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Research paper

Novel 1,6-naphthyridin-2(1*H*)-ones as potential anticancer agents targeting Hsp90

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ABSTRACT

Hsp90 is an ATP-dependent chaperone known to be overexpressed in many cancers. This way, Hsp90 is an important target for drug discovery. Novobiocin, an aminocoumarin antibiotic, was reported to inhibit Hsp90 targeting C-terminal domain, and showed anti-proliferative properties, leading to the development of new and more active compounds. Consequently, a new set of novobiocin analogs derived from 1,6-naphthyridin-2(1*H*)-one scaffold was designed, synthesized and evaluated against two breast cancer cell lines. Subsequently, cell cycle progression and apoptosis were conducted on best candidates, finally Western Blot analysis was performed to measure their ability to induce degradation of Hsp90 client proteins.

1. Introduction

The 90 kDa heat shock protein (Hsp90) is a highly conserved and specialized chaperone responsible for the maturation of a select clientele of proteins. Many of clients (Raf-1, Her2, Akt, etc.) are involved in multiple oncogenic pathways and associated with cancer cell survival, making Hsp90 an attractive target for cancer chemotherapy [1,2]. In terms of structure, Hsp90 exists as a homodimer with each monomer consisting of three domains; a N-terminal nucleotide binding domain, a middle domain with high affinity for co-chaperones and client proteins, and a C-terminal dimerization domain. In recent years, considerable effort in the discovery of Hsp90 inhibitors was performed. 17-AAG (tanespimycin), a semi-synthetic derivative of geldanamycin, was the first Hsp90 inhibitor to be evaluated in clinical trials and has shown significant anti-tumor activity at tolerable doses. In addition, 16 other molecules specifically targeting the N-terminal ATP-binding site have entered clinical trials for evaluation against various cancers [3]. These N-terminal inhibitors have demonstrated an ability to induce client protein degradation, which highlights the importance of Hsp90 as a promising cancer therapeutic target. Unfortunately, induction of a pro-survival heat shock response (HSR) at the same concentration needed to promote Hsp90 client protein degradation has compromised their future in therapeutic. This HSR expands the chaperone buffering capacity to contribute in the maturation of mutated and oncogenic clients leading to drug resistance and tumor metastasis [4]. In contrast to N-terminal inhibitors, recent studies have demonstrated that inhibitors of the C-terminal region do not induce the HSR and therefore provide an attractive alternative for the development of new Hsp90 modulators that exhibit better activities [5,6].

Novobiocin, an aminocoumarin member of the antibiotic family acting through inhibition of DNA gyrase, was the first natural product identified as Hsp90 C-terminal inhibitor that does not compete with other inhibitors for binding to the N-terminal domain [7]. Unfortunately, novobiocin displays poor anti-proliferative activity (IC_{50} ~ 700 μM against SKBr3 cells and 260 μM against MCF-7 cell line) and consequently has limited its therapeutic use. Although the binding mode of the N-terminal Hsp90 inhibitors is well established, the structural moieties required for interaction with the C-terminal domain are very poorly

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characterized [8]. Since to date, no cocrystal structure of Hsp90 bound to C-terminal inhibitors has been yet reported. Preliminary investigations on novobiocin analogs revealed structural key elements for elucidation of the Hsp90 C-terminal binding pocket and subsequent structure–activity relationships led to identify promising synthetic compounds that exhibit potent antiproliferative activity against a large panel of cancer cell lines [9–23]. The synthesis of these libraries has helped to highlight the following structure–activity relationships (SARs): (1) the lactone present in the coumarin core is not essential for Hsp90 inhibition while 4-hydroxy lowers the activity; (2) replacement of the noviose at the 7-position with hydrophilic amines residues is tolerated without compromising inhibitory activity (3) the aromatic side chain and the amide linker are critical for antiproliferative activity (Fig. 1).

On the basis of these SARs, we started a new medicinal chemistry program to design and synthesize novel novobiocin analogs as potent antiproliferative agents targeting the Hsp90 chaperone machinery.

First, we suggested that the coumarin core be replaced with a 1,6-naphthyridin-2(1*H*)-one scaffold. As the lactone is considered as non-essential for biological activity, bioisosteric exchange of the pyran-2-one ring with a pyridin-2-one core was realized. A pyridine ring is attached to complete the scaffold and to form the bicycle. To overcome problems with regard to solubility or reactivity of free N–H-containing 1,6-naphthyridones, we decided to investigate *N*-methylated 1,6-naphthyridones [24]. Then, a large variety of amides was introduced to the 3-position of a key amine intermediate, the 3-amino-7-chloro-1-methyl-1,6-naphthyridin-2(1*H*)-one, which was functionalized at the 7-position with hydrophilic amines.

2. Results and discussion

2.1. Chemistry

In previous works, we developed efficient one-pot syntheses of 3,7-disubstituted 1,6-naphthyridin-2(1*H*)-ones through regioselective palladium-catalyzed cross-coupling reactions [25,26]. In this paper, the same procedure was used to synthesize in multigram quantities an *ortho*-amino nicotinaldehyde (Scheme 1).

First, condensation of a commercial solution of diethyl 3-oxopentanedioate **1** with triethyl orthoformate led to an intermediate which was cyclized with aqueous ammonia to the ester **2**. Subsequent chlorination reaction was carried out with phosphorus(V) oxychloride and allowed isolation of the compound **3**. Regioselective nucleophilic substitution in the 4-position with methylamine provided monochloro compound **4** in 96% yield. Finally, a reduction/oxidation sequence afforded the desired aldehyde **5**. The key amine intermediate **6** was then obtained in a good yield from **5** and the condensation with ethyl glycinate.

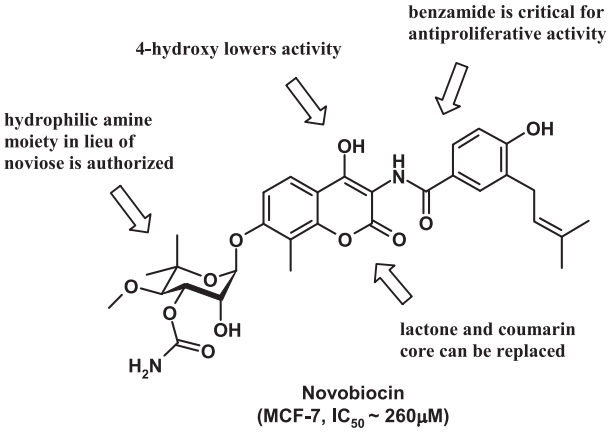


Fig. 1. SARs established from previous studies related to novobiocin analogs.

The target compounds were obtained in two steps from the 1,6-naphthyridin-2-(1*H*)-one **6**. Amides **7**–**18** were synthesized by reaction of amine **6** with functionalized acyl chlorides in anhydrous pyridine [27]. Introduction of various carboxamides (alkyle, cinnamoyle, aryles and heterocycles) in the 3-position sometimes required the preliminary synthesis of corresponding acyl chlorides. Finally, aromatic nucleophilic substitutions with aliphatic amines afforded analogs of novobiocin **19**–**45** in moderate to very good yields [28]. The structure elucidation of synthesized compounds was accomplished using extensive 1D and 2D NMR spectroscopic studies supplemented with MS analysis.

2.2. Biological evaluation

2.2.1. Antiproliferative activity (MTT)

To follow up on the synthesis of our naphthyridinones, an evaluation of their antiproliferative activity against MCF-7 (estrogen receptor positive breast cancer cells) and MDA-MB-468 (triple negative, estrogen and progesterone receptors negative, no HER2 expression) cell lines was conducted (Table 1).

The IC_{50} values of 17-AAG (N-terminal inhibitor) and novobiocin were consistent with those found in the literature.

Nine molecules (**19**, **20**, **21**, **23**, **25**, **27**, **28**, **32** and **44**) had no significant effect at a concentration greater than 100 µM on two cell lines tested, and three (**34**, **40**, **42**) exhibited antiproliferative effect only against MCF-7 cell line. Four analogs (**36**, **37**, **39**, **41**) displayed an activity less than 10 µM to both cell lines, but none is as active as 17-AAG. The p53 status of these cell lines, p53-wild type for MCF-7 and p53-mutant for MDA-MB-468, could generate different cellular responses. In view of these heterogeneous activities, we chose to undergo further biological assays to obtain more information regarding the antiproliferative mechanism of action of the most promising compounds.

2.2.2. Apoptosis detection

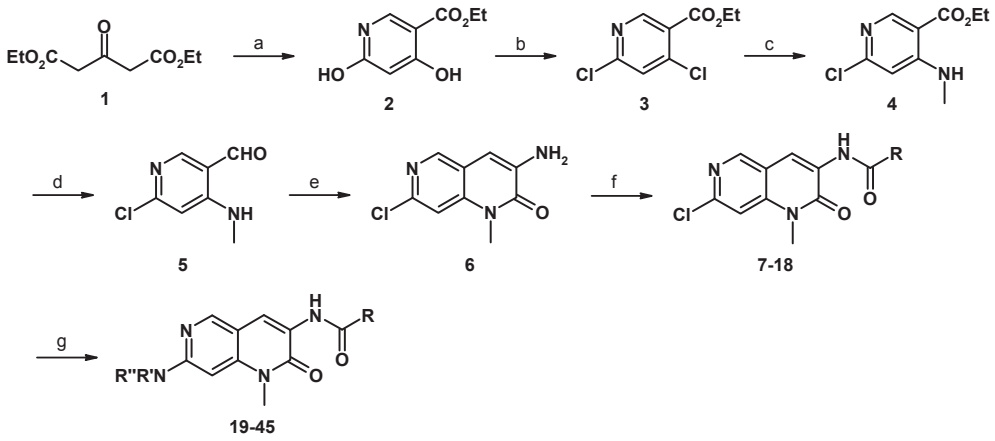
Seven compounds that have shown a significant antiproliferative activity on MCF-7 and MDA-MB-468 cell lines were selected for evaluation. For this purpose, the compounds **30**, **41** and **45** were chosen because they exhibited low IC_{50} values. All biphenyl derivatives **36**–**39** appeared promising and were also selected. Having demonstrated antiproliferative activity of these compounds, we investigated whether this effect could be the consequence of an induced cell death caused by these molecules.

Analyzes were performed on MDA-MB-468 cells and at concentrations corresponding to the IC_{50} of the compounds (Fig. 2).

The Annexin V/Propidium iodide (PI) protocol is a commonly used approach for studying the cell death and discriminate apoptotic from necrotic cells through differences in plasma membrane integrity and permeability. Three cell populations can be distinguished: viable cells (AV-, PI-), cells in early apoptosis (AV+, PI-) and cells in late apoptosis or necrosis (AV+, PI+). Among the seven analyzed molecules, only **30** and **38** have shown significant cytotoxicity with respective mortalities of 40 and 80% greater than that obtained with the 17-AAG. Molecules **36** and **39** did not induce cell death. Finally, compounds **37**, **41** and **45** presented a low level compared to 17-AAG. The antiproliferative activity of novobiocin on the MDA-MB-468 line is known to be low, so it was not included in cell death assays.

2.2.3. Cell cycle analysis

To complete these apoptosis assays, analysis of the cell cycle



Scheme 1. Synthesis of new analogs of novobiocin derived from 1,6-naphthyridin-2(1*H*)-one scaffold. *Reagents and conditions*: (a) $HC(OEt)_3$, Ac_2O, 120 °C, 1.5 h, then NH_3 (30%w), 0 °C, 70%; (b) $POCl_3$, 110 °C, 2 h, 95%; (c) CH_3NH_2 (40%w), CH_3CN, 0 °C, 30 min, then rt, 3 h, 96%; (d) $LiAlH_4$, THF, -78 °C, 3 h, then MnO_2, CH_2Cl_2, rt, 2 h, 84%; (e) Ethyl glycinate, K_2CO_3, anh. DMF, 90 °C, 5 h, 92%; (f) ClCOR, anh. Pyridine, 1–4 h, 33–84%; (g) R′R″NH_2, μ-wave, 130–170 °C, 1–8 h, 19–95%.

distribution of treated cancer cells was determined for each of the selected compounds. Cells were stained with PI and the proportion of cells in different phases of the cell cycle was recorded. MDA-MB-468 cells control (DMSO) presented a profile of cellular cycle conventionally observed in cancer cell lines in optimal growth conditions. With regard to the treated cells, a gradual accumulation of MDA-MB-468 cells and a blockage in G2/M phase for 17-AAG was observed after 48 h of incubation, which is consistent with the data from the literature [30]. Compounds **36**, **39**, **41**, **45** induced a significant increase in G2/M but not **37** (Fig. 3). No major change in the cell cycle for molecules **30** and **38** was observed, however a subG1 peak characteristic of apoptotic cells population was detected in agreement with previous apoptotic assays.

2.2.4. Western blot analysis

In order to ensure the antiproliferative activity through Hsp90 inhibition, Western Blot analyses of two Hsp90 client protein levels in MCF-7 and one in MDA-MB-468 were performed. As shown in Fig. 4, the Hsp90-dependent client proteins Raf-1 and ERα (only express in MCF-7 cells) were completely degraded in MCF-7 cell line upon treatment with 17-AAG. In addition, induction of a pro-survival heat shock response (HSR) was observed as described with other N-terminal inhibitors. These results were less pronounced in MDA-MB-468 cells. The heat shock response was also visible in MCF-7 cell line for **37** and **38** but to a lesser extent. All compounds induced degradation of client proteins in two cell lines, except for **45**. The analogue **39** seemed to be the most promising compound, exhibited important client proteins degradation in two cell lines, and was better than 17-AAG in MDA-MB-468 cells. The non-Hsp90-dependent protein, actin, was not altered upon administration of these compounds, indicating selective degradation of Hsp90-dependent proteins. In addition, Hsp90 levels remained unchanged except for **37** and **38**, which is consistent with other known Hsp90 C-terminal inhibitors [31].

A few brief comments on SARs reveals that all the seven tested compounds displaying the best activities bear an apolar core in the 3-position (i.e. benzothienyle, biphenyle, chlorophenyle and methoxyphenyle). Four of them are biphenyle derivatives but only compound **39** seems to be the most potent due to client proteins Raf-1 and ER-α inhibition on the two breast cancer cell lines. One might wonder why the protonatable nitrogen atom or the steric hindrance on polar chains like in compounds **36**–**38** are useful. Other pharmacomodulations should confirm this hypothesis.

3. Conclusion

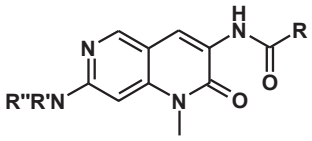
In summary, a library of 1,6-naphthyridinones derivatives based on novobiocin structure was synthesized and evaluated for their antiproliferative activities on MCF-7 and MDA-MB-468 breast cancer cell lines. Compounds having the biphenyl structure were the most convenient and all exhibited good activity on both cancer cell lines, suggesting the importance of a bulky benzamide group. Moreover, the addition of alkylamino substituents at the 7-position ensured an increased solubility for bioassays and this motif might be involved in the interaction with the target. Some compounds showed promising results in different biological assays (cell death, cell cycle distribution, expression of Hsp90 client proteins). Particularly, Western Blot analyses suggest these compounds manifest antiproliferative activity through Hsp90 inhibition. Investigations are currently underway to further develop this scaffold and the biological evaluation is still under progress to consider relevant SARs.

4. Experimental protocols

4.1. Chemistry

All commercial reagents were used without further purification. All solvents were reagent or HPLC grade. THF was distilled on Na/benzophenone system prior to use. Analytical TLC was performed on silica gel 60 F254 plates. Flash column chromatography was performed on silica gel 60 (70–230 mesh ASTM). Yields refer to chromatographically and spectroscopically pure compounds. Melting points were determined on an Electrothermal melting point apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO-d_6 or $CDCl_3$ on a 400 MHz spectrometer. Chemical shifts are reported as δ values in parts per million (ppm) relative to tetramethylsilane as internal standard and coupling constants (J) are given in hertz. Multiplicities are reported as follows: s = singlet, d = doublet, dd = doublet of doublets, ddd = doublet of doublet of doublets, t = triplet, m = multiplet, q = quartet, br s = broad singlet. Low resolution mass spectra were recorded using an Electrospray Ionization (ESI) Method with Waters ZQ 2000 spectrometer. UPLC column used was an Acquity UPLC® BEH Phenyl (2.1 mm i.d., 50 mm length, 1.7 μm particle size) from Waters. A linear mobile phase gradient was used with a mobile phase A as 100% of acetonitrile in water (at 2%) and mobile phase B as 100% acetonitrile. The gradient table was: 0–0.5 min, 0% B; 0.5–4.0 min 0 → 100% B;

Table 1
Antiproliferative activity of 1,6-naphthyridinones, novobiocin analogs.



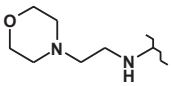
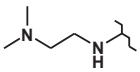
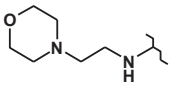
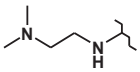
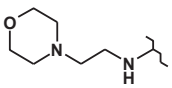
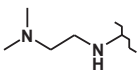
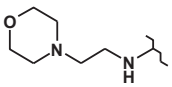
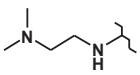
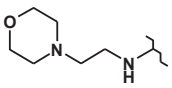
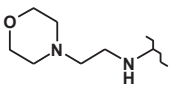
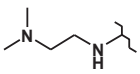
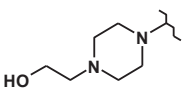
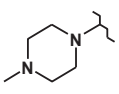
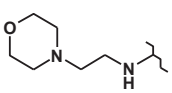
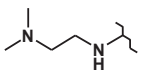
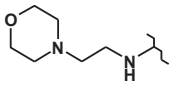
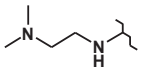
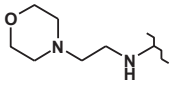
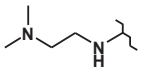
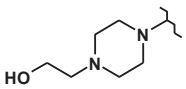
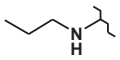
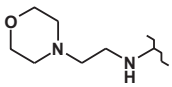
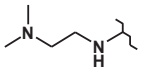
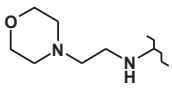
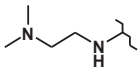
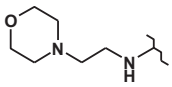
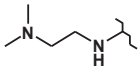
Compound	R	R'R″N	MCF-7 (IC_{50}, μM)[a]	MDA-MB-468 (IC_{50}, μM)[a]
17-AAG (tanespimycin)	–	–	0.029 ± 1.54 (Lit: 5 nM) [29]	1.57 ± 1.42
Novobiocin	–	–	293.3 ± 2.60 (Lit: 260 μM) [23]	338.40 ± 9.05
19	CH_3		>100	>100
20	CH_3		>100	>100
21	CH = CHPh		>100	>100
22	CH = CHPh		27.41 ± 1.68	12.05 ± 1.81
23	Ph		>100	>100
24	Ph		35.09 ± 1.24	18.27 ± 1.51
25	Ph-(*p*)NH_2		>100	>100
26	Ph-(*p*)NH_2		55.77 ± 1.91	20.41 ± 1.50
27	Ph-(*p*)OH		>100	>100
28	Ph-(*p*)OCH_3		>100	>100
29	Ph-(*p*)OCH_3		37.26 ± 1.58	15.71 ± 1.56
30	Ph-(*p*)OCH_3		8.59 ± 1.43	10.47 ± 3.30
31	Ph-(*p*)OCH_3		35.37 ± 0.33	12.55 ± 1.57
32	Ph-(*m*)OCH_3		>100	>100

Table 1 (*continued*)

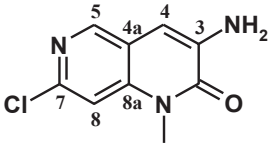
Compound	R	R'R''N	MCF-7 (IC_{50}, μM)[a]	MDA-MB-468 (IC_{50}, μM)[a]
33	Ph-(*m*)OCH_3		43.25 ± 1.38	17.44 ± 1.72
34	Ph-(*m*,*m*',*p*)OCH_3		48.56 ± 1.78	>100
35	Ph-(*m*,*m*',*p*)OCH_3		21.47 ± 1.40	15.59 ± 1.92
36	Ph-(*m*)Ph		4.85 ± 1.31	1.96 ± 1.83
37	Ph-(*m*)Ph		1.93 ± 1.55	1,68 ± 1,67
38	Ph-(*m*)Ph		15.20 ± 1.31	14.67 ± 1.70
39	Ph-(*m*)Ph		6.00 ± 1.34	1.87 ± 1.59
40	Ph-(*p*)Cl		6.35 ± 2.04	>100
41	Ph-(*p*)Cl		3.09 ± 2.04	1.64 ± 1.56
42	4-pyridyl		4.47 ± 4.12	>100
43	4-pyridyl		36.56 ± 2.48	8.36 ± 2.68
44	2-benzothienyl		>100	>100
45	2-benzothienyl		12.03 ± 1.40	3.57 ± 1.76

[a] Values represent mean ± standard deviation for at least three separate experiments performed in triplicate.

4.0–5.5% 100% B; 5.5–5.7 min 100 → 0% B; 5.7–7.5 min 0% B. at flow rate 0.5 mL min^{-1} and column temperature 35 °C. High resolution mass spectra were obtained by ESI/TOF. Microwave reactions were carried out in a CEM Discover microwave reactor in sealed vessels (monowave, maximum power 300 W, temperature control via IR-sensor, fixed temperature).

Compounds **2**–**5** were synthesized following the procedure previously described [25].

4.1.1. 3-Amino-7-chloro-1-methyl-1,6-naphthyridin-2(1H)-one (**6**)



To a mixture of **5** (2.00 g, 11.7 mmol) in anhydrous DMF (30 mL) were added ethyl glycinate (1.80 g, 12.9 mmol, 1.10 equiv.) and K_2CO_3 (3.23 g, 23.4 mmol, 2.0 equiv.). The reaction mixture was stirred at 90 °C for 5 h. After cooling, water was added, and the mixture was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Trituration with diisopropyl ether afforded **6**

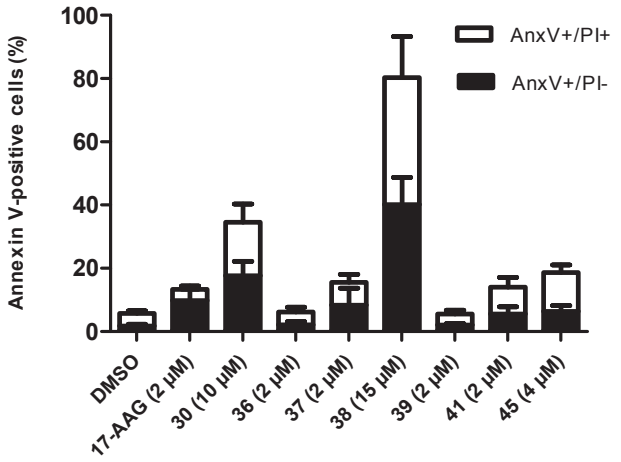


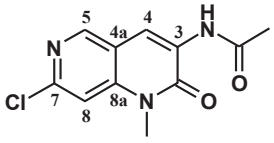
Fig. 2. Percentage of annexin V-positive cells in MDA-MB-468 cells treated for 48 h with selected compounds.

(2.25 g, 92% yield) as a pale yellow powder. Mp: 260–261 °C; IR, ν (cm^{-1}): 3445 and 3325 (ν N–H); 1622 (ν C=O); 1481, 1423 (ν C=C); 777 (ν C–Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (s, 1H, H_5), 7.48 (s, 1H, H_8), 6.80 (s, 1H, H_4), 5.94 (s, 2H, NH_2), 3.68 (s, 3H, NCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.96 (C_2), 145.72 (C_5), 144.47 (C_{8a}), 138.24 (C_7 or C_3), 138.19 (C_3 or C_7), 118.13 (C_{4a}), 107.98 (C_8), 101.58 (C_4), 29.71 (NCH_3); MS (ESI) *m/z* (%): 210.1 (100) $[M+H]^+$, 212.1 (40) $[M+H+2]^+$; UPLC purity = 96%, Rt 1.86 min; HRMS (ESI): calcd. for $C_9H_8ClN_3O$ $[M+H]^+$ 210.0429, found: 210.0421.

*4.1.2. General procedure for the synthesis of carboxamides **7**–**18***

Compound **6** (400 mg, 1.91 mmol) was dissolved in dry pyridine (10 mL). The corresponding acyl chloride (6.30 mmol, 3.3 equiv.) was added and the reaction mixture was refluxed for 1–5 h. After cooling, water was added and the organic layer was extracted with CH_2Cl_2. The organic layer was washed with 1 M HCl, brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was then purified by silica gel chromatography to provide **7**–**18**.

*4.1.2.1. N-(7-Chloro-1-methyl-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)acetamide (**7**).*



The reaction was carried out using amine **6** (400 mg, 1.91 mmol) and acetyl chloride (448 μL, 6.30 mmol) and refluxed in pyridine for 3 h. Purification by silica gel chromatography (dichloromethane/ethyl acetate, 90:10) afforded **7** (302 mg, 63% yield) as a white powder. Mp: 264–265 °C; IR, ν (cm^{-1}): 3361 (νN–H); 2986, 2970 (νC–H_{al}); 1771, 1645 (νC = O); 1498 (νC = C); 682 (νC–Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.70 (s, 1H, NH), 8.76 (s, 1H, H_5), 8.72 (s, 1H, H_4), 7.64 (s, 1H, H_8), 3.70 (s, 3H, NCH_3), 2.23 (s, 3H, $COCH_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.69 (NH–C=O), 157.29 (C_3–C=O), 149.21 (C_5), 148.48 (C_{8a}), 142.33 (C_7), 129.13 (C_3), 116.68 (C_4), 116.00 (C_{4a}), 108.40 (C_8), 30.07 (NCH_3), 24.11 ($COCH_3$); MS (ESI) *m/z* (%): 252.1 (100) $[M+H]^+$, 254.1 (40) $[M+H+2]^+$; UPLC purity = 98%, Rt 1.92 min; HRMS (ESI): calcd. for $C_{11}H_{10}ClN_3O_2$ $[M+H]^+$ 252.0534, found: 252.0525.

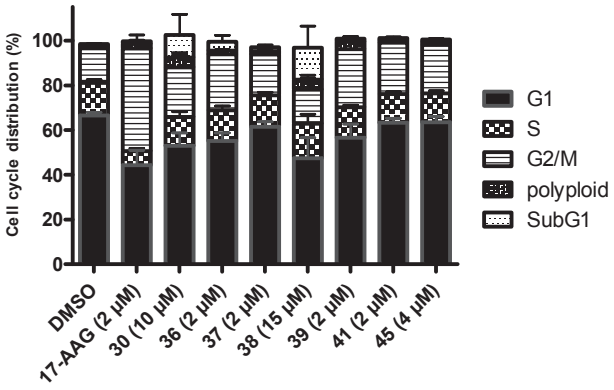
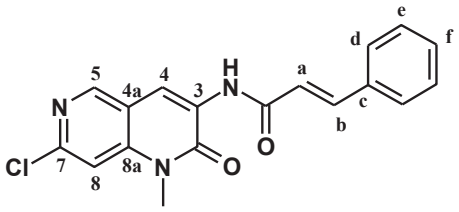


Fig. 3. Cell cycle distribution in MDA-MB-468 cells treated for 48 h with selected compounds.

*4.1.2.2. (2E)-N-(7-Chloro-1-methyl-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)-3-phenylprop-2-enamide (**8**).*



The reaction was carried out using amine **6** (400 mg, 1.91 mmol) and (2*E*)-3-phenylprop-2-enoyl chloride (1.15 g, 6.30 mmol) and

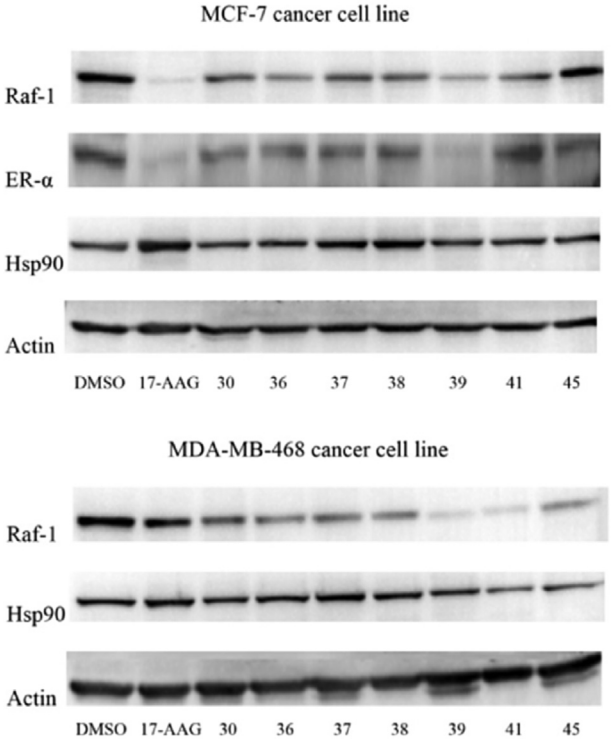
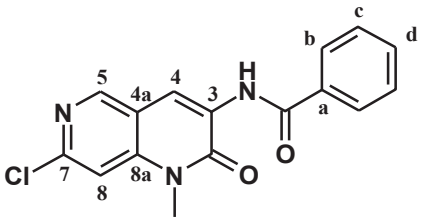


Fig. 4. Western Blot analyses of MCF-7 and MDA-MB-468 cell lysates for Hsp90 client protein degradation after 48 h of incubation. All compounds are tested at concentrations of 1.5 times their IC_{50} values (μM). 17-AAG (0.5 μM) and DMSO were employed, respectively, as positive and negative controls.

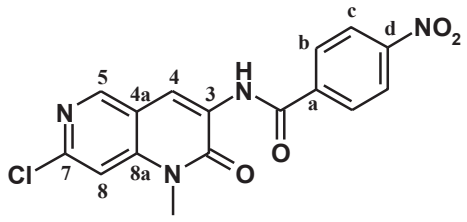
refluxed in pyridine for 3 h. Purification by silica gel chromatography (mixtures of dichloromethane/methanol of increasing polarity) and recrystallization from EtOH provided **8** (381 mg, 59% yield) as a brown powder. Mp: 254–255 °C; IR, ν (cm^{-1}): 3335 (νN–H); 3063 (νC–H_{ar}); 1682, 1643 (νC = O); 1574, 1512 (νC = C); 766 (νC–Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.94 (s, 1H, NH), 8.90 (s, 1H, H_5), 8.80 (s, 1H, H_4), 7.70–7.65 (m, 3H, H_b, H_e), 7.61 (s, 1H, H_8), 7.49–7.45 (m, 4H, H_a, H_d, H_f), 3.73 (s, 3H, NCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.90 (NH–C=O), 157.36 (C_3–C=O), 149.34 (C_5), 148.60 (C_{8a}), 142.42 (C_7), 141.13 (C_b), 134.69 (C_c), 129.94 (C_f), 129.29 (C_3), 128.94 (2C_e), 127.93 (2C_d), 122.06 (C_a), 117.12 (C_4), 116.05 (C_{4a}), 108.40 (C_8), 30.15 (NCH_3); MS (ESI) *m/z* (%): 340.2 (100) $[M + H]^+$, 342.2 (40) $[M + H + 2]^+$; UPLC purity = 96%, Rt 3.30 min; HRMS (ESI): calcd. for $C_{18}H_{14}ClN_3O_2$ $[M + H]^+$ 340.0847, found: 340.0835.

*4.1.2.3. N-(7-Chloro-1-methyl-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)benzamide (**9**).*



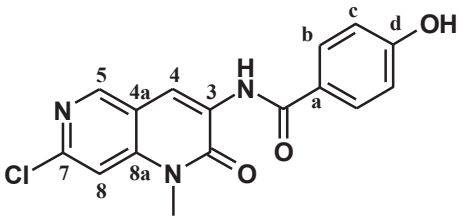
The reaction was carried out using amine **6** (400 mg, 1.91 mmol) and benzoyl chloride (731 μL, 6.30 mmol) and refluxed in pyridine for 3 h. Purification by silica gel chromatography (dichloromethane/ethyl acetate, 99.5:0.5) and recrystallization from EtOH provided **9** (425 mg, 71% yield) as a white powder. Mp: 225–226 °C; IR, ν (cm^{-1}): 3380 (νN–H); 1672, 1649 (νC = O); 1578, 1518 (νC = C); 704 (νC–Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.61 (s, 1H, NH), 8.86 (s, 1H, H_5), 8.83 (s, 1H, H_4), 7.99 (d, ^3J = 7.6 Hz, 2H, H_b), 7.71 (s, 1H, H_8), 7.70–7.60 (m, 3H, H_c, H_d), 3.75 (s, 3H, NCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.33 (NH–C=O), 157.64 (C_3–C=O), 149.59 (C_5), 148.92 (C_{8a}), 142.57 (C_7), 133.47 (C_a), 132.40 (C_d), 128.90 (2C_c), 128.54 (C_3), 127.29 (2C_b), 117.75 (C_4), 115.89 (C_{4a}), 108.70 (C_8), 30.29 (NCH_3); MS (ESI) *m/z* (%): 314.1 (100) $[M + H]^+$, 316.1 (40) $[M + H + 2]^+$; UPLC purity = 98%, Rt 3.07 min; HRMS (ESI): calcd. for $C_{16}H_{12}ClN_3O_2$ $[M + H]^+$ 314.0691, found: 314.0678.

*4.1.2.4. N-(7-Chloro-1-methyl-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)-4-nitrobenzamide (**10**).*



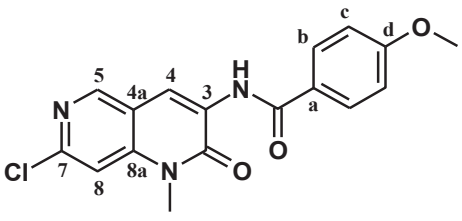
The reaction was carried out using amine **6** (400 mg, 1.91 mmol) and 4-nitrobenzoyl chloride (1.17 g, 6.30 mmol) and refluxed in pyridine for 1.5 h. After cooling, the precipitate was collected by filtration and rinsed with cold water to afford **10** (541 mg, 79% yield) as a pale yellow powder. Mp: 348–349 °C; IR, ν (cm^{-1}): 3385 (νN–H); 1738, 1639 (νC = O); 1587 (νC = C); 1512 ($\nu_{as}NO_2$), 1236 ($\nu_{sy}NO_2$); 710 (νC–Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.04 (s, 1H, NH), 8.90 (s, 1H, H_5), 8.86 (s, 1H, H_4), 8.43 (d, ^3J = 8.8 Hz, 2H, H_c), 8.23 (d, ^3J = 8.8 Hz, 2H, H_b), 7.74 (s, 1H, H_8), 3.76 (s, 3H, NCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.27 (NH–C=O), 156.54 (C_3–C=O), 150.91 (C_{8a}), 150.69 (C_5), 150.21 (C_d), 144.96 (C_7), 140.29 (C_a), 133.29 (C_3), 131.50 (2C_b), 125.86 (C_{4a}), 124.29 (2C_c), 114.41 (C_4), 109.17 (C_8), 30.00 (NCH_3); MS (ESI) *m/z* (%): 359.1 (100) $[M + H]^+$, 361.1 (40) $[M + H + 2]^+$; UPLC purity = 97%, Rt 3.09 min; HRMS (ESI): calcd. for $C_{16}H_{11}ClN_4O_4$ $[M + H]^+$ 359.0542, found: 359.0527.

*4.1.2.5. N-(7-Chloro-1-methyl-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)-4-hydroxybenzamide (**11**).*



The reaction was carried out using amine **6** (400 mg, 1.91 mmol) and 4-(acetyloxy)benzoyl chloride [32] (1.25 g, 6.30 mmol) and refluxed in pyridine for 5 h. After concentration under reduced pressure, NaOH (108 mg, 2.69 mmol, 1.4 equiv.) was added to a stirred solution of the crude product in MeOH/water 1:1 (30 mL). After 2 h at room temperature, the reaction mixture was acidified to pH 5 with concentrated HCl. The precipitate was collected by filtration and rinsed with cold EtOH to afford **11** (394 mg, 63% yield, 2 steps) as a yellow powder. Mp: 325–326 °C; IR, ν (cm^{-1}): 3264 (νN–H, νO–H); 1628, 1574 (νC = O); 1504 (νC = C); 756 (νC–Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.35 (s, 1H, OH), 9.41 (s, 1H, NH), 8.85 (s, 1H, H_5), 8.81 (s, 1H, H_4), 7.87 (d, ^3J = 8.5 Hz, 2H, H_b), 7.71 (s, 1H, H_8), 6.96 (d, ^3J = 8.5 Hz, 2H, H_c), 3.76 (s, 3H, NCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.77 (NH–C=O), 161.33 (C_d), 157.71 (C_3–C=O), 149.43 (C_5), 148.67 (C_{8a}), 142.34 (C_7), 129.38 (2C_b), 128.74 (C_3), 123.90 (C_a), 116.74 (C_4), 116.04 (C_{4a}), 115.48 (2C_c), 108.67 (C_8), 30.26 (NCH_3); MS (ESI) *m/z* (%): 330.1 (100) $[M + H]^+$, 332.1 (40) $[M + H + 2]^+$; UPLC purity = 98%, Rt 2.52 min; HRMS (ESI): calcd. for $C_{16}H_{12}ClN_3O_3$ $[M + H]^+$ 330.0640, found: 330.0635.

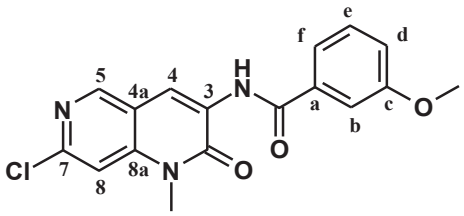
*4.1.2.6. N-(7-Chloro-1-methyl-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)-4-methoxybenzamide (**12**).*



The reaction was carried out using amine **6** (400 mg, 1.91 mmol) and 4-methoxybenzoyl chloride (1.08 g, 6.30 mmol) and refluxed in pyridine for 4 h. Purification by silica gel chromatography (dichloromethane/ethyl acetate, 99:5) provided **12** (499 mg, 76% yield) as a white powder. Mp: 229–230 °C; IR, ν (cm^{-1}): 3366 (νN–H); 2914, 2837 (νC–H_{al}); 1653, 1639 (νC = O); 1580, 1456 (νC = C); 682 (νC–Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.49 (s, 1H, NH), 8.85 (s, 1H, H_5), 8.81 (s, 1H, H_4), 7.97 (d, ^3J = 8.6 Hz, 2H, H_b), 7.71 (s, 1H, H_8), 7.14 (d, ^3J = 8.6 Hz, 2H, H_c), 3.89 (s, 3H, OCH_3), 3.75 (s, 3H, NCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.64 (NH–C=O), 162.49 (C_d), 157.67 (C_3–C=O), 149.47 (C_5), 148.75 (C_{8a}), 142.40 (C_7),

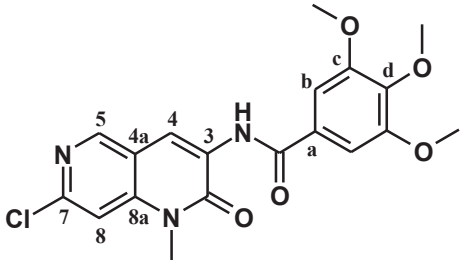
129.27 ($2C_b$), 128.65 (C_3), 125.49 (C_a), 117.14 (C_4), 115.97 (C_{4a}), 114.11 ($2C_c$), 108.66 (C_8), 55.50 (OCH_3), 30.25 (NCH_3); MS (ESI) *m/z* (%): 344.1 (100) $[M + H]^+$, 346.1 (40) $[M + H + 2]^+$; UPLC purity = 99%, Rt 3.12 min; HRMS (ESI): calcd. for $C_{17}H_{14}ClN_3O_3$ $[M + H]^+$ 344.0796, found: 344.0796.

*4.1.2.7. N-(7-Chloro-1-methyl-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)-3-methoxybenzamide (**13**).*



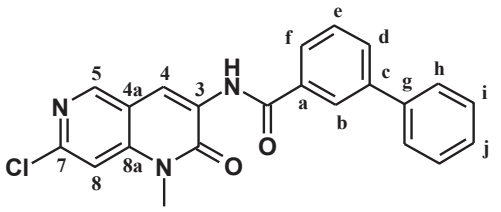
The reaction was carried out using amine **6** (400 mg, 1.91 mmol) and 3-methoxybenzoyl chloride (1.10 g, 6.30 mmol) and refluxed in pyridine for 1.5 h. Purification by silica gel chromatography (dichloromethane/ethyl acetate, 98:2) provided **13** (365 mg, 76% yield) as a pale yellow powder. Mp: 195–196 °C; IR, ν (cm^{-1}): 3375 (νN–H); 2941(νC–H_{al}); 1652, 1632 (νC = O); 1574, 1494 (νC = C); 765 (νC–Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.58 (s, 1H, NH), 8.85 (s, 1H, H_5), 8.80 (s, 1H, H_4), 7.71 (s, 1H, H_8), 7.54–7.53 (m, 2H, H_b, H_f), 7.49–7.48 (m, 1H, H_e), 7.27–7.24 (m, 1H, H_d), 3.89 (s, 3H, OCH_3), 3.74 (s, 3H, NCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.10 (NH–C=O), 159.14 (C_c), 157.62 (C_3–C=O), 149.59 (C_5), 148.94 (C_{8a}), 142.59 (C_7), 134.94 (C_a), 130.09 (C_e), 128.37 (C_3), 119.24 (C_b), 117.95 (C_4), 117.83 (C_d), 115.84 (C_{4a}), 112.59 (C_f), 108.70 (C_8), 55.35 (OCH_3), 30.34 (NCH_3); MS (ESI) *m/z* (%): 344.1 (100) $[M + H]^+$, 346.1 (40) $[M + H + 2]^+$; UPLC purity = 96%, Rt 3.13 min; HRMS (ESI): calcd. for $C_{17}H_{14}ClN_3O_3$ $[M + H]^+$ 344.0796, found: 344.0782.

*4.1.2.8. N-(7-Chloro-1-methyl-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)-3,4,5-trimethoxybenzamide (**14**).*



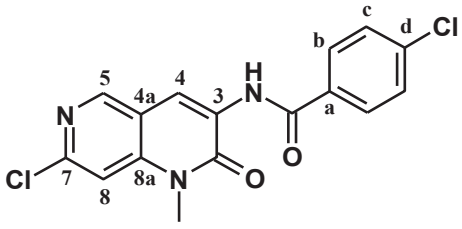
The reaction was carried out using amine **6** (400 mg, 1.91 mmol) and 3,4,5-trimethoxybenzoyl chloride (1.45 g, 6.30 mmol) and refluxed in pyridine for 3 h. Purification by silica gel chromatography (mixtures of dichloromethane/ethanol of increasing polarity) provided **14** (444 mg, 58% yield) as a white powder. Mp: 249–250 °C; IR, ν (cm^{-1}): 3674 (νN–H); 2963, 2901 (νC–H_{al}); 1717, 1639 (νC = O); 1584, 1489 (νC = C); 630 (νC–Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.63 (s, 1H, NH), 8.86 (s, 1H, H_5), 8.77 (s, 1H, H_4), 7.73 (s, 1H, H_8), 7.29 (s, 2H, H_b), 3.92 (s, 6H, $2C_c$–OCH_3), 3.79 (s, 3H, C_d–OCH_3), 3.76 (s, 3H, NCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.07 (NH–C=O), 157.69 (C_3–C=O), 152.84 ($2C_c$), 149.56 (C_5), 148.97 (C_{8a}), 142.69 (C_7), 140.91 (C_d), 128.91 (C_a), 128.64 (C_3), 118.50 (C_4), 115.85 (C_{4a}), 108.69 (C_8), 104.97 ($2C_b$), 60.13 (C_d–OCH_3), 56.09 ($2C_c$–OCH_3), 30.21 (NCH_3); MS (ESI) *m/z* (%): 404.1 (100) $[M + H]^+$, 406.1 (40) $[M + H + 2]^+$; UPLC purity = 97%, Rt 3.02 min; HRMS (ESI): calcd. for $C_{19}H_{18}ClN_3O_5$ $[M + H]^+$ 404.1008, found: 404.1014.

*4.1.2.9. N-(7-Chloro-1-methyl-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)biphenyl-3-carboxamide (**15**).*



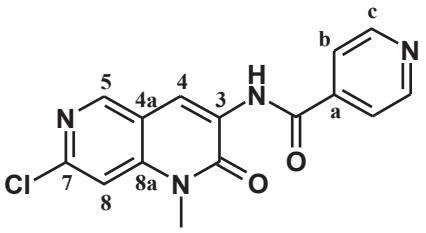
The reaction was carried out using amine **6** (400 mg, 1.91 mmol) and biphenyl-3-carbonyl chloride [33] (1.37 g, 6.30 mmol) and refluxed in pyridine for 1 h. Purification by silica gel chromatography (mixtures of dichloromethane/methanol of increasing polarity) provided **15** (626 mg, 84% yield) as a white powder. Mp: 213–214 °C; IR, ν (cm^{-1}): 3300 (νN–H); 2969 (νC–H_{al}); 1771, 1636 (νC = O); 1578, 1489 (νC = C); 766 (νC–Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.79 (s, 1H, NH), 8.85 (s, 1H, H_5), 8.82 (s, 1H, H_4), 8.20 (br s, 1H, H_b), 7.97–7.95 (m, 2H, H_d, H_f), 7.80–7.70 (m, 4H, H_8, H_e, H_h), 7.56 (appt, ^{app}J = 7.2 Hz, 2H, H_i), 7.47 (t, ^3J = 6.6 Hz, 1H, H_j), 3.74 (s, 3H, NCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.61 (NH–C=O), 157.84 (C_3–C=O), 149.79 (C_5), 149.17 (C_{8a}), 142.83 (C_7), 140.84 (C_c or C_g), 139.45 (C_g or C_c), 134.48 (C_a), 130.75 (C_d), 129.69 (C_e), 129.24 ($2C_i$), 128.80 (C_3), 128.14 (C_j), 127.09 ($2C_h$), 126.60 (C_b), 125.82 (C_f), 118.47 (C_4), 116.04 (C_{4a}), 108.85 (C_8), 30.41 (NCH_3); MS (ESI) *m/z* (%): 390.1 (100) $[M + H]^+$, 392.1 (40) $[M + H + 2]^+$; UPLC purity = 96%, Rt 3.88 min; HRMS (ESI): calcd. for $C_{22}H_{16}ClN_3O_2$ $[M + H]^+$ 390.1004, found: 390.0999.

*4.1.2.10. 4-Chloro-N-(7-chloro-1-methyl-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)benzamide (**16**).*



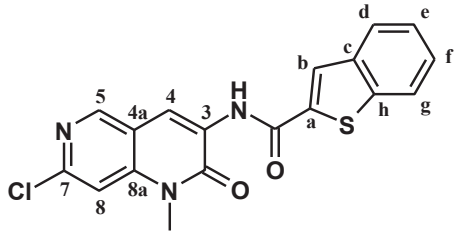
The reaction was carried out using amine **6** (400 mg, 1.91 mmol) and 4-chlorobenzoyl chloride (1.10 g, 6.30 mmol) and refluxed in pyridine for 3 h. Purification by silica gel chromatography (dichloromethane) and trituration with acetonitrile afforded **16** (350 mg, 53% yield) as a white powder. Mp: 278–279 °C; IR, ν (cm^{-1}): 3379 (νN–H); 1686, 1636 (νC = O); 1580, 1518, 1483 (νC = C); 743, 606 (νC–Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.72 (s, 1H, NH), 8.86 (s, 1H, H_5), 8.81 (s, 1H, H_4), 8.01 (d, ^3J = 8.2 Hz, 2H, H_c), 7.71 (s, 1H, H_8), 7.68 (d, ^3J = 8.2 Hz, 2H, H_b), 3.75 (s, 3H, NCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.56 (NH–C=O), 157.62 (C_3–C=O), 149.56 (C_5), 149.02 (C_{8a}), 142.72 (C_7), 137.15 (C_d), 132.30 (C_a), 129.40 ($2C_c$), 128.87 ($2C_b$), 128.51 (C_3), 118.42 (C_4), 115.82 (C_{4a}), 108.71 (C_8), 30.24 (NCH_3); MS (ESI) *m/z* (%): 348.0 (100) $[M + H]^+$, 350.0 (65) $[M + H + 2]^+$, 352.0 (10) $[M + H + 4]^+$; UPLC purity = 98%, Rt 3.44 min; HRMS (ESI): calcd. for $C_{16}H_{11}Cl_2N_3O_2$ $[M + H]^+$ 348.0301, found: 348.0287.

*4.1.2.11. N-(7-Chloro-1-methyl-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)pyridine-4-carboxamide (**17**).*



The reaction was carried out using amine **6** (400 mg, 1.91 mmol) and isonicotinoyl chloride hydrochloride (1.12 g, 6.30 mmol) and refluxed in pyridine for 2 h. After cooling, water was added, and the mixture was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The precipitate was collected by filtration and rinsed with cold water to afford **17** (239 mg, 39% yield) as a yellow powder. Mp: 308–309 °C; IR, ν (cm^{-1}): 3352 (νN–H); 2961, 2901 (νC–H_{al}); 1717, 1638 (νC = O); 1582, 1518, 1491 (νC = C); 743 (νC–Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.95 (s, 1H, NH), 8.87 (s, 1H, H_5), 8.84 (d, ^3J = 6.0 Hz, 2H, H_c), 8.83 (s, 1H, H_4), 7.88 (d, ^3J = 6.0 Hz, 2H, H_b), 7.72 (s, 1H, H_8), 3.74 (s, 3H, NCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.40 (NH–C=O), 157.53 (C_3–C=O), 150.52 ($2C_c$), 149.79 (C_5), 149.24 (C_{8a}), 142.94 (C_7), 140.63 (C_a), 128.29 (C_3), 121.29 ($2C_b$), 119.37 (C_4), 115.69 (C_{4a}), 108.73 (C_8), 30.23 (NCH_3); MS (ESI) *m/z* (%): 315.1 (100) $[M + H]^+$, 317.1 (40) $[M + H + 2]^+$; UPLC purity = 98%, Rt 2.03 min; HRMS (ESI): calcd. for $C_{15}H_{11}ClN_4O_2$ $[M + H]^+$ 315.0643, found: 315.0635.

*4.1.2.12. N-(7-Chloro-1-methyl-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)-1-benzothiophene-2-carboxamide (**18**).*

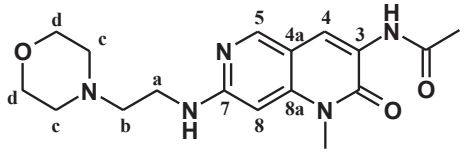


The reaction was carried out using amine **6** (400 mg, 1.91 mmol) and benzo[*b*]thiophene-2-carbonyl chloride (1.24 g, 6.30 mmol) and refluxed in pyridine for 3 h. After cooling, water was added, and the mixture was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The precipitate was collected by filtration and trituration with CH_3CN afforded **18** (234 mg, 33% yield) as a yellow powder. Mp: 287–288 °C; IR, ν (cm^{-1}): 3354 (νN–H); 1643, 1580 (νC = O); 1525 (νC = C); 617 (νC–Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.85 (s, 1H, NH), 8.87 (s, 1H, H_5), 8.78 (s, 1H, H_4), 8.48 (s, 1H, H_b), 8.13–8.07 (m, 2H, H_d, H_g), 7.73 (s, 1H, H_8), 7.59–7.51 (m, 2H, H_e, H_f), 3.77 (s, 3H, NCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.57 (NH–C=O), 157.51 (C_3–C=O), 149.67 (C_5), 149.08 (C_{8a}), 142.76 (C_7), 140.71 (C_a), 139.07 (C_h), 137.92 (C_c), 128.19 (C_3), 127.08 (C_b), 126.97 (C_e or C_f), 125.82 (C_d or C_g), 125.21 (C_f or C_e), 122.90 (C_g or C_d), 118.75 (C_4), 115.79 (C_{4a}), 108.73 (C_8), 30.27 (NCH_3); MS (ESI) *m/z* (%): 370.1 (100) $[M + H]^+$, 372.0 (40) $[M + H + 2]^+$; UPLC purity = 95%, Rt 3.65 min; HRMS (ESI): calcd. for $C_{18}H_{12}ClN_3O_2S$ $[M + H]^+$ 370.0412, found: 370.0398.

*4.1.3. Typical procedure for the synthesis of carboxamides **19**–**45***

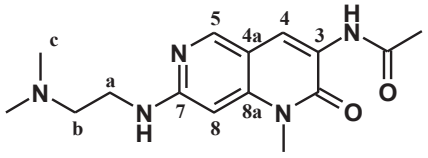
In a 10 mL vessel were introduced amide **11** (75 mg, 0.22 mmol) and *N*,*N*-dimethylethylenediamine (0.47 mL, 4.36 mmol). The tube was sealed, and the reaction mixture was heated under microwave irradiation at 130 °C for 6 h. After cooling, water was added, and the mixture was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (mixtures of dichloromethane/methanol of increasing polarity) and trituration with diisopropyl ether provided **35** (44 mg, 50% yield) as a pale yellow powder.

*4.1.3.1. N-(1-Methyl-7-{[2-(morpholin-4-yl)ethyl]amino}-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)acetamide (**19**).*



The reaction was carried out using amide **7** (75 mg, 0.30 mmol) and 2-(morpholin-4-yl)ethanamine (789 μL, 6.00 mmol) at 170 °C for 1 h. Purification by silica gel chromatography (mixtures of dichloromethane/methanol of increasing polarity) and trituration with diisopropyl ether afforded **19** (20 mg, 19% yield) as beige powder. Mp: 203–204 °C; IR, ν (cm^{-1}): 3291, 3237 (νN–H); 2970 (νC–H_{al}); 1620 (νC = O); 1589, 1514 (νC = C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (s, 1H, H_4), 8.36 (s, 1H, H_5), 8.33 (s, 1H, C_3–NH), 6.06 (s, 1H, H_8), 5.44 (br s, 1H, C_7–NH), 3.74 (t, ^3J = 4.4 Hz, 4H, H_d), 3.66 (s, 3H, NCH_3), 3.42–3.37 (m, 2H, H_a), 2.68 (t, ^3J = 5.6 Hz, 2H, H_b), 2.54–2.49 (m, 4H, H_c), 2.22 (s, 3H, $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.90 (NH–C=O), 158.82 (C_7), 158.53 (C_3–C=O), 149.86 (C_5), 143.18 (C_{8a}), 124.03 (C_3), 119.60 (C_4), 109.55 (C_{4a}), 87.66 (C_8), 66.85 ($2C_d$), 56.87 (C_b), 53.30 ($2C_c$), 38.38 (C_a), 29.78 (NCH_3), 24.78 ($COCH_3$); MS (ESI) *m/z* (%): 346.1 (100) $[M + H]^+$; UPLC purity = 98%, Rt 1.15 min; HRMS (ESI): calcd. for $C_{17}H_{23}N_5O_3$ $[M + H]^+$ 346.1874, found: 346.1873.

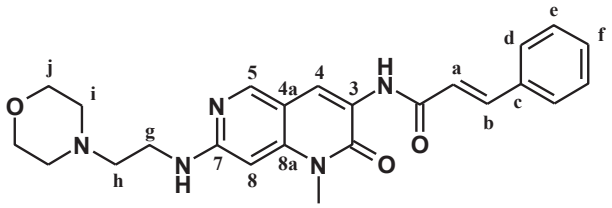
*4.1.3.2. N-(7-{[2-(dimethylamino)ethyl]amino}-1-methyl-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)acetamide (**20**).*



The reaction was carried out using amide **7** (75 mg, 0.30 mmol), *N*,*N*-dimethylethylenediamine (646 μL, 6.00 mmol) and CuI (28 mg, 0.15 mmol) at 130 °C for 5 h. Purification by silica gel chromatography (mixtures of dichloromethane/methanol of increasing polarity) afforded **20** (44 mg, 48% yield) as a beige powder. Mp: 132–133 °C; IR, ν (cm^{-1}): 3292, 3227 (νN–H); 2970 (νC–H_{al}); 1639 (νC = O); 1587, 1504 (νC = C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (s, 1H, H_4), 8.35 (s, 1H, H_5), 8.34 (s, 1H, C_3–NH), 6.10 (s, 1H, H_8), 5.34 (br s, 1H, C_7–NH), 3.64 (s, 3H, $CONCH_3$), 3.45–3.41 (m, 2H, H_a), 2.64 (t, ^3J = 5.8 Hz, 2H, H_b), 2.33 (s, 6H, H_c), 2.22 (s, 3H, $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.89 (NH–C=O), 158.84 (C_7), 158.58 (C_3–C=O), 149.77 (C_5), 143.09 (C_{8a}), 123.97 (C_3), 119.64 (C_4), 109.52 (C_{4a}), 88.14 (C_8), 57.77 (C_b), 45.02 ($2C_c$), 38.32 (C_a), 29.74 ($CONCH_3$), 24.77

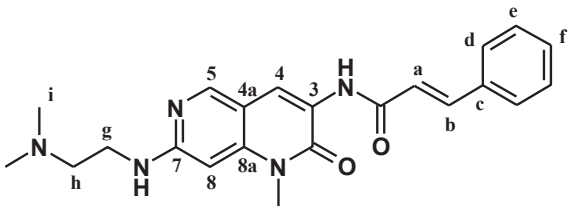
($CO\underline{C}H_3$); MS (ESI) *m/z* (%): 304.2 (100) $[M + H]^+$; UPLC purity = 96%, Rt 1.17 min; HRMS (ESI): calcd. for $C_{15}H_{21}N_5O_2$ $[M + H]^+$ 304.1768, found: 304.1767.

*4.1.3.3. (2E)-N-(1-Methyl-7-{[2-(morpholin-4-yl)ethyl]amino}-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)-3-phenylprop-2-enamide (**21**).*



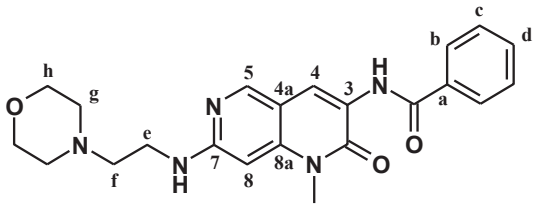
The reaction was carried out using amide **8** (75 mg, 0.22 mmol) and 2-(morpholin-4-yl)ethanamine (579 μL, 4.40 mmol) at 150 °C for 4 h. Purification by silica gel chromatography (mixtures of dichloromethane/methanol of increasing polarity) afforded **21** (36 mg, 38% yield) as a yellow powder. Mp: 231–232 °C; IR, ν (cm^{-1}): 3294 (νN–H); 2922 (νC–H_{al}); 1622 (νC = O); 1589, 1520 (νC = C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (s, 1H, H_4), 8.55 (s, 1H, C_3–NH), 8.38 (s, 1H, H_5), 7.72 (d, ^3J = 15.6 Hz, 1H, H_b), 7.56 (dd, ^3J = 8.0 Hz, ^4J = 2.4 Hz, 2H, H_e), 7.40–7.38 (m, 3H, H_d, H_f), 6.61 (d, ^3J = 15.6 Hz, 1H, H_a), 6.06 (s, 1H, H_8), 5.51 (br s, 1H, C_7–NH), 3.74 (t, ^3J = 4.4 Hz, 4H, H_j), 3.68 (s, 3H, NCH_3), 3.42–3.38 (m, 2H, H_g), 2.68 (t, ^3J = 5.8 Hz, 2H, H_h), 2.53–2.49 (m, 4H, H_i); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.34 (NH–C=O), 158.90 (C_3–$\underline{C}$=O), 158.59 (C_7), 150.03 (C_5), 143.23 (C_{8a}), 142.32 (C_b), 134.58 (C_c), 130.07 (C_f), 128.92 (2C_e), 128.05 (2C_d), 124.16 (C_3), 120.71 (C_a), 120.09 (C_4), 109.63 (C_{4a}), 87.56 (C_8), 66.90 (2C_j), 56.84 (C_h), 53.31 (2C_i), 38.43 (C_g), 29.83 (NCH_3); MS (ESI) *m/z* (%): 435.2 (100) $[M + H]^+$; UPLC purity = 94%, Rt 2.49 min; HRMS (ESI): calcd. for $C_{24}H_{27}N_5O_3$ $[M + H]^+$ 434.2187, found: 434.2201.

*4.1.3.4. (2E)-N-(7-{[2-(dimethylamino)ethyl]amino}-1-methyl-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)-3-phenylprop-2-enamide (**22**).*



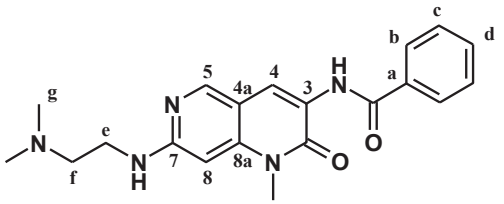
The reaction was carried out using amide **8** (75 mg, 0.22 mmol) and *N*,*N*-dimethylethylenediamine (474 μL, 4.40 mmol) at 130 °C for 3 h. Purification by silica gel chromatography (mixtures of dichloromethane/methanol of increasing polarity) afforded **22** (21 mg, 24% yield) as a yellow powder. Mp: 161–162 °C; IR, ν (cm^{-1}): 3354 (νN–H); 2922 (νC–H_{al}); 1649 (νC = O); 1587, 1514 (νC = C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 (s, 1H, H_4), 8.54 (s, 1H, C_3–NH), 8.38 (s, 1H, H_5), 7.73 (d, ^3J = 15.6 Hz, 1H, H_b), 7.56 (dd, ^3J = 6.0 Hz, ^4J = 2.4 Hz, 2H, H_e), 7.41–7.36 (m, 3H, H_d, H_f), 6.61 (d, ^3J = 15.6 Hz, 1H, H_a), 6.15 (s, 1H, H_8), 5.67 (br s, 1H, C_7–NH), 3.66 (s, 3H, $CONCH_3$), 3.52–3.57 (m, 2H, H_g), 2.71 (t, ^3J = 5.6 Hz, 2H, H_h), 2.38 (s, 6H, H_i); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.37 (NH–C=O), 158.90 (C_3–$\underline{C}$=O), 158.50 (C_7), 149.91 (C_5), 143.13 (C_{8a}), 142.28 (C_b), 134.59 (C_c), 130.09 (C_f), 129.01 (2C_e), 128.10 (2C_d), 124.11 (C_3), 120.76 (C_a), 120.07 (C_4), 109.71 (C_{4a}), 88.55 (C_8), 57.93 (C_h), 45.10 (2C_i), 39.10 (C_g), 29.79 ($CON\underline{C}H_3$); MS (ESI) *m/z* (%): 392.1 (100) $[M + H]^+$; UPLC purity = 95%, Rt 2.46 min; HRMS (ESI): calcd. for $C_{22}H_{25}N_5O_2$ $[M + H]^+$ 392.2081, found: 392.2077.

*4.1.3.5. N-(1-Methyl-7-{[2-(morpholin-4-yl)ethyl]amino}-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)benzamide (**23**).*



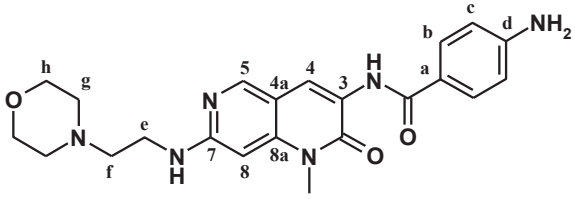
The reaction was carried out using amide **9** (75 mg, 0.24 mmol) and 2-(morpholin-4-yl)ethanamine (631 μL, 4.80 mmol) at 170 °C for 1 h. Purification by silica gel chromatography (mixtures of dichloromethane/methanol of increasing polarity) afforded **23** (91 mg, 93% yield) as a beige powder. Mp: 168–169 °C; IR, ν (cm^{-1}): 3663 (νN–H); 2986, 2970 (νC-H_{al}); 1632 (νC = O); 1591, 1514 (νC = C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.20 (s, 1H, C_3–NH), 8.82 (s, 1H, H_4), 8.41 (s, 1H, H_5), 7.94 (d, ^3J = 7.2 Hz, 2H, H_b), 7.58–7.49 (m, 3H, H_c, H_d), 6.08 (s, 1H, H_8), 5.48 (t, ^3J = 4.8 Hz, 1H, C_7–NH), 3.75 (t, ^3J = 4.6 Hz, 4H, H_h), 3.70 (s, 3H, NCH_3), 3.43–3.39 (m, 2H, H_e), 2.68 (t, ^3J = 6.0 Hz, 2H, H_f), 2.53–2.50 (m, 4H, H_g); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.71 (NH–C=O), 159.13 (C_3–$\underline{C}$=O), 158.64 (C_7), 150.02 (C_5), 143.28 (C_{8a}), 134.32 (C_a), 132.07 (C_d), 128.83 (2C_c), 127.14 (2C_b), 124.17 (C_3), 119.85 (C_4), 109.72 (C_{4a}), 87.85 (C_8), 66.97 (2C_h), 56.93 (C_f), 53.38 (2C_g), 38.61 (C_e), 29.94 (NCH_3); MS (ESI) *m/z* (%): 408.1 (100) $[M + H]^+$; UPLC purity = 95%, Rt 2.03 min; HRMS (ESI): calcd. for $C_{22}H_{25}N_5O_3$ $[M + H]^+$ 408.2030, found: 408.2040.

*4.1.3.6. N-(7-{[2-(dimethylamino)ethyl]amino}-1-methyl-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)benzamide (**24**).*



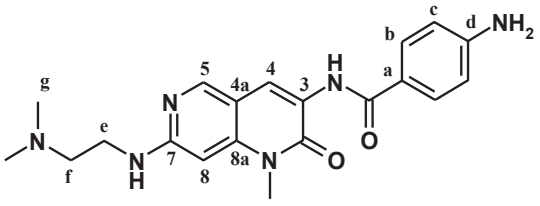
The reaction was carried out using amide **9** (75 mg, 0.24 mmol) and *N*,*N*-dimethylethylenediamine (517 μL, 4.80 mmol) at 130 °C for 6 h. Purification by silica gel chromatography (mixtures of dichloromethane/methanol of increasing polarity) afforded **24** (58 mg, 66% yield) as a yellow powder. Mp: 145–146 °C; IR, ν (cm^{-1}): 3383, 3250 (νN–H); 2978, 2949 (νC–H_{al}); 1641 (νC = O); 1593, 1518 (νC = C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.20 (s, 1H, C_3–NH), 8.81 (s, 1H, H_4), 8.41 (s, 1H, H_5), 7.94 (d, ^3J = 6.8 Hz, 2H, H_b), 7.58–7.48 (m, 3H, H_c, H_d), 6.12 (s, 1H, H_8), 5.50 (s, 1H, C_7–NH), 3.68 (s, 3H, $CONCH_3$), 3.44–3.40 (m, 2H, H_e), 2.62 (t, ^3J = 6.0 Hz, 2H, H_f), 2.31 (s, 6H, H_g); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.62 (NH–C=O), 159.11 (C_3–$\underline{C}$=O), 158.73 (C_7), 149.98 (C_5), 143.16 (C_{8a}), 134.33 (C_a), 132.05 (C_d), 128.81 (2C_c), 127.11 (2C_b), 124.04 (C_3), 119.90 (C_4), 109.52 (C_{4a}), 88.04 (C_8), 57.73 (C_f), 45.12 (2C_g), 39.49 (C_e), 29.76 ($CON\underline{C}H_3$); MS (ESI) *m/z* (%): 366.2 (100) $[M + H]^+$; UPLC purity = 98%, Rt 1.93 min; HRMS (ESI): calcd. for $C_{20}H_{23}N_5O_2$ $[M + H]^+$ 366.1925, found: 366.1921.

*4.1.3.7. 4-Amino-N-(1-methyl-7-{[2-(morpholin-4-yl)ethyl]amino}-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)benzamide (**25**).*



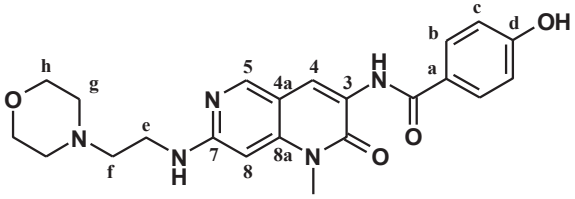
The reaction was carried out using amide **10** (75 mg, 0.21 mmol) and 2-(morpholin-4-yl)ethanamine (552 μL, 4.20 mmol) at 170 °C for 1 h. Purification by silica gel chromatography (mixtures of dichloromethane/methanol of increasing polarity) and trituration with diisopropyl ether afforded **25** (55 mg, 62% yield) as a yellow powder. Mp: 250–251 °C; IR, ν (cm^{-1}): 3383, 3350, 3240 (νN–H); 2968 (νC–H_{al}); 1627 (νC = O); 1587, 1508 (νC = C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.04 (s, 1H, C_3–NH), 8.56 (s, 1H, H_4), 8.42 (s, 1H, H_5), 7.66 (d, ^3J = 8.6 Hz, 2H, H_b), 6.76 (t, ^3J = 5.2 Hz, 1H, C_7–NH), 6.67 (d, ^3J = 8.6 Hz, 2H, H_c), 6.39 (s, 1H, H_8), 5.92 (s, 2H, NH_2), 3.64–3.61 (m, 7H, H_h, NCH_3), 3.50–3.45 (m, 2H, H_e), 2.56–2.54 (m, 2H, H_f), 2.49–2.45 (m, 4H, H_g); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.44 (NH–C=O), 158.69 (C_3–C=O), 158.44 (C_7), 152.57 (C_d), 149.03 (C_5), 142.39 (C_{8a}), 128.65 (2C_b), 123.35 (C_a), 119.98 (C_3), 119.12 (C_4), 112.90 (2C_c), 108.31 (C_{4a}), 88.60 (C_8), 66.15 (2C_h), 57.37 (C_f), 53.33 (2C_g), 38.10 (C_e), 29.43 (NCH_3); MS (ESI) *m/z* (%): 423.2 (100) $[M + H]^+$; UPLC purity = 97%, Rt 1.67 min; HRMS (ESI): calcd. for $C_{22}H_{26}N_6O_3$ $[M + H]^+$ 423.2139, found: 423.2153.

*4.1.3.8. 4-Amino-N-(7-{[2-(dimethylamino)ethyl]amino}-1-methyl-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)benzamide (**26**).*



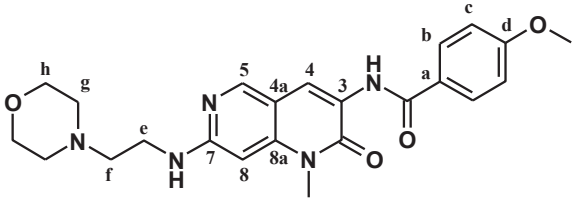
The reaction was carried out using amide **10** (75 mg, 0.21 mmol) and *N*,*N*-dimethylethylenediamine (452 μL, 4.20 mmol) at 130 °C for 8 h. Purification by silica gel chromatography (mixtures of dichloromethane/methanol of increasing polarity) and trituration with diisopropyl ether afforded **26** (15 mg, 19% yield) as an orange powder. Mp: 180–181 °C; IR, ν (cm^{-1}): 3379, 3345, 3335 (νN–H); 2949 (νC–H_{al}); 1626 (νC = O); 1532, 1505 (νC = C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.07 (s, 1H, C_3–NH), 8.77 (s, 1H, H_4), 8.38 (s, 1H, H_5), 7.78 (d, ^3J = 8.4 Hz, 2H, H_b), 6.71 (d, ^3J = 8.4 Hz, 2H, H_c), 6.14 (s, 1H, H_8), 5.57 (br s, 1H, C_7–NH), 4.04 (s, 2H, NH_2), 3.68 (s, 3H, $CONCH_3$), 3.48–3.44 (m, 2H, H_e), 2.67 (t, ^3J = 5.2 Hz, 2H, H_f), 2.35 (s, 6H, H_g); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.56 (NH–C=O), 159.67 (C_7), 158.62 (C_3–C=O), 150.20 (C_d), 149.65 (C_5), 143.05 (C_{8a}), 129.17 (2C_b), 124.75 (C_a), 123.91 (C_3), 119.28 (C_4), 114.44 (2C_c), 110.02 (C_{4a}), 88.57 (C_8), 58.06 (C_f), 45.23 (2C_g), 39.55 (C_e), 29.87 ($CONCH_3$); MS (ESI) *m/z* (%): 381.2 (100) $[M + H]^+$; UPLC purity = 95%, Rt 1.64 min; HRMS (ESI): calcd. for $C_{20}H_{24}N_6O_2$ $[M + H]^+$ 381.2034, found: 381.2033.

*4.1.3.9. N-(1-Methyl-7-{[2-(morpholin-4-yl)ethyl]amino}-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)-4-hydroxybenzamide (**27**).*



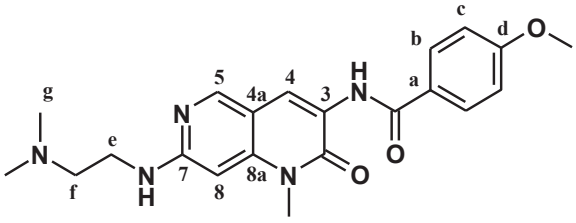
The reaction was carried out using amide **11** (75 mg, 0.23 mmol) and 2-(morpholin-4-yl)ethanamine (605 μL, 4.60 mmol) at 170 °C for 1.5 h. The reaction mixture was adjusted to pH 7 with a 1 M HCl solution. The precipitate was collected by filtration, rinsed with cold water and purified by silica gel chromatography (mixtures of dichloromethane/methanol of increasing polarity). Trituration with diisopropyl ether afforded **27** (49 mg, 53% yield) as a yellow powder. Mp: 232–233 °C; IR, ν (cm^{-1}): 3229 (νN–H, νO–H); 2943 (νC–H_{al}); 1591, 1578 (νC = O); 1528, 1503, 1445 (νC = C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.26 (s, 1H, OH), 9.19 (s, 1H, C_3–NH), 8.56 (s, 1H, H_4), 8.44 (s, 1H, H_5), 7.83 (d, ^3J = 8.4 Hz, 2H, H_b), 6.93 (d, ^3J = 8.4 Hz, 2H, H_c), 6.81 (s, 1H, C_7–NH), 6.40 (s, 1H, H_8), 3.62–3.61 (m, 7H, H_h, NCH_3), 3.48–3.46 (m, 2H, H_e), 3.21–3.20 (m, 2H, H_f), 2.49–2.44 (m, 4H, H_g); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.52 (NH–C=O), 161.14 (C_d), 159.15 (C_3–C=O), 158.72 (C_7), 149.53 (C_5), 142.86 (C_{8a}), 129.37 (2C_b), 124.63 (C_3), 123.33 (C_a), 120.48 (C_4), 115.64 (2C_c), 108.39 (C_{4a}), 88.84 (C_8), 66.40 (2C_h), 57.54 (C_f), 53.58 (2C_g), 38.29 (C_e), 29.75 (NCH_3); MS (ESI) *m/z* (%): 425.2 (100) $[M + H]^+$; UPLC purity = 95%, Rt 1.29 min; HRMS (ESI): calcd. for $C_{22}H_{25}N_5O_4$ $[M + H]^+$ 424.1979, found: 424.1970.

*4.1.3.10. N-(1-Methyl-7-{[2-(morpholin-4-yl)ethyl]amino}-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)-4-methoxybenzamide (**28**).*



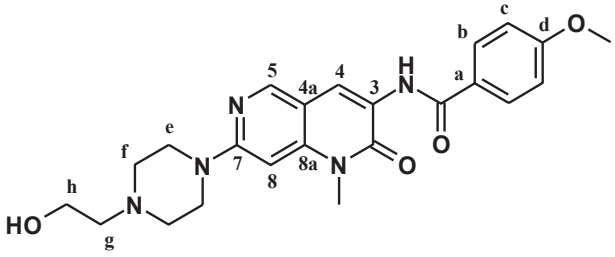
The reaction was carried out using amide **12** (75 mg, 0.22 mmol) and 2-(morpholin-4-yl)ethanamine (579 μL, 4.40 mmol) at 170 °C for 2 h. Purification by silica gel chromatography (mixtures of dichloromethane/methanol of increasing polarity) and trituration with diisopropyl ether afforded **28** (39 mg, 40% yield) as a beige powder. Mp: 195–196 °C; IR, ν (cm^{-1}): 3362 (νN–H); 2945 (νC–H_{al}); 1632 (νC = O); 1585, 1499, 1454 (νC = C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.12 (s, 1H, C_3–NH), 8.80 (s, 1H, H_4), 8.40 (s, 1H, H_5), 7.91 (d, ^3J = 8.8 Hz, 2H, H_b), 6.99 (d, ^3J = 8.8 Hz, 2H, H_c), 6.08 (s, 1H, H_8), 5.47 (br s, 1H, C_7–NH), 3.88 (s, 3H, OCH_3), 3.76–3.74 (m, 4H, H_h), 3.70 (s, 3H, NCH_3), 3.44–3.39 (m, 2H, H_e), 2.68 (t, ^3J = 5.5 Hz, 2H, H_f), 2.55–2.51 (m, 4H, H_g); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.21 (NH–C=O), 162.68 (C_d), 159.17 (C_3–C=O), 158.56 (C_7), 149.96 (C_5), 143.16 (C_{8a}), 129.03 (2C_b), 126.58 (C_a), 124.33 (C_3), 119.49 (C_4), 114.02 (2C_c), 109.69 (C_{4a}), 87.56 (C_8), 66.95 (2C_h), 56.83 (C_f), 55.50 (OCH_3), 53.32 (2C_g), 38.46 (C_e), 29.80 (NCH_3); MS (ESI) *m/z* (%): 438.2 (100) $[M + H]^+$; UPLC purity = 98%, Rt 2.20 min; HRMS (ESI): calcd. for $C_{23}H_{27}N_5O_4$ $[M + H]^+$ 438.2136, found: 438.2138.

*4.1.3.11. N-(7-{[2-(dimethylamino)ethyl]amino}-1-methyl-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)-4-methoxybenzamide (**29**).*



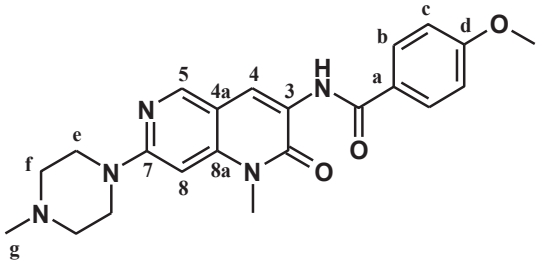
The reaction was carried out using amide **12** (75 mg, 0.22 mmol) and *N*,*N*-dimethylethylenediamine (474 μL, 4.40 mmol) at 130 °C for 2.5 h. Purification by silica gel chromatography (mixtures of dichloromethane/methanol of increasing polarity) and trituration with diisopropyl ether afforded **29** (46 mg, 53% yield) as a beige powder. Mp: 146–147 °C; IR, ν (cm^{-1}): 3397, 3240 (νN–H); 1641 (νC = O); 1593, 1497, 1462 (νC = C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.12 (s, 1H, C_3–NH), 8.78 (s, 1H, H_4), 8.39 (s, 1H, H_5), 7.91 (d, ^3J = 8.8 Hz, 2H, H_b), 6.98 (d, ^3J = 8.8 Hz, 2H, H_c), 6.15 (s, 1H, H_8), 5.63 (br s, 1H, C_7–NH), 3.88 (s, 3H, OCH_3), 3.68 (s, 3H, $CONCH_3$), 3.48–3.46 (m, 2H, H_e), 2.69 (t, ^3J = 5.6 Hz, 2H, H_f), 2.36 (s, 6H, H_g); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.20 (NH–C=O), 162.66 (C_d), 159.17 (C_7), 158.49 (C_3–C=O), 149.76 (C_5), 143.05 (C_{8a}), 129.02 ($2C_b$), 126.61 (C_a), 124.30 (C_3), 119.47 (C_4), 114.01 ($2C_c$), 109.73 (C_{4a}), 88.45 (C_8), 57.83 (C_f), 55.49 (OCH_3), 44.97 ($2C_g$), 39.16 (C_e), 29.78 ($CONCH_3$); MS (ESI) *m*/*z* (%): 396.3 (100) $[M + H]^+$; UPLC purity = 96%, Rt 1.90 min; HRMS (ESI): calcd. for $C_{21}H_{25}N_5O_3$ $[M + H]^+$ 396.2030, found: 396.2036.

*4.1.3.12. N-7-[4-(2-Hydroxyethyl)piperazin-1-yl]-1-methyl-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)-4-methoxybenzamide (**30**).*



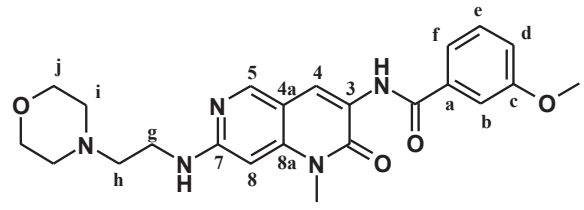
The reaction was carried out using amide **12** (75 mg, 0.22 mmol) and 1-(2-hydroxyethyl)piperazine (540 μL, 4.40 mmol) at 130 °C for 1 h. Purification by silica gel chromatography (mixtures of dichloromethane/methanol of increasing polarity) afforded **30** (67 mg, 70% yield) as a beige powder. Mp: 238–239 °C; IR, ν (cm^{-1}): 3385 (νN–H); 2920, 2849 (νC–H_{al}); 1641 (νC = O); 1591, 1501, 1437 (νC = C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.31 (s, 1H, NH), 8.61 (s, 1H, H_4), 8.56 (s, 1H, H_5), 7.94 (d, ^3J = 9.0 Hz, 2H, H_b), 7.13 (d, ^3J = 9.0 Hz, 2H, H_c), 6.62 (s, 1H, H_8), 4.50 (m, 1H, OH), 3.88 (s, 3H, OCH_3), 3.69 (s, 3H, NCH_3), 3.67–3.65 (m, 4H, H_e), 3.61–3.57 (m, 2H, H_h), 2.59–2.56 (m, 4H, H_f), 2.49 (t, ^3J = 6.2 Hz, 2H, H_g); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.40 (NH–C=O), 162.86 (C_d), 159.30 (C_3–C=O), 159.20 (C_7), 149.46 (C_5), 143.21 (C_{8a}), 129.19 ($2C_b$), 126.67 (C_a), 125.04 (C_3), 119.23 (C_4), 114.18 ($2C_c$), 109.98 (C_{4a}), 88.69 (C_8), 59.52 (C_h), 57.94 (C_g), 55.65 (OCH_3), 52.77 ($2C_f$), 45.57 ($2C_e$), 29.89 (NCH_3); MS (ESI) *m*/*z* (%): 438.3 (100) $[M + H]^+$; UPLC purity = 97%, Rt 2.01 min; HRMS (ESI): calcd. for $C_{23}H_{27}N_5O_4$ $[M + H]^+$ 438.2136, found: 438.2149.

*4.1.3.13. N-(1-Methyl-7-(4-methylpiperazin-1-yl)-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)-4-methoxybenzamide (**31**).*



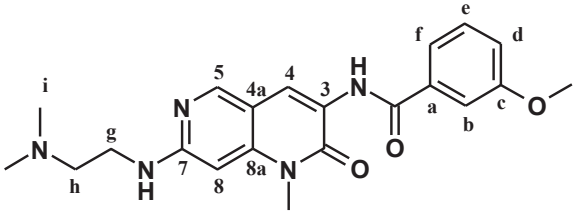
The reaction was carried out using amide **12** (75 mg, 0.22 mmol) and 1-methylpiperazine (488 μL, 4.40 mmol) at 150 °C for 1 h. Purification by silica gel chromatography (mixtures of dichloromethane/methanol of increasing polarity) and trituration with diisopropyl ether afforded **31** (46 mg, 51% yield) as a pale yellow powder. Mp: 248–249 °C; IR, ν (cm^{-1}): 3368 (νN–H); 2936 (νC–H_{al}); 1639 (νC = O); 1593, 1528, 1503 (νC = C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.15 (s, 1H, NH), 8.81 (s, 1H, H_4), 8.47 (s, 1H, H_5), 7.91 (d, ^3J = 8.4 Hz, 2H, H_b), 6.99 (d, ^3J = 8.4 Hz, 2H, H_c), 6.30 (s, 1H, H_8), 3.88 (s, 3H, OCH_3), 3.74–3.72 (m, 7H, H_e, $CONCH_3$), 2.69–2.63 (m, 4H, H_f), 2.44 (s, 3H, H_g); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.26 (NH–C=O), 162.72 (C_d), 159.14 (C_7), 158.91 (C_3–C=O), 149.30 (C_5), 143.07 (C_{8a}), 129.05 ($2C_b$), 126.51 (C_a), 124.97 (C_3), 119.03 (C_4), 114.03 ($2C_c$), 109.94 (C_{4a}), 88.69 (C_8), 55.50 (OCH_3), 54.56 ($2C_f$), 45.84 (C_g), 44.98 ($2C_e$), 29.77 ($CONCH_3$); MS (ESI) *m*/*z* (%): 408.3 (100) $[M + H]^+$; UPLC purity = 96%, Rt 2.01 min; HRMS (ESI): calcd. for $C_{22}H_{25}N_5O_3$ $[M + H]^+$ 408.2030, found: 408.2026.

*4.1.3.14. N-(1-Methyl-7-{[2-(morpholin-4-yl)ethyl]amino}-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)-3-methoxybenzamide (**32**).*



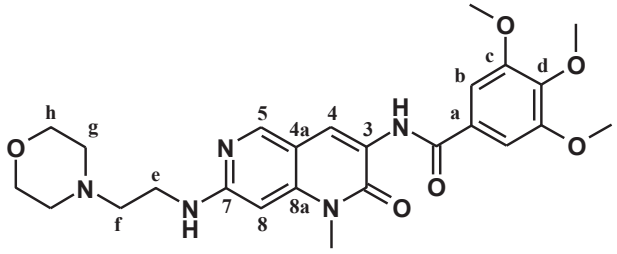
The reaction was carried out using amide **13** (75 mg, 0.22 mmol) and 2-(morpholin-4-yl)ethanamine (579 μL, 4.40 mmol) at 170 °C for 1 h. Trituration with diisopropyl ether afforded **32** (83 mg, 86% yield) as a beige powder. Mp: 183–184 °C; IR, ν (cm^{-1}): 3304, 3227 (νN–H); 2967 (νC–H_{al}); 1641 (νC = O); 1587, 1504 (νC = C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.17 (s, 1H, C_3–NH), 8.80 (s, 1H, H_4), 8.40 (s, 1H, H_5), 7.49–7.46 (m, 2H, H_b, H_f), 7.40 (appt, ^{app}J = 8.0 Hz, 1H, H_e), 7.09 (ddd, ^3J = 8.0 Hz, ^4J = 2.0 Hz, ^4J = 2.0 Hz, 1H, H_d), 6.09 (s, 1H, H_8), 5.50 (br s, 1H, C_7–NH), 3.88 (s, 3H, OCH_3), 3.75 (t, ^3J = 4.8 Hz, 4H, H_j), 3.69 (s, 3H, NCH_3), 3.44–3.40 (m, 2H, H_g), 2.69 (t, ^3J = 6.0 Hz, 2H, H_h), 2.54–2.51 (m, 4H, H_i); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.54 (NH–C=O), 162.02 (C_d), 159.08 (C_7), 158.56 (C_3–C=O), 149.97 (C_5), 143.27 (C_{8a}), 135.76 (C_a), 129.82 (C_e), 124.16 (C_3), 119.87 (C_4), 118.91 (C_b), 118.37 (C_d), 112.34 (C_f), 109.63 (C_{4a}), 87.82 (C_8), 66.73 ($2C_j$), 56.93 (C_h), 55.49 (OCH_3), 53.29 ($2C_i$), 38.28 (C_g), 29.82 (NCH_3); MS (ESI) *m*/*z* (%): 439.2 (100) $[M + H]^+$; UPLC purity = 95%, Rt 2.18 min; HRMS (ESI): calcd. for $C_{23}H_{27}N_5O_4$ $[M + H]^+$ 438.2136, found: 438.2154.

*4.1.3.15. N-(7-{[2-(dimethylamino)ethyl]amino}-1-methyl-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)-3-methoxybenzamide (**33**).*



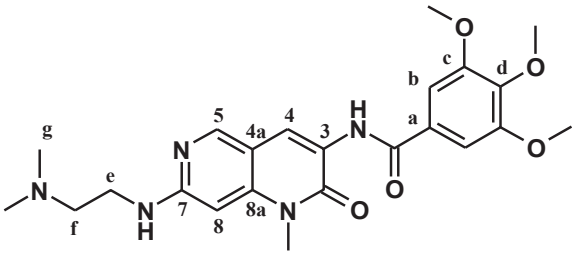
The reaction was carried out using amide **13** (75 mg, 0.22 mmol) and *N*,*N*-dimethylethylenediamine (474 μL, 4.40 mmol) at 130 °C for 6 h. Purification by silica gel chromatography (mixtures of dichloromethane/methanol of increasing polarity) and trituration with diisopropyl ether afforded **33** (44 mg, 50% yield) as a pale yellow powder. Mp: 154–155 °C; IR, ν (cm^{-1}): 3395 (νN–H); 2980, 2951 (νC–H_{al}); 1717, 1645 (νC = O); 1589, 1518, 1456 (νC = C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.18 (s, 1H, C_3–NH), 8.78 (s, 1H, H_4), 8.38 (s, 1H, H_5), 7.49–7.48 (m, 2H, H_b, H_f), 7.40 (appt, ^{app}J = 8.0 Hz, 1H, H_e), 7.09 (ddd, ^3J = 8.0 Hz, ^4J = 2.0 Hz, ^4J = 2.0 Hz, 1H, H_d), 6.22 (s, 1H, H_8), 5.86 (br s, 1H, C_7–NH), 3.88 (s, 3H, OCH_3), 3.67 (s, 3H, $CONCH_3$), 3.59–3.55 (m, 2H, H_g), 2.82 (t, ^3J = 5.4 Hz, 2H, H_h), 2.47 (s, 6H, H_i); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.53 (NH–C=O), 160.01 (C_c), 159.04 (C_7), 158.15 (C_3–C=O), 149.53 (C_5), 143.12 (C_{8a}), 135.75 (C_a), 129.81 (C_e), 124.29 (C_3), 119.67 (C_4), 118.91 (C_b), 118.37 (C_d), 112.33 (C_f), 109.88 (C_{4a}), 89.48 (C_8), 58.13 (C_h), 55.48 (OCH_3), 44.60 ($2C_i$), 38.30 (C_g), 29.86 ($CONCH_3$); MS (ESI) *m*/*z* (%): 396.3 (100) $[M + H]^+$; UPLC purity = 98%, Rt 1.84 min; HRMS (ESI): calcd. for $C_{21}H_{25}N_5O_3$ $[M + H]^+$ 396.2030, found: 396.2022.

*4.1.3.16. N-(1-Methyl-7-{[2-(morpholin-4-yl)ethyl]amino}-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)-3,4,5-trimethoxybenzamide (**34**).*



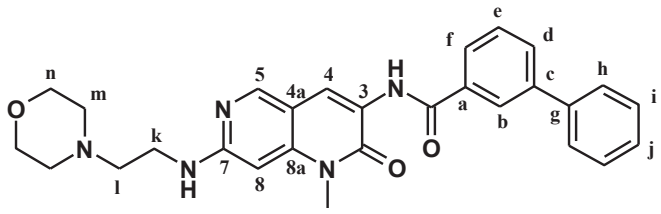
The reaction was carried out using amide **14** (75 mg, 0.19 mmol) and 2-(morpholin-4-yl)ethanamine (500 μL, 3.80 mmol) at 170 °C for 1 h. Purification by silica gel chromatography (mixtures of dichloromethane/methanol of increasing polarity) afforded **34** (52 mg, 55% yield) as a beige powder. Mp: 197–198 °C; IR, ν (cm^{-1}): 3238 (νN–H); 2922 (νC–H_{al}); 1634 (νC = O); 1587, 1495 (νC = C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.11 (s, 1H, C_3–NH), 8.79 (s, 1H, H_4), 8.41 (s, 1H, H_5), 7.15 (s, 2H, H_b), 6.09 (s, 1H, H_8), 5.51 (t, ^3J = 5.2 Hz, 1H, C_7–NH), 3.95 (s, 6H, $2C_c$-OCH_3), 3.92 (s, 3H, C_d–OCH_3), 3.75 (t, ^3J = 4.6 Hz, 4H, H_h), 3.70 (s, 3H, NCH_3), 3.43–3.39 (m, 2H, H_e), 2.69 (t, ^3J = 5.8 Hz, 2H, H_f), 2.53–2.51 (m, 4H, H_g); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.32 (NH–C=O), 159.14 (C_3–C=O), 158.66 (C_7), 153.36 ($2C_c$), 150.08 (C_5), 143.22 (C_{8a}), 141.44 (C_d), 129.68 (C_a), 124.08 (C_3), 119.90 (C_4), 109.57 (C_{4a}), 104.41 ($2C_b$), 87.57 (C_8), 66.93 ($2C_h$), 60.98 (C_d–OCH_3), 56.81 ($2C_c$-OCH_3), 56.36 (C_f), 53.31 ($2C_g$), 38.43 (C_e), 29.81 (NCH_3); MS (ESI) *m*/*z* (%): 498.3 (100) $[M + H]^+$; UPLC purity = 99%, Rt 1.53 min; HRMS (ESI): calcd. for $C_{25}H_{31}N_5O_6$ $[M + H]^+$ 498.2347, found: 498.2364.

*4.1.3.17. N-(7-{[2-(dimethylamino)ethyl]amino}-1-methyl-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)-3,4,5-trimethoxybenzamide (**35**).*



The reaction was carried out using amide **14** (75 mg, 0.19 mmol) and *N*,*N*-dimethylethylenediamine (409 μL, 3.80 mmol) at 130 °C for 3.5 h. Purification by silica gel chromatography (mixtures of dichloromethane/methanol of increasing polarity) afforded **35** (52 mg, 60% yield) as a beige powder. Mp: 188–189 °C; IR, ν (cm^{-1}): 3240 (νN–H); 2938 (νC–H_{al}); 1636 (νC = O); 1586, 1495 (νC = C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.11 (s, 1H, C_3–NH), 8.77 (s, 1H, H_4), 8.39 (s, 1H, H_5), 7.15 (s, 2H, H_b), 6.16 (s, 1H, H_8), 5.66 (t, ^3J = 5.6 Hz, 1H, C_7–NH), 3.95 (s, 6H, $2C_c$-OCH_3), 3.91 (s, 3H, C_d–OCH_3), 3.68 (s, 3H, $CONCH_3$), 3.50–3.46 (m, 2H, H_e), 2.70 (t, ^3J = 5.6 Hz, 2H, H_f), 2.37 (s, 6H, H_g); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.30 (NH–C=O), 159.13 (C_3–C=O), 158.58 (C_7), 153.35 ($2C_c$), 149.90 (C_5), 143.10 (C_{8a}), 141.41 (C_d), 129.70 (C_a), 124.05 (C_3), 119.88 (C_4), 109.62 (C_{4a}), 104.40 ($2C_b$), 88.45 (C_8), 60.98 (C_d–OCH_3), 57.83 (C_f), 56.35 ($2C_c$-OCH_3), 44.96 ($2C_g$), 39.13 (C_e), 29.79 ($CONCH_3$); MS (ESI) *m*/*z* (%): 456.3 (100) $[M + H]^+$; UPLC purity = 97%, Rt 1.44 min; HRMS (ESI): calcd. for $C_{23}H_{29}N_5O_5$ $[M + H]^+$ 456.2241, found: 456.2257.

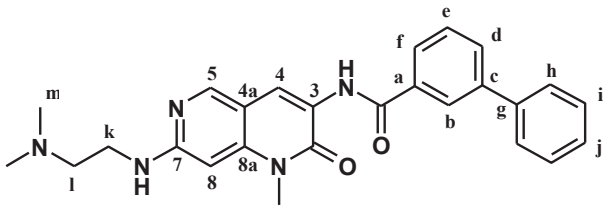
*4.1.3.18. N-(1-Methyl-7-{[2-(morpholin-4-yl)ethyl]amino}-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)biphenyl-3-carboxamide (**36**).*



The reaction was carried out using amide **15** (75 mg, 0.19 mmol) and 2-(morpholin-4-yl)ethanamine (500 μL, 3.80 mmol) at 170 °C for 1 h. Purification by silica gel chromatography (mixtures of dichloromethane/methanol of increasing polarity) and trituration with diisopropyl ether afforded **36** (65 mg, 71% yield) as a white powder. Mp: 171–172 °C; IR, ν (cm^{-1}): 3231 (νN–H); 1639 (νC = O); 1591, 1512, 1466 (νC = C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.25 (s, 1H, C_3–NH), 8.84 (s, 1H, H_4), 8.42 (s, 1H, H_5), 8.15 (s, 1H, H_b), 7.90 (d, ^3J = 7.6 Hz, 1H, H_f), 7.78 (d, ^3J = 8.0 Hz, 1H, H_d), 7.65 (d, ^3J = 7.6 Hz, 2H, H_h), 7.57 (appt, ^{app}J = 7.6 Hz, 1H, H_e), 7.48 (appt, ^{app}J = 7.4 Hz, 2H, H_i), 7.39 (t, ^3J = 7.2 Hz, 1H, H_j), 6.09 (s, 1H, H_8), 5.50 (br s, 1H, C_7–NH), 3.75 (t, ^3J = 4.0 Hz, 4H, H_n), 3.70 (s, 3H, NCH_3), 3.44–3.39 (m, 2H, H_k), 2.69 (t, ^3J = 5.2 Hz, 2H, H_l), 2.54–2.50 (m, 4H, H_m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.63 (NH–C=O), 159.11 (C_7), 158.66 (C_3–C=O), 150.09 (C_5), 143.27 (C_{8a}), 142.01 (C_c or C_g), 140.08 (C_g or C_c), 134.91 (C_a), 130.74 (C_d), 129.28 (C_e), 128.96 ($2C_i$), 127.87 (C_j), 127.26 ($2C_h$), 125.93 (C_b), 125.80 (C_f), 124.13 (C_3), 119.96 (C_4), 109.58 (C_{4a}), 87.59 (C_8), 66.92 ($2C_n$), 56.83 (C_l), 53.31 ($2C_m$), 38.42 (C_k), 29.81 (NCH_3); MS (ESI) *m*/*z* (%): 484.3 (100) $[M + H]^+$; UPLC purity = 96%, Rt 2.79 min; HRMS (ESI): calcd. for $C_{28}H_{29}N_5O_3$

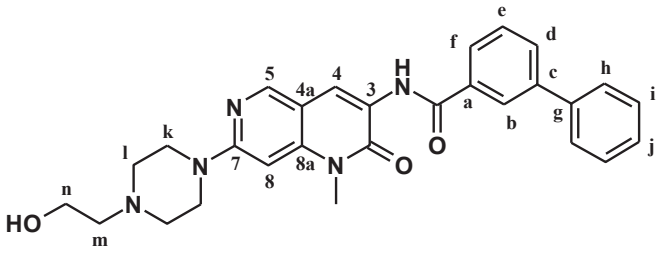
$[M + H]^+$ 484.2343, found: 484.2361.

*4.1.3.19. N-(7-{[2-(dimethylamino)ethyl]amino}-1-methyl-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)biphenyl-3-carboxamide (**37**).*



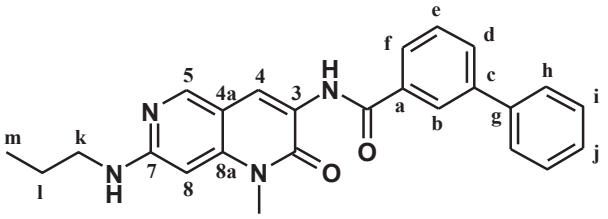
The reaction was carried out using amide **15** (75 mg, 0.19 mmol) and *N*,*N*-dimethylethylenediamine (409 μL, 3.80 mmol) at 130 °C for 1.5 h. Purification by silica gel chromatography (mixtures of dichloromethane/methanol of increasing polarity) and trituration with diisopropyl ether afforded **37** (54 mg, 64% yield) as a yellow powder. Mp: 136–137 °C; IR, ν (cm^{-1}): 3372 (νN–H); 2949 (νC–H_{al}); 1634 (νC = O); 1593, 1518, 1474 (νC = C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.26 (s, 1H, C_3–NH), 8.82 (s, 1H, H_4), 8.41 (s, 1H, H_5), 8.15 (s, 1H, H_b), 7.90 (d, ^3J = 7.5 Hz, 1H, H_f), 7.78 (d, ^3J = 7.6 Hz, 1H, H_d), 7.65 (d, ^3J = 7.2 Hz, 2H, H_h), 7.57 (appt, ^{app}J = 7.8 Hz, 1H, H_e), 7.48 (appt, ^{app}J = 7.4 Hz, 2H, H_i), 7.39 (t, ^3J = 7.4 Hz, 1H, H_j), 6.14 (s, 1H, H_8), 5.59 (br s, 1H, C_7–NH), 3.68 (s, 3H, $CONCH_3$), 3.48–3.43 (m, 2H, H_k), 2.66 (t, ^3J = 5.6 Hz, 2H, H_l), 2.34 (s, 6H, H_m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.62 (NH–C=O), 159.13 (C_7), 158.66 (C_3–C=O), 149.96 (C_5), 143.18 (C_{8a}), 142.00 (C_c or C_g), 140.09 (C_g or C_c), 134.92 (C_a), 130.72 (C_d), 129.27 (C_e), 128.95 ($2C_i$), 127.86 (C_j), 127.26 ($2C_h$), 125.93 (C_b), 125.80 (C_f), 124.08 (C_3), 119.99 (C_4), 109.59 (C_{4a}), 88.28 (C_8), 57.80 (C_l), 45.03 ($2C_m$), 39.29 (C_k), 29.79 ($CONCH_3$); MS (ESI) *m*/*z* (%): 442.3 (100) $[M + H]^+$; UPLC purity = 100%, Rt 2.73 min; HRMS (ESI): calcd. for $C_{26}H_{27}N_5O_2$ $[M + H]^+$ 442.2238, found: 442.2232.

*4.1.3.20. N-7-[4-(2-Hydroxyethyl)piperazin-1-yl]-1-methyl-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)biphenyl-3-carboxamide (**38**).*



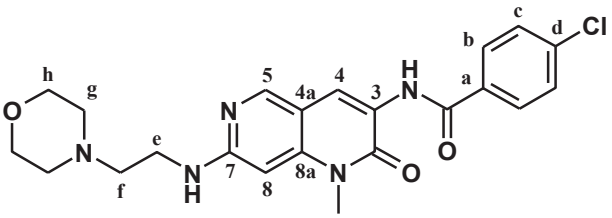
The reaction was carried out using amide **15** (75 mg, 0.19 mmol) and 1-(2-hydroxyethyl)piperazine (466 μL, 3.80 mmol) at 130 °C for 1 h. Purification by silica gel chromatography (mixtures of dichloromethane/methanol of increasing polarity) and trituration with diisopropyl ether afforded **38** (56 mg, 61% yield) as a pale yellow powder. Mp: 188–189 °C; IR, ν (cm^{-1}): 3374 (νN–H); 2833 (νC–H_{al}); 1638 (νC = O); 1589, 1517 (νC = C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.28 (s, 1H, C_3–NH), 8.85 (s, 1H, H_4), 8.50 (s, 1H, H_5), 8.15 (s, 1H, H_b), 7.90 (d, ^3J = 8.0 Hz, 1H, H_f), 7.79 (d, ^3J = 7.6 Hz, 1H, H_d), 7.65 (d, ^3J = 7.2 Hz, 2H, H_h), 7.58 (appt, ^{app}J = 7.6 Hz, 1H, H_e), 7.48 (appt, ^{app}J = 7.4 Hz, 2H, H_i), 7.40 (t, ^3J = 7.4 Hz, 1H, H_j), 6.30 (s, 1H, H_8), 3.72–3.68 (m, 9H, H_n, H_k, NCH_3), 2.69 (t, ^3J = 5.0 Hz, 4H, H_l), 2.64 (t, ^3J = 5.2 Hz, 2H, H_m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.68 (NH–C=O), 159.13 (C_7), 159.10 (C_3–C=O), 149.45 (C_5), 143.19 (C_{8a}), 142.03 (C_c or C_g), 140.07 (C_g or C_c), 134.86 (C_a), 130.79 (C_d), 129.30 (C_e), 128.96 ($2C_i$), 127.88 (C_j), 127.26 ($2C_h$), 125.94 (C_b), 125.80 (C_f), 124.69 (C_3), 119.57 (C_4), 109.72 (C_{4a}), 88.51 (C_8), 59.39 (C_n), 57.80 (C_m), 52.63 ($2C_l$), 45.41 ($2C_k$), 29.77 (NCH_3); MS (ESI) *m*/*z* (%): 484.3 (100) $[M + H]^+$; UPLC purity = 100%, Rt 2.59 min; HRMS (ESI): calcd. for $C_{28}H_{29}N_5O_3$ $[M + H]^+$ 484.2343, found: 484.2341.

*4.1.3.21. N-[1-Methyl-2-oxo-7-n-propylamino-1,2-dihydro-1,6-naphthyridin-3-yl]biphenyl-3-carboxamide (**39**).*



The reaction was carried out using amide **15** (75 mg, 0.19 mmol) and *n*-propylamine (312 μL, 3.80 mmol) in *N*-methyl-2-pyrrolidone (1 mL). CuI (19 mg, 0.10 mmol, 0.5 equiv.) was added and the mixture was heated at 130 °C for 5 h under microwave irradiation. Purification by silica gel chromatography (mixtures of dichloromethane/methanol of increasing polarity) and trituration with diisopropyl ether afforded **39** (45 mg, 57% yield) as a pale yellow powder. Mp: 144–145 °C; IR, ν (cm^{-1}): 3387, 3244 (νN–H); 3078 (νC–H_{ar}), 2961, 2936, 2876 (νC–H_{al}); 1643 (νC = O); 1587, 1512 (νC = C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.25 (s, 1H, C_3–NH), 8.84 (s, 1H, H_4), 8.40 (s, 1H, H_5), 8.15 (s, 1H, H_b), 7.90 (d, ^3J = 8.0 Hz, 1H, H_f), 7.78 (d, ^3J = 8.0 Hz, 1H, H_d), 7.65 (d, ^3J = 7.2 Hz, 2H, H_h), 7.58 (appt, ^{app}J = 7.8 Hz, 1H, H_e), 7.48 (appt, ^{app}J = 7.2 Hz, 2H, H_i), 7.40 (t, ^3J = 7.2 Hz, 1H, H_j), 6.05 (s, 1H, H_8), 4.90–4.87 (m, 1H, C_7–NH), 3.71 (s, 3H, NCH_3), 3.31–3.26 (m, 2H, H_k), 1.77–1.68 (m, 2H, H_l), 1.05 (t, ^3J = 7.4 Hz, 3H, H_m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.62 (NH–C=O), 159.13 (C_3–C=O), 158.89 (C_7), 150.13 (C_5), 143.35 (C_{8a}), 142.00 (C_c or C_g), 140.09 (C_g or C_c), 134.94 (C_a), 130.72 (C_d), 129.27 (C_e), 128.95 ($2C_i$), 127.86 (C_j), 127.26 ($2C_h$), 125.93 (C_b), 125.80 (C_f), 124.03 (C_3), 120.03 (C_4), 109.48 (C_{4a}), 86.86 (C_8), 44.45 (C_k), 29.80 (NCH_3), 22.58 (C_l), 11.62 (C_m); MS (ESI) *m*/*z* (%): 413.2 (100) $[M + H]^+$; UPLC purity = 100%, Rt 3.12 min; HRMS (ESI): calcd. for $C_{25}H_{24}N_4O_2$ $[M + H]^+$ 413.1972, found: 413.1962.

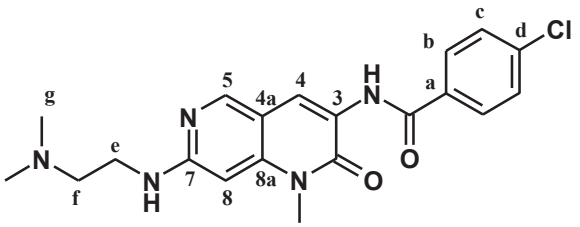
*4.1.3.22. 4-Chloro-N-(1-methyl-7-{[2-(morpholin-4-yl)ethyl]amino}-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)benzamide (**40**).*



The reaction was carried out using amide **16** (75 mg, 0.22 mmol) and 2-(morpholin-4-yl)ethanamine (579 μL, 4.40 mmol) at 170 °C for 1 h. Purification by silica gel chromatography (mixtures of dichloromethane/methanol of increasing polarity) and trituration with diisopropyl ether afforded **40** (77 mg, 79% yield) as a yellow powder. Mp: 209–210 °C; IR, ν (cm^{-1}): 3383 (νN–H); 2978, 2951 (νC–H_{al}); 1638 (νC = O); 1593, 1517, 1483 (νC = C); 613 (νC–Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.15 (s, 1H, C_3–NH), 8.78 (s, 1H, H_4), 8.41 (s,

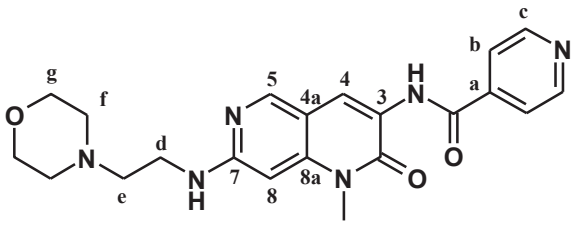
1H, H_5), 7.87 (d, ^3J = 6.8 Hz, 2H, H_c), 7.47 (d, ^3J = 6.8 Hz, 2H, H_b), 6.08 (s, 1H, H_8), 5.51 (br s, 1H, C_7–NH), 3.75 (t, ^3J = 4.6 Hz, 4H, H_h), 3.69 (s, 3H, NCH_3), 3.43–3.39 (m, 2H, H_e), 2.69 (t, ^3J = 5.8 Hz, 2H, H_f), 2.54–2.50 (m, 4H, H_g); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.51 (NH–C=O), 159.06 (C_3–C=O), 158.70 (C_7), 150.12 (C_5), 143.29 (C_{8a}), 138.40 (C_d), 132.66 (C_a), 129.10 (2C_c), 128.54 (2C_b), 123.90 (C_3), 120.07 (C_4), 109.48 (C_{4a}), 87.58 (C_8), 66.92 (2C_h), 56.82 (C_f), 53.31 (2C_g), 38.42 (C_e), 29.82 (NCH_3); MS (ESI) *m/z* (%): 442.1 (100) $[M + H]^+$, 444.1 (40) $[M + H + 2]^+$; UPLC purity = 97%, Rt 2.43 min; HRMS (ESI): calcd. for $C_{22}H_{24}ClN_5O_3$ $[M + H]^+$ 442.1640, found: 442.1638.

*4.1.3.23. 4-Chloro-N-(7-{[2-(dimethylamino)ethyl]amino}-1-methyl-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)benzamide (**41**).*



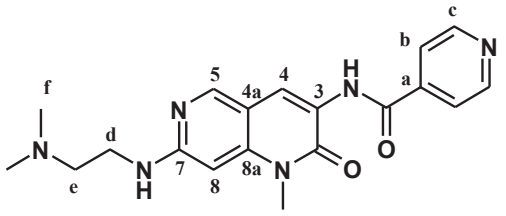
The reaction was carried out using amide **16** (75 mg, 0.22 mmol) and *N*,*N*-dimethylethylenediamine (474 μL, 4.40 mmol) at 130 °C for 5 h. Purification by silica gel chromatography (mixtures of dichloromethane/methanol of increasing polarity) and trituration with diisopropyl ether afforded **41** (62 mg, 70% yield) as a yellow powder. Mp: 183–184 °C; IR, ν (cm^{-1}): 3375 (νN–H); 2972 (νC–H_{al}); 1634 (νC = O); 1518, 1485 (νC = C); 613 (νC–Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.16 (s, 1H, C_3–NH), 8.77 (s, 1H, H_4), 8.40 (s, 1H, H_5), 7.87 (d, ^3J = 8.4 Hz, 2H, H_c), 7.48 (d, ^3J = 8.4 Hz, 2H, H_b), 6.14 (s, 1H, H_8), 5.60 (br s, 1H, C_7–NH), 3.68 (s, 3H, $CONCH_3$), 3.47–3.43 (m, 2H, H_e), 2.66 (t, ^3J = 5.6 Hz, 2H, H_f), 2.54 (s, 6H, H_g); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.50 (NH–C=O), 159.07 (C_7), 158.70 (C_3–C=O), 150.00 (C_5), 143.18 (C_{8a}), 138.38 (C_d), 132.68 (C_a), 129.09 (2C_c), 128.54 (2C_b), 123.84 (C_3), 120.09 (C_4), 109.48 (C_{4a}), 88.27 (C_8), 57.79 (C_f), 45.02 (2C_g), 39.27 (C_e), 29.80 ($CONCH_3$); MS (ESI) *m/z* (%): 400.1 (100) $[M + H]^+$, 402.1 (40) $[M + H + 2]^+$; UPLC purity = 99%, Rt 2.35 min; HRMS (ESI): calcd. for $C_{20}H_{22}ClN_5O_2$ $[M + H]^+$ 400.1535, found: 400.1524.

*4.1.3.24. N-(1-Methyl-7-{[2-(morpholin-4-yl)ethyl]amino}-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)pyridine-4-carboxamide (**42**).*



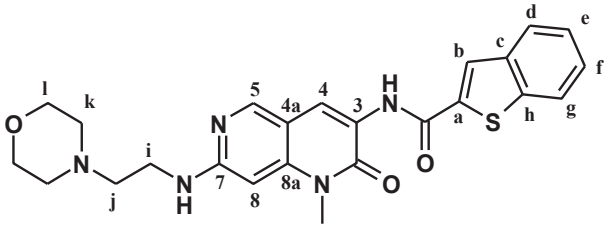
The reaction was carried out using amide **17** (75 mg, 0.24 mmol) and 2-(morpholin-4-yl)ethanamine (631 μL, 4.80 mmol) at 170 °C for 1 h. Purification by silica gel chromatography (mixtures of dichloromethane/methanol of increasing polarity) afforded **42** (44 mg, 45% yield) as a yellow powder. Mp: 235–236 °C; IR, ν (cm^{-1}): 3408, 3368 (νN–H); 2847 (νC–H_{al}); 1643 (νC = O); 1593, 1501, 1487 (νC = C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.24 (s, 1H, C_3–NH), 8.82 (d, ^3J = 6.0 Hz, 2H, H_c), 8.80 (s, 1H, H_4), 8.42 (s, 1H, H_5), 7.76 (d, ^3J = 6.0 Hz, 2H, H_b), 6.10 (s, 1H, H_8), 5.55 (br s, 1H, C_7–NH), 3.75 (t, ^3J = 4.4 Hz, 4H, H_g), 3.70 (s, 3H, NCH_3), 3.44–3.40 (m, 2H, H_d), 2.70 (t, ^3J = 6.0 Hz, 2H, H_e), 2.55–2.51 (m, 4H, H_f); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.53 (NH–C=O), 158.96 (C_7), 158.84 (C_3–C=O), 150.84 (2C_c), 150.29 (C_5), 143.45 (C_{8a}), 141.20 (C_a), 123.44 (C_3), 120.80 (2C_b, C_4), 109.30 (C_{4a}), 87.65 (C_8), 66.90 (2C_g), 56.85 (C_e), 53.35 (2C_f), 38.33 (C_d), 29.86 (NCH_3); MS (ESI) *m/z* (%): 409.3 (100) $[M + H]^+$; UPLC purity = 99%, Rt 0.98 min; HRMS (ESI): calcd. for $C_{21}H_{24}N_6O_3$ $[M + H]^+$ 409.1983, found: 409.1981.

*4.1.3.25. N-(7-{[2-(dimethylamino)ethyl]amino}-1-methyl-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)pyridine-4-carboxamide (**43**).*



The reaction was carried out using amide **17** (75 mg, 0.24 mmol) and *N*,*N*-dimethylethylenediamine (517 μL, 4.80 mmol) at 130 °C for 3 h. Purification by silica gel chromatography (mixtures of dichloromethane/methanol of increasing polarity) afforded **43** (53 mg, 60% yield) as a yellow powder. Mp: 169–170 °C; IR, ν (cm^{-1}): 3366, 3246 (νN–H); 2853 (νC–H_{al}); 1643 (νC = O); 1597, 1517 (νC = C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.24 (s, 1H, C_3–NH), 8.82 (dd, ^3J = 4.4 Hz, ^5J = 1.6 Hz, 2H, H_c), 8.78 (s, 1H, H_4), 8.41 (s, 1H, H_5), 7.76 (dd, ^3J = 4.4 Hz, ^5J = 1.6 Hz, 2H, H_b), 6.14 (s, 1H, H_8), 5.67 (br s, 1H, C_7–NH), 3.68 (s, 3H, $CONCH_3$), 3.49–3.45 (m, 2H, H_d), 2.68 (t, ^3J = 5.6 Hz, 2H, H_e), 2.36 (s, 6H, H_f); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.50 (NH–C=O), 158.93 (C_7), 158.82 (C_3–C=O), 150.88 (2C_c), 150.17 (C_5), 143.33 (C_{8a}), 141.24 (C_a), 123.37 (C_3), 120.77 (2C_b), 120.76 (C_4), 109.29 (C_{4a}), 88.34 (C_8), 57.75 (C_e), 44.99 (2C_f), 39.17 (C_d), 29.83 ($CONCH_3$); MS (ESI) *m/z* (%): 367.3 (100) $[M + H]^+$; UPLC purity = 96%, Rt 1.43 min; HRMS (ESI): calcd. for $C_{19}H_{22}N_6O_2$ $[M + H]^+$ 367.1877, found: 367.1872.

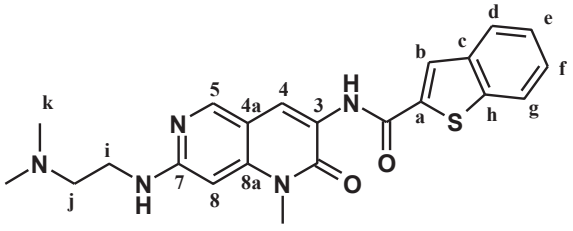
*4.1.3.26. N-(1-Methyl-7-{[2-(morpholin-4-yl)ethyl]amino}-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)-1-benzothiophene-2-carboxamide (**44**).*



The reaction was carried out using amide **18** (75 mg, 0.20 mmol) and 2-(morpholin-4-yl)ethanamine (526 μL, 4.00 mmol) at 170 °C for 1 h. Purification by silica gel chromatography (mixtures of dichloromethane/methanol of increasing polarity) afforded **44** (72 mg, 77% yield) as a yellow powder. Mp: 222–223 °C; IR, ν (cm^{-1}): 3320 (νN–H); 2845 (νC–H_{al}); 1634 (νC = O); 1585, 1530, 1487 (νC = C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.18 (s, 1H, C_3–NH), 8.76 (s, 1H, H_4), 8.40 (s, 1H, H_5), 7.93 (s, 1H, H_b), 7.90–7.88 (m, 2H, H_g, H_d), 7.48–7.41 (m, 2H, H_f, H_e), 6.09 (s, 1H, H_8), 5.22 (br s, 1H, C_7–NH), 3.75 (t, ^3J = 4.6 Hz, 4H, H_l), 3.71 (s, 3H, NCH_3), 3.43–3.39 (m, 2H, H_i),

2.69 (t, ^3J = 5.8 Hz, 2H, H_j), 2.53–2.51 (m, 4H, H_k); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.57 (NH–C=O), 158.90 (C_7), 158.70 (C_3–C=O), 150.14 (C_5), 143.30 (C_{8a}), 141.39 (C_a), 139.13 (C_h), 138.64 (C_c), 126.68 (C_3), 125.53 (C_b), 125.33 (C_f or C_e), 125.08 (C_g or C_d), 123.71 (C_e or C_f), 122.79 (C_d or C_g), 120.14 (C_4), 109.49 (C_{4a}), 87.58 (C_8), 66.92 ($2C_l$), 56.81 (C_j), 53.31 ($2C_k$), 38.41 (C_i), 29.87 (NCH_3); MS (ESI) *m/z* (%): 464.4 (100) $[M + H]^+$; UPLC purity = 95%, Rt 2.10 min; HRMS (ESI): calcd. for $C_{24}H_{25}N_5O_3S$ $[M + H]^+$ 464.1751, found: 464.1752.

*4.1.3.27. N-(7-{[2-(dimethylamino)ethyl]amino}-1-methyl-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)-1-benzothiophene-2-carboxamide (**45**).*



The reaction was carried out using amide **18** (75 mg, 0.20 mmol) and *N*,*N*-dimethylethylenediamine (431 μL, 4.00 mmol) at 130 °C for 3 h. Purification by silica gel chromatography (mixtures of dichloromethane/methanol of increasing polarity) afforded **45** (35 mg, 42% yield) as a yellow powder. Mp: 216–217 °C; IR, ν (cm^{-1}): 3244, 3360 (νN–H); 2970 (νC–H_{al}); 1638 (νC = O); 1593, 1522, 1503 (νC = C); ^1H NMR (400 MHz, $CDCl_3$) δ 9.18 (s, 1H, C_3–NH), 8.75 (s, 1H, H_4), 8.39 (s, 1H, H_5), 7.93 (s, 1H, H_b), 7.90–7.88 (m, 2H, H_g, H_d), 7.47–7.41 (m, 2H, H_d, H_e), 6.15 (s, 1H, H_8), 5.64 (br s, 1H, C_7–NH), 3.69 (s, 3H, $CONCH_3$), 3.49–3.45 (m, 2H, H_i), 2.67 (t, ^3J = 5.6 Hz, 2H, H_j), 2.35 (s, 6H, H_k); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.55 (NH–C=O), 158.90 (C_7), 158.69 (C_3–C=O), 150.00 (C_5), 143.18 (C_{8a}), 141.39 (C_a), 139.13 (C_h), 138.69 (C_c), 126.65 (C_3), 125.52 (C_b), 125.32 (C_f or C_e), 125.07 (C_g or C_d), 123.65 (C_e or C_f), 122.78 (C_f or C_g), 120.16 (C_4), 109.50 (C_{4a}), 88.36 (C_8), 57.79 (C_j), 45.01 ($2C_k$), 39.22 (C_i), 29.84 ($CONCH_3$); MS (ESI) *m/z* (%): 422.3 (100) $[M + H]^+$; UPLC purity = 97%, Rt 1.96 min; HRMS (ESI): calcd. for $C_{22}H_{23}N_5O_2S$ $[M + H]^+$ 422.1645, found: 422.1634.

4.2. Biological evaluation

4.2.1. Antiproliferative assay (MTT)

Cells were grown in DMEM medium supplemented with L-glutamine (2 mM), streptomycin (50 units/mL), penicillin (550 ng/mL) and 5% FBS. Cells were grown to confluence in a humidified atmosphere (37 °C, 5% CO_2), seeded (6000/well, 50 μL) in 96-well plates, and allowed to attach for 24 h. Compounds at varying concentrations in DMSO (1% DMSO final concentration) were added, and cells were returned to the incubator for 48 h. At 48 h, 50 μL of MTT solution (2.5 mg/mL in PBS) was added to each well and incubated for 3.5 h. Medium was subsequently removed from wells and resulting formazan crystals solubilized in 100 μL DMSO. Culture plates were rocked gently for 30 min to solubilize before optical density was measured using a microplate reader (BioRad) at 570 nm. Cells that incubated in 1% DMSO were used as 100% proliferation (i.e. DMSO = 100% growth) and the relative growth for each compound concentration was compared to 1% DMSO. IC_{50} values were calculated from separate experiments performed in triplicate using GraphPad Prism.

4.2.2. Annexin V cell death detection assay

MDA-MB-468 cells were seeded (40,000/well, 500 μL) on a 96-well plate and incubated in 5% CO_2 incubator at 37 °C for 24 h. Compounds at varying concentrations in DMSO (1% DMSO final concentration) were added, and cells were returned to the incubator for 48 h. Floating dead cells and adherent cells after trypsin treatment were harvested and washed once with PBS. Cells were pelleted and re-suspended in annexin V binding buffer containing 1 μL annexin V-FITC (Miltenyi Biotec) and 1 μL propidium iodide (Sigma–Aldrich) for 15 min on ice in the dark. Subsequently, cells were analyzed by flow cytometry, using a FACSCalibur operated by CellQuest software and at least 10 000 events were collected per sample. Cells were considered apoptotic if they stained positive for Annexin V.

4.2.3. Cell cycle assay

MDA-MB-468 cells were seeded (40,000/well, 500 μL) on a 24-well plate and incubated in 5% CO_2 incubator at 37 °C for 24 h. Compounds at varying concentrations in DMSO (1% DMSO final concentration) were added, and cells were returned to the incubator for 48 h. Cells were harvested using trypsin and gentle agitation, washed with PBS and fixed with 70% ethanol. Following rehydration in PBS, cells were stained in PBS containing 2 μg/mL propidium iodide. Subsequently, cells were analyzed by flow cytometry, using a FACSCalibur operated by CellQuest software and at least 10 000 events were collected per sample.

4.2.4. Western blot analysis

For Western Blot analysis MCF-7 and MDA-MB-468 cells were seeded on a 6-well plate and incubated in 5% CO_2 incubator at 37 °C for 24 h. Cells were treated with various concentrations of drug, 17-AAG in DMSO (1% DMSO final concentration), or vehicle (DMSO) for 48 h. After 48 h incubation with individual compounds, cells were washed by PBS and harvested. Cell lysates were obtained by 2 h treatment with CHiP cell lysis buffer containing protease inhibitors and phosphatase inhibitors on ice. After sonication, the protein concentration in the supernatant was measured by BCA assay. Equal amounts of protein were analyzed by SDS-PAGE, transferred into PVDF membrane and blocked for 1 h with Western Blocking Reagent (WBR) containing 0.05% Tween. The samples were subjected to immunoblotting with specific primary antibodies overnight at 4 °C, followed by washing with TBST for 30 min. The resulting membrane was exposed into HRP-conjugated secondary antibody for 1 h at room temperature. After 30 min washing with TBST, the membrane was developed by ECL prime solution and the chemiluminescent signal was measured by ChemiDoc MP imaging system.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2016.04.050.

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