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Synthesis and physicochemical characterization of novel 6-aminopyrido[3,4-*c*][1,9]phenanthrolines as aza-analogs of benzo[*c*]phenanthridines

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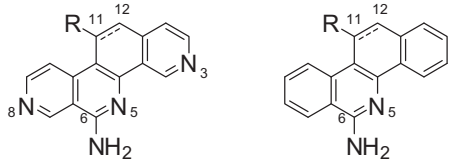
ABSTRACT

Based on the 6-aminobenzo[*c*]phenanthridines, a compound class showing noteworthy antitumor activity, an efficient one-step synthesis consisting of a base-catalyzed condensation of 2 equiv of 4-methylpyridine-3-carbonitrile and various aldehydes causing twofold ring closure is described. The obtained 6-amino-11,12-dihydropyrido[3,4-*c*][1,9]phenanthrolines could be aromatized with Pd/C at high temperatures to form 6-aminopyrido[3,4-*c*][1,9]phenanthrolines. All compounds were systematically characterized regarding both lipophilicity and solubility and a high cytotoxic potential was evaluated in preliminary in vitro studies. Compared to formerly described 6-aminobenzo[*c*]phenanthridines our newly developed phenanthrolines turned out to possess improved drugability, due to significantly increased water-solubility and decreased lipophilicity.

1. Introduction

Naturally occurring benzo[*c*]phenanthridine alkaloids have been extensively studied in the last decades and are of particular interest due to their antitumor, antimicrobial, and antiviral properties.[1,2] Regarding the antitumor activity, the alkaloids *nitidine* and *fagaronine* were determined as model compounds for the development of DNA topoisomerase I inhibitors with a non camptothecin skeleton.[3] Current papers set focus on the design of derivatives containing additional nitrogen atoms in the basic tetracyclic ring system and often exhibit an aminoalkyl side chain both to increase the cytotoxicity and higher the solubility in aqueous media.[4–6] The aza-analogous benzo[*c*]phenanthridines ARC-111 (Topovale) and its *N*-monomethyl derivative are presently in clinical trials.[3,7]

In recent years our laboratory developed an efficient method for the synthesis of 11-substituted 6-aminobenzo[*c*]phenanthridines and corresponding dihydro derivatives (Scheme 1).[8,9] The broad applicability and the simple handling of this procedure allowed us to prepare a variety of derivatives with different substituents through condensation of 2 equiv of 2-methyl-benzonitriles with aromatic aldehydes in the presence of potassium *tert*-butylate in 1,3-dimethyltetrahydropyrimidin-2-one (DMPU) as solvent.[10–12]



Scheme 1. Comparison of 6-aminopyrido[3,4-*c*][1,9]phenanthro-lines (left) and 6-aminobenzo[*c*]phenanthridines (right).

Moreover, applying this novel reaction method we successfully designed a four-step procedure to provide the benzo[*c*]phenanthridine skeleton.[13] Our compounds turned out to exhibit considerable cytotoxicity and antitumor properties in the in vitro anticancer screening and the in vivo hollow fiber assay of the National Cancer Institute, Maryland, USA.[13] Derivatives with di- or trimethoxyphenyl substituents in the 11-position led to compounds with enhanced cytotoxicity showing GI_{50}-values<1 μM over the whole cell line panel in the 60-tumor-cell-line-screening of the NCI.[14] However, an essential drawback of the 6-aminobenzo[*c*]phenanthridines and compounds possessing the benzo[*c*]phenanthridine skeleton in general that are known so far is their very poor water solubility limiting the application in test assays as well as subsequent pharmaceutical use.[15] Previous attempts dealing with the improvement of drugability and thus water solubility were focused on the modification of the 6-amino-function with basic or acidic substitutions.[16] In this work, we tried to overcome poor

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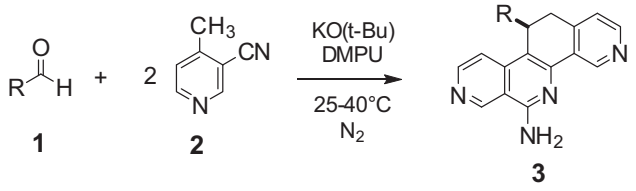
water solubility by introducing nitrogen atoms into the skeleton and decided to apply 4-methylpyridine-3-carbonitrile **2** as a pyridine-analog of 2-methylbenzonitrile as educt. The resulting compounds, 6-aminopyrido[3,4-*c*][1,9]phenanthrolines and their 11,12-dihydro derivatives, are novel heterocycles containing 3 nitrogen-atoms at ring positions 3, 5, and 8, as well as an amino-group at position 6.

2. Results & discussion

2.1. Synthesis

The synthesis was carried out using various aldehydes **1** and 2 equiv of 4-methylpyridine-3-carbonitrile **2** under nitrogen atmosphere and room temperature, leading to 6-amino-11,12-dihydropyrido[3,4-*c*][1,9]phenanthrolines **3** as racemates (Scheme 2). This procedure allows a wide range of different substituents in position 11 according to the specific aldehydes that were used. In this connection, aromatic aldehydes afforded the best yields, but also aliphatic aldehydes as well as *para*-formaldehyde could be used.

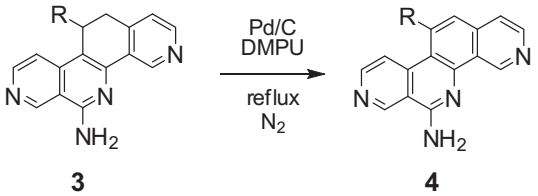


Scheme 2. Synthesis of 11-substituted 6-aminopyrido[3,4-*c*][1,9]phenanthrolines **3**.

Synthesized 6-amino-11,12-dihydropyrido[3,4-*c*][1,9]phenanthrolines 3a–p:

3a: R=phenyl	**3i**: R=thienyl
3b: R=3-methoxyphenyl	**3j**: R=3-bromophenyl
3c: R=4-methoxyphenyl	**3k**: R=4-bromophenyl
3d: R=2,3-dimethoxyphenyl	**3l**: R=3-chlorophenyl
3e: R=3,5-dimethoxyphenyl	**3m**: R=4-fluorophenyl
3f: R=3,4,5-trimethoxyphenyl	**3n**: R=biphenyl
3g: R=2,4,6-trimethoxyphenyl	**3o**: R=propyl
3h: R=furyl	**3p**: R=H

Raising the temperature up to 80 °C without inert gas we could determine the 11,12-dehydrogenated type as the main product but the conversion was not complete. Moreover, dehydrogenation of **3** to obtain the fully aromatic system using DDQ in various solvents[8,9,14] failed. Heating the compounds in the presence of 30% Pd/charcoal without a solvent,[17] the results were non-satisfying regarding the yields and long reaction times. However, we finally developed an oxidation method in boiling the 6-amino-11,12-dihydro-pyrido[3,4-*c*][1,9]phenanthrolines **3** with Pd (10%)/C in DMPU for 10–30 min under reflux and nitrogen atmosphere giving 6-aminopyrido[3,4-*c*][1,9]-phenanthrolines **4** in good yields (Scheme 3).



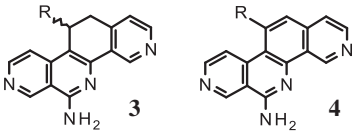
Scheme 3. Synthesis of 11-substituted 6-aminopyrido[3,4-*c*][1,9]-phenanthrolines **4**.

4a: R=phenyl	**4f**: R=3,4,5-trimethoxyphenyl
4b: R=3-methoxyphenyl	
4d: R=2,3-dimethoxyphenyl	**4h**: R=furyl

2.2. Determination of lipophilicity

Lipophilicity (log*P*) of phenanthroline derivatives was—with exception of the biphenyl derivative **3n** (log*P*: 5.78)—determined to be in the range of 2.34–4.91. The calculated lipophilicities (*clogP*) are in accordance to the log*P*-values determined experimentally for series **3** and **4** (Table 1). The lowest lipophilicity is observed for the

Table 1
Calculated and determined log*P* values of certain phenanthroline derivatives with different substituents in 11-position



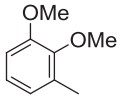
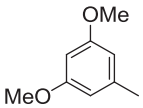
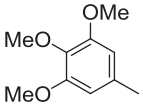
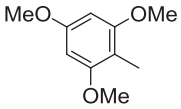
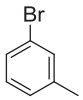
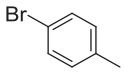
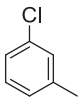
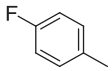
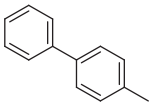
Compound	Residue	Yield [%]	log*P*[a]	*clogP*[a]
3a		55	3.34	3.34
4a		89	—	3.70
3b		42	3.77	3.26
4b		58	—	3.62
3d		33	3.74	2.60
4d		72	3.93	2.80
3e		69	3.35	3.35
4e		—	—	3.71
3f		28	2.34	2.64
4f		75	3.94	3.00
3g		63	4.78	2.54
4g		—	—	2.58
3h		67	2.72	2.52
4h		63	2.93	3.09
3i		54	2.84	2.99
4i		—	—	3.56
3j		19	4.20	4.21
4j		—	—	4.57

Table 1 (*continued*)

Compound	Residue	Yield [%]	log*P*[a]	*clogP*[a]
3k		37	4.60	4.21
4k		—	—	4.57
3l		32	4.91	4.06
4l		—	—	4.42
3m		30	3.58	3.49
4m		—	—	3.85
3n		28	5.78	5.23
4n		—	—	5.59
3o		21	4.65	3.36
4o		—	—	3.37

[a] For comparison log*P* and *clogP* are given for classes **3** and **4** (if determined).

3,4,5-trimethoxy derivative **3f** (2.34), followed by the furan derivative **3h** (2.72), and the thiophene derivative **3i** (2.84).

Moreover, we analogously investigated six 6-aminobenzo[*c*]-phenanthridines **BP** to draw a reasonable conclusion regarding reduction of lipophilicity. Results demonstrate a considerably decreased lipophilicity (mean: 3.58–5.28) compared to the formerly described **BP** derivatives (mean: 4.13–6.13). For details see Fig. 1.

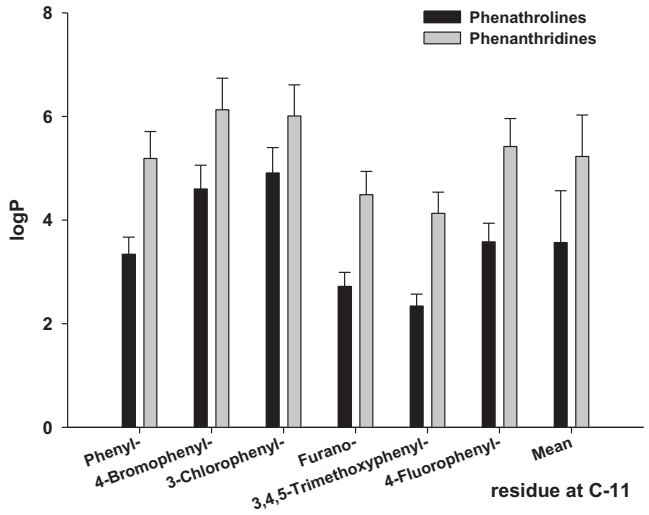


Fig. 1. Summary of log*P* values (**3** and **BP**) determined by HPLC analysis.

2.3. Determination of solubility

We set focus on the solubility of our newly discovered derivatives, because recently published 6-aminobenzo[*c*]phenanthridine derivatives are known to suffer from very poor solubility that often limits use in test assays and possibly reduces oral bioavailability.[15,16] Previous studies determined solubility of several 6-aminobenzo[*c*]phenanthridines to be in the nanomolar range.[16] For example, solubility of the phenanthridine analogues of **3d** and **3h** at pH 7.0 is $(16\pm2)\times10^{-9}$ mol L^{-1} and $(66\pm5)\times10^{-9}$ mol L^{-1}, respectively.[16] In this work, we tried to overcome this fundamental drawback by incorporating two additional nitrogen atoms into the aromatic ring systems and investigated solubility at different pH. Thereby, all compounds showed pH-dependent solubility with manifestly enhanced solubility especially in acidic media (Fig. 2, different scaling at the *y*-axis). On the basis of the significantly decreased lipophilicities, we also expected improved solubility at slight acidic and neutral media. At pH 7.4 solubility of **3d** and **3h** is determined to be $(8\pm2)\times10^{-6}$ mol L^{-1} and $(6.5\pm0.8)\times10^{-6}$ mol L^{-1}, respectively. Thus, compared to the data mentioned above, our 6-aminopyrido[3,4-*c*][1,9]phenanthrolines **3** show significantly improved solubility.

2.4. Evaluation of drug-likeness in silico

The drug-likeness of all derivatives was evaluated according to Lipinski's rule of five to get first hints regarding a possible oral bioavailability via passive absorption from the intestine. A compound is expected to be oral available if no more than one criteria is violated: molecular weight below 500 g mol^{-1}, number of hydrogen bond donors below 5, hydrogen bond acceptors below 10, and a log*P* not greater than 5.[18] However, all 6-amino-11,12-dihydropyrido[3,4-*c*][1,9]phenanthrolines **3** and **4** presented in this work are anticipated to possess sufficient oral bioavailability, because all parameters are in accordance to the rule of five.

2.5. Cytotoxicity

To evaluate the biological potential of the 6-amino-pyrido[3,4-*c*][1,9]phenanthrolines **4** and its 11,12-dihydro- derivatives **3** preliminary in vitro cytotoxicity studies were performed by the U.S. National Cancer Institute (NCI) revealing a high cytotoxic potential. In the 'NCI-60 DTP Human Tumor Cell Line Screening' 60 tumor cell lines from nine different types of cancer were treated with the substances to reveal dose–response-relations. As one important parameter determined in the test, the MG_MID (meangraph midpoint) is the mean of the logarithms of the GI_{50} values (growth inhibition 50%) and corresponds to an average response characteristic of all 60 cell lines to the test substance.[9]

Taking the antilogarithm of this value gives the mean of the GI_{50} values over all cell lines.[19] Table 2 indicates a cytostatic activity (expressed by the GI_{50}-value) for the two compounds **3l** (3.80 μM) and **3o** (4.02 μM) comparable to the natural occurring alkaloids *nitidine* (1.78 μM) and *fagaronine* (9.48 μM) and even better GI_{50}-values for the fully aromatized compounds **4f** (0.76 μM) and **4h** (1.74 μM) were found.[20]

3. Conclusions

In this work, we successfully designed a novel class of cytostatically active 6-amino-11,12-dihydropyrido[3,4-*c*][1,9]-phenanthroline derivatives **3**. The synthesis used allows a wide range of different substituents in 11-position that can lead to a large library of potentially cytostatic substances. These derivatives could be easily aromatized in good yields to 6-aminopyrido[3,4-*c*][1,9]phenanthrolines **4** with an optimized oxidation method. First investigations concerning lipophilicity and solubility show interesting results indicating a good suitability of this compound class as a pharmaceutical. Extended studies concerning the cytotoxicity are underway bearing promising data. Actual synthetic strategies in our group are focussed on the variation of the amino group in 6-position like the linking of (amino)alkyl-side chains or

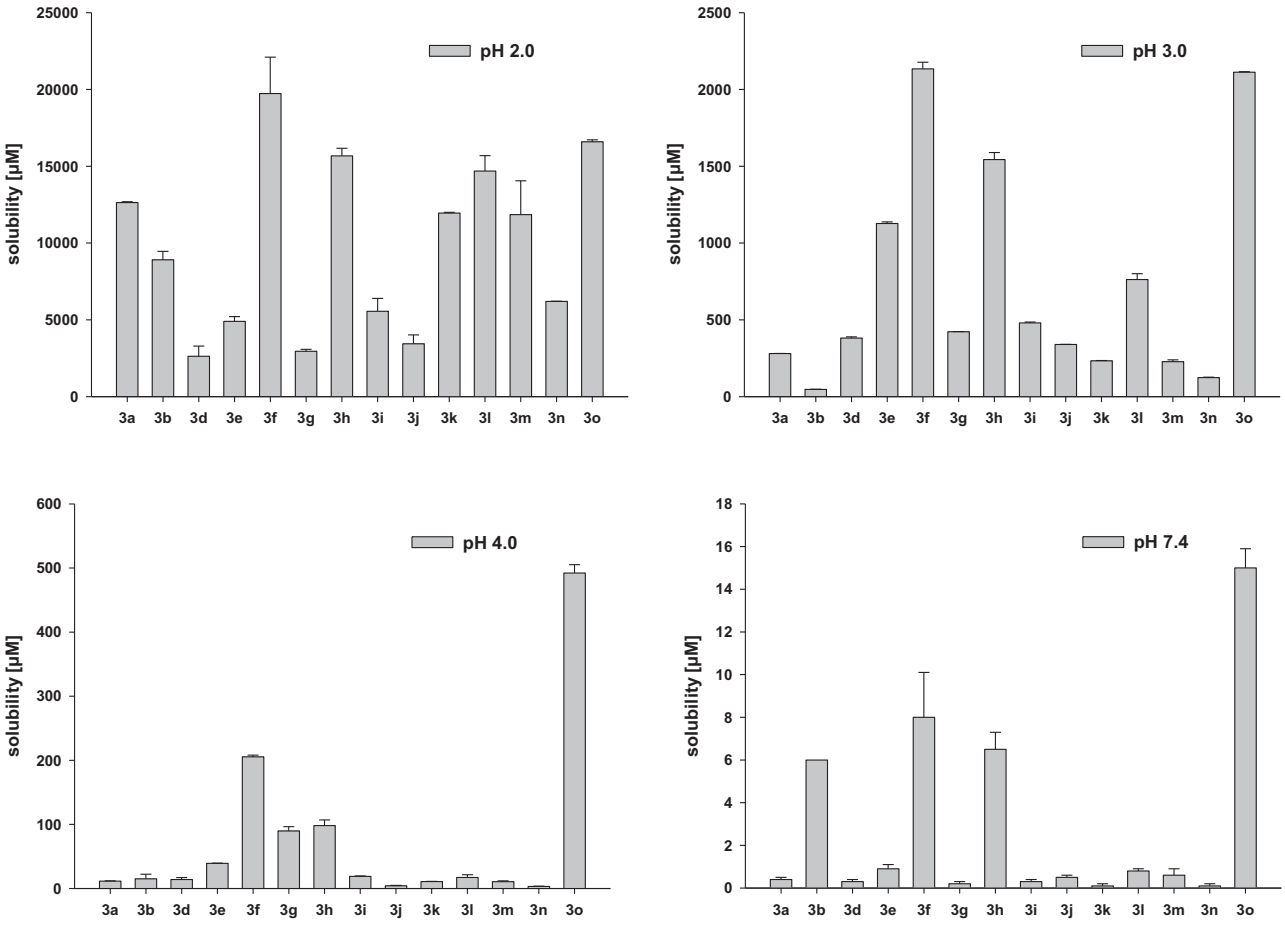


Fig. 2. Solubility of phenanthroline derivatives **3** in phosphate buffer at different pH values.

Table 2
Cytotoxic activity of phenanthroline derivatives **3** and **4** and selected derivatives from DTP-60 screening by the NCI[20]

Compound	MG_MID	$\sum GI_{50}/60$ [µM]
3l	−5.42	3.80
3o	−5.39	4.07
4f	−6.12	0.76
4h	−5.76	1.74
Fagaronine	−5.02	9.48
Nitidine	−5.75	1.78

its removal to obtain the unsubstituted heterocycles. Future studies will be performed dealing with efficacy to further characterize these newly identified compounds.

4. Experimental section

4.1. General

All reactions were carried out in oven-dried glassware. The solvents (except DMPU) were freshly distilled. All commercial reagents were used without purification. Melting points were determined on a Stuart Scientific SMP3 melting-point apparatus, and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance III 300 instrument at 300 K. Chemical shifts are given in parts per million relative to solvent residual peaks. Mass spectra were recorded on a Bruker Esquire∼LC instrument under ESI conditions or on a Hewlett Packard MS-Engine 5989 A under EI conditions (70 eV). IR-spectroscopy was carried out using a Perkin Elmer Spectrum 100 FTIR instrument with ATR attachment. Elemental analysis were performed by the Department of Inorganic Chemistry, Christian-Albrechts-University of Kiel with a CHNS Analyserr of HEKAtech GmbH, High resolution mass spectra were recorded on a LTQ Velos Orbitrap mass spectrometer equipped with a nano electrospray source in positive mode at the Institute of Experimental Medicine, University Hospital, Kiel.

4.2. General procedure for the synthesis of the 6-amino-11,12-dihydropyrido[3,4-*c*][1,9]phenanthrolines

A solution of **1** (2.1 mmol) and **2** (4.2 mmol) in 10 ml DMPU was added dropwise to KO(*t*-Bu) (500 mg, 4.4 mmol) in 5 ml DMPU while the solution was magnetically stirred under N_2 at 25–40 °C. The mixture was stirred for 1–3 h and then hydrolyzed in 80 ml of ice water. The precipitate formed during hydrolysis was collected by filtration. The filtrate was extracted with CH_2Cl_2 (3×50 ml). The combined organic extracts were dried (Na_2SO_4) and concentrated in vacuo. DMPU was removed by distillation at high vacuum at 100–110 °C. The resulting residue was collected by filtration. The combined crude products were either recrystallized from MeOH/CH_2Cl_2 or, if necessary, purified by column chromatography (silica gel, CH_2Cl_2/MeOH (5–20%)).

*4.2.1. 6-Amino-11-phenyl-11,12-dihydropyrido[3,4c][1,9]phenanthroline (**3a**).* The reaction of benzaldehyde (223 mg, 2.1 mmol) with **2** (500 mg, 4.2 mmol) at room temperature for 3 h afforded compound **3a**. $C_{21}H_{16}N_4$, yield: 373 mg (55%, recrystallized from MeOH/CH_2Cl_2); solid; mp 279 °C under decomp.; ^1H NMR

(300 MHz, DMSO-d_6): δ=3.15 (d, 1H, H-12a, $^2J_{H\text{-}12a/H\text{-}12b}$=16.5 Hz), 3.54 (dd, 1H, H-12b, $^2J_{H\text{-}12b/H\text{-}12a}$=16.3 Hz, $^3J_{H\text{-}12b/H\text{-}11}$=7.6 Hz), 4.86 (d, 1H, H-11, $^3J_{H\text{-}11/H\text{-}12b}$=7.3 Hz), 7.09 (m, 5H, phenyl), 7.18 (d, 1H, Ar–H, ^3J=4.9 Hz), 7.45 (br s, 2H, $-NH_2$), 7.55 (d, 1H, Ar–H, ^3J=6.0 Hz), 8.39 (d, 1H, Ar–H, ^3J=4.9 Hz), 8.54 (d, 1H, Ar–H, ^3J=5.9 Hz), 9.36 (s, 1H, Ar–H), 9.57 (s, 1H, Ar–H); ^{13}C NMR (75 MHz, DMSO-d_6): δ=35.2 (C-12), 35.9 (C-11), 112.8, 114.1, 115.7, 123.3, 126.3, 127.1 (2C), 128.4 (2C), 129.9, 139.3, 143.0, 144.1, 145.7, 145.9, 147.7, 149.1, 149.6, 157.1; IR (ATR, cm^{-1}): 3330, 3170, 3060, 2902, 1652, 1612, 1599, 1449, 1045; MS (ESI) *m*/*z*: 325 ([M+H]$^+$, 100%), 163 ([M+2H]$^{++}$, 35%); HRMS (ESI) *m*/*z* calcd for [M+H]$^+$ 325.14476, found 325.14465.

*4.2.2. 6-Amino-11-(3-methoxyphenyl)-11,12-dihydropyrido[3,4c][1,9]-phenanthroline (**3b**).* 3-Methoxybenzaldehyde (286 mg, 2.1 mmol), rt, 2.5 h, $C_{22}H_{18}N_4O$, yield: 312 mg (42%, recrystallized from MeOH/CH_2Cl_2); solid; mp 259 °C under decomp.; ^{1}H NMR (300 MHz, DMSO-d_6): δ=3.15 (d, 1H, H-12a, $^2J_{H\text{-}12a/H\text{-}12b}$=16.4 Hz), 3.52 (dd, 1H, H-12b, $^2J_{H\text{-}12b/H\text{-}12a}$=16.4 Hz, $^3J_{H\text{-}12b/H\text{-}11}$=7.7 Hz), 4.82 (d, 1H, H-11, ^3J=7.6 Hz), 6.46–6.53 (m, 1H, Ar–H, ^3J=7.8 Hz, ^4J=0.9 Hz), 6.55–6.60 (m, 1H, Ar–H), 6.61–6.68 (m, 1H, Ar–H, ^3J=8.1 Hz, ^4J=0.8 Hz), 7.01 (m, 1H, Ar–H, ^3J=7.9 Hz), 7.19 (d, 1H, Ar–H, ^3J=4.9 Hz), 7.45 (br s, 2H, $-NH_2$), 7,56 (d, 1H, Ar–H, ^3J=5.9 Hz), 8.40 (d, 1H, Ar–H, ^3J=4.9 Hz), 8.55 (d, 1H, Ar–H, ^3J=5.9 Hz), 9.35 (s, 1H, Ar–H), 9.57 (s, 1H, Ar–H); ^{13}C NMR (75 MHz, DMSO-d_6): δ=35.1 (C-12), 35.9 (C-11), 54.7 (OCH_3), 111.1, 112.7, 113.5, 114.1, 115.7, 119.3, 123.3, 129.4, 129.9, 139.3, 144.2, 144.6, 145.7, 145.9, 147.7, 149.1, 149.6, 157.1, 159.1; IR (ATR, cm^{-1}): 3326, 3170, 3082, 2930, 1651, 1598, 1454, 1061, 821; MS (ESI) *m*/*z*: 355 ([M+H]$^+$, 100%), 178 ([M+2H]$^{++}$, 16%), 129 (13%), 124 ([M−3-methoxybenzene+2H]$^{++}$, 4%). Anal. calcd for $C_{22}H_{18}N_4O$: C, 74.56; H, 5.12; N, 15.81, found: C, 74.04; H, 4.78; N, 15.75.

*4.2.3. 6-Amino-11-(4-methoxyphenyl)-11,12-dihydropyrido[3,4-c][1,9]-phenanthroline (**3c**).* 4-Methoxybenzaldehyde (285 mg, 2.1 mmol), rt, 2.5 h, $C_{22}H_{18}N_4O$, yield: 252 mg (33%, recrystallized from MeOH/CH_2Cl_2); solid; mp 282 °C under decomp.; ^{1}H NMR (300 MHz, DMSO-d_6): δ=3.09 (d, 1H, H-12a, $^2J_{H\text{-}12a/H\text{-}12b}$=15.8 Hz), 3.48 (dd, 1H, H-12b, $^2J_{H\text{-}12b/H\text{-}12a}$=16.1 Hz, $^3J_{H\text{-}12b/H\text{-}11}$=7.9 Hz), 3.58 (s, 3H, OCH_3), 4.78 (d, 1H, H-11, $^3J_{H\text{-}11/H\text{-}12b}$=6.8 Hz), 6.66 (m, 2H, H-3′, H-5′), 6.87 (m, 2H, H-2′, H-6′), 7.16 (d, 1H, Ar–H, ^3J=4.9 Hz), 7.39 (br s, 2H, $-NH_2$), 7.53 (d, 1H, Ar–H, ^3J=5.7 Hz), 8.38 (d, 1H, Ar–H, ^3J=4.9 Hz), 8.52 (d, 1H, Ar–H, ^3J=5.9 Hz), 9.33 (s, 1H, Ar–H), 9.55 (s,1H, Ar–H); ^{13}C NMR (75 MHz, DMSO-d_6): δ=35.1 (C-12), 35.4 (C-11), 54.8 (OCH_3), 112.8, 113.7 (2C), 114.5, 115.7, 123.3, 128.1 (2C), 129.9, 134.8, 129.3, 144.2, 145.7, 145.8, 147.6, 149.0, 149.6, 157.0, 157.7; IR (ATR, cm^{-1}): 3324, 3172, 2950, 2830, 1650, 1456, 1182, 1034; MS (EI) *m*/*z*: 354 M$^+$ (55%), 353 [M−H]$^+$ (11%), 247 [M−4-methoxy-benzene]$^+$ (100%), 230 (19%), 177 (10%), 155 (8%), 121 (6%). Anal. calcd for $C_{22}H_{18}N_4O$: C, 74.56; H, 5.12; N, 15.81, found: C, 74.89; H, 5.51; N, 15.74.

*4.2.4. 6-Amino-11-(2,3-dimethoxyphenyl)-11,12-dihydropyrido[3,4-c]-[1,9]phenanthroline (**3d**).* 2,3-Dimethoxybenzaldehyde (349 mg, 2.1 mmol), rt, 2 h, $C_{23}H_{20}N_4O_2$, yield: 492 mg (61% recrystallized from MeOH/CH_2Cl_2); solid, mp 268 °C under decomp.; ^{1}H NMR (300 MHz, DMSO-d_6): δ=3.05 (d, 1H, H-12a, $^2J_{H\text{-}12a/H\text{-}12b}$=16.4 Hz), 3.54 (dd, 1H, H-12b, $^2J_{H\text{-}12b/H\text{-}12a}$=16.4 Hz, $^3J_{H\text{-}12b/H\text{-}11}$=8.1 Hz), 3.79 (s, 3H, OCH_3), 4.02 (s, 3H, OCH_3), 5.10 (d, 1H, H-11, $^3J_{H\text{-}11/H\text{-}12b}$=7.9 Hz), 5.87 (dd, 1H, Ar–H, ^3J=7.9 Hz, ^4J=1.3 Hz), 6.58 (t, 1H, Ar–H, ^3J=8.0 Hz), 6.78 (dd, 1H, Ar–H, ^3J=8.2 Hz, ^4J=1.3 Hz), 7.16 (d, 1H, Ar–H, ^3J=4.9 Hz), 7.38 (d, 1H, Ar–H, ^3J=5.9 Hz), 7.43 (br s, 2H, $-NH_2$), 8.39 (d, 1H, Ar–H, ^3J=4.9 Hz), 8.54 (d, 1H, Ar–H, ^3J=5.9 Hz), 9.37 (s, 1H, Ar–H), 9.56 (s, 1H, Ar–H); ^{13}C NMR (75 MHz, DMSO-d_6): δ=34.9 (C-12), 35.2 (C-11), 55.6 (OCH_3), 60.6 (OCH_3), 111.9, 113.1, 114.6, 119.1, 119.2, 124.0, 126.8, 131.9, 134.5, 137.2, 138.1, 141.5, 142.8, 144.0, 145.2, 147.7, 152.5, 156.6, 158.3; IR (ATR, cm^{-1}): 3415, 3306, 3148, 2929, 1648, 1601, 1453, 1075, 1005; MS (ESI) *m*/*z*: 385 ([M+H]$^+$, 100%), 247 ([M−2,3-dimethoxybenzene]$^+$, 8%), 193 ([M+2H]$^{++}$, 57%), 185 (8%), 124 ([M−2,3-dimethoxybenzene+2H]$^{++}$, 14%); HRMS (ESI) *m*/*z* calcd for [M+H]$^+$ 385.16589, found 385.16565.

*4.2.5. 6-Amino-11-(3,5-dimethoxyphenyl)-11,12-dihydropyrido[3,4-c]-[1,9]phenanthroline (**3e**).* 3,5-Dimethoxybenzaldehyde (349 mg, 2.1 mmol), rt, 2 h, $C_{23}H_{20}N_4O_2$, yield: 557 (69%, recrystallized from MeOH/CH_2Cl_2); solid, mp 258 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ=3.14 (d, 1H, H-12a, $^2J_{H\text{-}12a/H\text{-}12b}$=16.3 Hz), 3.50 (dd, 1H, H-12b, $^2J_{H\text{-}12b/H\text{-}12a}$=16.2 Hz, $^3J_{H\text{-}12b/H\text{-}11}$=7.6 Hz), 3.56 (s, 6H, 2× OCH_3), 4.77 (d, 1H, H-11, $^3J_{H\text{-}11/H\text{-}12b}$=7.3 Hz), 6.11 (m, 2H, H-2′, H-6′, ^4J=2.2 Hz), 6.23 (m, 1H, H-4′, ^4J=2.2 Hz), 7.20 (d, 1H, Ar–H, ^3J=4.9 Hz), 7.46 (br s, 2H, $-NH_2$), 7.57 (d, 1H, Ar–H, ^3J=5.9 Hz), 8.41 (d, 1H, Ar–H, ^3J=4.9 Hz), 8.56 (d, 1H, Ar–H, ^3J=5.9 Hz), 9.35 (s, 1H, Ar–H), 9.58 (s, 1H, Ar–H); ^{13}C NMR (75 MHz, DMSO-d_6): δ=35.0 (C-12), 36.1 (C-11), 54.8 (2C, 2× OCH_3), 97.3, 105.6 (2C, C-2′, C-6′), 112.7, 114.1, 115.7, 123.3, 130.0, 139.3, 144.3, 145.4, 145.6, 145.9, 147.7, 149.1, 159.6, 157.1, 160.3 (2C, C-3′, C-5′); IR (ATR, cm^{-1}): 3325, 3168, 2969, 2828, 1650, 1595, 1452, 1318, 1067. MS (ESI) *m*/*z*: 385 ([M+H]$^+$, 100%), 247 (M−3,5-dimethoxybenzene$^+$, 2%), 193 ([M+2H]$^{++}$, 73%); Anal. calcd for $C_{23}H_{20}N_4O_2$: C, 71.86; H, 5.24; N, 14.57, found: C, 71.34; H, 5.22; N, 14.43.

*4.2.6. 6-Amino-11-(3,4,5-trimethoxyphenyl)-11,12-dihydropyrido[3,4-c][1,9]phenanthroline (**3f**).* 3,4,5-Trimethoxybenzaldehyde (392 mg, 2.1 mmol), 35 °C, 3 h, $C_{24}H_{22}N_4O_3$, yield: 87 mg (28%, silica gel, CH_2Cl_2/MeOH (5–20%)), mp 219 °C under decomp.; ^{1}H NMR (300 MHz, DMSO-d_6): δ=3.18 (d, 1H, H-12a, $^2J_{H\text{-}12a/H\text{-}12b}$=16.2 Hz), 3.53 (s, 6H, 2× OCH_3), 3.57 (s, 3H, OCH_3), 4.82 (d, 1H, H-11, $^3J_{H\text{-}11/H\text{-}12b}$=6.9 Hz), 6.34 (s, 2H, H-2′, H-6′), 7.26 (d, 1H, Ar–H, ^3J=4.8 Hz), 7.47 (br s, 2H, $-NH_2$), 7.70 (d, 1H, Ar–H, ^3J=5.9 Hz), 8.46 (d, 1H, Ar–H, ^3J=4.8 Hz), 8.62 (d, 1H, Ar–H, ^3J=5.9 Hz), 9.40 (s, 1H, Ar–H), 9.62 (s, 1H, Ar–H). The signal of proton 12b is overlayed by the signals of the methoxygroups at 3.53–3.57 ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ=35.2 (C-12), 36.2 (C-11), 55.6 (2C, 2× OCH_3), 59.8 (OCH_3), 104.5 (2C, C-2′, C-6′), 112.7, 114.5, 115.8, 123.4, 130.0, 136.1, 138.7, 139.4, 144.7, 145.4, 145.8, 147.6, 149.0, 152.5 (2C, C-3′, C-5′), 157.1; IR (ATR, cm^{-1}): 3300, 3122, 3006, 2932, 1670, 1612, 1600, 1463, 1124; MS (ESI) *m*/*z*: 415 ([M+H]$^+$, 100%), 247 ([M−3,4,5-trimethoxybenzene]$^+$, 4%), 208 ([M+2H]$^{++}$, 7%), 124 ([M−3,4,5-trimethoxybenzene+2H]$^{++}$, 16%); Anal. calcd for $C_{24}H_{22}N_4O_3$: C, 69.55; H, 5.35; N, 13.52, found: C, 69.18; H, 4.93; N, 13.49.

*4.2.7. 6-Amino-11-(2,4,6-trimethoxyphenyl)-11,12-dihydropyrido[3,4-c][1,9]phenanthroline (**3g**).* 2,4,6-Trimethoxybenzaldehyde (412 mg, 2.1 mmol), 2 h, $C_{24}H_{22}N_4O_3$, yield: 547 mg (63%, recrystallized from MeOH/CH_2Cl_2); mp 265 °C under decomp.; ^{1}H NMR (300 MHz, DMSO-d_6): δ=2.84 (d, 1H, H-12a, $^2J_{H\text{-}12a/H\text{-}12b}$=17.0 Hz), 3.43 (br s, 6H, 2× OCH_3), 3.53 (dd, 1H, H-12b, $^2J_{H\text{-}12b/H\text{-}12a}$=17.0 Hz, $^3J_{H\text{-}12b/H\text{-}11}$=10.2 Hz), 3.72 (s, 3H, OCH_3), 5.25 (d, 1H, H-11, $^3J_{H\text{-}11/H\text{-}12b}$=10.2 Hz), 6.15 (s, 2H, H-3′, H-5′), 7.17 (d, 1H, Ar–H, ^3J=4.9 Hz), 7.26 (br s, 2H, $-NH_2$), 7.52 (d, 1H, Ar–H, ^3J=5.9 Hz), 8.44 (d, 1H, Ar–H, ^3J=4.9 Hz), 8.51 (d, 1H, Ar–H, ^3J=5.9 Hz), 9.44 (s, 1H, Ar–H), 9.53 (s, 1H, Ar–H); ^{13}C NMR (75 MHz, DMSO-d_6): δ=24.9 (C-11), 33.5 (C-12), 55.1 (3C, 2× OCH_3, OCH_3), 91.1 (2C), 112.2, 112.6, 114.3, 119.4, 124.7, 132.4, 136.4, 137.3, 141.2, 143.1, 143.6, 148.1, 157.5, 158.0, 158.7, 159.9 (2C); IR (ATR, cm^{-1}): 3115, 3060, 2839, 1655, 1600, 1541, 1413, 1203, 1115; MS (ESI) *m*/*z*: 415 ([M+H]$^+$, 100%) 247 ([M−2,4,6-trimethoxybenzene]$^+$, 6%), 208 ([M+2H]$^{++}$, 74%); Anal. calcd for $C_{24}H_{23}N_4O_3\cdot 3HCl\cdot 1H_2O$: C, 53.20; H, 5.02; N, 10.34, found: C, 53.25; H, 5.06; N, 10.50.

*4.2.8. 6-Amino-11-furyl-11,12-dihydropyrido[3,4-c][1,9]phenanthroline (**3h**).* Furfural (201 mg, 2.1 mmol), 2 h, $C_{19}H_{14}N_4O$, yield: 441 mg (67%, recrystallized from MeOH/CH_2Cl_2); solid, mp 267 °C

under decomp.; ^{1}H NMR (300 MHz, DMSO-d_6): δ=3.05 (d, 1H, H-12a, $^2J_{H\text{-}12a/H\text{-}12b}$=16.4 Hz), 3.54 (dd, 1H, H-12b, $^2J_{H\text{-}12b/H\text{-}12a}$=16.4 Hz, $^3J_{H\text{-}12b/H\text{-}11}$=8.1 Hz), 4.36 (m, 2H, ^3J=4.0 Hz), 4.92 (m, 1H, ^3J=4.0 Hz), 5.60 (m, 1H, ^3J=3.2 Hz, ^4J=1.9 Hz, ^4J=0.9 Hz), 6.12 (m, ^3J=1.8 Hz), 7.34 (d, 1H, Ar–H, ^3J=4.9 Hz), 7.39 (m, 1H, ^3J=1.8 Hz, ^4J=0.8 Hz), 7.45 (br s, 2H, $-NH_2$), 7.78 (d, 1H, Ar–H, ^3J=5.9 Hz), 8.44 (d, 1H, Ar–H, ^3J=4.9 Hz), 8.62 (d, 1H, Ar–H, ^3J=5.9 Hz), 9.29 (s, 1H, Ar–H), 9.57 (s, 1H, Ar–H); ^{13}C NMR (75 MHz, DMSO-d_6): δ=30.6 (C-12), 31.9 (C-11), 106.4, 110.6, 112.9, 113.0, 116.4, 123.5, 123.6, 139.8, 142.4, 145.0, 146.1, 146.4, 148.2, 149.5, 150.1, 156.1, 157.7; IR (ATR, cm^{-1}): 3325, 3164, 2893, 1649, 1599, 1508, 1456, 1047, 822; MS (ESI) *m*/*z*: 315 ([M+H]$^+$, 50%), 218 (100%), 158 ([M+2H]$^{++}$, 35%); HRMS (ESI) *m*/*z* calcd for [M+H]$^+$ 315.12403, found 315.12372.

*4.2.9. 6-Amino-11-thienyl-11,12-dihydropyrido[3,4-c][1,9]phenanthroline (**3i**).* Thiophen-2-carbaldehyde (236 mg, 2.1 mmol), 2 h, $C_{19}H_{14}N_4S$, yield: 374 mg (54%, recrystallized from MeOH/CH_2Cl_2); solid, mp 269 °C under decomp.; ^{1}H NMR (300 MHz, DMSO-d_6): δ=3.27 (d, 1H, H-12a, $^2J_{H\text{-}12a/H\text{-}12b}$=16.2 Hz), 3.51 (dd, 1H, H-12b, $^2J_{H\text{-}12b/H\text{-}12a}$=16.0 Hz, $^3J_{H\text{-}12b/H\text{-}11}$=6.9 Hz), 5.16 (d, 1H, H-11, $^3J_{H\text{-}11/H\text{-}12b}$=6.9 Hz), 6.68–6.80 (m, 2H, ^3J=5.2 Hz, ^4J=1.3 Hz), 7.11 (dd, 1H, ^3J=5.2 Hz, ^4J=1.3 Hz), 7.30 (d, 1H, Ar–H,=4.9 Hz), 7.45 (br s, 2H, $-NH_2$), 7.78 (d, 1H, Ar–H, ^3J=5.9 Hz), 8.44 (d, 1H, Ar–H, ^3J=4.9 Hz), 8.62 (d, 1H, Ar–H, ^3J=5.9 Hz), 9.29 (s, 1H, Ar–H), 9.58 (s, 1H, Ar–H); ^{13}C NMR (75 MHz, DMSO-d_6): δ=31.1 (C-11), 35.8 (C-12), 112.7, 115.6, 119.3, 124.8, 125.3, 126.7, 126.9, 131.4, 138.1, 139.0, 142.3, 142.6, 144.6, 145.1, 146.7, 155.3, 158.4; IR (ATR, cm^{-1}): 3325, 3167, 3070, 2971, 1650, 1598, 1541, 1454, 1043, 825; MS (ESI) *m*/*z*: 331 ([M+H]$^+$, 100%), 218 (100%), 166 ([M+2H]$^{++}$, 43%), 124 ([M−thiophene+2H]$^{++}$, 14%). HRMS (ESI) *m*/*z* calcd for [M+H]$^+$ 331.10118, found 331.10071.

*4.2.10. 6-Amino-11-(3-bromophenyl)-11,12-dihydropyrido[3,4-c][1,9-]-phenanthroline (**3j**).* 3-Bromobenzaldehyde (389 mg, 2.1 mmol), 3 h, $C_{21}H_{15}N_4Br$, yield: 161 mg (19%, recrystallized from MeOH/CH_2Cl_2); solid, mp 268 °C under decomp.; ^{1}H NMR (300 MHz, DMSO-d_6): δ=3.15 (d, 1H, H-12a, $^2J_{H\text{-}12a/H\text{-}12b}$=16.3 Hz), 3.53 (dd, 1H, H-12b, $^2J_{H\text{-}12b/H\text{-}12a}$=16.2 Hz, $^3J_{H\text{-}12b/H\text{-}11}$=7.4 Hz), 4.89 (d, 1H, H-11, $^3J_{H\text{-}11/H\text{-}12b}$=7.4 Hz), 6.87 (d, 1H, Ar–H, ^3J=7.7 Hz), 7.05 (t, 1H, Ar–H, ^3J=7.7 Hz), 7.20 (d, 1H, Ar–H, ^3J=4.8 Hz), 7.27 (d, 1H, Ar–H, ^3J=7.7 Hz), 7.29 (s, 1H, Ar–H), 7.49 (br s, 2H, $-NH_2$), 7.56 (d, 1H, Ar–H, ^3J=5.9 Hz), 8.41 (d, 1H, Ar–H, ^3J=5.0 Hz), 8.56 (d, 1H, Ar–H, ^3J=5.8 Hz), 9.37 (s, 1H, Ar–H), 9.59 (s, 1H, Ar–H); ^{13}C NMR (75 MHz, DMSO-d_6): δ=35.0 (C-12), 35.5 (C-11), 112.7, 113.4, 115.5, 121.7, 123.3, 126.1, 129.3, 129.8, 130.0, 130.5, 139.2, 143.8, 145.7, 145.8, 146.1, 147.9, 149.1, 149.8, 157.3; IR (ATR, cm^{-1}): 3325, 3169, 3052, 2969, 1650, 1599, 1457, 1041, 818; MS (ESI) *m*/*z*: 405 ([M+H]$^+$, ^{81}Br, 70%), 403 ([M+H]$^+$, ^{79}Br, 71%), 203 ([M+2H]$^{++}$, ^{81}Br, 94%), 202 ([M+2H]$^{++}$, ^{79}Br, 100%), 163 (4%), 157 (10%); Anal. calcd for $C_{21}H_{15}N_4Br$: C, 62.54; H, 3.75; N, 13.89, found: C, 61.85; H, 3.81; N, 13.39.

*4.2.11. 6-Amino-11-(4-bromophenyl)-11,12-dihydropyrido[3,4-c][1,9-]-phenanthroline (**3k**).* 4-Bromobenzaldehyde (389 mg, 2.1 mmol), 3 h, $C_{21}H_{15}N_4Br$, yield: 313 mg (37%, recrystallized from MeOH/CH_2Cl_2); solid, mp 278 °C under decomp.; ^{1}H NMR (300 MHz, DMSO-d_6): δ=3.12 (d, 1H, H-12a, $^2J_{H\text{-}12a/H\text{-}12b}$=16.5 Hz), 3.52 (dd, 1H, H-12b, $^2J_{H\text{-}12b/H\text{-}12a}$=16.2 Hz, $^3J_{H\text{-}12b/H\text{-}11}$=7.5 Hz), 4.86 (d, 1H, H-11, $^3J_{H\text{-}11/H\text{-}12b}$=7.3 Hz), 6.94 (m, 2H, H-2′, H-6′, ^3J=8.4 Hz), 7.18 (d, 1H, Ar–H, ^3J=4.9 Hz), 7.31 (m, 2H, H-3′, H-5′, ^3J=8.4 Hz), 7.48 (br s, 2H, $-NH_2$), 7.53 (d, 1H, Ar–H, ^3J=6.0 Hz), 8.40 (d, 1H, Ar–H, ^3J=4.9 Hz), 8.54 (d, 1H, Ar–H, ^3J=5.9 Hz), 9.36 (s, 1H, Ar–H), 9.58 (s, 1H, Ar–H); ^{13}C NMR (75 MHz, DMSO-d_6): δ=35.0 (C-12), 35.3 (C-11), 112.7, 113.6, 115.5, 119.5, 123.3, 129.4 (2C), 129.8, 131.2 (2C), 139.2, 142.3, 143.8, 145.8, 146.0, 147.8, 149.1, 149.7, 157.2; IR (ATR, cm^{-1}): 3327, 3171, 2899, 1649, 1600, 1543, 1456, 1012, 818; MS (ESI) *m*/*z*: 405 ([M+H]$^+$, ^{81}Br, 64%), 403 ([M+H]$^+$, ^{79}Br, 63%), 203 ([M+2H]$^{++}$, ^{81}Br, 100%), 202 ([M+2H]$^{++}$, ^{79}Br, 95%), 193 (5%), 124 ([M−3-bromobenzene+2H]$^{++}$, 10%). Anal. calcd for $C_{21}H_{15}N_4Br$: C, 62.54; H, 3.75; N, 13.89, found: C, 62.47; H, 3.99; N, 14.17.

*4.2.12. 6-Amino-11-(3-chlorophenyl)-11,12-dihydropyrido[3,4-c][1,9-]-phenanthroline (**3l**).* 3-Chlorbenzaldehyde (295 mg, 2.1 mmol), 2.5 h, $C_{21}H_{15}N_4Cl$, yield: 241 mg (32%, recrystallized from MeOH/CH_2Cl_2); solid, mp 307 °C under decomp.; ^{1}H NMR (300 MHz, DMSO-d_6): δ=3.16 (d, 1H, H-12a, $^2J_{H\text{-}12a/H\text{-}12b}$=16.4 Hz), 3.53 (dd, 1H, H-12b, $^2J_{H\text{-}12b/H\text{-}12a}$=16.4 Hz, $^3J_{H\text{-}12b/H\text{-}11}$=7.4 Hz), 4.90 (d, 1H, H-11, $^3J_{H\text{-}11/H\text{-}12b}$=7.3 Hz), 6.80–6.90 (m, 1H, Ar–H), 7.07–7.16 (m, 3H, Ar–H), 7.20 (d, 1H, Ar–H, ^3J=4.9 Hz), 7.51 (br s, 2H, $-NH_2$), 7.56 (d, 1H, Ar–H, ^3J=6.0 Hz), 8.41 (d, 1H, Ar–H, ^3J=4.9 Hz), 8.56 (d, 1H, Ar–H, ^3J=5.9 Hz), 9.36 (s, 1H, Ar–H), 9.59 (s, 1H, Ar–H); ^{13}C NMR (75 MHz, DMSO-d_6): δ=35.4 (C-12), 36.0 (C-11), 113.2, 113.9, 116.0, 123.8, 126.2, 127.0, 127.6, 130.3, 130.7, 133.5, 139.7, 144.3, 146.1, 146.3, 146.6, 148.4, 149.6, 150.3, 157.8; IR (ATR, cm^{-1}): 3305, 3121, 2970, 2811, 1666, 1598, 1459, 1375, 820; MS (ESI) *m*/*z*: 361 ([M+H]$^+$, ^{37}Cl, 35%), 359 ([M+H]$^+$, ^{35}Cl, 100%), 181 ([M+2H]$^{++}$, ^{37}Cl, 17%), 180 ([M+2H]$^{++}$, ^{35}Cl, 46%), 124 ([M−3-chlorobenzene+2H]$^{++}$, 7%); HRMS (ESI) *m*/*z* calcd for [M+H]$^+$, ^{37}Cl: 359.10579, found 359.10526.

*4.2.13. 6-Amino-11-(4-fluorophenyl)-11,12-dihydropyrido[3,4-c][1,9-]-phenanthroline (**3m**).* 4-Fluorobenzaldehyde (261 mg, 2.1 mmol), 3 h, $C_{21}H_{15}N_4F$, yield: 215 mg (30%, recrystallized from MeOH/CH_2Cl_2); solid, mp 286 °C under decomp.; ^{1}H NMR (300 MHz, DMSO-d_6+1% acetic-acid-d_4): δ=3.50 (d, 1H, H-12a, $^2J_{H\text{-}12a/H\text{-}12b}$=16.7 Hz), 3.76 (dd, 1H, H-12b, $^2J_{H\text{-}12b/H\text{-}12a}$=16.9 Hz, $^3J_{H\text{-}12b/H\text{-}11}$=7.5 Hz), 5.09 (d, 1H, H-11, $^3J_{H\text{-}11/H\text{-}12b}$=7.3 Hz), 6.87–6.97 (m, 2H, Ar–H, AA′-part, $^4J_{HF}$=8.8 Hz), 7.02–7.13 (m, 2H, Ar–H, XX′-part, $^3J_{HF}$=8.9 Hz), 7.84 (d, 1H, Ar–H, ^3J=5.8 Hz), 7.96 (d, 1H, Ar–H, ^3J=6.6 Hz), 8.66 (d, 1H, Ar–H, ^3J=6.6 Hz), 8.77 (d, 1H, Ar–H, ^3J=5.8 Hz), 9.39 (s, 1H, Ar–H), 9.99 (s, 1H, Ar–H); ^{13}C NMR (75 MHz, DMSO-d_6+1% acetic-acid-d_4): δ=34.6 (C-11), 35.7 (C-12), 113.0, 114.7, 115.1, 115.4, 118.7, 126.2, 129.0, 120.1, 131.4, 137.4, 138.7, 140.1, 142.2, 143.0, 145.1, 154.4, 158.1, 159.4, 162.7; IR (ATR, cm^{-1}): 3333, 3164, 3061, 2888, 1655, 1598, 1508, 1456, 826; MS (ESI) *m*/*z*: 343 ([M+H]$^+$, 51%), 172 ([M+2H]$^{++}$, 100%), 124 ([M−4-fluorobenzene +2H]$^{++}$, 10%); Anal. calcd for $C_{21}H_{15}N_4F$: C, 73.67; H, 4.42; N, 16.36, found: C, 74.03; H, 4.69; N, 16.69.

*4.2.14. 6-Amino-11-(p-biphenyl)-11,12-dihydropyrido[3,4-c][1,9-]phenanthroline (**3n**).* Biphenyl-4-carboxaldehyde (383 mg, 2.1 mmol), 2.5 h, $C_{27}H_{20}N_4$, yield: 235 mg (28%, recrystallized from MeOH/CH_2Cl_2); solid, mp 265 °C under decomp.; ^{1}H NMR (300 MHz, DMSO-d_6): δ=3.21 (d, 1H, H-12a, $^2J_{H\text{-}12a/H\text{-}12b}$=16.3 Hz), 3.57 (dd, 1H, H-12b, $^2J_{H\text{-}12b/H\text{-}12a}$=16.3 Hz, $^3J_{H\text{-}12b/H\text{-}11}$=7.5 Hz), 4.91 (d, 1H, H-11, $^3J_{H\text{-}11/H\text{-}12b}$=7.4 Hz), 7.08 (m, 2H, Ar–H, ^3J=8.2 Hz), 7.21 (d, 1H, Ar–H, ^3J=4.9 Hz) 7.25–7.48 (m, 5H, Ar–H; br s, 2H, $-NH_2$), 7.51 (m, 2H, Ar–H, ^3J=8.1 Hz), 7.59 (d, 1H, Ar–H, ^3J=5.9 Hz), 8.41 (d, 1H, Ar–H, ^3J=4.9 Hz), 8.56 (d, 1H, Ar–H, ^3J=5.9 Hz), 9.39 (s, 1H, Ar–H), 9.58 (s, 1H, Ar–H); ^{13}C NMR (75 MHz, DMSO-d_6+1% acetic-acid-d_4): δ=34.7 (C-11), 35.8 (C-12), 113.2, 114.9, 120.0, 126.4 (2C), 127.0 (2C), 127.4, 127.8 (2C), 128.8 (2C), 131.8, 131.9, 137.1, 137.6, 138.9, 139.4, 140.5, 141.5, 143.2, 143.8, 147.3, 156.7, 158.3; IR (ATR, cm^{-1}): 3327, 3029, 2970, 2898, 1652, 1598, 1455, 1044. 821; MS (ESI) *m*/*z*: 401 ([M+H]$^+$, 59%), 201 ([M+2H]$^{++}$, 100%), 124 ([M−*p*-phenylbenzene+2H]$^{++}$, 8%); HRMS (ESI) *m*/*z* calcd for [M+H]$^+$ 401.17606, found 401.17587.

*4.2.15. 6-Amino-11-propyl-11,12-dihydropyrido[3,4-c][1,9]phenanthroline (**3o**).* Butyraldehyde (151 mg, 2.1 mmol), 3 h, $C_{18}H_{18}N_4$, yield: 128 mg (21%, recrystallized from MeOH/CH_2Cl_2); solid, mp 231 °C under decomp.; ^{1}H NMR (300 MHz, DMSO-d_6): δ=0.78 (t,

3H, $-CH_3$, ^3J=6.9 Hz), 1.12–1.48 (m, 4H, $-CH_2-CH_2-$), 2.92–3.14 (m, 2H, H-12), 3.42–3.56 (m, 1H, H-11), 7.30 (br s, 2H, $-NH_2$), 7.34 (d, 1H, Ar–H, ^3J=4.9 Hz), 7.75 (d, 1H, Ar–H, ^3J=5.9 Hz), 8.46 (d, 1H, Ar–H, ^3J=4.9 Hz), 8.64 (d, 1H, Ar–H, ^3J=5.9 Hz), 9.27 (s, 1H, Ar–H), 9.56 (s, 1H, Ar–H); ^{13}C NMR (75 MHz, DMSO-d_6): δ=13.9 ($-CH_3$), 19.9 ($-CH_2-$), 29.9 (C-11), 30.8 (C-12), 35.3 ($-CH_2-$), 112.5, 115.5, 117.1, 123.5, 129.6, 138.9, 144.5, 144.9, 145.7, 147.5, 149.2, 149.5, 156.6; IR (ATR, cm^{-1}): 3325, 3164, 3045, 2953, 2930, 2870, 1651, 1598, 1455, 1043, 821; MS (ESI) *m*/*z*: 291 ([M+H]$^+$, 59%), 146 ([M+2H]$^{++}$, 25%), 124 ([M−propane +2H]$^{++}$, 100%). HRMS (ESI) *m*/*z* calcd for [M+H]$^+$ 291.16041, found 291.16046.

*4.2.16. 6-Amino-11,12-dihydropyrido[3,4-c][1,9]phenanthroline (**3p**).* Paraformaldehyde (64 mg, 2.1 mmol), 3 h, $C_{15}H_{12}N_4$, yield: 150 mg (29%, silica gel, CH_2Cl_2/MeOH (5–20%)); solid; mp>310 °C ^{1}H NMR (300 MHz, DMSO-d_6): δ=3.00 (m, 4H, CH_2-CH_2), 7.25 (br s, 2H, NH_2), 7.31 (d, 1H, Ar–H, ^3J=4.9 Hz), 7.75 (d, 1H, Ar–H, ^3J=5.8 Hz), 8.44 (d, 1H, Ar–H, ^3J=4.9 Hz), 8.62 (d, 1H, Ar–H, ^3J=5.8 Hz), 9.25 (s, 1H, Ar–H), 9.54 (s, 1H, Ar–H).^{13}C NMR (75 MHz, DMSO-d_6): δ=35.1 (2C), 112.4, 115.6, 122.6, 129.9, 139.2, 145.3, 145.9, 146.3, 147.5, 149.0, 149.2, 155.9, 156.6; IR (ATR, cm^{-1}): 3306, 3136, 1670, 1612, 1568, 1462; MS (EI) *m*/*z*:=248 M$^+$ (100), 247 [M−H]$^+$ (97), 230 (21). HRMS (ESI) *m*/*z* calcd for [M+H]$^+$ 249.11346, found 249.11319.

4.3. General procedure for the dehydration of the 6-amino-11,12-dihydropyrido[3,4-*c*][1,9]phenanthrolines to 6-aminopyrido-[3,4-*c*][1,9]phenanthrolines

200 mg of **3** were dissolved in 5–10 ml of DMPU and Palladium (10%)/charcoal (30% by weight) was added. The solution was refluxed under N_2 for 10–30 min. The reaction vessel was washed with 50 ml of CH_2Cl_2 and the Palladium/charcoal was filtered off. The filtrate was concentrated in vacuo and DMPU could be removed by distillation at high vacuum at 90–110 °C. The resulting residue was collected by filtration and purified via column chromatography (silica gel, CH_2Cl_2/MeOH (10%)).

*4.3.1. 6-Amino-11-phenylpyrido[3,4c][1,9]phenanthroline (**4a**).* The reaction of **3a** (200 mg, 0.62 mmol) with 60 mg Pd/C for 10 min afforded compound **4a**. $C_{21}H_{14}N_4$, yield: 178 mg (89%); solid; mp 221 °C under decomp.; ^{1}H NMR (300 MHz, D_2O+2% DCl in D_2O): δ=7.05–7.11 (m, 2H, Ar–H), 7.29–7.51 (m, 5H, Ar–H), 8.02 (d, 1H, Ar–H, ^3J=6.6 Hz), 8.21 (d, 1H, Ar–H, ^3J=7.0 Hz), 8.53 (d, 1H, Ar–H, ^3J=6.6 Hz), 9.55 (s, 1H, Ar–H), 9.84 (s, 1H, Ar–H); ^{13}C NMR (75 MHz, DMSO-d_6): δ=113.7, 114.5, 123.1, 123.2 (2C), 124.4, 128.2 (3C), 129.8 (2C), 135.0, 136.5, 139.2, 141.4, 142.0, 143.1, 143.8, 148.8, 159.2, 156.8, IR (ATR, cm^{-1}): 3360, 3300, 3049, 1675, 1605, 1560, 1462, 1053, 860; MS (ESI) *m*/*z*: 323 ([M+H]$^+$, 100%), 162 ([M+2H]$^{++}$, 32%). HRMS (ESI) *m*/*z* calcd for [M+H]$^+$ 323.12911, found 323.12891.

*4.3.2. 6-Amino-11-(3-methoxyphenyl)pyrido[3,4c][1,9]phenanthroline (**4b**).* The reaction of **3b** (200 mg, 0.56 mmol) with 60 mg Pd/C for 10 min afforded compound **4b**. $C_{22}H_{16}N_4O$, yield: 116 mg (58%); solid; mp 260 °C under decomp.; ^{1}H NMR (300 MHz, DMSO-d_6): δ=3.79 (s, 3H, OCH_3), 6.96–7.03 (m, 1H, Ar–H, ^3J=7.5 Hz, ^4J=0.9 Hz), 7.04–7.07 (m, 1H, Ar–H), 7.08–7.15 (m, 1H, Ar–H; d, 1H, Ar–H, ^3J=5.9 Hz), 7.45 (m, 1H, Ar–H, ^3J=7.9 Hz), 7.52 (s, 1H, Ar–H, H-12), 7.78–7.94 (d, 1H, Ar–H, ^3J=5.9 Hz; br s, 2H, $-NH_2$), 8.38 (d, 1H, Ar–H, ^3J=5.9 Hz), 8.67 (d, 1H, Ar–H, ^3J=6.0 Hz), 9.68 (br s, 1H, Ar–H), 10.33 (br s, 1H, Ar–H); ^{13}C NMR (75 MHz, DMSO-d_6+2% DCl in D_2O): δ=55.4, 113.5, 113.6, 115.0, 115.1, 120.5, 121.9, 122.7, 123.4, 124.4, 130.9, 135.2, 137.7, 141.1, 141.9, 142.5, 143.1, 143.4, 148.1, 149.2, 157.3, 160.0; IR (ATR, cm^{-1}): 3358, 3272, 3047, 1673, 1606, 1559, 1462, 786; MS (ESI) *m*/*z*: 353 ([M+H]$^+$, 100%), 177 ([M+2H]$^{++}$, 12%), 169 (17%). Anal. calcd for $C_{22}H_{16}N_4O$: C, 74.89; H, 4.58; N, 15.90, found: C, 74.33; H, 4.66; N, 15.57.

*4.3.3. 6-Amino-11-(2,3-dimethoxyphenyl)-pyrido[3,4c][1,9]phenanthroline (**4d**).* The reaction of **3d** (200 mg, 0.52 mmol) with 60 mg Pd/C for 10 min afforded compound **4d**. $C_{23}H_{18}N_4O_2$, yield: 144 mg (72%); solid; mp 231 °C under decomp.; ^{1}H NMR (300 MHz, DMSO-d_6+2% DCl in D_2O): δ=3.35 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 7.04 (m, 1H, Ar–H, J=4.6 Hz), 7.36 (m, 2H, Ar–H, J=4.6 Hz), 7.53 (dd, 1H, Ar–H, ^3J=6.7 Hz, ^4J=0.6 Hz), 7.89 (s, 1H, Ar–H, H-12), 8.53 (dd, 1H, Ar–H, ^3J=6.5 Hz, ^4J=0.8 Hz), 8.69 (dd, 1H, Ar–H, ^3J=6.7 Hz, ^4J=0.6 Hz), 8.93 (dd, 1H, Ar–H, ^3J=6.5 Hz, ^4J=0.8 Hz), 10.21 (m, 1H, Ar–H), 10.23 (m, 1H, Ar–H); ^{13}C NMR (75 MHz, DMSO-d_6): δ=56.0 ($-OCH_3$), 60.1 ($-OCH_3$), 114.2, 114.4, 114.6, 120.0, 120.8, 123.0, 123.6, 124.2, 125.6, 126.2, 129.0, 134.9, 135.0, 140.6 (2C), 142.1, 142.8, 144.8 (2C), 144.9, 148.1, 153.0, 157.1; IR (ATR, cm^{-1}): 3328, 3163, 2952, 1641, 1606, 1553, 1407, 1267, 1050; MS (ESI) *m*/*z*: 383 ([M+H]$^+$, 100%), 184 (19%), 169 (14%); 163 (13%). HRMS (ESI) *m*/*z* calcd for [M+H]$^+$ 383.15024, found 383.14987.

*4.3.4. 6-Amino-11-(3,4,5-trimethoxyphenyl)-pyrido[3,4c][1,9]phenanthroline (**4f**).* The reaction of **3f** (200 mg, 0.48 mmol) with 60 mg Pd/C for 10 min afforded compound **4f**. $C_{24}H_{20}N_4O_3$, yield: 149 mg (75%); solid; mp 246 °C under decomp.; ^{1}H NMR (300 MHz, DMSO-d_6): δ=3.73 (s, 6H, 2× OCH_3), 3.79 (s, 3H, $-CH_3$), 6.79 (s, 2H, H-2′, H6′), 7.18 (d, 1H, Ar–H, ^3J=6.0 Hz), 7.58 (s, 1H, Ar–H, H-12), 7.78–7.93 (d, 1H, Ar–H; br s, 2H, $-NH_2$) 8.46 (d, 1H, Ar–H, ^3J=6.0 Hz), 8.67 (d, 1H, Ar–H, ^3J=6.1 Hz), 9.68 (br s, 1H, Ar–H), 10.33 (br s, 1H, Ar–H); ^{13}C NMR (75 MHz, DMSO-d_6): δ=56.1 (2× OCH_3), 60.3 (OCH_3), 106.0 (2C, C-2′, C-6′), 113.2, 113.8, 118.6, 119.8, 122.4, 123.6, 136.2, 137.4, 138.1, 138.3, 142.3, 145.6, 145.9, 147.2, 148.7, 149.4, 153.4 (2C, C-3′, C-5′), 156.3; IR (ATR, cm^{-1}): 3330, 3196, 2832, 1644, 1580, 1502, 1462, 1420; MS (EI) *m*/*z*: 412 (M$^+$, 100%), 397 ([M−CH_3]$^+$, 31%), 337 (17%), 294 (13%), 283 (10%). Anal. calcd for $C_{24}H_{20}N_4O_3$: C, 69.89; H, 4.89; N, 13.58, found: C, 69.99; H, 5.43; N, 13.75.

*4.3.5. 6-Amino-11-furylpyrido[3,4c][1,9]phenanthroline (**4h**).* The reaction of **3h** (200 mg, 0.64 mmol) with 60 mg Pd/C for 15 min afforded compound **4h**. $C_{19}H_{12}N_4O$, yield: 126 mg (63%); solid; mp 248 °C under decomp.; ^{1}H NMR (300 MHz, DMSO-d_6+2% DCl in D_2O): δ=6.96 (m, ^3J=1.9 Hz), 7.12 (dd, 1H, Ar–H, ^3J=6.8 Hz, ^4J=0.7 Hz), 7.27 (dd, 1H, Ar–H, ^3J=3.4 Hz, ^4J=0.8 Hz), 8.03 (m, 1H, ^3J=1.8 Hz, ^4J=0.8 Hz), 8.13 (s, 1H, Ar–H, H-12), 8.54 (dd, 1H, ^3J=6.7 Hz, ^4J=0.8 Hz), 8.88 (dd, 1H, ^3J=6.8 Hz, ^4J=0.6 Hz), 8.97 (dd, 1H, ^3J=6.5 Hz, ^4J=0.8 Hz), 10.11 (m, 1H, Ar–H), 10.31 (m, 1H, Ar–H); ^{13}C NMR (75 MHz, DMSO-d_6): δ=111.7, 112.5, 113.1, 114.4, 120.9, 121.6, 123.6, 124.5, 135.1, 135.3, 138.8, 141.2, 142.1, 142.3, 143.8, 145.3. 149.1, 151.4, 157.2; IR (ATR, cm^{-1}): 3359, 3278, 3049, 2822, 1674, 1610, 1559, 1408, 863; MS (ESI) *m*/*z*: 313 ([M+H]$^+$, 100%), 157 ([M+2H]$^{++}$, 8%), 143 (6%); HRMS (ESI) *m*/*z* calcd for [M+H]$^+$ 313.10838, found 313.10803.

4.4. Solubility

1–2 mg were suspended in 100 mM potassium phosphate buffer (pH 2.0, pH 3.0, pH 4.0, and pH 7.4), shaken for 45 min at room temperature, and centrifuged twice at 13,000 rpm for 15 min. Supernatants were analyzed by HPLC and concentrations of the dissolved compounds were calculated by a calibration of the respective compound in 0.1% TFA in acetonitrile.

4.5. HPLC-analytics

We used the Waters Alliance HPLC system consisting of Waters e2695 XC separation module, Waters 2998 photodiode array

detector, column heater, and Empower 2 software. Compounds were separated on a LiChrospher® 60 RP-select B (125×4 mm, 5 μm) with a C18 guard column (4×4 mm). The mobile phase consisted of 0.1% TFA and acetonitrile. The flow rate was set to 1 mL min^{-1}.

4.6. Separation of 6-aminopyrido[3,4-*c*][1,9]phenanthroline-derivatives 3

The following gradient was adjusted to ensure separation within 12 min. The acetonitrile percentage was elevated from 26% to 50% (0–8 min), then reduced to 26% (8–9.5 min), and maintained at 26% for another 2.5 min. The detection wavelength was set to 265 nm. Retention times: **3a**: (2.5±0.1) min; **3b**: (2.2±0.1) min; **3d**: (3.2±0.1) min; **3e**: (3.1±0.1) min; **3f**: (2.2±0.1) min; **3g**: (3.7±0.1) min; **3h**: (1.7±0.1) min; **3i**: (2.1±0.1) min; **3j**: (3.9±0.1) min; **3k**: (3.4±0.1) min; **3l**: (3.2±0.1) min; **3m**: (2.8±0.1) min; **3n**: (5.2±0.1) min; **3o**: (1.6±0.1) min.

4.7. Separation of 6-aminobenzo[*c*]phenanthridine-derivatives BP

Elution was carried out isocratically. The mobile phase contained 40% acetonitrile and the detection wavelength was set to 220 nm. Compounds were eluted within 8 min. Retention times: **BP-1**: (4.7±0.1) min; **BP-7**: (3.4±0.1) min; **BP-9**: (3.7±0.1) min; **BP-32**: (5.1±0.1) min; **BP-34**: (6.7±0.1) min.

4.8. Experimental determination of lipophilicity (log*P*)

Lipophilicity was determined by using liquid chromatography. Analytics were carried out on a LiChrospher® 100 RP-8 (125×4 mm, 5 μm) with a C18 guard column (4×4 mm) and a mobile phase consisting of 20 mM potassium phosphate buffer pH 7.4 and methanol (40/60). A calibration curve was generated by analyzing 9 standard compounds with log*P* values within the range of 2.5–6.1. Test compounds were dissolved in the mobile phase in a concentration of 100 μM. All compounds were eluted within 30 min at a flow rate of 1 mL min^{-1}. Lipophilicity was calculated on the basis of retention times and calibration curve. The following standard compounds were used (log*P*): *p*-chlorotoluene (2.5), toluene (2.7), chlorobenzene (2.8), benzophenone (3.2), testosterone (3.2), thymol (3.3), biphenyl (4.0), ketoconazole (4.5), and clotrimazole (6.1).

4.9. Calculation of lipophilicity (*clogP*)

Lipophilicity of all compounds was calculated in their uncharged state using the software 'ChemBioDraw Ultra' (CambridgeSoft, Cambridge, MA, USA).

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