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Design and Synthesis of a Focused Library of Novel Aryl- and Heteroaryl-Ketopiperazides

1-Phenyl-4-piperazinyl-carbonyl-substituted nitrogen-containing heterocycles were discovered at Zentaris as a new class of potent, synthetic, small molecule tubulin inhibitors with strong antiproliferative activity. The lead structure of this class, **D-24203**, proved to be a potent inhibitor of *in vivo* tumor growth in different xenograft models including mammary and renal cancers. As part of our efforts in the lead optimization process to expand structural diversity as well as to optimize bioavailability parameters such as solubility and metabolic stability for these compounds, we produced and evaluated a focused library containing 320 compounds. Five new heterocyclic compound classes with comparable activity properties in the cytotoxicity and tubulin polymerization assay could be identified. *In silico* calculated bioavailability parameters for selected library members provides new compound classes with improved solubility properties.
Library design, development of adequate solution phase methodology, and synthesis will be presented, as well as results of lead optimization.

Keywords: Ketopiperazides; Focused library; Automated synthesis; Cytotoxic activity; Tubulin polymerization inhibitor

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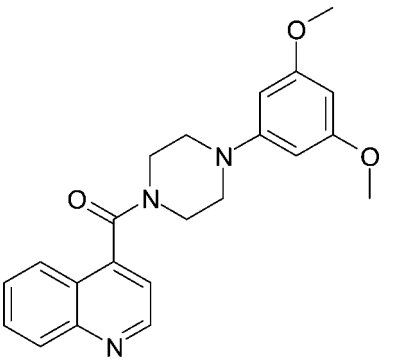
Introduction

General

The class of substituted aryl and heteroaryl piperazinyl carbonyl compounds has long been known to exhibit interesting biological activity. The activity profile of various heterocyclic derivatives with a carbonylpiperazidic side chain encompasses antispasmolytic [1], antihypertensive [2], antipyretic [3], antiinflammatoric [4], and antitumor properties [5].

In the field of antitumor agents tubulin inhibitors have played a pivotal role during the last decades. Several tubulin affecting agents, natural product or small synthetic molecule inhibitors, are described in the literature [6]. An attack on the cytoskeleton and especially on the microtubule system is a common way to stop proliferating cells. By interfering with the natural dynamics of tubulin polymerization and depolymerization antimitotic agents inhibit cell proliferation by arresting dividing cells in the cell cycle. Drugs with antimitotic activity have been in clinical use for more than 20 years. These medicines are effective against breast, ovarian, and other wide-spread cancers. In search of new antitumor agents with tubulin inhibitor properties, we have recently found that certain substituted 1-phenyl-4-piperazinyl-carbonyl-substituted heterocyclic compounds possess potent cytotoxic properties [7].



D-24203

Figure 1. Lead structure.

Lead structure **D-24203** (Figure 1) has been identified by a cell-based high throughput screening assay on four tumor cell lines (SKOV3, SF268, KB/HeLa, NCI-H460) on account of its very high cytotoxic activity. With regard to the mode of action it was found that the lead structure acts as a tubulin interacting agent which inhibits polymerization with an IC_{50} value of 1.6 μM for tubulin with 30% MAP (microtubule associated proteine). In contrast to the antitumor agents paclitaxel and vincristine this new inhibitor of microtubule forma-

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Full Paper

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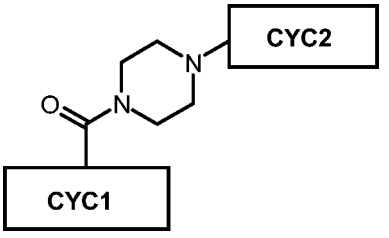

Figure 2. Generalized scaffold with two points of variation, CYC1 and CYC2.

tion is not a substrate for phosphoglycoproteine pgp170, and, consequently, retains the antitumoral efficacy in cell lines with multi drug resistance (MDR) phenotypes.

A very important part of our chemical optimization process is the improvement of biological activity and bioavailability parameters of **D-24203** such as solubility. To achieve this goal, we have designed, synthesized, and tested a compound library based on the ketopiperazide scaffold with a variety of substituents in position CYC1 and CYC2 (Figure 2).

Library design

Library design was directed by a "focused library approach". In order to define suitable substituents for the CYC1 and CYC2 positions, docking experiments of a variety of compounds on tubulin were carried out, in which either the CYC1 or CYC2 substituent of Figure 2 was varied while the other substituent was held constant.

Experimental data [7] suggest that piperazinyl-carbonyl substituted heterocycles bind at the colchicine binding site of αβ-tubulin. Bai et al. [8] indicated that this binding site is situated near the Cys239 and Cys354 residues. Recently, Ravelli et al. [9] solved the crystal structure of the β-tubulin-stathmin complex and showed that the colchicine binding site is situated at the interface between α- and β-tubulin and, indeed, in the vicinity of the above mentioned cysteine residues. We have analyzed the x-ray crystal structure of αβ-tubulin as determined by Löw et al. (Brookhaven code 1JFF) [10] and the structure of the colchicines-tubulin-stathmin complex (Brookhaven code 1SA0, 1SA1) [10] for possible common interaction sites of colchicine and piperazinyl-carbonyl substituted heterocycles, and could define a putative binding pocket for our compounds situated in the vicinity of the cysteine residues of β-tubulin. A virtual library containing appropriate aryl-/heteroaryl-scaffolds (CYC1) and piperazine residues (CYC2) was constructed with SYBYL [11] taking into account availability of reagents as well as calculated physicochemical parameters such as solubility, CNS activity, logP, etc. calculated with the programme QikProp [12]. The structures were initially optimized using the AM1 hamiltonian [13], and were subsequently docked into the putative binding site of β-tubulin by using the standard default settings of the program GOLD [14]. The GoldScore function was used for ranking the results, however, the conformations used in analyzing the results were not always the ones having the highest score, as it is well known that docking algorithms supply well fitted structures in a binding cavity which, however, do not necessarily correlate with biological function. The resulting receptor-ligand complexes were energy minimized with the MMFF94 force-field [11].

The "best" docked conformation of **D-24203** was used as a guide in selecting further potential binders. Since we knew that **D-24203** exhibited good biological activity we expected that compounds which bind in a similar manner would also show equivalent activity. We have thus established the following criteria for defining good binders: firstly, similarity of binding conformation and mode to that of **D-24203**; secondly, steric hindrance within the receptor pocket; thirdly, ability to build hydrogen bonds to receptor residues; fourthly, fitness parameter; and finally, protein-ligand interaction energy after energy minimization.

The results from docking experiments were used to select "interesting" compounds. Based on this procedure, suitable starting materials were initially evaluated. In parallel, reaction parameters of the chemical synthesis were optimized and scope and limitations within the selected group of substituents were explored. Additional filters such as commercial availability and contribution to diversity were subsequently used for the selection of starting materials. Sixteen carboxylic acids with aryl- or heteroaryl-scaffolds (for example acridines, anthracenes, quinolines, indoles, pyridines, etc.) and 20 piperazine derivatives with diverse substitution patterns (for example alkyl, aryl, or heteroaryl residues) were either purchased from commercial suppliers or synthesized according to literature procedures [15].

Library synthesis

The general reaction sequence for the production of the library is shown in Scheme 1.

In preparation for the automated library synthesis an investigation of suitable reaction parameters was carried out. The parameters for the amidation reaction were optimized by taking quinoline-4-carboxylic acid

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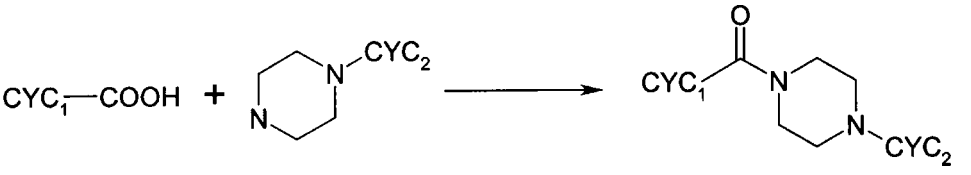

Scheme 1. General reaction sequence.

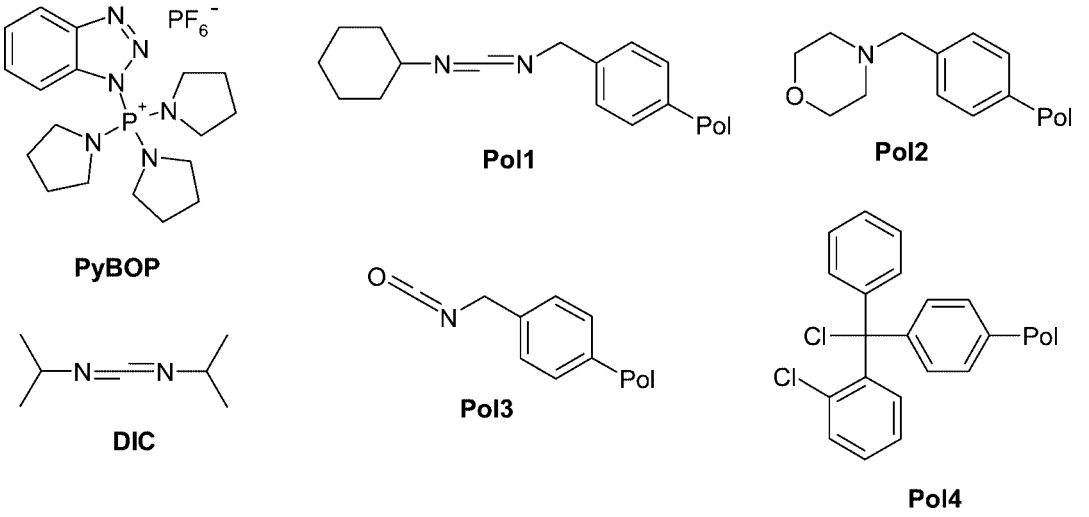

Figure 3. Used reagents and resin-bound scavengers.

and 1-(3-,5-dimethoxyphenyl)-piperazine as model reactants. PyBOP, DIC, and resin-bound DCC (**Pol1**) were tested as coupling reagents together with free and resin-bound N-methylmorpholine (**Pol2**) as base (Figure 3). To remove excess amine or acid, resin-bound phenyl isocyanate (**Pol3**) or o-chloro trityl (**Pol4**) were examined as scavengers.

The best reaction conditions turned out to the following:

1.0 eq quinoline-4-carboxylic acid, 0.8 eq of 1-(3-,5-dimethoxyphenyl)-piperazine and 1.6 eq resin-bound DCC in dichloromethane as solvent yielded approx. 70% of the corresponding product at ambient temperature after 24 h. The UV purity according to HPLC/MS was approx. 80%. Scope and limitations of this set of reaction parameters were examined by performing another 20 reactions with a variety of piperazine and acid derivatives. The desired products could be isolated in good purities for 18 examples, albeit sometimes in rather moderate yields. However, enough material could be obtained for determination of cytotoxic activity. The results were encouraging and led to the synthesis of the entire library.

Library synthesis was performed on a Chemspeed ASW 2000 Synthesizer (Figure 4).

In four runs, each of 80 reactions, overall 320 conversions were performed in two weeks. The conversion of the reactions was detected by HPLC/MS analysis.

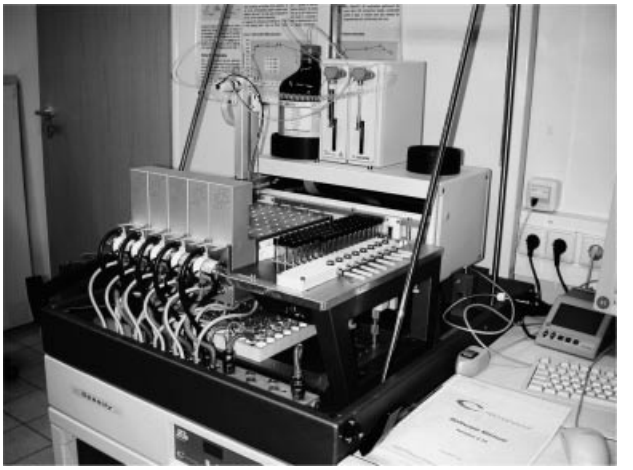

Figure 4. Chemspeed ASW 2000 Synthesizer.

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Table 1. List of the obtained compound numbers per run.

	Run 1 (COOH1-8, PIP1-10)	Run 2 (COOH8-16, PIP1-10)	Run 3 (COOH1-8, PIP11-20)	Run 4 (COOH8-16, PIP11-20)	Sum	Action
>85% UV-purity	0	23	30	10	63	Compound accepted
>20–85% UV-purity	52	49	47	57	205	Mixture for purification
>0–20% UV-purity	26	2	2	11	41	Residue not accepted
0% UV-purity	2	6	1	2	11	No reaction
Sum	80	80	80	80	320	

Reaction mixtures containing at least 20% of the desired product were selected for purification by chromatography.

The numbers of pure compounds, mixtures for purification and rejected residues are given in Table 1.

Purification of the appropriate raw products was achieved by means of parallel flash chromatography on a CombiFlash Optix 10 System (Isco Inc., USA). 155 out of 205 purified mixtures were obtained with a degree of purity higher than 85%. In total, 218 compounds out of 320 reactions (68%) were obtained in a highly automated production process in approximately one month (Figure 5). Between 1 and 50 mg of product were isolated, which was sufficient for biological testing.

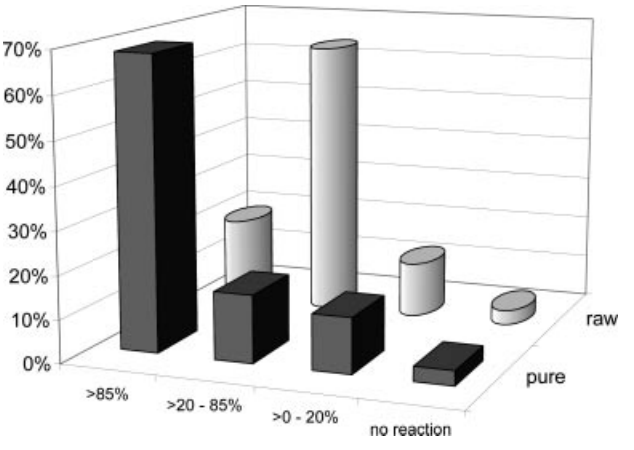

Figure 5. Overview of the purity of obtained compounds before and after purification.

Results and discussion

After registration in the corporate database, compounds were re-dissolved in dimethylsulfoxide and distributed to assay-ready microtiter plates (MTPs) for biological testing. The compounds were initially tested for cytotoxic activity in an XTT assay. Four tumor cell lines were used in the cell-based HTS assay, namely SKOV3 (human ovarian carcinoma), SF268 (CNS cancer, glioma), KB/HeLa (human cervix carcinoma), and NCI-H460 (non-small cell lung cancer). For selected compounds the results of the *in vitro* cytotoxic screening are given in Table 2.

The *in vitro* cytotoxic screening experiments clearly showed that several of the investigated aryl and heteroaryl scaffolds with ketopiperazide side chains exhibited remarkably high activity in the XTT assay. In particular, the compounds **1**–**4** out of the acridine structural class possess higher cytotoxic activity as the lead structure **D-24203**.

In general, compounds with high cytotoxic potency were further examined for inhibition of tubulin polymerization and competition of [^{3}H]-colchicine binding at the tubulin binding site (Table 3).

The results of the second screening indicate that specific variations of the substitution pattern lead to compounds with high cytotoxicity and inhibitory activity on tubulin polymerization. In the tubulin polymerization assay acridine derivatives showed outstanding inhibition values in comparison to **D-24203**. Also in the colchicines competition assay for this type of compounds lower IC_{50} values as for the lead structure **D-24203** could be found, suggesting a stronger binding affinity to the colchicine binding site.

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Table 2. Cytotoxic activity of selected compounds against a panel of tumor cell lines; XTT (Sodium 3′-[1-(phenylamino-carbonyl)-3,4-tetrazolium]-bis(4-methoxy-6-nitro)benzene sulfonic acid hydrate).

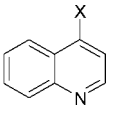
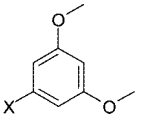
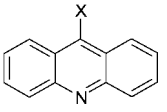
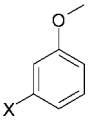
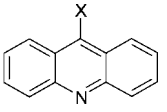
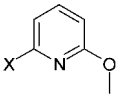
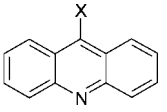
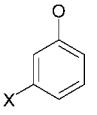
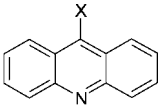
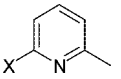
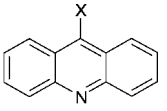
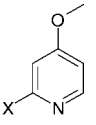
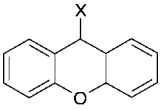
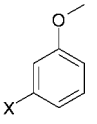
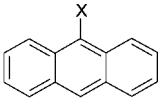
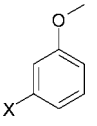
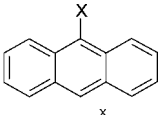
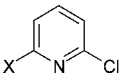
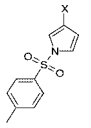
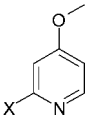
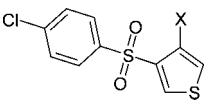
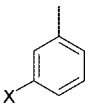
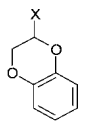
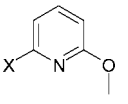
No	CYC1	CYC2	XTT-Assay; Cytotoxic Activity IC_{50} [μM] KB/HeLa	SKOV3	SF268	NCI-H460
D-24203			0.08	0.03	0.09	0.02
1			0.01	0.02	0.01	0.02
2			0.02	0.02	0.03	0.03
3			0.03	0.02	0.03	0.02
4			0.03	0.02	0.04	0.03
5			0.3	0.11	0.2	0.17
6			1.57	0.73	1.02	0.72
7			0.14	0.13	0.15	0.26
8			1.14	0.66	0.74	0.7
9			0.25	0.1	0.12	0.23
10			0.71	0.38	0.42	0.62
11			1.93	1.24	1.3	2.39
paclitaxel	–	–	0.01	0.01	0.01	0.01
colchicine	–	–	0.03	0.05	0.05	0.07

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Table 3. Biological activity of selected compounds in a tubulin polymerization and a colchicine competition assay (n.c.: no competition; n.d.: not determined; Solubility: −: <1 μg/mL; ○: 1 − 5 μg/mL; +: >5 μg/mL)

Compound	Tubulin-Polymerization IC_{50} [μM]	Competition Binding [H^3H]-colchicine IC_{50} [μM]	Calculated Solubility (QikProp)
D-24203	1.6	2.03	○
1	1.6	0.59	−
2	1.57	1.03	−
3	1.58	0.76	−
4	1.58	0.91	−
5	6.37	n.d.	−
6	1.83	2.43	○
7	5.59	4.51	−
8	6.65	n.d.	−
9	>10	n.d.	+
19	>10	n.d.	−
11	>10	n.d.	+
paclitaxel	>10	n.c.	○
colchicine	2.6	1.26	+

Table 4. Cytotoxic activity of selected library members against wildtype and MDR cancer cell lines in comparison to **D-24203** and reference compounds [LT12 (leukemia, rat); L1210 (leukemia, mouse); P388 (leukemia, mouse)].

	XTT-Assay; Cytotoxic Activity (IC_{50} [μM)]					
Compound	LT12	LT12MDR	L1210	L1210VCR	P388	P388ADR
1	0.02	0.04	0.03	0.03	0.01	0.02
4	0.04	0.07	0.05	0.03	0.03	0.05
7	0.32	0.26	0.48	0.37	0.28	0.25
9	0.16	0.16	0.25	0.24	0.12	0.16
D-24203	0.04	0.05	0.05	0.06	0.05	0.05
paclitaxel	0.01	0.4	0.06	>5	0.04	>5
vincristine	0.002	0.134	0.02	>5	0.004	0.93

Furthermore, compound **6**, a xanthene derivative, showed promising results in the tubulin polymerization assay comparable to lead structure **D-24203**. In addition, the calculated solubility data for this class of compounds (Table 3) are in the same range as for **D-24203**. These data allow the assumption that this compound class possesses similar water solubility and bioavailability.

Selected compounds of the synthesized library, that showed high activity in the first *in vitro* cytotoxicity screening, were tested in multi drug resistant (MDR) cell lines for further characterization (Table 4).

Comparison with lead structure **D-24203** and reference compounds paclitaxel and colchicine revealed similar *in vitro* profiles for the shown compounds. These results afford information about alternative heterocyclic scaffolds and substitution patterns with cytotoxic properties against resistant tumor cell lines comparable to the quinolines.

In conclusion, based on modelling results as well as on calculated physico-chemical properties and information about synthetic usability, 16 carboxylic acids and 20 piperazine derivatives were selected as suitable starting materials for the production of a focused

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library. In a highly automated process, 320 reactions yielded 218 compounds in milligram scale with UV-purities higher than 85 %. Cytotoxicity and tubulin polymerization assays revealed novel compound classes with improved or at least comparable *in vitro* activity profiles. Furthermore, novel active compounds were found with a calculated solubility similar to **D-24203**. We expect that these selected compounds will also have good water solubility and bioavailability properties.

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Experimental

Thin-layer chromatography (TLC) was performed on Silical gel plates (Merck F_{254}, Merck, Darmstadt, Germany). Visualization of spots was accomplished by UV-illumination at 254 nm. Chromatographic separations were performed on silica gel (Merck Kieselgel 60) or on a parallel flash chromatography system (CombiFlash Optix 10) with RediSep™ silicagel flash columns (Isco Inc., USA, http://www.combichemlab.com). HPLC/MS analyses using a HP1100 with UV/VIS and MS detectors (FAB method, M+1, Agilent) and a Gilson pipettor were performed by AQura GmbH, Analytical Solutions, Hanau, Germany.

Nuclear magnetic resonance (NMR) spectra were recorded on a DRX 600 instrument (Bruker, Karlsruhe, Germany), using d_6-dimethylsulfoxide as solvent and tetramethylsilane as internal standard. NMR signals are reported in ppm on a δ scale.

The optimization of reaction conditions for parallel synthesis was carried out on Bohdan Miniblocks (Mettler-Toledo, Giessen, Germany). Scavenger resins for work-up procedures were purchased from Merck Biosciences (Schwalbach, Germany). Chemicals and solvents were obtained from commercial sources.

For aliquotation of compounds to assay MTP's a Zinsser aliquotation robot was used and the biological testing was performed on a Biomek 2000 system (Beckman Coulter Inc., Fullerton CA, USA).

Tumor cell lines

The various human and rat tumor cell lines were acquired from commercial sources. The murine cell lines L1210 and L1210 VCR [16] were prepared as published. Rat LT12 cells and the LT12/MDR subline [17] were provided by Dr. Nooter (Univ. Hosp. Rotterdam, NL). Cell lines were cultivated as published.

Cytotoxicity assays

A MTP-based screening for cytotoxic agents is routinely done on a Biomek 2000 robotic system. Test compounds at final concentrations of 1 mg/mL or 10mM in DMSO, were added to the tumor cells and incubated for 48 h. The XTT assay was used to determine proliferation [18]. IC_{50}-values were obtained by nonlinear regression (GraphPad Prism; GraphPad Software, San Diego, CA, USA). The cytotoxicity of selected compounds against these cells with and without induction of $p27^{kip1}$ was determined in an XTT assay [11].

Tubulin polymerization assay

A modified tubulin polymerization assay according to [19] was used. Lyophilized bovine brain tubulin (*ML113*−MAP rich or *TL238*−MAP free, Cytoskeleton, Denver, CO, USA) was reconstituted according to the manufacturer's protocol. Tubulin heterodimers (*ML113*−2 mg/mL, *TL238*−5 mg/mL) were incubated with different compounds in 96-well half area plates (Nunc, Roskilde, Denmark). The tubulin polymerization was determined at 340 nm in a Spectramax 190 plate reader (Molecular Devices, Sunnyvale, CA, USA). The areas under the curves were used to calculate the inhibition of tubulin polymerization as compared to controls by nonlinear regression (GraphPad Prism).

^{3}H colchicine competition-binding assay

The [^{3}H]-colchicine competition assay was performed according to [20]. [^{3}H]-colchicine was diluted and biotin-labeled tubulin (*T333*, Cytoskeleton) was reconstituted according to the manufacturer's protocol. The diluted compounds and the [^{3}H]-colchicine were transferred to a 96-well isoplate (PE-Wallac, Boston, MA, USA), buffer and the reconstituted biotin-labeled tubulin were added. After incubation, streptavidin-coated yttrium SPA beads (Amersham Pharmacia Biotech, Piscataway, NJ, USA) were added and the bound radioactivity was determined using a MicroBeta Trilux Microplate scintillation counter (PE-Wallac). IC_{50}-values were obtained by nonlinear regression (GraphPad Prism).

General procedure for automated synthesis

A Chemspeed ASW 2000 synthesizer with five reactor blocks containing 16 reaction vessels (13 mL of usable volume) was used for the automated synthesis. 0.4 mmol N-cyclohexylcarbodiimide bound to N'-methyl polystyrene HL (200-400 mesh) were placed in the reaction vessels. The integrated Gilson pipettor added 0.25 mmol (2.5 mL of 0.1 mol/L solution in dichloromethane) carboxylic acid and the mixture was vortexed for 30 min at room temperature. 0.20 mmol (1.0 mL of 0.2 mol/L solution in dichloromethane) piperazine were added and the mixture was again vortexed for 16 h at room temperature. The reactions were monitored by TLC performed automatically by the Gilson pipettor. The resin was separated by filtration. An aliquot of the filtrate was taken for HPLC/MS analysis and kept in a bar-coded vial. The filtrates were concentrated in an evaporator (Genevac HT4) and the residues stored in bar-coded vials. Chromatographic separations of the reaction mixtures with positive HPLC/MS analysis were carried out on a parallel flash chromatography purification system CombiFlash Optix 10.

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*Acridin-9-yl-[4-(3-methoxy-phenyl)-piperazin-1-yl]-methanone (**1**)*

Yield: 44 mg (56%); ^{1}H-NMR (DMSO-d_6) δ = 2.95 (m, 2H), 3.08–3.13 (m, 2H), 3.45–3.49 (m, 2H), 3.69 (s, 3H), 4.08–4.12 (m, 2H), 6.38 (d, 1H), 6.43 (s, 1H), 6.48 (d, 1H), 7.09 (t, 1H), 7.68 (t, 2H), 7.87 (t, 2H), 7.96 (d, 2H), 8.25 (d, 2H) ppm. MS m/z: 398.1

*Acridin-9-yl-[4-(6-methoxy-pyridin-2-yl)-piperazin-1-yl]-methanone (**2**)*

Yield: 42 mg (42%); ^{1}H-NMR (DMSO-d_6) δ = 3.07–3.13 (m, 2H), 3.25–3.33 (m, 2H), 3.74 (s, 3H), 3.79–3.86 (m, 2H), 4.05–4.12 (m, 2H), 6.08 (d, 1H, *J* = 7.9 Hz), 6.32 (d, 1H, *J* = 7.9 Hz), 7.46 (t, 1H, *J* = 7.9 Hz), 7.70 (t, 2H, *J* = 7.5 Hz), 7.92 (t, 2H, *J* = 7.3 Hz), 7.99 (d, 2H, *J* = 8.4 Hz), 8.25 (d, 2H, *J* = 8.8 Hz) ppm. MS m/z: 399.1

*Acridin-9-yl-[4-(3-hydroxy-phenyl)-piperazin-1-yl]-methanone (**3**)*

Yield: 11 mg (12%); ^{1}H-NMR (DMSO-d_6) δ = 2.87–2.90 (m, 2H), 3.09–3.12 (m, 2H), 3.38–3.41 (m, 2H), 4.08–4.11 (m, 2H), 6.24 (d, 1H, *J* = 7.9 Hz), 6.29 (s, 1H), 6.36 (d, 1H, *J* = 7.9 Hz), 6.98 (t, 1H, *J* = 8.2 Hz), 7.71 (t, 2H, *J* = 7.5 Hz), 7.92 (t, 2H, *J* = 7.3 Hz), 7.98 (d, 2H, *J* = 8.4 Hz), 8.24 (d, 2H, *J* = 8.8 Hz), 9.18 (s, 1H), ppm. MS m/z: 384.1

*Acridin-9-yl-[4-(6-methyl-pyridin-2-yl)-piperazin-1-yl]-methanone (**4**)*

Yield: 17 mg (18%); ^{1}H-NMR (DMSO-d_6) δ = 3.03–3.13 (m, 2H), 3.28–3.30 (m, 2H), 3.33 (m, 3H), 3.80–3.83 (m, 2H), 4.06–4.09 (m, 2H), 6.55 (d, 1H, *J* = 7.3 Hz), 6.60 (d, 1H, *J* = 8.4 Hz), 7.44 (t, 1H, *J* = 7.9 Hz), 7.70 (t, 2H, *J* = 7.5 Hz), 7.92 (t, 2H, *J* = 7.3 Hz), 7.99 (d, 2H, *J* = 8.4 Hz), 8.24 (d, 2H, *J* = 8.8 Hz) ppm. MS m/z: 383.1

*Acridin-9-yl-[4-(4-methoxy-pyridin-2-yl)-piperazin-1-yl]-methanone (**5**)*

Yield: 50 mg (50%); ^{1}H-NMR (DMSO-d_6) δ = 3.03–3.13 (m, 2H), 3.28–3.30 (m, 2H), 3.77 (s, 3H), 3.80–3.83 (m, 2H), 4.05–4.09 (m, 2H), 6.26–6.40 (m, 3H, *J* = 7.3 Hz), 7.70 (t, 1H, *J* = 7.6 Hz), 7.89–7.95 (m, 3H), 7.99 (d, 2H, *J* = 8.4 Hz), 8.24 (d, 2H, *J* = 8.8 Hz) ppm. MS m/z: 399.1

*[4-(3-Methoxy-phenyl)-piperazin-1-yl]-(9H-xanthen-9-yl)-methanone (**6**)*

Yield: 0.5 mg (0.5%); ^{1}H-NMR (DMSO-d_6) δ = 3.08–3.10 (m, 2H), 3.18–3.20 (m, 2H), 3.59–3.61 (m, 2H), 3.73 (s, 3H), 4.05–4.07 (m, 2H), 5.74 (s, 1H), 6.42 (d, 1H, *J* = 8.1 Hz), 6.50 (s, 1H), 6.56 (d, 1H, *J* = 8.1 Hz), 7.08–7.17 (m, 5H), 7.23 (d, 2H, *J* = 7.7 Hz), 7.30 (t, 2H, *J* = 7.9 Hz) ppm. MS m/z: 401.1

*Anthracen-9-yl-[4-(3-methoxy-phenyl)-piperazin-1-yl]-methanone (**7**)*

Yield: 9.1 mg (9%); ^{1}H-NMR (DMSO-d_6) δ = 2.90–2.94 (m, 2H), 3.07–3.11 (m, 2H), 3.42–3.46 (m, 2H), 3.69 (s, 3H), 4.08–4.12 (m, 2H), 6.39 (d, 1H, *J* = 8.2 Hz), 6.44 (s, 1H), 6.51 (d, 1H, *J* = 8.2 Hz), 7.10 (t, 1H, *J* = 8.2 Hz), 7.55–7.64 (m, 4H), 7.90 (d, 2H, *J* = 8.7 Hz), 8.18 (d, 2H, *J* = 7.7 Hz), 8.71 (s, 1H) ppm. MS m/z: 397.1

*Anthracen-9-yl-[4-(6-chloro-pyridin-2-yl)-piperazin-1-yl]-methanone (**8**)*

Yield: 11 mg (11%); ^{1}H-NMR (DMSO-d_6) δ = 3.03–3.07 (m, 2H), 3.26–3.30 (m, 2H), 3.81–3.85 (m, 2H), 4.06–4.10 (m, 2H), 6.70 (d, 1H, *J* = 7.7 Hz), 6.77 (d, 1H, *J* = 8.4 Hz), 7.53–7.62 (m, 5H), 7.91 (d, 2H, *J* = 8.1 Hz), 8.18 (d, 2H, *J* = 8.4 Hz), 8.72 (s, 1H) ppm. MS m/z: 403.1

*[4-(4-Methoxy-pyridin-2-yl)-piperazin-1-yl]-[1-(toluene-4-sulfonyl)-1H-pyrrol-3-yl]-methanone (**9**)*

Yield: 14.1 mg (13%); ^{1}H-NMR (DMSO-d_6) δ = 2.41 (s, 3H), 3.50–3.58 (m, 4H), 3.60–3.68 (m, 4H), 3.79 (s, 3H), 6.29–6.34 (m, 2H), 6.51–6.54 (m, 1H), 7.41 (t, 1H, *J* = 2.8 Hz), 7.49 (d, 2H, *J* = 8.1 Hz), 7.66 (t, 1H, *J* = 2.0 Hz), 7.92–7.97 (m, 3H) ppm. MS m/z: 441.1

*[4-(4-Chloro-benzenesulfonyl)-thiophen-3-yl]-(4-m-tolyl-piperazin-1-yl)-methanone (**10**)*

Yield: 27.7 mg (24%); ^{1}H-NMR (DMSO-d_6) δ = 2.26 (s, 3H), 3.07–3.11 (m, 2H), 3.20–3.23 (m, 2H), 3.27–3.31 (m, 2H), 3.71–3.75 (m, 2H), 6.64 (d, 1H, *J* = 7.3 Hz), 6.75 (d, 1H, *J* = 8.2 Hz), 6.78 (s, 1H), 7.10 (t, 1H, *J* = 7.8 Hz), 7.71 (d, 2H, *J* = 8.8 Hz), 7.86 (s, 1H), 7.96 (d, 2H, *J* = 8.7 Hz), 8.68 (s, 1H) ppm. MS m/z: 462.1

*(2,3-Dihydro-benzo[1,4]dioxin-2-yl)-[4-(6-methoxy-pyridin-2-yl)-piperazin-1-yl]-methanone (**11**)*

Yield: 9.5 mg (11%); ^{1}H-NMR (DMSO-d_6) δ = 3.41–3.78 (m, 8H), 3.79 (s, 3H), 4.25 (dd, 1H, *J* = 11.8/6.5 Hz), 4.41 (d, 1H, *J* = 11.8 Hz), 5.29 (d, 1H, *J* = 6.5 Hz), 6.09 (d, 1H, *J* = 7.8 Hz), 6.36 (d, 1H, *J* = 8.1 Hz), 6.83–6.89 (m, 2H), 6.91–6.95 (m, 1H), 7.48 (t, 1H, *J* = 7.9 Hz) ppm. MS m/z: 356.1

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