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β-Carboline and chloroquine hybrids as potent antiplasmodial agents: Design, synthesis and biological evaluation

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ABSTRACT

A series of thirteen novel *β*-carboline and chloroquine (CQ) hybrids was designed and synthesized. The compounds were evaluated for *in vitro* antiplasmodial activity against *Plasmodium falciparum* strains 3D7 (CQ-sensitive) and Dd2 (multidrug-resistant). All compounds exhibited potent activity, with IC_{50} values in the nanomolar to low micromolar range, while retaining potency against the resistant strain (1.0 to 365.5 nmol L^{-1} for 3D7 and from 2.2 to 2415.3 nmol L^{-1} for Dd2 strain). Several compounds were more active against Dd2, then against 3D7 strain with resistance index (RI) lower than 1. Moreover, nearly all compounds showed lower RI values compared to CQ which additionally underscores their outstanding activity. At the highest tested concentration (250 μmol L^{-1}), no cytotoxicity was observed in HepG2 cells, resulting in favourable selectivity indices expressed as lower limits. Overall, the obtained results confirm strong antiplasmodial activity of the *β*-carboline and chloroquine hybrids as promising antiplasmodial candidates, particularly due to their activity against resistant strains and improved selectivity.

Keywords: *β*-carboline, quinoline, hybrids, synthesis, antiplasmodial, cytotoxicity

INTRODUCTION

Malaria is a widespread parasitic disease caused by *Plasmodium* protozoa and transmitted by infected female *Anopheles* mosquitoes. Among the species capable of infecting humans (*P. falciparum*, *P. vivax*, *P. ovale*, *P. knowlesi*, and *P. malariae*), *P. falciparum* is responsible for the most malaria-related deaths worldwide, whereas *P. vivax* represents a major cause of malaria morbidity in Southeast Asia and South America (1, 2).

In 2024, 282 million malaria cases were reported worldwide, with 610,000 estimated deaths reflecting a persistent global increase in malaria incidence and burden observed

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since 2015. The reasons for this increase are driven by multiple factors, including population growth in endemic regions and disruptions of health services. Additionally, it is presumed that improvement in case detection may increase the number of reported malaria cases in the coming years (3). At the same time, emergence of resistant parasites to antimalarial drugs, along with increasing insecticide resistance in mosquito vectors continues to threaten malaria control efforts and drive the search for new antimalarial agents (2).

Recent advances in antimalarial drug discovery have identified several structurally diverse chemotypes active against *P. falciparum*, among which the compounds presented in Fig. 1 are currently in advanced phase of clinical development (4).

Cipargamin, a spiroindolone derivative structurally related to *β*-carboline, has demonstrated strong potency against *P. falciparum* by inhibiting the parasite P-type ATPase *Pf*ATP4, a Na^+ efflux pump essential for ionic homeostasis, leading to intracellular Na^+ accumulation and rapid parasite death (5–7).

Among quinoline-based antimalarial candidates in advanced clinical development, M5715 that is an elongator factor-2 inhibitor is currently being evaluated in combination with pyronaridine, whereas ferroquine, an inhibitor of hemozoin formation, is being investigated in combination with ZY19489 (4).

Ganaplacide is an imidazolopiperazine antimalarial candidate that exhibits potent multistage activity against *Plasmodium* parasites and is currently in late-stage clinical development in combination with lumefantrine and triple combination with cipargamin (8). Its mechanism of action is not fully elucidated but appears to involve disruption of the parasite intracellular secretory pathway, with resistance linked to mutations in the *Pf*CARL locus (9).

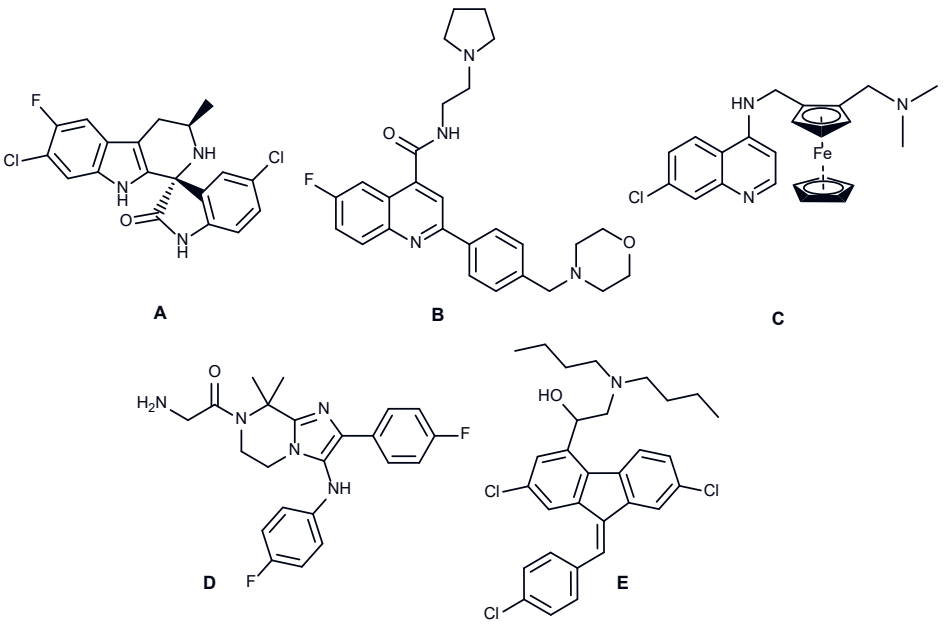


Fig. 1. Cipargamin (A), M5715 (B), ferroquine (C), ganaplacide (D) and lumefantrine (E).

The continued emergence of new antimalarial chemotypes highlights the importance of exploring heterocyclic scaffolds (10, 11).

Quinoline-based antimalarials, including chloroquine, primaquine, and tafenoquine, continue to play a key role in malaria treatment by targeting different stages of the parasite life cycle through distinct mechanisms of action (12, 13). β-Carbolines represent another biologically active heteroaromatic scaffold with reported antiparasitic and DNA-interacting properties (14). In this context, quinoline derivatives represent a well-established antimalarial pharmacophore, while β-carbolines constitute biologically active heterocycles with reported antiparasitic activity, making their molecular hybridization an attractive strategy for the development of novel antiplasmodial agents.

While combination therapy is a cornerstone in the treatment of infectious diseases due to its ability to improve efficacy and limit resistance, molecular hybridization offers a promising alternative by incorporating multiple pharmacophores into a single molecule, enabling improved metabolic stability, reduced cytotoxicity, and activity against resistant parasites (15). Consequently, the molecular hybridization of β-carboline and quinoline motifs represents a rational strategy to combine two privileged antiplasmodial scaffolds within a single molecule, potentially enabling multitarget activity with the aim of antiplasmodial activity (16).

Encouraged by the exceptional antiplasmodial activity of the second generation harmiquins bridged at the nitrogen at the position 9 of the β-carboline ring with quinoline from our previous work (17), we have decided to explore additional functionalities on the β-carboline, while maintaining the preferred position for bridging quinoline moiety and the amide/triazole linker.

EXPERIMENTAL

Chemistry. 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, USA) in the range from 450 to 4000 cm^{-1}. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance III HD operating at 300, 400 or 600 MHz for the ^1H and 75, 101 or 151 MHz for the ^{13}C nuclei (Bruker, 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 ^1H 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 6400 Series Triple Quad (Agilent Technologies, USA). The mobile phase consisted of Milli Q water with 0.1 % formic acid as component A and MeOH (HPLC grade, J. T. Baker) as component B. Separation was performed on a Zorbax XDB C18 column (4.6 × 75 mm, 3.5 μm, Agilent Technologies, USA) at 25 °C. Gradient elution was used at a flow rate of 0.5 mL min^{-1}, and 5 μL of analyte solution was injected per analysis. The starting conditions and gradient steepness were adjusted according to the analyte polarity. A 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^{-1}. For the MS data analysis Agilent MassHunter software (Agilent Technologies) was used. HPLC purity of the final compounds was determined by liquid chromatography with UV diode-array detection at 254 nm, with compound identity confirmed by LC-MS using electrospray ionization in positive and negative ion modes, and was ≥ 95 %. 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 or cyclohexane/EtOAc/MeOH as the solvent system. Spots were visualized by UV light (λ = 254 nm; 365 nm). Column chromatography was performed on silica gel 0.063–0.200 mm (Sigma-Aldrich, USA) with the same eluents used for TLC. All chemicals and solvents were of analytical grade and purchased from commercial sources.

7-Chloroquinoline-based azide **1** and carboxylic acid **2**, *β*-carboline phenol **5**, *β*-carbolines and their respective alkynes and amines **11–13**, **16**, **20–21**, **23**, **25**, **31**, **33** and **34** were prepared according to procedures published by us or others (17–20).

*General procedure for the synthesis of β-carbolines **6** and **7***

To a stirred solution of *β*-carboline phenol **5** in anhydrous DMF (5 mL), under an argon atmosphere, Cs_2CO_3 was added. The resulting suspension was stirred at room temperature for 20 min, followed by the addition of an appropriate alkyl bromide. The reaction mixture was stirred at room temperature, then at 40 °C, cooled down, poured into water (50 mL) and extracted with ethyl acetate (3×50 mL). The collected organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered, and evaporated under the reduced pressure. The crude product was purified by column chromatography (DCM:MeOH = 9:1) and triturated with diethyl ether.

*6-Ethoxy-1-methyl-9H-pyrido[3,4-b]indole (**6**).* – *β*-Carboline phenol **5**: 0.567 g, 2.86 mmol; Cs_2CO_3: 1.305 g, 4.00 mmol, 1.4 equiv.; ethyl bromide: 0.747 g, 6.86 mmol, 2.4 equiv.; 2 h at 40 °C; yield: 0.336 g (52 %); mp > 200 °C; IR (ATR, ν/cm^{-1}) 2982, 1600, 1564, 1497, 1465, 1289, 1208, 1120, 1070, 1041, 985, 880, 850, 818, 702, 622, 579; ^{1}H NMR (DMSO-d_6) δ 11.36 (s, 1H), 8.15 (d, 1H, J = 5.3 Hz), 7.89 (d, 1H, J = 5.3 Hz), 7.73 (d, 1H, J = 2.5 Hz), 7.50 (d, 1H, J = 8.9 Hz), 7.17 (dd, 1H, J = 8.8, 2.5 Hz), 4.12 (q, 2H, J = 7.0 Hz), 2.74 (s, 3H), 1.39 (t, 3H, J = 7.0 Hz); ^{13}C NMR (DMSO-d_6) δ 155.16, 144.81, 139.50, 137.89, 137.74, 129.43, 124.08, 120.93, 115.39, 107.07, 66.27, 23.05, 17.54; ESI-MS: m/z 227.1 $(M + 1)^+$.

*6-Isopropoxy-1-methyl-9H-pyrido[3,4-b]indole (**7**).* – Phenol **5**: 0.500 g, 2.52 mmol; Cs_2CO_3: 3.452 g, 10.594 mmol, 4.2 equiv.; isopropyl bromide: 2.482 g, 20.178 mmol, 8 equiv.; 23 h at room temperature; yield: 0.400 g (66 %); mp 161.5–168 °C; IR (ATR, ν/cm^{-1}) 3123, 2975, 1578, 1496, 1370, 1334, 1287, 1204, 1124, 1062, 987, 961, 877, 845, 744, 699, 626, 578; ^{1}H NMR (DMSO-d_6) δ 11.35 (s, 1H), 8.15 (d, 1H, J = 5.3 Hz), 7.90 (d, 1H, J = 5.3 Hz), 7.75 (d, 1H, J = 2.3 Hz), 7.49 (d, 1H, J = 8.8 Hz), 7.15 (dd, 1H, J = 8.8, 2.4 Hz), 4.65 (hept, 1H, J = 6.0 Hz), 2.74 (s, 3H), 1.31 (d, 6H, J = 6.0 Hz); ^{13}C NMR (DMSO-d_6) δ 151.61, 142.57, 137.35, 135.80, 135.57, 127.18, 122.02, 119.99, 113.21, 113.13, 107.34, 70.70, 22.47, 20.87; ESI-MS: m/z 241.2 $(M + 1)^+$.

*General procedure for the synthesis of β-carboline-based alkynes **8**, **9**, **14**, **15**, **22**, **26**, **32***

An appropriate *β*-carboline (**6**, **7**, **11**, **12**, **20**, **25**, **30**) was dissolved in dry DMF (1 mL/0.1 g; 3 mL). Under an argon atmosphere, 60 % dispersion of sodium hydride in mineral oil

(60 % NaH) (2.66 equiv.; 1.5 equiv. for **26** and **32**) or Cs_2CO_3 (2.88 equiv. for **22**) was added, followed by a dropwise addition of 80 % solution of propargyl bromide in toluene (3 equiv.; 1.44 equiv. for **22**; 1.5 equiv. for **26** and **32**). The reaction was stirred at r.t. and under an argon atmosphere for 2 h. Upon completion, the reaction mixture was poured into 30 mL of water. The product was extracted with ethyl acetate (3×30 mL). Organic layers were collected and washed with water and brine, dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The crude product was purified by column chromatography (DCM:MeOH = 95:5 or 9:1) and triturated with diethyl ether/petroleum ether/MeOH.

*6-Ethoxy-1-methyl-9-(prop-2-yn-1-yl)-9H-pyrido[3,4-b]indole (**8**).* – Compound **6**: 0.100 g, 0.44 mmol; 60 % NaH: 0.047 g, 1.18 mmol; 80 % solution of propargyl bromide in toluene: 148 μL, 0.197 g, 1.33 mmol; yield: 0.031 g (26 %); mp 187.0–188.0 °C; IR (ATR, ν/cm^{-1}) 3146, 2978, 2108, 1739, 1562, 1488, 1455, 1394, 1289, 1226, 1199, 1150, 1114, 1050, 987, 910, 847, 813, 797, 756, 692, 626, 615, 521; ^{1}H NMR (DMSO-d_6) δ 8.24 (d, 1H, J = 4.9 Hz), 7.99 (d, 1H, J = 4.8 Hz), 7.81 (s, 1H), 7.72 (d, 1H, J = 8.9 Hz), 7.21 (d, 1H, J = 8.6 Hz), 5.76 (d, 2H, J = 6.1 Hz), 4.13 (q, 2H, J = 6.5 Hz), 3.37 (s, 1H), 2.98 (s, 3H), 1.39 (t, 3H, J = 6.7 Hz); ^{13}C NMR (DMSO-d_6) δ 153.51, 142.39, 138.27, 135.29, 134.59, 128.51, 121.99, 118.31, 113.07, 112.38, 104.52, 86.04, 63.64, 23.22, 14.79; ESI-MS: m/z 265.20 $(M + 1)^+$.

*6-Isopropoxy-1-methyl-9-(prop-2-yn-1-yl)-9H-pyrido[3,4-b]indole (**9**).* – Compound **7**: 0.160 g, 0.67 mmol; 60 % NaH: 0.070 g, 1.77 mmol; 80 % solution of propargyl bromide in toluene: 223 μL, 0.297 g, 2.00 mmol; yield: 0.108 g (58 %); mp 126.5–138.0 °C; IR (ATR, ν/cm^{-1}) 3173, 2988, 2935, 2125, 2110, 1584, 1564, 1485, 1451, 1384, 1332, 1288, 1198, 1122, 1110, 1032, 987, 921, 863, 816, 800, 737, 624, 594, 507; ^{1}H NMR (DMSO-d_6) δ 8.21 (d, 1H, J = 5.2 Hz), 7.99 (d, 1H, J = 5.3 Hz), 7.81 (d, 1H, J = 2.4 Hz), 7.69 (d, 1H, J = 9.1 Hz), 7.24 (dd, 1H, J = 8.9, 2.5 Hz), 5.42 (d, 2H, J = 2.3 Hz), 4.72–4.66 (m, 1H), 3.36 (t, 1H, J = 2.3 Hz), 3.05 (s, 3H), 1.32 (d, 6H, J = 6.0 Hz); ^{13}C NMR (DMSO-d_6) δ 151.80, 141.69, 137.72, 135.79, 134.71, 119.46, 113.06, 111.23, 106.68, 96.35, 85.91, 70.11, 34.19, 22.49, 21.87; ESI-MS: m/z 279.20 $(M + 1)^+$.

*6-Methoxy-9-(prop-2-yn-1-yl)-1-(trifluoromethyl)-9H-pyrido[3,4-b]indole (**14**).* – Compound **11**: 0.150 g, 0.57 mmol; 60 % NaH: 0.060 g, 1.50 mmol; 80 % solution of propargyl bromide in toluene: 189 μL, 0.252 g, 1.70 mmol; yield: 0.104 g (61 %); mp 90.7–92.5 °C; IR (ATR, ν/cm^{-1}) 3278, 3183, 1592, 1490, 1458, 1416, 1345, 1288, 1238, 1193, 1178, 1147, 1101, 1082, 1039, 886, 834, 800, 771, 714, 657, 627, 595, 523; ^{1}H NMR (DMSO-d_6) δ 8.57 (d, 1H, J = 5.0 Hz), 8.52 (d, 1H, J = 5.0 Hz), 7.99 (d, 1H, J = 2.6 Hz), 7.78 (d, 1H, J = 9.0 Hz), 7.40 (dd, 1H, J = 9.0, 2.6 Hz), 5.30 (d, 1H, J = 2.4 Hz), 3.91 (d, 3H, J = 2.5 Hz), 3.28 (t, 1H, J = 2.3 Hz); ^{13}C NMR (DMSO-d_6) δ 154.91, 137.20, 136.68, 132.78, 132.59, 128.66 (q, J = 41.1 Hz), 121.42, 121.14, 119.63, 119.19, 112.40, 103.93, 86.17, 75.05, 55.80, 34.89 (q, J = 5.3 Hz); ESI-MS: m/z 305.20 $(M + 1)^+$.

*9-(Prop-2-yn-1-yl)-1-(trifluoromethyl)-9H-pyrido[3,4-b]indole (**15**).* – Compound **12**: 0.120 g, 0.51 mmol; 60 % NaH: 0.054 g, 1.35 mmol; 80 % solution of propargyl bromide in toluene: 170 μL, 0.227 g, 1.52 mmol; yield: 0.062 g (44 %); mp 73.5–75.0 °C; IR (ATR, ν/cm^{-1}) 3047, 2967, 1624, 1490, 1468, 1452, 1441, 1422, 1366, 1349, 1331, 1308, 1293, 1193, 1166, 1147, 1135, 1101, 1077, 1066, 1054, 940, 908, 893, 857, 836, 778 , 750, 738, 729, 694, 623, 576, 552; ^{1}H NMR (DMSO-d_6) δ 8.59 (d, 1H, J = 2.1 Hz), 8.42 (d, 1H, J = 7.7 Hz), 7.84 (d, 1H, J = 8.4 Hz), 7.73 (t, 1H, J = 7.7 Hz), 7.46 (t, 1H, J = 7.4 Hz), 7.29–7.17 (m, 1H), 5.78 (d, 1H, J = 6.2 Hz), 3.36 (s, 1H); ^{13}C NMR (DMSO-d_6)

δ 141.79, 138.17, 132.66, 132.31, 130.04, 129.16 (q, *J* = 35.4, 34.9 Hz), 122.07, 121.78, 121.25, 120.52, 119.02, 111.74, 94.97 (q, *J* = 4.4 Hz), 85.98, 34.83 (q, *J* = 5.1 Hz); ESI-MS: *m*/*z* 275.2 $(M + 1)^+$.

*Methyl 1-methyl-9-(prop-2-yn-1-yl)-9H-pyrido[3,4-b]indole-3-carboxylate (**22**).* – Compound **20**: 0.150 g, 0.62 mmol; Cs_2CO_3: 0.586 g, 1.798 mmol, 2.88 equiv.; 80 % solution of propargyl bromide in toluene: 100 μL, 0.134 g, 0.899 mmol, 1.44 equiv.; yield: 0.132 g (76 %); mp 166.5–170.5 °C; IR (ATR, ν/cm^{-1}) 3220, 1709, 1433, 1350, 1272, 1237, 1128, 1060, 780, 743, 724, 601, 525, 514; ^{1}H NMR (DMSO-d_6) δ 8.82 (s, 1H), 8.43 (dt, 1H, *J* = 7.8, 1.0 Hz), 7.93–7.85 (m, 1H), 7.69 (ddd, 1H, *J* = 8.4, 7.1, 1.2 Hz), 7.38 (ddd, 1H, *J* = 7.9, 7.1, 0.9 Hz), 5.56 (d, 2H, *J* = 2.4 Hz), 3.92 (s, 3H), 3.46 (t, 1H, *J* = 2.4 Hz), 3.14 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 165.79, 141.83, 141.21, 136.62, 135.78, 128.93, 128.62, 122.07, 121.07, 120.97, 116.01, 110.79, 76.11, 52.01, 34.38, 22.66; ESI-MS: *m*/*z* 279.2 $(M + 1)^+$.

*7-Fluoro-1-methyl-9-(prop-2-yn-1-yl)-9H-pyrido[3,4-b]indole (**26**).* – Compound **25**: 0.180 g, 0.90 mmol; 60 % NaH: 0.054 g, 1.349 mmol, 1.5 equiv.; 80 % solution of propargyl bromide in toluene: 151 μL, 0.201 g, 1.349 mmol, 1.5 equiv.; yield: 0.140 g (65 %); mp 141.0–143.0 °C; IR (ATR, ν/cm^{-1}) 3121, 2106, 1628, 1565, 1494, 1440, 1401, 1351, 1334, 1298, 1248, 1167, 1129, 974, 935, 924, 820, 803, 732, 667, 647, 615, 593, 551; ^{1}H NMR (DMSO-d_6) δ 8.31–8.27 (m, 2H), 8.01 (d, 1H, *J* = 5.2 Hz), 7.74 (dd, 1H, *J* = 10.5, 2.1 Hz), 7.16 (td, 1H, *J* = 9.4, 2.2 Hz), 5.47 (d, 2H, *J* = 2.3 Hz), 3.40 (t, 1H, *J* = 2.3 Hz), 3.07 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 162.89 (d, *J* = 242.1 Hz), 141.87 (d, *J* = 12.9 Hz), 141.70, 138.86, 134.88 (d, *J* = 2.0 Hz), 128.41, 123.38, 117.55, 112.90, 108.52 (d, *J* = 24.6 Hz), 97.28 (d, *J* = 27.5 Hz), 75.84, 34.49, 22.50; ESI-MS: *m*/*z* 239.3 $(M + 1)^+$.

*1-Bromo-9-(prop-2-yn-1-yl)-9H-pyrido[3,4-b]indole (**32**).* – Compound **30**: 0.180 g, 0.729 mmol; 60 % NaH: 0.044 g, 1.093 mmol, 1.5 equiv.; 80 % solution of propargyl bromide in toluene: 122 μL, 0.163 g, 1.093 mmol, 1.5 equiv.; yield: 0.107 g (52 %); mp 137.5–139.0 °C; IR (ATR, ν/cm^{-1}) 3217, 1624, 1534, 1489, 1446, 1434, 1414, 1329, 1298, 1275, 1207, 1193, 1157, 1135, 1056, 927, 843, 834, 798, 769, 742, 724, 700, 624, 594, 553; ^{1}H NMR (DMSO-d_6) δ 8.34 (d, 1H, *J* = 7.8 Hz), 8.28 (d, 1H, *J* = 5.0 Hz), 8.22 (d, 1H, *J* = 5.0 Hz), 7.86 (d, 1H, *J* = 8.4 Hz), 7.72 (t, 1H, *J* = 7.7 Hz), 7.41 (t, 1H, *J* = 7.5 Hz), 5.68 (d, 2H, *J* = 1.9 Hz), 3.34 (t, 2H, *J* = 3.2 Hz); ^{13}C NMR (DMSO-d_6) δ 141.40, 139.23, 132.88, 131.76, 129.43, 122.29, 121.97, 121.24 120.36, 115.13, 111.05, 75.68, 33.75; ESI-MS: *m*/*z* 285.0 $(M + 1)^+$.

*General procedure for the synthesis of β-carboline based Boc-protected amines **17** and **27***

To a stirred solution of compounds **13** or **25** in anhydrous DMF (1 mL/0.1 g; 6 mL) at 90 °C under an argon atmosphere, 60 % NaH (8 equiv.) was added. The resulting suspension was stirred at 90 °C for 20 min, followed by the addition of 2-(Boc-amino)ethyl bromide (3–4 equiv.). The reaction mixture was stirred at 90 °C for 18 h, cooled down, poured into water (60 mL) and extracted with ethyl acetate (3×60 mL). The collected organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered, and evaporated under the reduced pressure. The crude product was purified by column chromatography (DCM:MeOH = 9:1) and triturated with diethyl ether.

*Tert-butyl (2-(1-methyl-9H-pyrido[3,4-b]indol-9-yl)ethyl)carbamate (**17**).* – Compound **13**: 0.300 g, 1.65 mmol; 60 % NaH: 0.527 g, 13.17 mmol; 2-(Boc-amino)ethyl bromide: 1.107 g, 4.94 mmol, 3 equiv.; yield: 0.258 g (48 %); mp 158.5–159.5 °C; IR (ATR, ν/cm^{-1}) 3215, 2984, 1700, 1621, 1548, 1448, 1408, 1379, 1364, 1312, 1278, 1253, 1201, 1174, 1129, 1036, 971, 910, 872, 823, 776, 752, 739, 630, 587, 520; ^{1}H NMR (DMSO-d_6) δ 8.25–8.20 (m, 1H), 7.99 (d, 1H, *J* = 5.2 Hz),

7.68 (d, 1H, *J* = 8.3 Hz), 7.59 (ddd, 1H, *J* = 8.3, 7.0, 1.2 Hz), 7.27 (t, 1H, *J* = 7.4 Hz), 7.06 (t, 1H, *J* = 6.0 Hz), 4.62 (t, 1H, *J* = 6.7 Hz), 3.32 (q, 1H, *J* = 6.6 Hz), 2.99 (s, 2H), 1.31 (s, 8H), 1.00 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 155.71, 141.30, 137.51, 134.60, 128.11, 128.04, 121.45, 120.68, 119.54, 112.94, 110.21, 77.87, 43.86, 40.24, 28.11, 23.26; ESI-MS: *m*/*z* 326.4 $(M + 1)^+$.

Tert-butyl (2-(7-fluoro-1-methyl-9H-pyrido[3,4-b]indol-9-yl)ethyl)carbamate (**27**). – Compound **25**: 0.188 g, 0.939 mmol; 60 % NaH: 0.300 g, 7.512 mmol; 2-(Boc-amino)ethyl bromide: 0.842 g, 3.756 mmol, 4 equiv.; yield: 0.181 g (56 %); mp 146.0–147.0 °C; IR (ATR, ν/cm^{-1}) 2979, 1698, 1632, 1570, 1532, 1495, 1446, 1409, 1366, 1348, 1308, 1277, 1245, 1174, 1126, 1082, 1011, 979, 949, 858, 825, 803, 793, 748, 682, 620, 602, 581, 547; ^{1}H NMR (DMSO-d_6) δ 8.29–8.19 (m, 2H), 7.98 (d, 1H, *J* = 5.2 Hz), 7.50 (dd, 1H, *J* = 10.6, 2.3 Hz), 7.10 (td, 1H, *J* = 9.1, 2.2 Hz), 7.00 (t, 1H, *J* = 6.0 Hz), 4.58 (t, 1H, *J* = 6.3 Hz), 3.33 (s, 1H), 2.96 (s, 1H), 1.26 (s, 8H), 0.94 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 162.71 (d, *J* = 241.4 Hz), 155.62, 142.36 (d, *J* = 12.7 Hz), 141.18, 138.04, 135.26, 127.99, 123.12 (d, *J* = 10.6 Hz), 117.37, 112.77, 107.86 (d, *J* = 24.6 Hz), 96.95 (d, *J* = 26.9 Hz), 77.81, 44.18, 40.29, 28.03, 23.17; ESI-MS: *m*/*z* 344.2 $(M + 1)^+$.

*General procedure for the synthesis of β-carboline based amines **18** and **28***

A solution of the Boc-protected amine (**17** or **27**) and 4 mol L^{-1} HCl in MeOH (10 equiv.) was stirred at 50 °C for 4 h (**18**) or 18 h (**28**). Upon completion, solvent was removed under the reduced pressure. The residue was dissolved in water, basified to pH 12 with 5 % NaOH, and extracted with ethyl acetate (5×20 mL). The collected organic layers were dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography (DCM:MeOH = 9:1, **18**) and/or triturated with diethyl ether.

2-(1-Methyl-9H-pyrido[3,4-b]indol-9-yl)ethan-1-amine (***18***). – Compound **17**: 0.200 g, 0.615 mmol; 4 mol L^{-1} HCl in MeOH: 0.1536 mL, MeOH: 3.75 mL; yield: 0.073 g (53 %); mp 114.5–118.5 °C; IR (ATR, ν/cm^{-1}) 3215, 2984, 1700, 1621, 1548, 1448, 1408, 1379, 1364, 1312, 1278, 1253, 1201, 1174, 1129, 1036, 971, 910, 872, 823, 776, 752, 739, 630, 587, 520; ^{1}H NMR (DMSO-d_6) δ 8.23 (d, 1H, *J* = 7.9 Hz), 8.21 (d, 1H, *J* = 5.2 Hz), 7.99 (d, 1H, *J* = 5.1 Hz), 7.76 (d, 1H, *J* = 8.4 Hz), 7.59 (t, 1H, *J* = 7.7 Hz), 7.26 (t, 1H, *J* = 7.4 Hz), 4.57 (t, 2H, *J* = 7.2 Hz), 3.00 (s, 2H), 2.92 (t, 2H, *J* = 7.2 Hz); ^{13}C NMR (DMSO-d_6) δ 141.30, 137.31, 134.65, 127.86, 127.77, 121.27, 120.44, 119.30, 112.74, 110.44, 82.58, 47.24, 42.12, 23.31; ESI-MS: *m*/*z* 226.2 $(M + 1)^+$.

2-(7-fluoro-1-methyl-9H-pyrido[3,4-b]indol-9-yl)ethan-1-amine (***28***). – Compound **27**: 0.170 g, 0.495 mmol; 4 mol L^{-1} HCl in MeOH: 0.1237 mL, MeOH: 3.19 mL; yield: 0.061 g (51 %); mp 148.5–151.5 °C; IR (ATR, ν/cm^{-1}) 1628, 1443, 1408, 1228, 1148, 1128, 952, 834, 805, 622, 550; ^{1}H NMR (DMSO-d_6) δ 8.25 (dd, 1H, *J* = 8.6, 5.7 Hz), 8.22 (d, 1H, *J* = 5.2 Hz), 7.98 (d, 1H, *J* = 5.2 Hz), 7.66 (dd, 1H, *J* = 10.7, 2.3 Hz), 7.10 (ddd, 1H, *J* = 9.5, 8.6, 2.3 Hz), 4.53 (t, 2H, *J* = 7.1 Hz), 2.98 (s, 3H), 2.92 (t, 2H, *J* = 7.1 Hz); ^{13}C NMR (DMSO-d_6) δ 162.76 (d, *J* = 241.2 Hz), 142.33 (d, *J* = 12.9 Hz), 141.29, 137.94, 135.29 (d, *J* = 1.8 Hz), 127.74, 123.09 (d, *J* = 10.9 Hz), 117.16, 112.75, 107.81 (d, *J* = 24.7 Hz), 97.31 (d, *J* = 27.1 Hz), 47.46, 42.17, 23.29; ESI-MS: *m*/*z* 244.1 $(M + 1)^+$.

*General procedure for the synthesis of AT hybrids **35**–**37***

A suspension of a 7-chloroquinoline-based carboxylic acid **2**, appropriate amine (**18**, **28** or **34**; 1.1 equiv.) and TEA (2 equiv.) in dry DMF (1 mL) was stirred at room temperature for 10 min, followed by the dropwise addition of T3P (≥ 50 % in ethyl acetate, 1 equiv.). The

reaction mixture was stirred at room temperature for 18 h, or 72 h for **35**. Afterwards, the reaction mixture was placed in an ultrasonic bath and 5 % NaOH was added dropwise until the formation of white precipitate was completed. In the synthesis of **35** and **37**, the reaction mixture was extracted 7× (**35**) or 1× (**37**) with an appropriate amount of ethyl acetate. The collected organic layers were washed with water and brine, dried over anhydrous sodium sulfate, filtered, and evaporated under the reduced pressure. In the synthesis of **36**, the formed precipitate was filtered off. The crude product was purified by column chromatography (DCM:MeOH = 85:15 for **35**, DCM:MeOH = 8:2 for **36** and DCM:MeOH = 75:25 for **37**) and trituration with diethyl ether/petroleum ether/MeOH.

*2-((7-Chloroquinolin-4-yl)amino)-N-(2-(1-methyl-9H-pyrido[3,4-b]indol-9-yl)ethyl)acetamide (**35**).* – Carboxylic acid **2**: 0.067 g, 0.222 mmol; amine **18**: 0.070 g, 0.311 mmol; TEA: 0.057 g, 0.566 mmol; T3P: 0.100 g, 0.283 mmol; yield: 0.020 g (19 %); 262.0–262.5 °C; IR (ATR, ν/cm^{-1}) 3294, 1657, 1582, 1563, 1448, 1407, 1349, 1286, 1237, 1204, 1148, 1082, 983, 879, 849, 793, 747, 731, 639, 597, 561, 545; ^{1}H NMR (DMSO-d_6) δ 8.37–8.29 (m, 2H), 8.28–8.18 (m, 3H), 8.00 (d, 1H, J = 4.1 Hz), 7.82 (s, 1H), 7.73 (d, 2H, J = 6.9 Hz), 7.60 (t, 1H, J = 7.0 Hz), 7.50 (d, 1H, J = 8.2 Hz), 7.28 (t, 1H, J = 6.8 Hz), 6.08 (d, 1H, J = 5.4 Hz), 4.64 (t, 1H, J = 7.1 Hz), 3.85 (d, 1H, J = 4.4 Hz), 3.52 (q, J = 6.8 Hz, 1H, 2'), 3.00 (s, 1H, 13); ^{13}C NMR (DMSO-d_6) δ 169.47, 151.77, 150.19, 148.79, 141.36, 141.23, 137.65, 134.50, 133.54, 128.13, 128.10, 127.40, 124.37, 124.17, 121.54, 120.66, 119.66, 117.51, 112.97, 110.19, 98.98, 45.84, 43.35, 38.99, 23.24; ESI-MS: m/z 444.4 $(M + 1)^+$; HPLC purity 98.8 %.

*2-((7-Chloroquinolin-4-yl)amino)-N-(2-(7-fluoro-1-methyl-9H-pyrido[3,4-b]indol-9-yl)ethyl) acetamide (**36**).* – Carboxylic acid **2**: 0.044 g, 0.189 mmol; amine **28**: 0.050 g, 0.206 mmol; TEA: 0.038 g, 0.374 mmol; T3P: 0.060 g, 0.187 mmol; yield: 0.020 g (11 %); mp 252.0–255.0 °C; IR (ATR, ν/cm^{-1}) 3288, 1656, 1630, 1585, 1447, 1408, 1370, 1351, 1306, 1266, 1253, 1211, 1173, 1151, 1133, 1078, 956, 878, 850, 828, 809, 762, 651, 623, 549; ^{1}H NMR (DMSO-d_6) δ 8.39 (t, 1H, J = 6.1 Hz), 8.34 (d, 1H, J = 5.5 Hz), 8.31–8.27 (m, 2H), 8.24 (d, 1H, J = 5.2 Hz), 7.99 (d, 2H, J = 5.2 Hz), 7.84 (d, 1H, J = 2.2 Hz), 7.59 (dd, 1H, J = 10.5, 2.3 Hz), 7.53 (dd, 1H, J = 9.0, 2.3 Hz), 7.12 (ddd, 1H, J = 9.4, 8.5, 2.2 Hz), 6.11 (d, 1H, J = 5.6 Hz), 4.59 (t, 2H, J = 7.1 Hz), 3.88 (d, 2H, J = 5.8 Hz), 3.51 (q, 2H, J = 6.8 Hz), 2.99 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 169.28, 162.78 (d, J = 241.7 Hz), 150.82, 150.76, 147.67, 142.17 (d, J = 12.8 Hz), 141.22, 138.12, 135.15 (d, J = 1.8 Hz), 134.02, 127.96, 126.49, 124.65, 124.38, 123.32 (d, J = 10.8 Hz), 117.33 (d, J = 11.1 Hz), 112.85, 108.08 (d, J = 24.6 Hz), 98.92, 96.95 (d, J = 27.1 Hz), 45.80, 43.61, 38.72, 23.07; ESI-MS: m/z 462.4 $(M + 1)^+$.

*N-(2-(9H-pyrido[3,4-b]indol-9-yl)ethyl)-2-((7-chloroquinolin-4-yl)amino) acetamide (**37**).* – Carboxylic acid **2**: 0.072 g, 0.306 mmol; amine **34**: 0.071 g, 0.336 mmol; TEA: 0.062 g, 0.611 mmol; T3P: 0.097 g, 0.306 mmol; yield: 0.020 g (15 %); mp 263.0–265.5 °C; IR (ATR, ν/cm^{-1}) 3282, 1664, 1582, 1453, 1368, 1247, 1213, 1151, 849, 815, 747, 727, 550; ^{1}H NMR (DMSO-d_6) δ 9.04 (s, 1H), 8.40 (d, 1H, J = 5.2 Hz), 8.29 (t, 1H, J = 2.7 Hz), 8.28 (s, 1H), 8.20 (dd, 2H, J = 7.2, 1.7 Hz), 8.15 (dd, 1H, J = 5.2, 1.1 Hz), 7.81 (d, 1H, J = 6.5 Hz), 7.79 (d, 1H, J = 2.3 Hz), 7.70 (d, 1H, J = 8.3 Hz), 7.62 (ddd, 1H, J = 8.3, 7.0, 1.2 Hz), 7.48 (dd, 1H, J = 9.0, 2.2 Hz), 7.34–7.27 (m, 1H), 5.85 (d, 1H, J = 5.5 Hz), 4.58 (t, 2H, J = 6.1 Hz), 3.75 (d, 2H, J = 6.0 Hz), 3.58 (q, 2H, J = 6.0 Hz); ^{13}C NMR (DMSO-d_6) δ 169.66, 151.48, 150.98, 148.45, 141.39, 138.99, 136.76, 134.25, 132.96, 128.78, 127.80, 127.22, 124.94, 124.72, 122.46, 120.94, 120.01, 117.76, 115.08, 110.41, 99.28, 46.11, 42.45, 38.59; ESI-MS: m/z 430.4 $(M + 1)^+$; HPLC purity 98.2 %.

*General procedure for the synthesis of TT hybrids **38**–**47***

To a solution of an alkyne and the 7-chloroquinoline-based azide **1** (1.1 equiv.) in MeOH (4–5 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 an additional Al_2O_3 layer to remove Cu-salts) with DCM:MeOH = 85:15, cyclohexane:ethyl acetate:methanol = 1:1:0.5 for **40** and **41** or DCM:MeOH = 75:25 for **46** as a mobile phase. The crude product was triturated with diethyl ether or MeOH.

*7-Chloro-N-(2-(4-((6-ethoxy-1-methyl-9H-pyrido[3,4-b]indol-9-yl)methyl)-1H-1,2,3-triazol-1-yl)ethyl)quinolin-4-amine (**38**).* – Alkyne **8**: 0.045 g, 0.170 mmol; azide **1**: 0.046 g, 0.187 mmol; yield: 0.022 g (25 %); mp > 200 °C; IR (ATR, ν/cm^{-1}) 2971, 1579, 1448, 1368, 1324, 1244, 1220, 1194, 1141, 1103, 1049, 986, 879, 841, 819, 807, 741, 693, 622, 533; ^{1}H NMR (DMSO-d_6) δ 8.32 (d, 1H, J = 5.3 Hz), 8.18 (d, 1H, J = 5.2 Hz), 8.03 (d, 1H, J = 9.0 Hz), 7.96 (d, 1H, J = 5.2 Hz), 7.94 (s, 1H), 7.80 (d, 1H, J = 2.0 Hz), 7.77 (d, 1H, J = 2.3 Hz), 7.63 (d, 1H, J = 9.0 Hz), 7.42 (dd, 1H, J = 9.0, 2.1 Hz), 7.35 (t, 1H, J = 5.6 Hz), 7.14 (dd, 1H, J = 8.9, 2.4 Hz), 6.44 (d, 1H, J = 5.4 Hz), 5.82 (s, 2H), 4.55 (t, 2H, J = 5.9 Hz), 4.12 (q, 2H, J = 6.9 Hz), 3.69 (q, 2H, J = 5.7 Hz), 2.99 (s, 3H), 1.40 (t, 3H, J = 6.9 Hz); ^{13}C NMR (DMSO-d_6) δ 153.38, 152.26, 149.99, 149.41, 144.41, 142.22, 137.65, 136.27, 135.34, 133.87, 128.31, 127.96, 124.72, 124.22, 123.82, 121.62, 118.57, 117.80, 113.45, 111.88, 104.77, 99.18, 64.09, 48.30, 42.69, 23.65 15.27; ESI-MS: m/z 512.20 $(M + 1)^+$; HPLC purity 100.0 %.

*7-Chloro-N-(2-(4-((6-isopropoxy-1-methyl-9H-pyrido[3,4-b]indol-9-yl)methyl)-1H-1,2,3-triazol-1-yl)ethyl)quinolin-4-amine (**39**).* – Alkyne **9**: 0.026 g, 0.093 mmol; azide **1**: 0.025 g, 0.103 mmol; yield: 0.046 g (41 %); mp 80.0–89.5 °C; IR (ATR, ν/cm^{-1}) 2971, 1579, 1489, 1448, 1324, 1292, 1244, 1220, 1194, 1141, 1103, 1049, 986, 909, 879, 841, 819, 807, 741, 622, 533; ^{1}H NMR (DMSO-d_6) δ 8.32 (d, 1H, J = 5.1 Hz), 8.17 (d, 1H, J = 5.1 Hz), 8.05 (d, 1H, J = 9.0 Hz), 7.97 (s, 1H), 7.96 (s, 1H), 7.80 (d, 1H, J = 2.2 Hz), 7.78 (d, 1H), 7.61 (d, 1H, J = 8.9 Hz), 7.44 (d, 2H, J = 7.0 Hz), 7.12 (dd, 1H, J = 8.9, 2.0 Hz), 6.45 (d, 1H, J = 5.4 Hz), 5.81 (s, 2H), 4.65 (hept, 1H, J = 5.8, 5.3 Hz), 4.55 (t, 2H, J = 5.8 Hz), 3.70 (q, 2H, J = 6.0 Hz), 2.99 (s, 3H), 1.31 (d, 6H, J = 6.0 Hz); ^{13}C NMR (DMSO-d_6) δ 151.56, 151.35, 149.74, 148.43, 143.89, 141.62, 137.09, 135.84, 134.83, 133.56, 127.77, 127.07, 124.32, 123.77, 123.32, 121.18, 119.32, 117.19, 112.98, 111.31, 106.48, 98.63, 70.08, 47.78, 42.19, 23.07, 21.89; ESI-MS: m/z 526.20 $(M + 1)^+$; HPLC purity 100.0 %.

*7-Chloro-N-(2-(4-((6-methoxy-1-(trifluoromethyl)-9H-pyrido[3,4-b]indol-9-yl)methyl)-1H-1,2,3-triazol-1-yl)ethyl)quinolin-4-amine (**40**).* – Alkyne **14**: 0.060 g, 0.197 mmol; azide **1**: 0.054 g, 0.217 mmol; yield: 0.026 g (24 %); mp 133.5–139.0 °C; IR (ATR, ν/cm^{-1}) 2919, 1579, 1489, 1345, 1288, 1190, 1136, 1111, 1065, 835, 720, 557; ^{1}H NMR (DMSO-d_6) δ 8.55 (d, 1H, J = 4.9 Hz), 8.49 (d, 1H, J = 5.0 Hz), 8.31 (d, 1H, J = 5.4 Hz), 8.02 (d, 1H, J = 9.1 Hz), 7.94 (d, 1H, J = 2.6 Hz), 7.78 (s, 2H), 7.60 (d, 1H, J = 9.0 Hz), 7.40 (dd, 1H, J = 9.0, 2.2 Hz), 7.37 (t, 1H, J = 5.6 Hz), 7.21 (dd, 1H, J = 9.0, 2.6 Hz), 6.41 (d, 1H, J = 5.5 Hz), 5.73 (s, 2H), 4.53 (t, 2H, J = 6.0 Hz), 3.88 (s, 3H), 3.68 (q, 2H, J = 5.9 Hz); ^{13}C NMR (DMSO-d_6) δ 154.61, 151.53, 149.75, 148.65, 142.72, 136.91, 136.59, 133.59, 132.97, 132.37, 128.44 (q, J = 34.9 Hz), 127.26, 124.31, 123.80, 123.26, 121.50, 120.97, 119.34, 119.04, 117.28, 112.60, 103.68, 98.69, 55.75, 47.90, 44.48, 42.25; ESI-MS: m/z 552.4 $(M + 1)^+$; HPLC purity 97.7 %.

*7-chloro-N-(2-(4-((1-(trifluoromethyl)-9H-pyrido[3,4-b]indol-9-yl)methyl)-1H-1,2,3-triazol-1-yl)ethyl)quinolin-4-amine (**41**).* – Alkyne **15**: 0.062 g, 0.226 mmol; azide **1**: 0.062 g, 0.249 mmol;

yield: 0.030 g (41 %); mp 234.0–237.5 °C; IR (ATR, ν/cm^{-1}) 2922, 1613, 1584, 1429, 1347, 1323, 1295, 1193, 1142, 1120, 1068, 838, 803, 745, 726, 642, 557; ^{1}H NMR (DMSO-d_6) δ 8.58 (d, 1H, J = 5.0 Hz), 8.54 (d, 1H, J = 4.9 Hz), 8.39 (d, 1H, J = 7.8 Hz), 8.30 (d, 1H, J = 5.5 Hz), 8.05 (d, 1H, J = 9.0 Hz), 7.83 (s, 1H), 7.79 (d, 1H, J = 2.2 Hz), 7.70 (d, 1H, J = 8.4 Hz), 7.60 (ddd, 1H, J = 8.3, 7.0, 1.2 Hz), 7.46 (d, 1H, J = 5.9 Hz), 7.42 (dd, 1H, J = 9.0, 2.2 Hz), 7.40–7.36 (m, 1H), 6.42 (d, 1H, J = 5.5 Hz), 5.77 (s, 2H), 4.54 (t, 2H, J = 6.0 Hz), 3.69 (q, 2H, J = 5.9 Hz), 3.20 (s, 1H), 2.63 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 151.26, 149.95, 148.33, 142.61, 142.10, 137.22, 133.74, 132.71, 132.59, 129.73, 128.42 (q, J = 35.1 Hz), 127.01, 124.42, 123.87, 123.33, 121.78, 121.49, 121.18, 120.43, 119.00, 117.23, 111.64, 98.68, 47.91, 44.49, 42.27; ESI-MS: m/z 522.4 (M + 1)$^+$; HPLC purity 97.6 %.

*7-Chloro-N-(2-(4-((1-methyl-9H-pyrido[3,4-b]indol-9-yl)methyl)-1H-1,2,3-triazol-1-yl) ethyl)quinolin-4-amine (**42**).* – Alkyne **16**: 0.060 g, 0.272 mmol; azide **1**: 0.074 g, 0.300 mmol; yield: 0.040 g (31 %); mp 178.0–181.0 °C; IR (ATR, ν/cm^{-1}) 2957, 1616, 1582, 1445, 1405, 1354, 1327, 1300, 1245, 1200, 1178, 1141, 1084, 1047, 999, 973, 911, 875, 840, 775, 750, 733, 688, 646, 584, 558, 519; ^{1}H NMR (DMSO-d_6) δ 8.25 (s, 1H), 8.23 (d, 1H, J = 7.9 Hz), 8.11 (d, 1H, J = 8.9 Hz), 8.01 (d, 1H, J = 4.8 Hz), 7.98 (s, 1H), 7.84 (s, 1H), 7.73 (d, 1H, J = 8.3 Hz), 7.65 (d, 1H, J = 7.0 Hz), 7.52 (t, 1H, J = 7.7 Hz), 7.46 (d, 1H, J = 9.0 Hz), 7.26 (t, 1H, J = 7.4 Hz), 6.51 (s, 1H), 5.86 (s, 2H), 4.56 (t, 2H, J = 6.0 Hz), 3.73 (q, 2H, J = 6.0 Hz), 3.00 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 150.47, 149.88, 143.93, 143.42, 141.76, 140.96, 137.70, 135.52, 134.20, 128.16, 128.11, 126.85, 126.34, 124.80, 124.08, 123.47, 121.47, 120.77, 119.81, 113.06, 110.54, 103.51, 47.88, 42.36, 23.21; ESI-MS: m/z 468.5 (M + 1)$^+$; HPLC purity 100.0 %.

*Methyl 9-((1-(2-((7-chloroquinolin-4-yl)amino)ethyl)-1H-1,2,3-triazol-4-yl)methyl)-1-methyl-9H-pyrido[3,4-b]indole-3-carboxylate (**43**).* – Alkyne **22**: 0.060 g, 0.216 mmol; azide **1**: 0.059 g, 0.237 mmol; yield: 0.036 g (32 %); mp > 240 °C; IR (ATR, ν/cm^{-1}) 1713, 1578, 1430, 1350, 1273, 1240, 1140, 1050, 910, 875, 840, 786, 749, 730, 532; ^{1}H NMR (DMSO-d_6) δ 8.81 (s, 1H), 8.40 (d, J = 7.8 Hz, 1H), 8.31 (d, J = 7.7 Hz, 1H), 8.06 (d, J = 9.0 Hz, 1H), 8.03 (s, 1H), 7.81 (d, J = 8.1 Hz, 2H), 7.64 (s, 1H), 7.58 (t, J = 7.7 Hz, 1H), 7.46 (d, J = 8.9 Hz, 1H), 7.33 (t, J = 7.5 Hz, 1H), 6.47 (d, J = 5.3 Hz, 1H), 5.93 (s, 2H), 4.57 (t, J = 5.9 Hz, 2H), 3.91 (s, 3H), 3.73 (q, J = 5.9 Hz, 2H), 3.06 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 165.89, 150.47, 143.54, 141.89, 141.36, 136.14, 136.06, 134.18, 128.65, 128.17, 127.61, 126.33, 124.77, 124.03, 123.65, 121.89, 120.97, 120.76, 115.99, 111.01, 110.00, 98.72, 51.98, 47.91, 42.33, 23.29; ESI-MS: m/z 526.3 (M + 1)$^+$; HPLC purity 100.0 %.

*(9-((1-(2-((7-Chloroquinolin-4-yl)amino)ethyl)-1H-1,2,3-triazol-4-yl)methyl)-1-methyl-9H--pyrido[3,4-b]indol-3-yl)methanol (**44**).* – Alkyne **23**: 0.065 g, 0.260 mmol; azide **1**: 0.071 g, 0.286 mmol; yield: 0.042 g (33 %); mp 159.5–166.5 °C; IR (ATR, ν/cm^{-1}) 3079, 1622, 1580, 1456, 1427, 1360, 1336, 1297, 1256, 1197, 1137, 1123, 1031, 1014, 983, 914, 842, 808, 762, 732, 643, 627, 580, 567; ^{1}H NMR (DMSO-d_6) δ 8.30 (d, 1H, J = 5.4 Hz), 8.21 (d, 1H, J = 7.8 Hz), 8.10 (d, 1H, J = 8.8 Hz), 8.01 (s, 1H), 8.00 (s, 1H), 7.79 (d, 1H, J = 2.2 Hz), 7.71 (d, 1H, J = 8.3 Hz), 7.51 (ddd, 1H, J = 8.3, 7.0, 1.2 Hz), 7.46 (d, 1H, J = 5.6 Hz), 7.42 (dd, 1H, J = 9.0, 2.3 Hz), 7.24 (t, 1H, J = 7.4 Hz), 6.43 (d, 1H, J = 5.5 Hz), 5.84 (s, 2H), 5.38 (s, 1H), 4.67 (s, 2H), 4.56 (, 2H t, J = 6.0 Hz), 3.69 (q, 2H, J = 6.0 Hz), 2.98 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 151.80, 150.24, 149.68, 148.96, 143.92, 141.31, 140.51, 133.48, 133.42, 129.03, 128.02, 127.47, 124.31, 123.98, 123.44, 121.37, 120.93, 119.67, 117.41, 110.52, 109.06, 98.73, 64.35, 47.89, 42.27, 23.08; ESI-MS: m/z 498.10 (M + 1)$^+$; HPLC purity 99.0 %.

*7-chloro-N-(2-(4-((7-fluoro-1-methyl-9H-pyrido[3,4-b]indol-9-yl)methyl)-1H-1,2,3-triazol--1-yl)ethyl)quinolin-4-amine (**45**).* – Alkyne **26**: 0.060 g, 0.252 mmol; azide **1**: 0.069 g, 0.277 mmol;

yield: 0.054 g (44 %); mp 234.5–250.5 °C; IR (ATR, ν/cm^{-1}) 3063, 1628, 1578, 1444, 1331, 1244, 1161, 1128, 1039, 927, 803, 764, 619, 549; ^{1}H NMR (DMSO-d_6) δ 8.31 (d, 1H, J = 5.5 Hz), 8.28–8.25 (m, 1H), 8.25–8.22 (m, 1H), 8.06 (d, 1H, J = 9.0 Hz), 8.03 (s, 1H), 7.98 (d, 1H, J = 5.3 Hz), 7.80 (d, 1H, J = 2.3 Hz), 7.66 (dd, 1H, J = 10.5, 2.3 Hz), 7.58 (t, 1H, J = 5.8 Hz), 7.43 (dd, 1H, J = 8.9, 2.2 Hz), 7.11 (td, 1H, J = 9.0, 2.3 Hz), 6.48 (d, 1H, J = 5.6 Hz), 5.84 (s, 2H), 4.58 (t, 2H, J = 6.0 Hz), 3.73 (q, 2H, J = 5.9 Hz), 3.01 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 162.74 (d, J = 241.8 Hz), 150.73, 150.29, 147.69, 143.52, 141.95 (d, J = 12.8 Hz), 141.72, 138.31, 135.12, 134.02, 127.98, 126.52, 124.63, 123.91, 123.58, 123.20 (d, J = 10.9 Hz), 117.46, 117.12, 112.81, 108.21 (d, J = 24.5 Hz), 98.71, 97.40 (d, J = 27.1 Hz), 47.87, 42.33, 39.10, 23.14; ESI-MS: m/z 486.4 (M + 1)$^+$; HPLC purity 100.0 %.

*N-(2-(4-((9H-pyrido[3,4-b]indol-9-yl)methyl)-1H-1,2,3-triazol-1-yl)ethyl)-7-chloroquinolin--4-amine (**46**).* – Alkyne **31**: 0.027 g, 0.131 mmol; azide **1**: 0.036 g, 0.144 mmol; yield: 0.026 g (44 %); mp 223.5–225.5 °C; IR (ATR, ν/cm^{-1}) 1578, 1452, 1328, 1033, 841, 746, 728, 557; ^{1}H NMR (DMSO-d_6) δ 9.17 (s, 1H), 8.40 (d, 1H, J = 5.2 Hz), 8.30 (d, 1H, J = 5.6 Hz), 8.26 (dt, 1H, J = 7.8, 1.0 Hz), 8.18 (s, 1H), 8.14–8.10 (m, 2H), 7.84–7.80 (m, 2H), 7.72 (s, 1H), 7.57 (ddd, 1H, J = 8.3, 7.1, 1.2 Hz), 7.46 (dd, 1H, J = 9.0, 2.2 Hz), 7.28 (ddd, 1H, J = 7.9, 7.0, 0.9 Hz), 6.49 (d, 1H, J = 5.7 Hz), 5.76 (s, 2H), 4.59 (t, 2H, J = 6.0 Hz), 3.74 (q, 2H, J = 6.0 Hz); ^{13}C NMR (DMSO-d_6) δ 150.53, 150.45, 142.75, 140.46, 138.68, 135.79, 134.11, 133.02, 128.27, 127.41, 126.31, 124.72, 124.13, 123.92, 121.90, 120.92, 120.49, 119.69, 117.11, 114.56, 110.52, 98.72, 47.81, 42.36, 37.72; ESI-MS: m/z 454.4 (M + 1)$^+$; HPLC purity 97.1 %.

*N-(2-(4-((1-bromo-9H-pyrido[3,4-b]indol-9-yl)methyl)-1H-1,2,3-triazol-1-yl)ethyl)-7-chloro-quinolin-4-amine (**47**).* – Alkyne **32**: 0.060 g, 0.210 mmol; azide **1**: 0.057 g, 0.231 mmol; yield: 0.009 g (8 %); mp 165.0–169.0 °C; IR (ATR, ν/cm^{-1}) 3295, 2921, 2852, 1612, 1579, 1536, 1487, 1450, 1434, 1354, 1330, 1194, 1141, 1051, 912, 842, 768, 749, 728, 639, 596, 556; ^{1}H NMR (DMSO-d_6) δ 8.30 (d, 1H, J = 7.8 Hz), 8.25 (d, 2H, J = 5.0 Hz), 8.20 (d, 1H, J = 10.0 Hz), 8.17 (d, 1H, J = 5.0 Hz), 8.00 (s, 1H), 7.88 (s, 1H), 7.78 (d, 1H, J = 8.4 Hz), 7.60 (t, 1H, J = 7.7 Hz), 7.53 (d, 1H, J = 9.0 Hz), 7.34 (t, 1H, J = 7.4 Hz), 6.52 (s, 1H), 6.07 (s, 2H), 4.60 (t, 2H, J = 5.9 Hz), 3.81 (q, 2H, J = 5.9 Hz); ^{13}C NMR (DMSO-d_6) δ 152.11, 150.94, 144.66, 143.45, 141.54, 138.62, 135.42, 132.95, 131.31, 129.12, 125.47, 124.50, 123.65, 122.13, 121.75, 121.57, 121.15, 120.83, 120.12, 115.03, 111.26, 98.49, 47.87, 42.51, 39.01; ESI-MS: m/z 533.95 (M + 1)$^+$.

Prediction in SwissADME tool

An open-access web page, SwissADME, was used as a tool for prediction of selected drug-like properties of the title compounds **35**–**47** (21).

Parasite culture and in vitro *antiplasmodial activity against erythrocytic stages of* P. falciparum

Parasite culture and the antiplasmodial activity of novel hybrids **35**–**47** was evaluated against two *P. falciparum* strains (3D7, CQ-sensitive, and Dd2, multi-drug resistant) as previously described, using the histidine-rich protein 2 (HRP2) assay (22, 23). 96-Well plates were pre-coated with test compounds in a three-fold serial dilution, after which ring-stage parasites were added in complete culture medium at a haematocrit of 1.5 % and a parasitaemia of 0.05 %. Plates were incubated for 72 h at 37 °C under an atmosphere of 5 % CO_2 and 5 % O_2, then frozen until analysis by HRP2-ELISA. All compounds were tested in

duplicate in at least two independent experiments. IC_{50} values were determined by non-linear regression analysis of log concentration–response curves using the drc package (v0.9.0) in R (v2.6.1) (24).

HepG2 culture and in vitro *cytotoxicity in HepG2 cells*

Human hepatocellular carcinoma cell line (HepG2) was maintained, and cytotoxicity assay was performed using the neutral red uptake assay as described in (22, 25). Briefly, cells were seeded in 96-well plates in complete culture medium; on the following day, serial dilutions of the test compounds were added. After 24 h of incubation, cytotoxicity was assessed by addition of neutral red, followed by cell lysis and measurement of absorbance at 540 nm using a plate reader (CLARIOstar, BMG Labtech, Germany). IC_{50} values were determined as described for the antiplasmodial assay. The selectivity index (SI) was calculated as the ratio of IC_{50} values for HepG2 cells and the *P. falciparum* 3D7 strain.

RESULTS AND DISCUSSION

Chemistry

The three amide-type (AT) (**35**–**37**) and ten triazole-type (TT) (**38**–**47**) *β*-carboline and CQ hybrids bearing various substituents at the positions 1, 3, 6 and/or 7 of the *β*-carboline ring (Fig. 2) were synthesized *via* two distinctive strategies.

The quinoline azide **1** and carboxylic acid **2** (Fig. 3) were prepared according to previously described modified procedures (26–28).

To this end, the following *β*-carbolines and *β*-carboline-based building blocks are reported herein for the first time: *β*-carbolines **6**–**7**, their corresponding alkynes **8**–**9**,

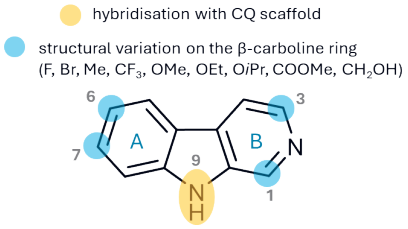


Fig. 2. Structural variety of the *β*-carboline in the title compounds.

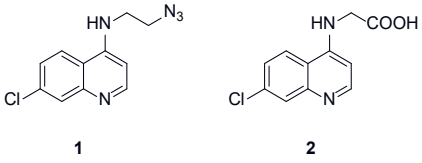
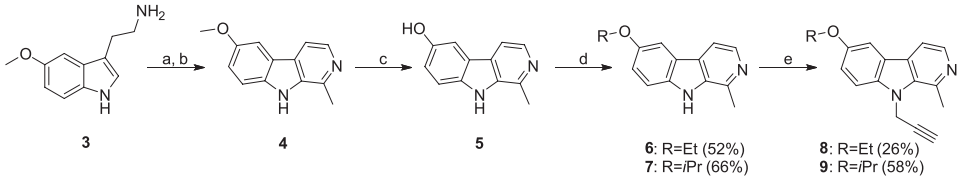


Fig. 3. 7-chloroquinoline-based intermediates **1** and **2**.



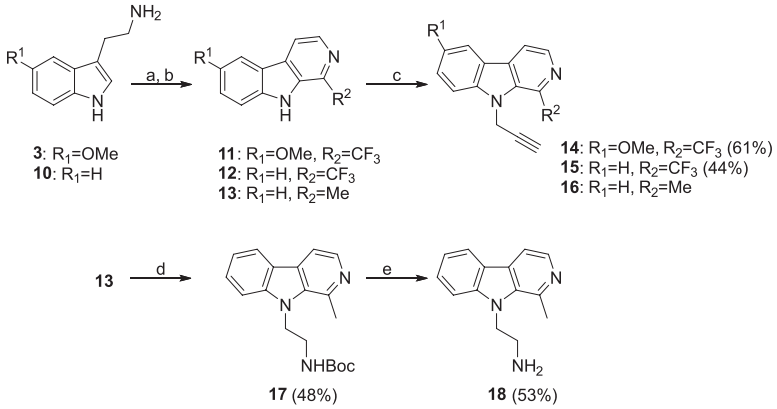
(a) $(CH_3O)_2CHCH_3$, TFA, ACN, MW, 110 °C, 10 min; (b) Pd/C, Li_2CO_3, EtOH, MW, 150 °C, 25 min; (c) HBr/AcOH, MW, 150 °C, 25 min; (d) R-Br (2.4 or 8 equiv), Cs_2CO_3 (1.4 or 4.2 equiv), DMF, 40 °C, 2 h (**8**); 23 h (**9**); (e) propargyl bromide (3 equiv), NaH (2.66 equiv), DMF, rt, 2 h.

Scheme 1

alkynes **14**, **15**, **22**, **26** and **32**, Boc-protected amines **17** and **27** and their corresponding amines **18** and **28** (Schemes 1–5).

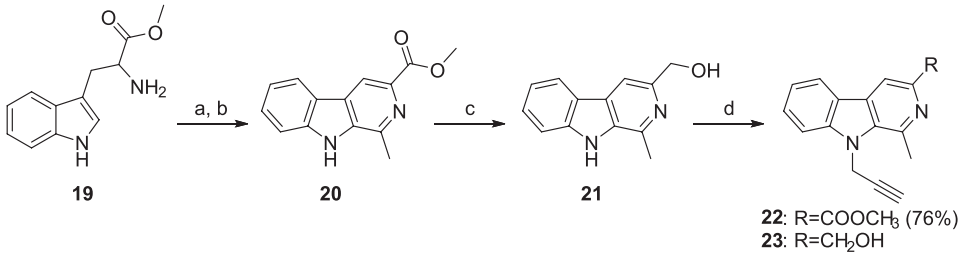
β-carbolines **4**, **13**, **20** and **25** were obtained according to previously described procedures (19), while the *β*-carbolines **11–12** were synthesized following modified literature procedures (18, 19). Phenol **5** was prepared according to a modified previously described procedure (29). *β*-carboline-based building blocks **21** and **23** were synthesised according to procedures previously reported by our group (30), while **16** (31), **31** (32), **33** and **34** (33), although previously described, were prepared following procedures reported in our earlier work (17) (Schemes 1–5).

Synthesis of *β*-carboline-based building blocks was conducted in multiple reaction steps. Alkylation of phenol **5** with ethyl bromide (2.4 equiv.) using cesium carbonate (1.4 equiv.) as a base afforded novel *β*-carboline **6**. The reaction was initiated with 1.2 equiv. of ethyl bromide and stirred at room temperature. Heating the reaction mixture to 40 °C did not improve conversion, and additional 1.2 equiv. of ethyl bromide and continued stirring at 40 °C, resulted in the appearance of by-products, thus the reaction was therefore stopped. Alkylation of phenol **5** with isopropyl bromide (8 equiv.) using cesium carbonate (4.2



(a) $CF_3CH(OH)OCH_2CH_3$, 110 °C, 3-4 h (**11-12**) or $(CH_3O)_2CHCH_3$, TFA, ACN, MW, 110 °C, 6 min (**13**); (b) Pd/C, EtOH, MW, 150 °C, 25 min or Pd/C, Li_2CO_3, EtOH, MW, 150 °C, 25 min (**13**); (c) propargyl bromide (3 equiv), NaH (2.66 equiv), DMF, rt, 2 h (**14-15**) or 24 h (**16**), (d) $BocNH(CH_2)_2Br$ (3 equiv), NaH (8 equiv), DMF, 90 °C, 21 h; (e) HCl/MeOH (10 equiv), 50 °C, 4 h.

Scheme 2

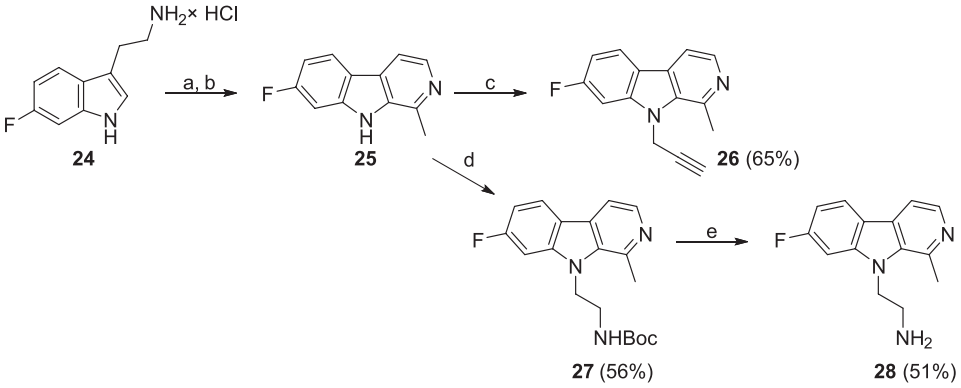


(a) $(CH_3O)_2CHCH_3$, TFA, ACN, MW, 110 °C, 12 min; (b) Pd/C, Li_2CO_3, EtOH, MW, 150 °C, 25 min; (c) $LiAlH_4$, THF, r.t., 30 min; (d) propargyl bromide (1.44 equiv for **22**), Cs_2CO_3 (2.88 equiv for **22**), DMF, rt, 24 h.

Scheme 3

equiv.) as a base afforded novel *β*-carboline **7**. The *β*-carboline **6** was obtained in 52 % yield, whereas *β*-carboline **7** was synthesized at room temperature over 23 h in 66 % yield.

The corresponding alkynes were obtained in moderate to high yields (**8**: 26 % and **9**: 58 %) by alkylation at *N*-9 with propargyl-bromide (3 equiv.) using 60 % NaH (2.66 equiv.) as a base at room temperature for 2 h. The same procedure was applied for the synthesis of alkynes **14** (61 %) and **15** (44 %) which were also obtained in moderate to high yields. Alkynes **26** and **32** were obtained in a similar way in moderate to high yields (**26**: 65 %; **32**: 52 %) by alkylation of the corresponding *β*-carboline at *N*-9 with propargyl-bromide (1.5 equiv.) using 60 % NaH (1.5 equiv.) as a base at room temperature for 2 h. Alkyne **22** was synthesised in high yield (76 %) by reaction with propargyl bromide (1.44 equiv.) in the presence of cesium carbonate as the base (2.88 equiv.). The reaction was initially carried out using 1.44 equiv. of cesium carbonate. TLC monitoring after 2 h indicated incomplete conversion and the presence of a considerable amount of starting material; consequently, a further 1.44. equiv. of cesium carbonate was added. The reaction was completed within 24 h at room temperature.



(a) $(CH_3O)_2CHCH_3$, TFA, ACN, MW, 110 °C, 10 min; (b) Pd/C, Li_2CO_3, EtOH, MW, 150 °C, 25 min; (c) propargyl bromide (1.5 equiv), NaH (1.5 equiv), DMF, rt, 2 h; (d) $BocNH(CH_2)_2Br$ (4 equiv), NaH (8 equiv), DMF, 90 °C, 3 h; (e) HCl/MeOH (10 equiv), 50 °C, 18 h.

Scheme 4

N-9 *β*-carboline-based amines **18** and **28** were synthesised *via* a two-step procedure. Initially, *β*-carbolines **13** and **25** were alkylated with 2-(Boc-amino)ethyl bromide (3–4 equiv.) using 60 % NaH (8 equiv.) at 90 °C for 18 h to afford Boc-protected intermediates **17** (48 %) and **27** (56 %). Subsequent acidic deprotection of the Boc group yielded the corresponding primary amines **18** and **28**. The deprotection step was carried out at 50 °C for 4 h or 18 h, respectively, affording the desired amines in 53 % and 51 % yields (Schemes 2, 4 and 5).

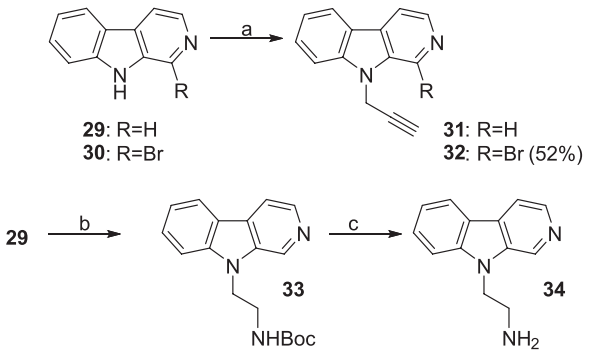
The AT hybrids (**35**–**37**) (Scheme 6) were obtained through a coupling reaction between the *β*-carboline-based amines (**18**, **28** and **34**) and 7-chloroquinoline-based carboxylic acid **2** using propylphosphonic anhydride (T3P), which has shown to be an efficient coupling reagent, in the presence of triethylamine (TEA). The reactions proceeded smoothly at room temperature over 18 h, affording the desired products in low yields (11–19 %).

In contrast, the TT hybrids (**38**–**47**) (Scheme 6) were synthesized *via* Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) also known as "*click*" reaction. The reactions were carried out using copper(II) acetate as a precatalyst in methanol at room temperature overnight, providing the corresponding hybrids in moderate to good yields (24–44 %). This methodology proved advantageous due to its operational simplicity, mild conditions, and tolerance toward various functional groups present in the *β*-carboline fragments.

Comparison of the two synthetic approaches indicates that the CuAAC strategy generally afforded higher yields than the coupling route. Moreover, the triazole linker introduces additional rigidity and potential for interactions with the target, while maintaining metabolic stability, which may contribute to the enhanced biological activity observed for several triazole derivatives.

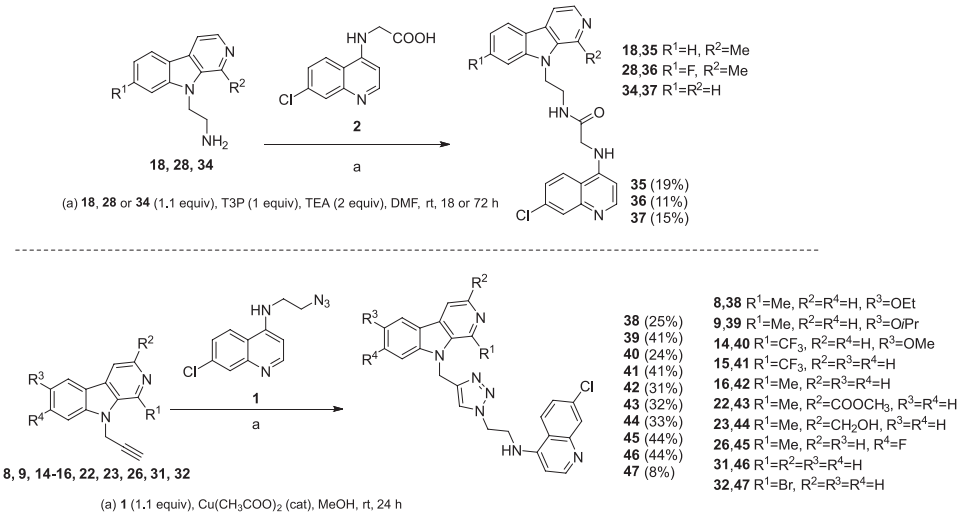
Both synthetic routes enabled efficient access to structurally diverse hybrids, allowing systematic variation of substituents on the *β*-carboline core and facilitating subsequent structure-activity relationship studies.

The synthesis of some intermediates, particularly alkynes and Boc-protected amines, also resulted in moderate to low yields, indicating incomplete conversions.



(a) propargyl bromide (1.5 equiv for **32**), NaH (1.5 equiv for **32**), DMF, rt, 2 h; (b) $BocNH(CH_2)_2Br$, Cs_2CO_3, DMF, 90 °C, 21 h; (c) HCl/MeOH, 50 °C, 27 h

Scheme 5



Scheme 6

In contrast, the CuAAC approach was more robust and reproducible, resulting in the higher number of final compounds; however, it is limited to substrates bearing azide and alkyne functionalities, requiring additional synthetic steps for intermediates preparation.

Table I. Analytical and MS data for AT and TT hybrids ***35–47***

Compd.	Yield (%)	t_t (°C)	Molecular formula	M_r	MS (*m*/*z*)	HPLC purity (%)
35	19	262.0–262.5	$C_{25}H_{22}ClN_5O$	443.93	444.4 $(M + 1)^+$	98.8
36	11	252.0–255.0	$C_{25}H_{21}ClFN_5O$	461.92	462.4 $(M + 1)^+$	n.d.[a]
37	15	263.0–265.5	$C_{24}H_{20}ClN_5O$	429.90	430.4 $(M + 1)^+$	98.2
38	25	> 200	$C_{28}H_{26}ClN_7O$	512.01	512.20 $(M + 1)^+$	100.0
39	41	80.0–89.5	$C_{29}H_{28}ClN_7O$	526.03	526.20 $(M + 1)^+$	100.0
40	24	133.5–139.0	$C_{27}H_{21}ClF_3N_7O$	551.95	552.4 $(M + 1)^+$	97.7
41	41	234.0–237.5	$C_{26}H_{19}ClF_3N_7$	521.92	522.4 $(M + 1)^+$	97.6
42	31	178.0–181.0	$C_{26}H_{22}ClN_7$	467.95	468.5 $(M + 1)^+$	100.0
43	32	> 240	$C_{28}H_{24}ClN_7O_2$	525.99	526.3 $(M + 1)^+$	100.0
44	33	159.5–166.5	$C_{27}H_{24}ClN_7O$	497.98	498.10 $(M + 1)^+$	99.0
45	44	234.5–250.5	$C_{26}H_{21}ClFN_7$	485.94	486.4 $(M + 1)^+$	100.0
46	44	223.5–225.5	$C_{25}H_{20}ClN_7$	453.93	454.4 $(M + 1)^+$	97.1
47	8	165.0–169.0	$C_{25}H_{19}BrClN_7$	532.82	533.95 $(M + 1)^+$	n.d.

[a] *n.d.* not determined

Overall, while the developed synthetic routes are versatile, they could be further optimized to improve yields and streamline access to more demanding derivatives.

Structures of AT and TT hybrids were confirmed by MS, IR, ^{1}H, and ^{13}C NMR spectra. Analytical and spectral data are given in Tables I and II.

SwissADME prediction

Physicochemical descriptors of the title compounds **35**–**47** were assessed by using SwissADME tool. The determined parameters are outlined in Table III: molecular weight (MW), heavy atoms (HA), number of rotatable bonds (RB), number of hydrogen bond acceptors (HBA) and donors (HBD), topological polar surface area (TPSA), consensus partition coefficient (cLog P) and Lipinski's (LV) and Veber's violations (VV). The number of HBA and HBD and cLogP are in line with the Lipinski's rule of five. However, some compounds (**38**–**41**, **43**, **45**–**47**) have molecular weight slightly above the limit of 500. TPSA represents the sum of the surface areas of all polar atoms in one molecule indicating its cellular permeability. All *β*-carboline and CQ hybrids have TPSA values within the suggested range (20–130 Å^2). In addition, all compounds are in line with Veber's rules (10 or less RB and TPSA equal to or less than

Table II. IR, ^{1}H and ^{13}C NMR spectroscopic data for AT and TT hybrids **35**–**47**

Compd.	IR (ATR, ν/cm^{-1})	^{1}H NMR (300, 400 or 600 MHz, DMSO-d_6) δ ppm	^{13}C NMR (75, 101 or 151 MHz, DMSO-d_6) δ ppm
35	3294, 1657, 1582, 1563, 1448, 1407, 1349, 1286, 1237, 1204, 1148, 1082, 983, 879, 849, 793, 747, 731, 639, 597, 561, 545	8.37–8.29 (m, 2H, 7/8′), 8.28–8.18 (m, 3H, 13′/3/3′), 8.00 (d, 1H, J = 4.1 Hz, 6′), 7.82 (s, 1H, 10′), 7.73 (d, 2H, J = 6.9 Hz, 6/12), 7.60 (t, 1H, J = 7.0 Hz, 1), 7.50 (d, 1H, J = 8.2 Hz, 12′), 7.28 (t, 1H, J = 6.8 Hz, 1), 6.08 (d, 1H, J = 5.4 Hz, 7′), 4.64 (t, 1H, J = 7.1 Hz, 1′), 3.85 (d, 1H, J = 4.4 Hz, 5′), 3.52 (q, J = 6.8 Hz, 1H, 2′), 3.00 (s, 1H, 13)	169.47 (4′), 151.77 (8′), 150.19 (15′), 148.79 (9′), 141.36 (8), 141.23 (11), 137.65 (7), 134.50 (9), 133.54 (11′), 128.13 (10′), 128.10 (4), 127.40 (13′), 124.37 (12′), 124.17 (1), 121.54 (3), 120.66 (14′), 119.66 (2), 117.51 (5), 112.97 (6), 110.19 (12), 98.98 (7′), 45.84 (5′), 43.35 (1′), 38.99 (2′), 23.24 (13)
36	3288, 1656, 1630, 1585, 1447, 1408, 1370, 1351, 1306, 1266, 1253, 1211, 1173, 1151, 1133, 1078, 956, 878, 850, 828, 809, 762, 651, 623, 549	8.39 (t, 1H, J = 6.1 Hz, 8′), 8.34 (d, 1H, J = 5.5 Hz, 7′), 8.31–8.27 (m, 2H, 6/13′), 8.24 (d, 1H, J = 5.2 Hz, 6), 7.99 (d, 2H, J = 5.2 Hz, 3/3′), 7.84 (d, 1H, J = 2.2 Hz, 10′), 7.59 (dd, 1H, J = 10.5, 2.3 Hz, 12′), 7.53 (dd, 1H, J = 9.0, 2.3 Hz, 2), 7.12 (ddd, 1H, J = 9.4, 8.5, 2.2 Hz, 12), 6.11 (d, 1H, J = 5.6 Hz, 7′), 4.59 (t, 2H, J = 7.1 Hz, 1′), 3.88 (d, 2H, J = 5.8 Hz, 5′), 3.51 (q, 2H, J = 6.8 Hz, 2′), 2.99 (s, 3H, 13)	169.28 (4′), 162.78 (d, J = 241.7 Hz, 1), 150.82 (8′), 150.76 (15′), 147.67 (9′), 142.17 (d, J = 12.8 Hz, 8), 141.22 (4), 138.12 (7), 135.15 (d, J = 1.8 Hz, 11), 134.02 (11′), 127.96 (5), 126.49 (10′), 124.65 (13′), 124.38 (12), 123.32 (d, J = 10.8 Hz, 6), 117.33 (d, J = 11.1 Hz, 9), 112.85 (12′), 108.08 (d, J = 24.6 Hz, 3), 98.92 (7′), 96.95 (d, J = 27.1 Hz, 2), 45.80 (5′), 43.61 (1′), 38.72 (2′), 23.07 (13)
37	3282, 1664, 1582, 1453, 1368, 1247, 1213, 1151, 849, 815, 747, 727, 550	9.04 (s, 1H, 8), 8.40 (d, 1H, J = 5.2 Hz, 8′), 8.29 (t, 1H, J = 2.7 Hz, 3′), 8.28 (s, 1H, 6′), 8.20 (dd, 2H, J = 7.2, 1.7 Hz, 3/7), 8.15 (dd, 1H, J = 5.2, 1.1 Hz, 12), 7.81 (d, 1H, J = 6.5 Hz, 6), 7.79 (d, 1H, J = 2.3 Hz, 10′), 7.70 (d, 1H, J = 8.3 Hz, 13′), 7.62 (ddd, 1H, J = 8.3, 7.0, 1.2 Hz, 1), 7.48 (dd, 1H, J = 9.0, 2.2 Hz, 12′), 7.34–7.27 (m, 1H, 2), 5.85 (d, 1H, J = 5.5 Hz, 7′), 4.58 (t, 2H, J = 6.1 Hz, 1′), 3.75 (d, 2H, J = 6.0 Hz, 5′), 3.58 (q, 2H, J = 6.0 Hz, 2′)	169.66 (4′), 151.48 (8′), 150.98 (15′), 148.45 (9′), 141.39 (11), 138.99 (7), 136.76 (9), 134.25 (11′), 132.96 (8), 128.78 (10′), 127.80 (5), 127.22 (13′), 124.94 (12′), 124.72 (1), 122.46 (3), 120.94 (4), 120.01 (2), 117.76 (14′), 115.08 (6), 110.41 (12), 99.28 (7′), 46.11 (5′), 42.45 (1′), 38.59 (2′)

Compd.	IR (ATR, ν/cm^{-1})	^{1}H NMR (300, 400 or 600 MHz, DMSO-d_6) δ ppm	^{13}C NMR (75, 101 or 151 MHz, DMSO-d_6) δ ppm
38	2971, 1579, 1448, 1368, 1324, 1244, 1220, 1194, 1141, 1103, 1049, 986, 879, 841, 819, 807, 741, 693, 622, 533	8.32 (d, 1H, *J* = 5.3 Hz, 16′), 8.18 (d, 1H, *J* = 5.2 Hz, 7), 8.03 (d, 1H, *J* = 9.0 Hz, 11′), 7.96 (d, 1H, *J* = 5.2 Hz, 6), 7.94 (s, 1H, 5′), 7.80 (d, 1H, *J* = 2.0 Hz, 3), 7.77 (d, 1H, *J* = 2.3 Hz, 14′), 7.63 (d, 1H, *J* = 9.0 Hz, 12), 7.42 (dd, 1H, *J* = 9.0, 2.1 Hz, 13′), 7.35 (t, 1H, *J* = 5.6 Hz, 18′), 7.14 (dd, 1H, *J* = 8.9, 2.4 Hz, 1), 6.44 (d, 1H, *J* = 5.4 Hz, 8′), 5.82 (s, 2H, 3′), 4.55 (t, 2H, *J* = 5.9 Hz, 6′), 4.12 (q, 2H, *J* = 6.9 Hz, 1′), 3.69 (q, 2H, *J* = 5.7 Hz, 7′), 2.99 (s, 3H, 13), 1.40 (t, 3H, *J* = 6.9 Hz, 2′)	153.38 (10'), 152.26 (16'), 149.99 (15'), 149.41 (2), 144.41 (8), 142.22 (9), 137.65 (7), 136.27 (13'), 135.34 (1), 133.87 (4'), 128.31 (4), 127.96 (14'), 124.72 (5'), 124.22 (11'), 123.82 (12'), 121.62 (5), 118.57 (6), 117.80 (10), 113.45 (1), 111.88 (12), 104.77 (3), 99.18 (17'), 64.09 (1'), 48.30 (3), 42.69 (6'/7'), 23.65 (13), 15.27 (2')
39	2971, 1579, 1489, 1448, 1324, 1292, 1244, 1220, 1194, 1141, 1103, 1049, 986, 909, 879, 841, 819, 807, 741, 622, 533	8.32 (d, 1H, *J* = 5.1 Hz, 7), 8.17 (d, 1H, *J* = 5.1 Hz, 16′), 8.05 (d, 1H, *J* = 9.0 Hz, 11′), 7.97 (s, 1H, 6), 7.96 (s, 1H, 5′), 7.80 (d, 1H, *J* = 2.2 Hz, 3), 7.78 (d, 1H, 14′), 7.61 (d, 1H, *J* = 8.9 Hz, 12), 7.44 (d, 2H, *J* = 7.0 Hz, 12'/8'), 7.12 (dd, 1H, *J* = 8.9, 2.0 Hz, 1), 6.45 (d, 1H, *J* = 5.4 Hz, 17′), 5.81 (s, 2H, 3′), 4.65 (hept, 1H, *J* = 5.8, 5.3 Hz, 1′), 4.55 (t, 2H, *J* = 5.8 Hz, 6′), 3.70 (q, 2H, *J* = 6.0 Hz, 7′), 2.99 (s, 3H, 13), 1.31 (d, 6H, *J* = 6.0 Hz, 2′)	151.56 (9'), 151.35 (16'), 149.74 (2), 148.43 (15'), 143.89 (8), 141.62 (9), 137.09 (7), 135.84 (13'), 134.83 (11), 133.56 (4'), 127.77 (4), 127.07 (14'), 124.32 (11'), 123.77 (5'), 123.32 (12'), 121.18 (5), 119.32 (6), 117.19 (10'), 112.98 (1), 111.31 (12), 106.48 (3), 98.63 (17'), 70.08 (1'), 47.78 (3'), 42.19 (6'/7'), 23.07 (13), 21.89 (2')
40	2919, 1579, 1489, 1345, 1288, 1190, 1136, 1111, 1065, 835, 720, 557	8.55 (d, 1H, *J* = 4.9 Hz, 7), 8.49 (d, 1H, *J* = 5.0 Hz, 15′), 8.31 (d, 1H, *J* = 5.4 Hz, 6), 8.02 (d, 1H, *J* = 9.1 Hz, 10′), 7.94 (d, 1H, *J* = 2.6 Hz, 3), 7.78 (s, 2H, 4′/13′), 7.60 (d, 1H, *J* = 9.0 Hz, 12), 7.40 (dd, 1H, *J* = 9.0, 2.2 Hz, 11′), 7.37 (t, 1H, *J* = 5.6 Hz, 16′), 7.21 (dd, 1H, *J* = 9.0, 2.6 Hz, 1), 6.41 (d, 1H, *J* = 5.5 Hz, 7′), 5.73 (s, 2H, 2′), 4.53 (t, 2H, *J* = 6.0 Hz, 5′), 3.88 (s, 3H, 1′), 3.68 (q, 2H, *J* = 5.9 Hz, 6′)	154.61 (8), 151.53 (15'), 149.75 (7'), 148.65 (2), 142.72 (14'), 136.91 (11), 136.59 (7), 133.59 (12'), 132.97 (9), 132.37 (3'), 128.44 (q, *J* = 34.9 Hz, 5), 127.26 (13'), 124.31 (10'), 123.80 (4'), 123.26 (11'), 121.50 (9'), 120.97 (4), 119.34 (1), 119.04 (3), 117.28 (13), 112.60 (6), 103.68 (12), 98.69 (16'), 55.75 (1'), 47.90 (2), 44.48 (5'), 42.25 (6')
41	2922, 1613, 1584, 1429, 1347, 1323, 1295, 1193, 1142, 1120, 1068, 838, 803, 745, 726, 642, 557	8.58 (d, 1H, *J* = 5.0 Hz, 7), 8.54 (d, 1H, *J* = 4.9 Hz, 14′), 8.39 (d, 1H, *J* = 7.8 Hz, 9′), 8.30 (d, 1H, *J* = 5.5 Hz, 6), 8.05 (d, 1H, *J* = 9.0 Hz, 3), 7.83 (s, 1H, 3′), 7.79 (d, 1H, *J* = 2.2 Hz, 12′), 7.70 (d, 1H, *J* = 8.4 Hz, 12), 7.60 (ddd, 1H, *J* = 8.3, 7.0, 1.2 Hz, 1), 7.46 (d, 1H, *J* = 5.9 Hz, 15′), 7.42 (dd, 1H, *J* = 9.0, 2.2 Hz, 2), 7.40–7.36 (m, 1H, 10′), 6.42 (d, 1H, *J* = 5.5 Hz, 6′), 5.77 (s, 2H, 1′), 4.54 (t, 2H, *J* = 6.0 Hz, 4′), 3.69 (q, 2H, *J* = 5.9 Hz, 5′)	151.26 (14'), 149.95 (8), 148.33 (7'), 142.61 (13'), 142.10 (11), 137.22 (7), 133.74 (11'), 132.71 (9), 132.59 (2'), 129.73 (12'), 128.42 (q, *J* = 35.1 Hz, 5), 127.01 (9'), 124.42 (3'), 123.87 (10'), 123.33 (1), 121.78 (3), 121.49 (4), 121.18 (2), 120.43 (8'), 119.00 (6), 117.23 (13), 111.64 (12), 98.68 (15'), 47.91 (1'), 44.49 (4'), 42.27 (5')
42	2957, 1616, 1582, 1445, 1405, 1354, 1327, 1300, 1245, 1200, 1178, 1141, 1084, 1047, 999, 973, 911, 875, 840, 775, 750, 733, 688, 646, 584, 558, 519	8.25 (s, 1H, 7), 8.23 (d, 1H, *J* = 7.9 Hz, 14′), 8.11 (d, 1H, *J* = 8.9 Hz, 9′), 8.01 (d, 1H, *J* = 4.8 Hz, 6), 7.98 (s, 1H, 3′), 7.84 (s, 1H, 12′), 7.73 (d, 1H, *J* = 8.3 Hz, 3), 7.65 (d, 1H, *J* = 7.0 Hz, 15′), 7.52 (t, 1H, *J* = 7.7 Hz, 2), 7.46 (d, 1H, *J* = 9.0 Hz, 10′), 7.26 (t, 1H, *J* = 7.4 Hz, 1), 6.51 (s, 1H, 6′), 5.86 (s, 2H, 1′), 4.56 (t, 2H, *J* = 6.0 Hz, 4′), 3.73 (q, 2H, *J* = 6.0 Hz, 5′), 3.00 (s, 3H, 13)	150.47 (7'), 149.88 (14'), 143.93 (13'), 143.42 (8), 141.76 (11), 140.96 (9), 137.70 (7), 135.52 (11), 134.20 (2'), 128.16 (12'), 128.11 (4), 126.85 (8'), 126.34 (9'), 124.80 (3'), 124.08 (10'), 123.47 (3), 121.47 (1), 120.77 (5), 119.81 (2), 113.06 (6), 110.54 (12), 103.51 (15'), 47.88 (1'), 42.36 (4'/5'), 23.21 (13)

Compd.	IR (ATR, ν/cm^{-1})	^{1}H NMR (300, 400 or 600 MHz, DMSO-d_6) δ ppm	^{13}C NMR (75, 101 or 151 MHz, DMSO-d_6) δ ppm
43	1713, 1578, 1430, 1350, 1273, 1240, 1140, 1050, 910, 875, 840, 786, 749, 730, 532	8.81 (s, 1H, 6), 8.40 (d, 1H, *J* = 7.8 Hz, 11'), 8.31 (d, 1H, *J* = 7.7 Hz, 3), 8.06 (d, 1H, *J* = 9.0 Hz, 16'), 8.03 (s, 1H, 5'), 7.81 (d, 2H, *J* = 8.1 Hz, 12'/12), 7.64 (s, 1H, 14'), 7.58 (t, 1H, *J* = 7.7 Hz, 1), 7.46 (d, 1H, *J* = 8.9 Hz, 17'), 7.33 (t, 1H, *J* = 7.5 Hz, 2), 6.47 (d, 1H, *J* = 5.3 Hz, 8'), 5.93 (s, 2H, 3'), 4.57 (t, 2H, *J* = 5.9 Hz, 6'), 3.91 (s, 3H, 1'), 3.73 (q, 2H, *J* = 5.9 Hz, 7'), 3.06 (s, 3H, 13)	165.89 (2'), 150.47 (9'), 143.54 (15), 141.89 (7), 141.36 (8), 136.14 (11), 136.06 (9), 134.18 (5), 128.65 (16'), 128.17 (4'), 127.61 (4), 126.33 (14'), 124.77 (11'), 124.03 (5'), 123.65 (12'), 121.89 (1), 120.97 (10'), 120.76 (3), 115.99 (2), 111.01 (6), 110.00 (12), 98.72 (17), 51.98 (1'), 47.91 (3'), 42.33 (6'/7'), 23.29 (13)
44	3079, 1622, 1580, 1456, 1427, 1360, 1336, 1297, 1256, 1197, 1137, 1123, 1031, 1014, 983, 914, 842, 808, 762, 732, 643, 627, 580, 567	8.30 (d, 1H, *J* = 5.4 Hz, 16'), 8.21 (d, 1H, *J* = 7.8 Hz, 11'), 8.10 (d, 1H, *J* = 8.8 Hz, 3), 8.01 (s, 1H, 6), 8.00 (s, 1H, 5'), 7.79 (d, 1H, *J* = 2.2 Hz, 14'), 7.71 (d, 1H, *J* = 8.3 Hz, 12), 7.51 (ddd, 1H, *J* = 8.3, 7.0, 1.2 Hz, 1), 7.46 (d, 1H, *J* = 5.6 Hz, 17'), 7.42 (dd, 1H, *J* = 9.0, 2.3 Hz, 12'), 7.24 (t, 1H, *J* = 7.4 Hz, 2), 6.43 (d, 1H, *J* = 5.5 Hz, 8'), 5.84 (s, 2H, 3'), 5.38 (s, 1H, 2'), 4.67 (s, 2H, 1'), 4.56 (t, 2H, *J* = 6.0 Hz, 6'), 3.69 (q, 2H, *J* = 6.0 Hz, 7'), 2.98 (s, 3H, 13)	151.80 (16'), 150.24 (9'), 149.68 (15'), 148.96 (8'), 143.92 (7), 141.31 (11), 140.51 (9), 133.48 (13'), 133.42 (4'), 129.03 (4), 128.02 (14'), 127.47 (11'), 124.31 (5'), 123.98 (12'), 123.44 (1), 121.37 (3), 120.93 (10'), 119.67 (2), 117.41 (5), 110.52 (12), 109.06 (6), 98.73 (17'), 64.35 (1'), 47.89 (3'), 42.27 (6'/7'), 23.08 (13)
45	3063, 1628, 1578, 1444, 1331, 1244, 1161, 1128, 1039, 927, 803, 764, 619, 549	8.31 (d, 1H, *J* = 5.5 Hz, 14'), 8.28–8.25 (m, 1H, 7), 8.25–8.22 (m, 1H, 9'), 8.06 (d, 1H, *J* = 9.0 Hz, 3), 8.03 (s, 1H, 3'), 7.98 (d, 1H, *J* = 5.3 Hz, 6), 7.80 (d, 1H, *J* = 2.3 Hz, 12'), 7.66 (dd, 1H, *J* = 10.5, 2.3 Hz, 10'), 7.58 (t, 1H, *J* = 5.8 Hz, 6'), 7.43 (dd, 1H, *J* = 8.9, 2.2 Hz, 2), 7.11 (td, 1H, *J* = 9.0, 2.3 Hz, 12), 6.48 (d, 1H, *J* = 5.6 Hz, 15'), 5.84 (s, 2H, 1'), 4.58 (t, 2H, *J* = 6.0 Hz, 4'), 3.73 (q, 2H, *J* = 5.9 Hz, 5'), 3.01 (s, 3H, 13)	162.74 (d, *J* = 241.8 Hz, 1), 150.73 (14'), 150.29 (7'), 147.69 (13'), 143.52 (8), 141.95 (d, *J* = 12.8 Hz, 9), 141.72 (11), 138.31 (7), 135.12 (11'), 134.02 (2'), 127.98 (4), 126.52 (9'), 124.63, 123.91 (3'), 123.58 (10'), 123.20 (d, *J* = 10.9 Hz, 3), 117.46 (8'), 117.12 (5), 112.81 (6), 108.21 (d, *J* = 24.5 Hz, 12), 98.71 (15'), 97.40 (d, *J* = 27.1 Hz, 2), 47.87 (1'), 42.33 (4'), 39.10 (5'), 23.14 (13)
46	1578, 1452, 1328, 1033, 841, 746, 728, 557	9.17 (s, 1H, 8), 8.40 (d, 1H, *J* = 5.2 Hz, 7), 8.30 (d, 1H, *J* = 5.6 Hz, 14'), 8.26 (dt, 1H, *J* = 7.8, 1.0 Hz, 3), 8.18 (s, 1H, 3'), 8.14–8.10 (m, 2H, 9'/15'), 7.84–7.80 (m, 2H, 6'/10'), 7.72 (s, 1H, 12'), 7.57 (ddd, 1H, *J* = 8.3, 7.1, 1.2 Hz, 1), 7.46 (dd, 1H, *J* = 9.0, 2.2 Hz, 12), 7.28 (ddd, 1H, *J* = 7.9, 7.0, 0.9 Hz, 2), 6.49 (d, 1H, *J* = 5.7 Hz, 6'), 5.76 (s, 2H, 1'), 4.59 (t, 2H, *J* = 6.0 Hz, 4'), 3.74 (q, 2H, *J* = 6.0 Hz, 5')	150.53 (14'), 150.45 (7'), 142.75 (13'), 140.46 (11), 138.68 (7), 135.79 (9), 134.11 (11'), 133.02 (8), 128.27 (12'), 127.41 (2'), 126.31 (9'), 124.72 (10'), 124.13 (3'), 123.92 (2), 121.90 (1), 120.92 (4) 120.49 (5), 119.69 (3), 117.11 (8'), 114.56 (6), 110.52 (12), 98.72 (15'), 47.81 (1'), 42.36 (4'), 37.72 (5')
47	3295, 2921, 2852, 1612, 1579, 1536, 1487, 1450, 1434, 1354, 1330, 1194, 1141, 1051, 912, 842, 768, 749, 728, 639, 596, 556	8.30 (d, 1H, *J* = 7.8 Hz, 14'), 8.25 (d, 2H, *J* = 5.0 Hz, 3/7), 8.20 (d, 1H, *J* = 10.0 Hz, 9'), 8.17 (d, 1H, *J* = 5.0 Hz, 6), 8.00 (s, 1H, 3'), 7.88 (s, 1H, 12'), 7.78 (d, 1H, *J* = 8.4 Hz, 12), 7.60 (t, 1H, *J* = 7.7 Hz, 1), 7.53 (d, 1H, *J* = 9.0 Hz, 10'), 7.34 (t, 1H, *J* = 7.4 Hz, 2), 6.52 (s, 1H, 6'), 6.07 (s, 2H, 1'), 4.60 (t, 2H, *J* = 5.9 Hz, 4'), 3.81 (q, 2H, *J* = 5.9 Hz, 5')	152.11 (7'), 150.94 (13'), 144.66 (8), 143.45 (11), 141.54 (11'), 138.62 (14'), 135.42 (2'), 132.95 (9), 131.31 (4), 129.12 (7), 125.47 (9'), 124.50 (3), 123.65 (12'), 122.13 (8'), 121.75 (1), 121.57 (10'), 121.15 (12), 120.83 (3), 120.12 (5), 115.03 (2), 111.26 (6), 98.49 (15'), 47.87 (1'), 42.51 (4'), 39.01 (5')

140 Å^2 or 12 or fewer HBD and HBA), which indicates high probability of their good oral bioavailability (34).

Table III. Drug-like properties based on Lipinski's and Veber's rules

Compd.	MW	HA	RB	HBA	HBD	TPSA/Å^2	cLogP	LV	VV
35	443.93	32	7	3	2	71.84	3.82	0	0
36	461.92	33	7	4	2	71.84	4.09	0	0
37	429.9	31	7	3	2	71.84	2.14	0	0
38	512.01	37	8	5	1	82.68	4.39	1	0
39	526.03	38	8	5	1	82.68	4.68	1	0
40	551.95	39	8	8	1	82.68	4.73	1	0
41	521.92	37	7	7	1	73.45	4.67	1	0
42	467.95	34	6	4	1	73.45	4.05	0	0
43	525.99	38	8	6	1	99.75	4.04	1	0
44	497.98	36	7	5	2	93.68	3.72	0	0
45	485.94	35	6	5	1	73.45	4.32	0	0
46	453.93	33	6	4	1	73.45	3.68	0	0
47	532.82	34	6	4	1	73.45	4.32	1	0

MW, molecular weight; HA, number of heavy atoms; RB, number of rotatable bonds; HBA, number of H-bond acceptors; HBD, number of H-bond donors; TPSA, polar surface area; cLogP, partition coefficient; LV, number of violations of the Lipiski rules; VV, number of the violations of the Veber rules.

Antiplasmodial activity

The antiplasmodial activity of the synthesized hybrids (**35**–**47**) was evaluated against the two *P. falciparum* strains (3D7 and Dd2), and the results are summarized in Table IV. All compounds exhibited activity in the nanomolar to low micromolar range, with IC_{50} values spanning from 1.0 to 365.5 nmol L^{-1} for 3D7 and from 2.2 to 2415.3 nmol L^{-1} for Dd2 strain. The most potent compounds against 3D7 strain were **36** (IC_{50} = 1.0 ± 0.2 nmol L^{-1}), **45** (1.2 ± 0.2 nmol L^{-1}), and **42** (2.9 ± 0.1 nmol L^{-1}), showing superior activity compared to the reference drug chloroquine (CQ, IC_{50} = 6.9 ± 0.0 nmol L^{-1}). Compounds **35** (4.6 ± 0.3 nmol L^{-1}) and **43** (11.9 ± 3.0 nmol L^{-1}) demonstrated potency comparable to CQ. Compounds **38**–**41**, **43**, **44**, **46**, and **47**, were less active compared to CQ. In the case of multi-drug resistant *P. falciparum* strain Dd2, only three compounds were less active than CQ (**37**, **44** and **46**), which strongly

Table IV. In vitro *antiplasmodial activity of hybrids* ***35***–***47*** *against* P. falciparum *erythrocytic stage (Pf3D7 and PfDd2 strains) and resistance index (RI) calculated as ratio between multi-drug resistant (PfDd2) and CQ sensitive (Pf3D7)* P. falciparum *strains*

Compd.	IC_{50}[a] (nmol L^{-1})		RI[e]	Position 1 substitution	Ring A substitution	*β*-carboline HBD/HBA[f]	Halogen
	*Pf*3D7	*Pf*Dd2					
35	4.6 ± 0.3[b]	10.4 ± 1.8	2.26	Me	–	–	–
36	1.0 ± 0.2	5.1 ± 0.6	5.10	Me	F	–	F
37	124.4 ± 72.8	455.2 ± 182.4	3.66	-	–	–	–
38	50.2 ± 2.4	35.5 ± 17.1	0.71	Me	OEt	HBA	–
39	44.7 ± 9.1	32.6 ± 13.5	0.73	Me	O*i*Pr	HBA	–
40	365.5 ± 6.8	70.6 ± 40.2	0.19	CF_3	OMe	HBA	CF_3
41	49.7 ± 29.3	64.2 ± 2.6	1.29	CF_3	–	–	CF_3
42	2.9 ± 0.1	92.4 ± 9.5	31.86	Me	–	–	–
43	11.9 ± 3.0	6.1 ± 2.3	0.51	Me	–	HBA	–
44	74.0 ± 6.1	2415.3 ± 531.9	32.64	Me	–	HBA, HBD	–
45	1.2 ± 0.2	2.2 ± 1.7	1.83	Me	F	–	F
46	59.8 ± 11.8	439.5 ± 76.1	7.35	-	–	–	–
47	67.6 ± 40.0	60.5 ± 4.2	0.89	Br	–	–	Br
CQ[c]	6.9 ± 0.0	148.6 ± 37.1	21.54				
harmine	8250 ± 2830[d]	> 27777.8	> 3.37				

[a] IC_{50}, the concentration of the tested compound that is necessary for 50 % growth inhibition; [b] the results are expressed as mean ± SD ($n \geq 2$); [c] CQ, chloroquine; [d] (20); [e] RI, resistance index expressed as ratio between IC_{50} obtained for multi-drug resistant (Dd2) and CQ-sensitive (3D7) *P. falciparum* strain; IC_{50}(*Pf*Dd2)/IC_{50}(*Pf*3D7); [f]HBD/HBA, hydrogen bond donors/hydrogen bond acceptors present in the *β*-carboline.

confirms the overall activity of the whole series, resulting in the resistance ratios significantly lower than for CQ.

By introducing various scaffolds in the *β*-carboline ring, we were once again able to establish SAR. Compounds **38** and **39**, structurally resembling to the novel hybrids from our previous work, were compared to the compound **39** bearing *O*-methyl group at the position 6 of the *β*-carboline ring from the previous series (17). Substitution of methyl group with ethyl (**38**) or *i*-propyl (**39**) in the new series, led to reduction in activity against 3D7 and increase in activity against Dd2 strain.

The compounds could be divided in five subgroups based on their structural features:

1) Amide-type (AT, **35**–**37**) and triazole-type (TT, **38**–**47**)
2) Compounds bearing substituent at the position 1 (CH_3 – **35**, **36**, **38**, **39**, **42**–**45**; CF_3 – **40**, **41**; Br – **47**)
3) Compounds without substituents on the ring A (amide **35**, **37** and triazoles **41**–**43**, **46** and **47**)
4) Compounds bearing hydrogen bond donors and/or acceptors (**38**–**40**, **43** and **44**)
5) Halogenated compounds bearing bromine (**47**), fluorine (**36** and **45**) or CF_3 group (**40**)

(1) Looking at amides and triazoles, it is clear that the substituent at the position 1 was essential for the strong activity in both groups, since the compounds without substituent at the position 1 are by far the least active.

(2) If we observe the compounds with the substituent at the position 1, the activity notably depended on the type of substituent. The methyl substituent has shown the best for the activity, and almost all compounds with methyl substituent at the position 1 have demonstrated strong activity in low nanomolar range.

(3) The substituents on the ring A (Fig. 2) influenced differently on the antiplasmodial activity, depending on the position on the ring A and the type of the substituent, the highest activity was shown for C-7 fluorinated compounds (**36** and **45**).

(4) Comparation of compounds rich in hydrogen bond donors (**44**) and acceptors (**43** and **44**) at the position 3 of the *β*-carboline ring, has shown that ester group was much more active than alcohol, therefore indicating the significance of the presence of hydrogen bond acceptors at the position 3 of the *β*-carboline ring.

(5) Halogenation influenced differently on the antiplasmodial activity, once again highlighting the presence of the fluorine as the group crucial for the strong antiplasmodial activity.

Almost all compounds demonstrated resistance ratios better than CQ, with the exception of **42** and **44**. Compounds **38**, **39**, **40**, **43** and **47** were more active against multi-drug resistant *P. falciparum* strain Dd2, then against CQ sensitive 3D7, with resistance ratios spanning from 0.19 for **40** to 0.9 for **47**, respectively.

In vitro *cytotoxicity screening*

Cytotoxicity of the title compounds was evaluated against the human HepG2 cell line. The results are presented in Table V. No significant cytotoxic effects were observed up to the highest tested concentration ($IC_{50} > 250$ μmol L^{-1}) for all tested compounds. Consequently, selectivity indices (SI), defined as the ratio of IC_{50} (HepG2) to IC_{50} (*P. falciparum* 3D7), are reported as lower limits. All tested compounds displayed very high selectivity, with SI values exceeding 10^3 for the most of compounds.

Harmine, used as a reference, exhibited significantly weaker antiplasmodial activity ($IC_{50} = 8250 \pm 2830$ nmol L^{-1}), resulting in a markedly lower selectivity index (SI > 30). This finding highlights the importance of hybridization with the CQ derived 4-amino-7--chloroquinoline moiety for enhancing both potency and selectivity.

Table V. In vitro *cytotoxicity screening of the novel compounds* ***35***–***47*** *against HepG2 and calculated selectivity indices*

Compd.	IC_{50}[a] (μmol L^{-1})	SI[d]
35	> 250	> 54349
36	> 250	> 263158
37	> 250	> 2009
38	> 250	> 4980
39	> 250	> 5593
40	> 250	> 687
41	> 250	> 5030
42	> 250	> 86207
43	> 250	> 21008
44	> 250	> 3378
45	> 250	> 217391
46	> 250	> 4181
47	> 250	> 3698
harmine	> 250[c]	> 30[e]

[a] IC_{50}, the concentration of the tested compound that is necessary for 50 % growth inhibition; [b] the results are expressed as mean ± SD ($n \geq 2$); [c] the precise IC_{50} value couldn't be determined as the activity was only detectable at the highest concentration tested (20); [d] SI (selectivity index) = IC_{50} (HepG2)/IC_{50} (Pf3D7); [e] the precise Selectivity Index (SI) couldn't be obtained due to the inability to determine the exact IC_{50} value

CONCLUSIONS

Overall, the obtained results demonstrate that the newly synthesized *β*-carboline and CQ hybrids combine outstanding antiplasmodial potency with low cytotoxicity toward human cells and favorable drug-like properties, highlighting compounds **36**, **42**, and **45** as the most promising candidates for further investigation with the aim of elucidating their mechanism of action.

Acronyms and abbreviations. – AT – amide-type, Boc – *tert*-butyloxycarbonyl, CQ – chloroquine, CuAAC – copper(I)-catalyzed azide-alkyne cycloaddition, DCM – dichloromethane, DMF – *N*,*N*-dimethylformamide, DMSO – dimethyl sulfoxide, ESI – electrospray ionization, FTIR-ATR – attenuated total reflectance Fourier transform infrared spectroscopy, HepG2 – human hepatocellular carcinoma cell line, HRP2 – histidine-rich protein 2, IC_{50} – the concentration of the tested compound necessary for 50 % growth inhibition, MeOH – methanol, NMR – nuclear magnetic resonance, *Pf*ATP4 – *P. falciparum* P-type ATPase, a Na^+ efflux pump essential for ionic homeostasis, *Pf*3D7 – chloroquine-sensitive strain of *P. falciparum*, *Pf*Dd2 – multidrug-resistant strain of *P. falciparum*, *Pf*CARL – *P. falciparum* cyclic amine resistance locus, RI – resistance index, SI – selectivity index, T3P – propylphosphonic anhydride, TEA – triethylamine, TMS – tetramethylsilane, TT – triazole-type, TWC – total wavelength chromatogram.

Conflicts of interest. – The authors declare no conflict of interest

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