acid; yield: 0.062 g (77%); mp 230.5–232.5 °C; IR (ATR, ν/cm^{-1}) 3646, 3171, 3062, 2975, 2939, 2879, 2792, 2613, 1664, 1620, 1566, 1501, 1460, 1384, 1345, 1287, 1199, 1110, 1072, 970, 856, 811, 781, 736, 667, 631, 561; ^{1}H NMR (DMSO-d_6) δ 11.40 (s, 1H), 8.43 (t, 1H, J = 5.5 Hz), 8.16 (d, 1H, J = 5.4 Hz), 7.92 (d, 1H, J = 5.4 Hz), 7.81 (d, 1H, J = 2.3 Hz), 7.79 (t, 1H, J = 1.8 Hz), 7.59-7.56 (m, 2H), 7.52 (d, 1H, J = 8.8 Hz), 7.45 (d, 1H, J = 15.8 Hz), 7.38 (t, 1H, J = 7.9 Hz), 7.22 (dd, 1H, J = 8.8, 2.4 Hz), 6.78 (d, 1H, J = 15.8 Hz), 4.17 (t, 2H, J = 5.5 Hz), 3.64 (q, 2H, J = 5.4 Hz), 2.75 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 164.92, 152.33, 142.09, 137.50, 137.19, 136.68, 135.42, 135.05, 131.97, 131.02, 130.04, 126.83, 126.40, 123.76, 122.25, 121.38, 118.37, 112.76, 104.74, 67.08, 38.71, 20.27; ESI-MS: *m/z* 450.3 $(M+1)^+$, 452.3 $(M+1)^+$; HPLC purity > 99.5%.

*4.1.16.4. (E)-N-(2-((1-methyl-9H-pyrido[3,4-b]indol-6-yl)oxy)ethyl)-3-(3-(trifluoromethyl)phenyl)acrylamide (**23d**).* CAD: 0.054 g of *p*-(trifluoromethyl)cinnamic acid; yield: 0.058 g (64%); mp 216.5–218.0 °C; IR (ATR, ν/cm^{-1}) 3676, 3222, 3100, 3067, 2988, 2937, 2881, 2820, 2611, 1665, 1624, 1545, 1501, 1440, 1331, 1286, 1200, 1167, 1117, 1073, 1031, 971, 863, 805, 738, 691, 658, 630, 574, 465; ^{1}H NMR (DMSO-d_6) δ 11.41 (s, 1H), 8.46 (t, 1H, J = 5.6 Hz), 8.16 (d, 1H, J = 5.4 Hz), 7.93-7.88 (m, 3H), 7.81 (d, 1H, J = 2.6 Hz), 7.73 (d, 1H, J = 7.8 Hz), 7.66 (t, 1H, J = 7.8 Hz), 7.57 (d, 1H, J = 15.8 Hz), 7.52 (d, 1H, J = 8.8 Hz), 7.22 (dd, 1H, J = 8.8, 2.5 Hz), 6.88 (d, 1H, J = 15.9 Hz), 4.18 (t, 2H, J = 5.5 Hz), 3.65 (q, 2H, J = 5.6 Hz), 2.75 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 164.88, 152.34, 142.10, 137.14, 136.69, 136.13, 135.43, 135.06, 131.30, 130.06, 129.73 (q, J = 32.3 Hz), 126.83, 125.73 (q, J = 3.0 Hz), 124.23, 124.04 (q, J = 273.7 Hz), 123.88 (q, J = 3.0 Hz), 121.38, 118.37, 112.76, 104.74, 67.09, 38.74, 20.27; ESI-MS: *m/z* 440.1 $(M+1)^+$; Anal. Calcd. for $C_{24}H_{20}F_3N_3O_2$: C, 65.60; H, 4.59; N, 9.56; found C, 65.80; H, 4.37; N, 9.71.

*4.1.16.5. (E)-3-(4-fluorophenyl)-N-(2-((1-methyl-9H-pyrido[3,4-b]indol-6-yl)oxy)ethyl)acrylamide (**23e**).* CAD: 0.041 g of *p*-fluorocinnamic acid; yield: 0.065 g (80%); mp 226.5–227.5 °C; IR (ATR, ν/cm^{-1}) 3659, 3311, 3225, 3099, 3045, 2990, 2938, 2165, 1870, 1667, 1625, 1542, 1502, 1422, 1347, 1286, 1208, 1160, 1122, 1074, 976, 897, 824, 740, 630, 576, 501, 459; ^{1}H NMR (DMSO-d_6) δ 11.40 (s, 1H), 8.43 (t, 1H, J = 5.4 Hz), 8.16 (d, 1H, J = 5.4 Hz), 7.92 (d, 1H, J = 5.3 Hz), 7.81 (d, 1H, J = 2.2 Hz), 7.66-7.62 (m, 2H), 7.53-7.46 (m, 2H), 7.28-7.21 (m, 3H), 6.68 (d, 1H, J = 15.8 Hz), 4.17 (t, 2H, J = 5.5 Hz), 3.64 (q, 2H, J = 5.4 Hz), 2.75 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 165.21, 162.68 (d, J = 247.0 Hz), 152.35, 142.10, 137.65, 136.71, 135.41, 135.05, 131.53 (d, J = 2.6 Hz), 129.68 (d, J = 8.4 Hz), 126.82, 121.97, 121.39, 118.36, 115.90 (d, J = 21.7 Hz), 112.76, 104.72, 67.11, 38.68, 20.28; ESI-MS: *m/z* 390.4 $(M+1)^+$; HPLC purity > 99.5%.

*4.1.16.6. (E)-3-(4-chlorophenyl)-N-(2-((1-methyl-9H-pyrido[3,4-b]indol-6-yl)oxy)ethyl)acrylamide (**23f**).* CAD: 0.045 g of *p*-chlorocinnamic acid; yield: 0.071 g (84%); mp 228.5–229.5 °C; IR (ATR, ν/cm^{-1}) 3642, 3231, 3066, 2989, 2939, 2164, 2113, 1872, 1665, 1621, 1548, 1502, 1406, 1342, 1288, 1208, 1075, 976, 938, 897, 809, 742, 630, 575, 490; ^{1}H NMR (DMSO-d_6) δ 11.40 (s, 1H), 8.46 (t, 1H, J = 5.3 Hz), 8.16 (d, 1H, J = 5.3 Hz), 7.91 (d, 1H, J = 5.3 Hz), 7.80 (d, 1H, J = 2.0 Hz), 7.60 (d, 2H, J = 8.4 Hz), 7.51 (d, 1H, J = 8.9 Hz), 7.49-7.45 (m, 3H), 7.22 (dd, 1H, J = 8.8, 2.3 Hz), 6.73 (d, 1H, J = 15.8 Hz), 4.17 (t, 2H, J = 5.4 Hz), 3.64 (q, 2H, J = 5.3 Hz), 2.74 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 165.08, 152.33, 142.11, 137.49, 136.72, 135.41, 135.05, 133.87, 129.22, 128.97, 126.81, 122.88, 121.39, 118.35, 112.76, 104.73, 67.09, 38.70, 20.29; ESI-MS: *m/z* 406.3 $(M+1)^+$; Anal. Calcd. for $C_{23}H_{20}ClN_3O_2$: C, 68.06; H, 4.97; N, 10.35; found C, 68.16; H, 4.78; N, 10.25.

*4.1.16.7. (E)-3-(4-methoxyphenyl)-N-(2-((1-methyl-9H-pyrido[3,4-b]indol-6-yl)oxy)ethyl)acrylamide (**23g**).* CAD: 0.044 g of *p*-methoxycinnamic acid; yield: 0.063g (75%); mp 241.5–242.5 °C; IR (ATR, ν/cm^{-1}) 3210, 3133, 3058, 2971, 2941, 2873, 2775, 2601, 2164, 1983, 1886, 1651, 1602, 1554, 1514, 1461, 1425, 1353, 1284, 1230, 1205, 1175, 1125, 1064, 1033, 983, 862, 827, 761, 699, 624, 555, 521; ^{1}H NMR (DMSO-d_6) δ 11.39 (s, 1H), 8.34 (t, 1H, J = 5.5 Hz), 8.16 (d, 1H, J = 5.4 Hz), 7.91 (d, 1H, J = 5.3 Hz), 7.80 (d, 1H, J = 2.4 Hz), 7.53-7.50 (m, 3H), 7.43 (d, 1H, J = 15.8 Hz), 7.22 (dd, 1H, J = 8.8, 2.5 Hz), 6.98 (d, 2H, J = 8.8 Hz), 6.58 (d, 1H, J = 15.8 Hz), 4.16 (t, 2H, J = 5.6 Hz), 3.79 (s, 3H), 3.63 (q, 2H, J = 5.5 Hz), 2.74 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 165.59, 160.32, 152.35, 142.13, 138.54, 136.80, 135.37, 135.06, 129.09, 127.45, 126.77, 121.40, 119.58, 118.30, 114.38, 112.74, 104.71, 67.16, 55.24, 38.63, 20.33; ESI-MS: *m/z* 402.4 $(M+1)^+$; HPLC purity > 99.5%.

*4.1.16.8. (E)-N-(2-((1-methyl-9H-pyrido[3,4-b]indol-6-yl)oxy)ethyl)-3-(4-(trifluoromethyl)phenyl)acrylamide (**23h**).* CAD: 0.054 g of *p*-(trifluoromethyl)cinnamic acid; yield: 0.057 g (62%); mp 231.5–233.5 °C; IR (ATR, ν/cm^{-1}) 3645, 3269, 3061, 2932, 2875, 1667, 1628, 1559, 1501, 1461, 1417, 1384, 1328, 1286, 1234, 1202, 1166, 1119, 1067, 1016, 976, 953, 882, 829, 814, 728, 708, 614, 591, 562, 522, 495; ^{1}H NMR (DMSO-d_6) δ 11.41 (s, 1H), 8.54 (t, 1H, J = 5.5 Hz), 8.16 (d, 1H, J = 5.3 Hz), 7.92 (d, 1H, J = 5.3 Hz), 7.81-7.76 (m, 5H), 7.57-7.51 (m, 2H), 7.22 (dd, 1H, J = 8.9, 2.5 Hz), 6.86 (d, 1H, J = 15.8 Hz), 4.18 (t, 2H, J = 5.5 Hz), 3.65 (q, 2H, J = 5.4 Hz), 2.75 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 164.82, 152.34, 142.09, 139.00, 137.19, 136.65, 135.44, 135.05, 129.20 (q, J = 32.3 Hz), 128.16, 126.85, 125.80 (q, J = 3.0 Hz), 124.87, 124.11 (q, J = 272.7 Hz), 121.38, 118.39, 112.78, 104.74, 67.06, 38.75, 20.25; MS: *m/z* 440.1 $(M+1)^+$; Anal. Calcd. for $C_{24}H_{20}F_3N_3O_2$: C, 65.60; H, 4.59; N, 9.56; found: C, 65.72; H, 4.37; N, 9.21.

*4.1.17. General procedure for the synthesis of harmicines **27a,b***

Harmicines **27a**,**b** were prepared according to the published procedure [26,27]. In short, a solution of a corresponding CAD (0.182 mmol), DIEA (0.063 mL, 0.364 mmol) and HATU (0.069 g, 0.182 mmol) in DCM (4 mL) was stirred at rt for 20 min, followed by the addition of amine **26** (0.042 g, 0.165 mmol). The resulting solution was stirred at rt for 1 h. The formed precipitate was filtered off. After purification by column chromatography (DCM/MeOH 8:1) and trituration with diethyl ether/petroleum ether mixture compounds **27a**,**b** were obtained.

*4.1.17.1. (E)-N-(2-(7-methoxy-1-methyl-9H-pyrido[3,4-b]indol-9-yl)ethyl)-3-(3-(trifluoromethyl)phenyl)acrylamide (**27a**).* CAD: 0.039 g

of *m*-(trifluoromethyl)cinnamic acid; yield: 0.033 g (44%); mp 220.5–222.5 °C; IR (ATR, ν/cm^{-1}) 3177, 2978, 2930, 1679, 1623, 1570, 1500, 1450, 1409, 1377, 1343, 1328, 1281, 1270, 1253, 1221, 1199, 1185, 1169, 1139, 1114, 1096, 1079, 1043, 1018, 991, 981, 803, 694, 659, 556, 530; ^{1}H NMR (DMSO-d_6) δ 8.42 (t, 1H, J = 6.0 Hz), 8.18 (d, 1H, J = 5.2 Hz), 8.09 (d, 1H, J = 8.5 Hz), 7.91-7.87 (m, 3H), 7.74 (dd, 1H, J = 7.7, 1.6 Hz), 7.66 (t, 1H, J = 7.8 Hz), 7.54 (d, 1H, J = 15.8 Hz), 7.27 (d, 1H, J = 2.2 Hz), 6.87 (dd, 1H, J = 8.6, 2.2 Hz), 6.68 (d, 1H, J = 15.8 Hz), 4.68 (t, 2H, J = 7.0 Hz), 3.90 (s, 3H), 3.61 (q, 2H, J = 6.6 Hz), 2.99 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 165.35, 160.52, 142.92, 140.66, 137.87, 137.51, 135.98, 134.68, 131.26, 130.09, 129.76 (q, J = 31.7 Hz), 128.51, 125.86 (q, J = 3.6 Hz), 124.06 (q, J = 3.6 Hz), 124.04 (q, J = 272.5 Hz), 123.81, 122.41, 114.33, 112.29, 109.37, 93.57, 55.41, 43.37, 39.13, 23.10; ESI-MS: *m/z* 454.2 (M+1)$^+$; HPLC purity 98.2%.

*4.1.17.2. (E)-N-(2-(7-methoxy-1-methyl-9H-pyrido[3,4-b]indol-9-yl) ethyl)-3-(4-(trifluoromethyl)phenyl)acrylamide (**27b**).* CAD: 0.039 g of *p*-(trifluoromethyl)cinnamic acid; yield: 0.034 g (45%); mp 252.0–254.5 °C; IR (ATR, ν/cm^{-1}) 3188, 3005, 2962, 2844, 1680, 1633, 1569, 1445, 1412, 1321, 1253, 1199, 1165, 1108, 1066, 974, 831, 801, 597, 573; ^{1}H NMR (DMSO-d_6) δ 8.50 (t, 1H, J = 6.0 Hz), 8.18 (d, 1H, J = 5.1 Hz), 8.09 (d, 1H, J = 8.6 Hz), 7.89 (d, 1H, J = 5.2 Hz), 7.78 (s, 4H), 7.53 (d, 1H, J = 15.8 Hz), 7.27 (d, 1H, J = 2.2 Hz), 6.87 (dd, 1H, J = 8.6, 2.2 Hz), 6.68 (d, 1H, J = 15.8 Hz), 4.68 (t, 2H, J = 6.9 Hz), 3.90 (s, 3H), 3.61 (q, 2H, J = 6.7 Hz), 3.00 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 165.27, 160.52, 142.92, 140.62, 138.83, 137.83, 137.51, 134.64, 129.29 (q, J = 31.8 Hz), 128.52, 128.21, 125.81 (q, J = 3.5 Hz), 124.44, 124.09 (q, J = 272.0 Hz), 122.40, 114.30, 112.28, 109.40, 93.51, 55.40, 43.32, 39.13, 23.06; ESI-MS: *m/z* 454.1 (M+1)$^+$; HPLC purity 99.1%.

4.2. In vitro drug sensitivity assay against P. falciparum erythrocytic stages

Antiplasmodial activity of harmicines **5**, **7**, **14**–**16**, **20** and **23** was evaluated against two laboratory *P. falciparum* strains (3D7, CQ-sensitive strain, and Dd2, CQ-resistant strain), as previously described, using the histidine-rich protein 2 (HRP2) assay [23,24]. Briefly, 96-well plates were pre-coated with the tested compounds in a three-fold dilution before ring-stage parasites were added in complete culture medium at a haematocrit of 1.5% and a parasitaemia of 0.05%. After three days of incubation at 37 °C, 5% CO_2 and 5% oxygen, plates were frozen until analysed by HRP2-ELISA. All compounds were evaluated in duplicate in at least two independent experiments. The IC_{50} was determined by nonlinear regression analysis of log concentration-response curves using the drc-package v0.9.0 of R v2.6.1 [25].

4.3. In vitro activity against P. berghei hepatic stages

In vitro activity of harmicines **5**, **7**, **14**–**16**, **20** and **23** against the liver stage of *P. berghei* infection was assessed as previously described [26,27]. Briefly, Huh7 cells were routinely cultured in 1640 Roswell Park Memorial Institute (RPMI) medium supplemented with 10% (*v*/*v*) fetal bovine serum, 1% (*v*/*v*) glutamine, 1% (*v*/*v*) penicillin/streptomycin, 1% non-essential amino acids, and 10 mM 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethane-1-sulphonic acid (HEPES). For drug screening experiments, Huh7 cells were seeded at 1 × 10^4 cell/well of a 96-well plate and incubated overnight at 37 °C with 5% CO_2. 10 mM stock solutions of test compounds were prepared in DMSO and were serially diluted in infection medium, *i.e.* culture medium supplemented with gentamicin (50 μg/ml) and amphotericin B (0.8 μg/ml), to obtain the test concentrations. On the day of the infection, the culture medium was replaced by the serial dilutions of test compounds and incubated for 1 h at 37 °C with 5% CO_2. Next, 1 × 10^4 firefly luciferase-expressing *P. berghei* sporozoites, freshly isolated from the salivary glands of female infected *Anopheles stephensi* mosquitoes, were added to the cultures, plates were centrifuged at 1800×*g* for 5 min at room temperature and incubated at 37 °C with 5% CO_2. To assess the effect of each compound concentration on cell viability, cultures were incubated with Alamar Blue (Invitrogen, UK) at 46 h post infection (hpi), according to the manufacturer's recommendations. Parasite load was then assessed by a bioluminescence assay (Biotium, Fremont, CA, USA), using a multi-plate reader Infinite M200 (Tecan, Switzerland). Nonlinear regression analysis was employed to fit the normalized results of the dose-response curves, and IC_{50} values were determined using GraphPad Prism 6.0 (GraphPad software, La Jolla, CA, USA).

4.4. In vitro cytotoxicity assay

Cytotoxicity against a human cell line (HepG2) was evaluated using the neutral red assay [29]. In brief, human cells were seeded to a 96 well plate in a complete culture medium, before on the following day a serial dilution of the respective compound was added. After one day incubation, cytotoxicity was evaluated by the addition of Neutral Red, subsequent lysis of cells and measurement of absorbance in a plate reader. The IC_{50} was determined as for the *in vitro* drug assay against *P. falciparum*. To assess the safety of a compound, SI was calculated as the fractional ratio between the IC_{50}s for HepG2 and *P. falciparum* 3D7 strain.

4.5. Molecular dynamics simulations

MD simulations were performed on a *Pf*Hsp90 *N*-terminal domain X-ray structure collected from the Protein Data Bank (accession code 3K60). Ligands (ADP and SO_4^{2-}) were removed from the model and selected compounds were placed in the ATP binding pocket, including harmine as a reference. Original crystal waters were removed so that the water molecules from the bulk solvent could diffuse into the protein during equilibration and production MD runs. The investigated ligands were parameterized by performing the geometry optimization and RESP charge calculations in the Gaussian 16 program [39] at the HF/6–31G(d) level to be consistent with the employed GAFF force field, while the *Pf*Hsp90 protein was modelled with the AMBER ff14SB force field. Such protein complexes were solvated in a truncated octahedral box of TIP3P water molecules (10 Å-thick buffer), neutralized by Na^+ ions and submitted to geometry optimization in the AMBER 16 program [39] by employing periodic boundary conditions in all directions. Optimized systems were gradually heated from 0 to 300 K and equilibrated during 30 ps using NVT conditions, followed by productive and unconstrained MD simulations of 300 ns by employing a time step of 2 fs at a constant pressure (1 atm) and temperature (300 K), with the latter held constant using a Langevin thermostat with a collision frequency of 1 ps^{-1}. Bonds involving hydrogen atoms were constrained using the SHAKE algorithm [40] while the long-range electrostatic interactions were calculated employing the Particle Mesh Ewald method [41] The nonbonded interactions were truncated at 10.0 Å.

The binding free energies, ΔG_{BIND}, of each ligand within the *Pf*Hsp90 ATP binding site were calculated using the established MM-GBSA protocol [42,43] available in AmberTools16 [39], and in line with our earlier reports [10,15,44,45]. For that purpose, 1000 snapshots collected from the last 30 ns of the corresponding MD trajectories were utilized. The calculated MM-GBSA binding free energies were decomposed into specific residue contributions on a per-residue basis according to the established procedure [46,47]. This protocol calculates contributions to ΔG_{BIND} arising from each amino

acid residue and identifies the nature of the energy change in terms of interaction and solvation energies or entropic contributions.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations

ADMP	2-azido-1,3-dimethylimidazolinium hexafluorophosphate
AT	amide-type
ATR	attenuated total reflection
Boc	*tert*-butyloxycarbonyl
CAD	cinnamic acid derivative;
CQ	chloroquine;
DBU	1,8-diazabicyclo(5.4.0)undec-7-ene
DCM	dichloromethane
DIEA	*N,N*-diisopropylethylamine;
DMF	*N,N*-dimethylformamide;
ESI	electrospray ionization
EtOAc	ethyl acetate
EtOH	ethanol
ΔG_{BIND}	binding free energies
HAR	harmine
HATU	1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo [4,5-*b*]pyridinium-3-oxidhexafluorophosphate
HEPES	2-[4-(2-hydroxyethyl)piperazin-1-yl]ethane-1-sulphonic acid; hpi, hours post infection
HRP2	histidine-rich protein 2
HepG2	human liver hepatocellular carcinoma cell line;
IC_{50}	the concentration of the tested compound necessary for 50% growth inhibition
MD	molecular dynamics
MeOH	methanol
*Pf*3D7	chloroquine-sensitive strain of *P. falciparum*
*Pf*Dd2	chloroquine-resistant strain of *P. falciparum*
*Pf*Hsp90	*P. falciparum* heat shock protein 90
PQ	primaquine
SI	selectivity index
t-BuOH	*t*-buthanol
TEA	triethylamine
TMS	tetramethylsilane
TT	triazole-type
TWC	total wavelength chromatogram

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2021.113687.

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