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Synthesis and structure–activity relationships of *N*-aryl(indol-3-yl)glyoxamides as antitumor agents

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ABSTRACT

The synthesis and study of the structure–activity relationships of cytotoxic compounds based on *N*-pyridinyl or *N*-aryl-2-(1-benzylindol-3-yl)glyoxamide skeleton, represented by the lead structures **D-24241** and **D-24851**, are described. The presence of *N*-(pyridin-4-yl) moiety was crucial for activity and 2-[1-(4-chloro-3-nitrobenzyl)-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-4-yl)acetamide (**55**), the most potent derivative, showed IC_{50} = 39 nM, 51 nM and 11 nM against HeLa/KB (human cervix carcinoma), L1210 (murine leukemia) and SKOV3 (human ovarian carcinoma) cell lines proliferation assay, respectively, as active as the lead compounds.

1. Introduction

Microtubules are involved in a wide number of cellular functions including motility, process of separating duplicated chromosomes before cell division, shape maintenance and transport of vesicles. Microtubules are highly dynamic polymers of heterodimers of α and β tubulin.[1] Tubulin-binding molecules interfere with the dynamic instability of microtubules and thereby disrupt microtubules inducing cell cycle arrest in the M-phase, forming abnormal mitotic spindles, and finally leading to apoptotic cell death. Therefore the crucial involvement of microtubules in mitosis makes them an important target for anticancer drugs. A large number of chemically diverse substances has been identified to interfere with the tubulin system.[2] Among these antimitotic molecules, the Vinca alkaloids exemplified by vincristine, vinblastine, vindesine, and vinorelbine or the taxanes from natural sources exemplified by paclitaxel and docetaxel, are widely used in the clinic in monotherapy or in combinations to the treatment of cancer. But toxicity, development of drug resistance, complex galenic formulations due to the lack of oral bioavailability show the necessity to develop new antimitotic agents without the cited drawbacks.

Several small synthetic molecules with indole nucleus as core structure, that act as tubulin inhibitors, have been identified over the past few years.[3] These include 2-aroylindoles (**1**),[4] 3-aroylindoles (**2**),[5] 3-formyl-2-phenylindoles (**3**),[6] 2,3-diarylindoles (**4**),[7] 2-aryl-3-aroylindoles (**5**),[7] arylthioindoles (**6**)[8] and (indol-3-yl)glyoxamides (**7**)[9] (Fig. 1).

D-24241 and analogs were previously reported as antiasthmatic, antiallergic and immunosuppressant/immunomodulating agents[10] but results from the cellular cytotoxicity screen suggested their interest as potent antitumor agents.[9d–f]

(Indol-3-yl)glyoxamide **D-24851**[9b,c] (Fig. 1), known as indibulin,[11] was described as novel synthetic anticancer agent with significant antitumoral activity targeting the tubulin system. **D-24851** destabilizes microtubules via a direct interaction with tubulin at a binding site distinct from those of the known destabilizing tubulin agents vincristine or colchicine. **D-24851** blocks cell cycle transition specifically at G2/M-phase and leads to subsequent cell death. Its oral bioavailability, its potent in vitro and in vivo antitumoral activity, its lack of neurotoxicity, and efficacy against multidrug resistant tumors make it a promising drug for cancer therapy. **D-24851** is currently in phase I clinical trials.

The aim of the present study was the identification of the minimum structural requirements of this class of (indol-3-yl)glyoxamides (**7**) to exhibit potent activity against cancer cells, based on lead structures **D-24241** and **D-24851** (Fig. 1). The *N*-1 indole position is mainly substituted by various benzyl groups and we show the favorable incidence of the presence of the *N*-(pyridin-4-yl) moiety on the glyoxamide side chain. Initial structure–activity relationships for antitumor activity will be established.

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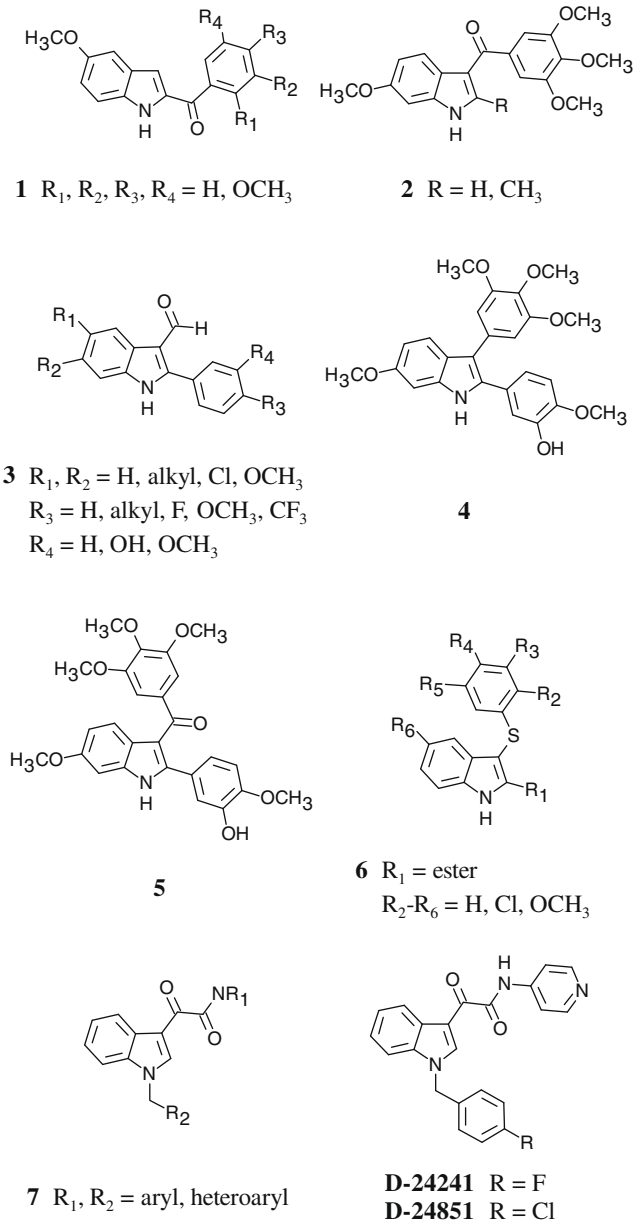
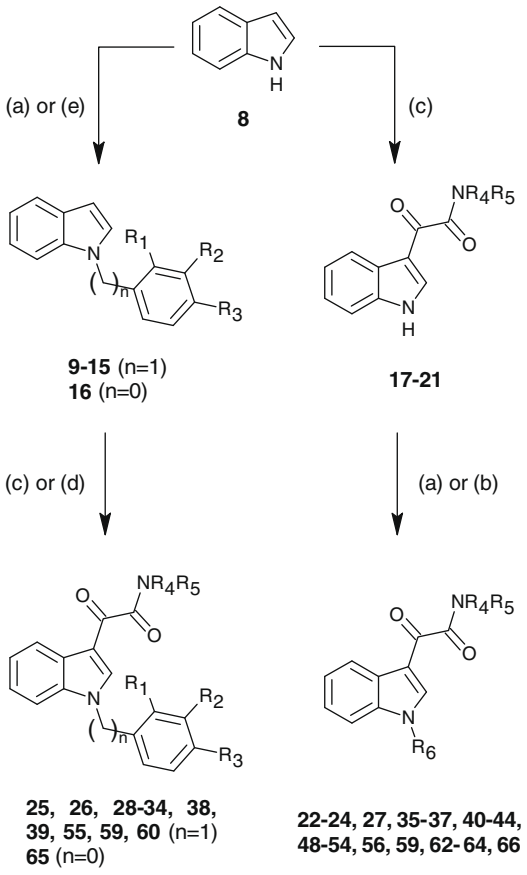


Figure 1. Indole derivatives as inhibitors of tubulin polymerization.

2. Chemistry

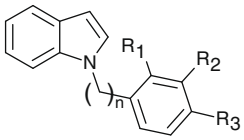
The general method for the synthesis of *N*-substituted (indol-3-yl)gloxamides **22–66** is depicted in Scheme 1. *N*-Benzylindoles **9–14** (Table 1) were synthesized, in moderate to good yields, by heating indole 1 with various benzyl halides in the presence of sodium hydride in anhydrous DMSO (method A).[12] The benzyl halides were commercially available or prepared starting from the desired benzyl alcohols or toluene derivatives after a bromination step involving phosphorus tribromide[13] or *N*-bromosuccinimide,[14] respectively. Surprisingly, we did not succeed in obtaining compound **15** (Table 1), only degradation of the reaction mixture was observed.

The glyoxamide group was then introduced into the 3-position of the indole ring by reaction with oxalyl chloride in diethyl ether,[15] followed by treatment with the suitable amines in THF affording compounds **25**, **26**, **28–34**, **38**, **39**, **55**, **59**, **60** and **65** (Tables 3 and 4) in 29–92% yields. For the last step the amine, was added in large excess (route C), or in stoichiometric conditions in the presence of a base, namely triethylamine (route D).



Scheme 1. Reagents: (a) benzyl halide, NaH, DMSO; (b) benzyl halide or 4-fluorobenzoyl chloride (for **66**), K_2CO_3, acetone; (c) (1) oxalyl chloride, Et_2O, N_2 (2) HNR_4R_5 (2.1 equiv), THF, N_2; (d) (1) oxalyl chloride, Et_2O, N_2 (2) HNR_4R_5 (1.2 equiv), Et_3N (1.2 equiv), THF, N_2; (e) 4-fluoroiodobenzene, Cu, K_2CO_3, DMF (for **16**).

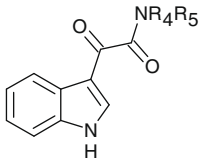
Table 1
N-Benzyl-1*H*-indole intermediates **9**–**16**

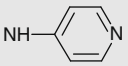
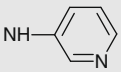
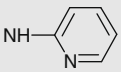
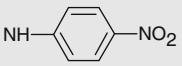
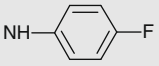


Compound	*n*	R_1	R_2	R_3	Route
9	1	H	H	F	A
10	1	H	F	H	A
11	1	F	H	H	A
12	1	H	OCH_3	Cl	A
13	1	OCH_3	H	Cl	A
14	1	H	NO_2	Cl	A
15	1	NO_2	H	Cl	A (failure)
16	0	H	H	F	—

Compounds **22–24**, **27**, **35–37**, **40–44**, **48–54**, **56**, **59**, **62–64** and **66** (Tables 3 and 4) were prepared by inverting the reaction sequence (Scheme 1). Indole **8** was reacted with oxalyl chloride and amines prior to alkylation of indole nitrogen. As shown in Scheme 1, the 3-glyoxamide functionality already in place (compounds **17–21**, Table 2) enhanced the hydrogen lability at the level of the nitrogen and alkylation step was carried out not only with the couple NaH/DMSO (route A) at room temperature but also with K_2CO_3 in refluxing acetone (route B). We succeeded in improving the yield for the obtention of compound **59** (33–53%) using the second

Table 2
Synthetic route of 2-(1*H*-indol-3-yl)glyoxamide intermediates **17**–**21** unsubstituted at position 1 of the indole ring



Compound	NR_4R_5	Route
17		C
18		C
19		C
20		C
21		C

synthetic route (acylation–benzylation) instead of the first one (benzylation–acylation).

2-[1-(4-Fluorophenyl)-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-4-yl)acetamide **65** was prepared, in the first step, by Ullmann-type coupling[16] of 4-fluoroiodobenzene with indole **8** in the presence of copper-catalyst and K_2CO_3 in refluxing DMF, followed by the introduction of the glyoxamide chain on 1-(4-fluorophenyl)-1*H*-indole **16** (Table 1) as previously described.

2-[1-(4-Fluorobenzoyl)-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-4-yl) acetamide **66** was obtained by usual *N*-acylation of 2-(1*H*-indol-3-yl)-2-oxo-*N*-(pyridin-4-yl) acetamide **17** with K_2CO_3 and 4-fluorobenzoyl chloride in acetone.

Boron tribromide demethylation[17] of **44** and **59** gave the corresponding *N*-(hydroxybenzyl)indoles **45** and **61**, respectively (Scheme 2). Surprisingly, the deprotection failed with compound **60**, in the same experimental conditions only starting material was recovered.

2-[1-(4-Nitrobenzyl)-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-4-yl)acetamide **42** was reduced to the amine **46** by hydrogenation in dioxane over Raney nickel catalyst.[18] Acetylation of amine **46** were carried out by treatment with acetic anhydride[19] to furnish acetamide **47**.

Reduction of the *N*-(nitrobenzyl)-3-glyoxylindole derivatives **55** and **56** was achieved by tin(II) chloride in acidic medium[20] to afford compounds **57** and **58** in moderate yield.

3. Results and discussion

3.1. Anti-proliferative activity

The synthesized compounds **22**–**66** were evaluated for their cytotoxic activities against three cancer cell lines, namely HeLa/KB (human cervix carcinoma), L1210 (murine leukemia) and SKOV3 (human ovarian carcinoma) in a common proliferation assay (Tables 3 and 4).[21] The lead compound **D-24241** shows IC_{50} values in the range of 54 nM (KB), 455 nM (L1210) and 455 nM (SKOV3). Best compound is so far the clinical candidate **D-24851** with antiproliferative activity of 44 nM (KB), 44 nM (L1210) and 77 nM (SKOV3).

We first evaluated the effect of the replacement of the 4-aminopyridyl moiety on the glyoxamide chain for cytotoxic activity.

Shifting the position of the nitrogen on the pyridine from *para* (**D-24241**) to *meta* (**22**) or *ortho* (**23**) led to loss of activity suggesting that the 4-aminopyridine scaffold is crucial for the binding to the tubulin protein, as confirmed by James and co-workers.[22] Replacement of the pyridine heterocycle of **D-24241** by monosubstituted phenyl ring (cf. **24–28**, Table 3) or disubstituted phenyl ring (cf. **31–34**) produced inactive compounds.

We attempted the introduction of a piperazine ring in the place of the nitrogen of the glyoxamide function. This chemical modification produced only inactive compound (cf. **29**, Table 3).

We introduced a trimethoxy substitution pattern, a common structural feature of colchicine, combretastatin A-4, and other tubulin inhibitors (Fig. 1). Compound **30** (Table 3) displayed some activity; its IC_{50} value of 378 nM was comparable to that of the lead compound **D-24241** (IC_{50} = 455 nM) against L1210 cells. Nevertheless, 3,4,5-trimethoxyphenyl derivative **30** was seven to eight-fold less potent (IC_{50} = 378 nM and 3784 nM) than **D-24241** against KB and SKOV3 tumor cells, respectively.

As the trimethoxy group is present in most of tubulin inhibitors which target the colchicine binding site, unlike **D-24851**, it would be interesting to investigate compound **30** for colchicine competition assay to get better idea of its activity.

To evaluate the role of the indole nitrogen substitution pattern in *N*-(pyridin-4-yl)-2-(indol-3-yl)glyoxamide series, we synthesized various derivatives of the indole building block (cf. **17**, **35–66**, Table 4). The precursor compound **17** and unsubstituted benzyl (**35**) derivatives proved to be inactive.

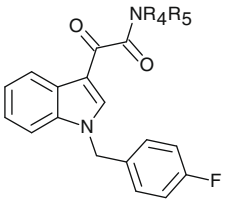
For the identification of the structural requirements for cytotoxic or antiproliferative activity, nature and position of the halogen on the benzyl group were changed first. The structure–activity relationship (SAR) information indicates that a chlorine located at the C-4 position (**D-24851**) results in the highest activity; 4-fluoro (**D-24241**) and 4-bromo (**40**) derivatives were 10-fold less potent. 3-Chloro derivative (**36**) was not active but it was possible to retain activity against L1210 or SKOV3 cells by substitution with a fluorine at the C-3 position (**38**) of the benzyl group.

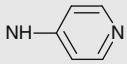
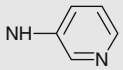
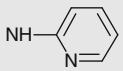
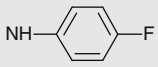
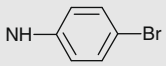
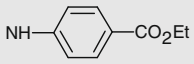
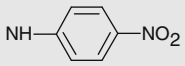
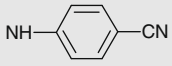
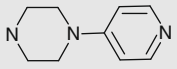
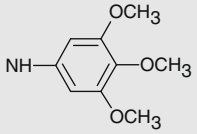
Shifting to the C-2 position (**37**, **39**, **41**) decreased the activity drastically. By comparing the effect of electron-donating or withdrawing groups at the benzyl-4-position, no clear influence could be noted. Except for compound **48** (CH_3) with IC_{50} values of 474 nM against the three tested cell lines, the introduction of nitro (**42**), trifluoromethyl (**43**), methoxy (**44**), hydroxy (**45**), amino (**46**), acetamide (**47**) or more bulky phenyl (**49**) furnished only inactive compounds.

Replacement of the benzyl moiety by a pyridine azaheterocycle did not improve the activity since (pyridin-4-yl)methyl (**50**), (pyridin-3-yl)methyl (**51**) and (pyridin-2-yl)methyl (**52**) derivatives showed no cytotoxic activity. For the benzyl disubstituted series, additional halogen keeping the chlorine in position 4 did not improve the antiproliferative effect (cf. **53**, **54**).

Interestingly, the introduction of a nitro group in *meta* position of the 4-chlorobenzyl substituent afforded a very active compound **55** whereas its analogue **56** in *ortho* position was inactive. 2-[1-(4-Chloro-3-nitrobenzyl)-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-4-yl)acetamide **55**, was as active as **D-24851** against the Hela/KB cells (IC_{50} = 39 nM) and L1210 cells (IC_{50} = 51 nM), and seven times more active than the reference against the SKOV3 cells (IC_{50} = 11 nM). Reducing the nitro group of compounds **55** and **56** gave access to amine derivatives **57** and **58**, respectively, with moderate cytotoxic activity (IC_{50}'s in 338–1213 nM range).

Table 3
Synthetic route and in vitro cytotoxicity of 2-[1-(4-fluorobenzyl)-1*H*-indol-3-yl]glyoxamide analogs **22**–**30**



Compound	NR_4R_5	Route	Hela/KB[a] IC_{50} (nM ± SD[b])	L1210[a] IC_{50} (nM ± SD[b])	SKOV3[a] IC_{50} (nM ± SD[b])
D-24241			54 ± 6	455 ± 22	455 ± 32
22		B	4687 ± 490	4687 ± 520	4687 ± 540
23		B	>10,000	8302 ± 350	>10,000
24		B	7941 ± 350	4483 ± 310	>10,000
25		C	3878 ± 390	3878 ± 410	3878 ± 420
26		D	3937 ± 310	3937 ± 360	3937 ± 380
27		B	>10,000	>10,000	>10,000
28		C	>10,000	>10,000	>10,000
29		D	>10,000	3955 ± 270	>10,000
30		D	378 ± 31	378 ± 42	3784 ± 390

[a] Each experiment was performed at least in duplicate.
[b] SD: standard deviation.

Compounds **59** and **60**, bearing a methoxy group at the position 3 or 2 of the 4-chlorobenzyl moiety, remained inactive. Hydroxy analogue **61** was found to be inactive.

On the basis of the structure of natural products combretastatin A-4, colchicine or podophyllotoxin (Fig. 1), we decided to introduce 3,4,5-trimethoxyphenyl (cf. **62**), 3,5-dimethoxyphenyl (cf. **63**) or 6-chloro-1,3-benzodioxole (cf. **64**) groups at the level of the nitrogen indole benzyl position but, unfortunately, the corresponding compounds were found to be inactive.

Introduction of 4-fluorophenyl (**65**) or 4-fluorobenzoyl (**66**) group, modifying the flexibility of the appendage and the possible interaction with the target, provided compounds with no cytotoxic activity in comparison to the lead **D-24241**.

3.2. Effect on cell cycle progression

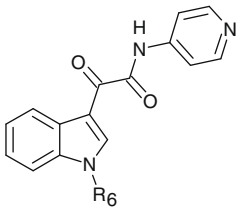
Previous studies with **D-24851**[9b,c,11] suggested that this glyoxamide exerts its cytotoxic effect via tubulin as the intracellular target. Both the inhibition of tubulin polymerization and stabilization of microtubules can lead to an arrest of the cell cycle in the G2/M-phase. Thus, **D-24851**, **D-24241** and compound **55** were examined for their effects on the cell cycle progression with Hela/KB cells.

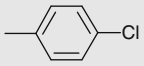
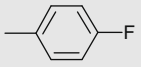
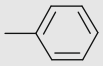
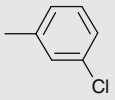
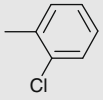
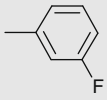
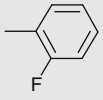
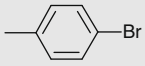
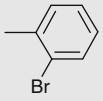
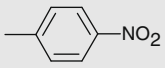
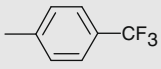
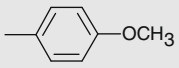
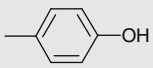
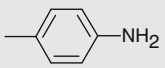
By using fluorescence activated cell sorting (FACS) analysis, a concentration-dependent arrest of Hela/KB cells in the G2/M-phase was observed for **D-24851** and **D-24241** but not for compound **55** as indicated by the dose–response curve (Fig. 2).

In summary, the antiproliferative activity of compound **55** did not correlate with its effect on cell cycle progression, suggesting an other target than the tubulin.

A slight modification at the level of the benzyl side chain, i.e. introduction of a nitro group at position 3 would modify the binding mode of the molecule to the tubulin. This result suggests that not only 4-pyridyl ring but also benzyl appendage would be involved in the binding mode to the tubulin target. The presence of a hydrophobic pocket in close proximity, where 4-fluoro or

Table 4
Synthetic route and in vitro cytotoxicity of *N*-(pyridin-4-yl)-2-[1*H*-indol-3-yl]glyoxamide analogs **17**, **35–66**



Compound	R_6	Route	Hela/KB[a] IC_{50} (nM ± SD[b])	L1210[a] IC_{50} (nM ± SD[b])	SKOV3[a] IC_{50} (nM ± SD[b])
D-24851			44 ± 7	44 ± 5	77 ± 8
D-24241			54 ± 6	455 ± 22	455 ± 32
17	H	C	>10,000	>10,000	>10,000
35		B	141 ± 15	253 ± 34	4924 ± 390
36		B	4489 ± 290	4489 ± 310	4489 ± 350
37		B	4489 ± 340	4489 ± 350	6618 ± 590
38		C	469 ± 26	469 ± 31	469 ± 37
39		C	4687 ± 320	4687 ± 340	>10,000
40		B	403 ± 22	403 ± 27	403 ± 34
41		B	4029 ± 310	>10,000	>10,000
42		B	>10,000	>10,000	>10,000
43		B	7464 ± 610	7464 ± 650	7464 ± 670
44		B	4541 ± 310	454 ± 36	4541 ± 340
45			>10,000	>10,000	>10,000
46			>10,000	3510 ± 190	>10,000

(*continued on next page*)

Table 4 (*continued*)

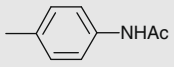
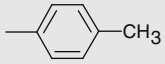
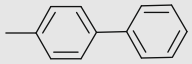
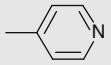
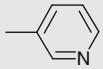
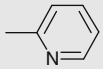
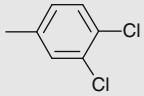
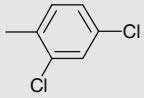
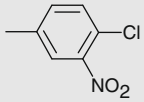
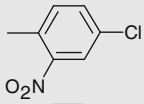
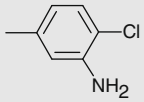
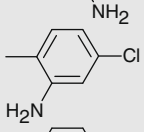
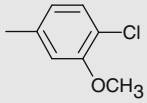
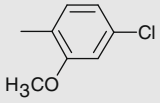
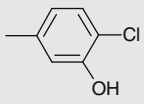
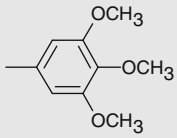
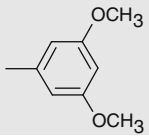
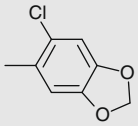
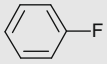
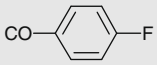
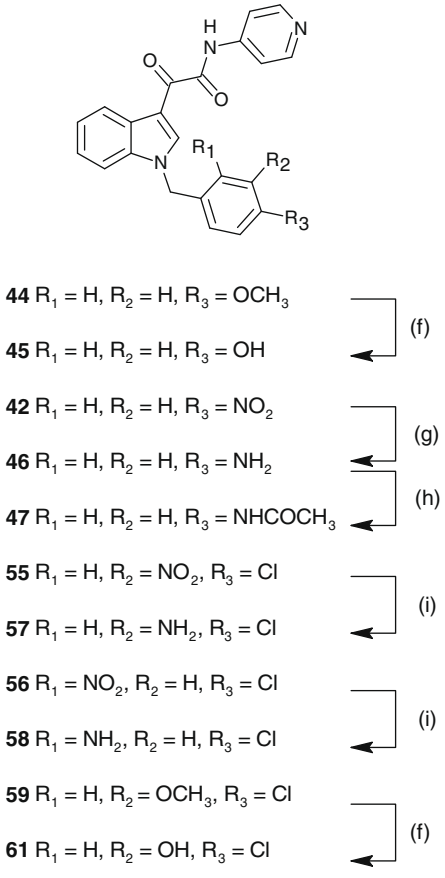
Compound	R_6	Route	Hela/KB[a] IC_{50} (nM ± SD[b])	L1210[a] IC_{50} (nM ± SD[b])	SKOV3[a] IC_{50} (nM ± SD[b])
47			>10,000	>10,000	>10,000
48		B	474 ± 42	474 ± 48	471 ± 54
49		B	>10,000	>10,000	>10,000
50		B	>10,000	6580 ± 560	>10,000
51		B	>10,000	4910 ± 430	>10,000
52		B	4910 ± 460	>10,000	>10,000
53		B	>10,000	>10,000	>10,000
54		B	7448 ± 670	7448 ± 680	7448 ± 690
55		C	39 ± 4	51 ± 8	11 ± 2
56		B	>10,000	>10,000	>10,000
57			701 ± 55	706 ± 64	1213 ± 120
58			462 ± 47	574 ± 52	338 ± 34
59		C/A	3132 ± 160	6145 ± 510	2451 ± 260
60		C	4023 ± 440	6154 ± 580	5340 ± 510
61			729 ± 97	>10,000	6111 ± 580
62		B	>10,000	>10,000	>10,000
63		B	>10,000	>10,000	>10,000

Table 4 (*continued*)

Compound	R_6	Route	Hela/KB[a] IC_{50} (nM ± SD[b])	L1210[a] IC_{50} (nM ± SD[b])	SKOV3[a] IC_{50} (nM ± SD[b])
64		B	7284 ± 650	7284 ± 670	7284 ± 690
65		C	>10,000	>10,000	>10,000
66		B	>10,000	>10,000	>10,000

[a] Each experiment was performed at least in duplicate.
[b] SD: standard deviation.



Scheme 2. Reagents: (f) BBr_3, CH_2Cl_2 (g) H_2, Ni Raney, dioxane; (h) Ac_2O; (i) $SnCl_2$, HCl.

4-chlorobenzyl moieties fit well but not 3-nitro derivative, would explain the lack of binding affinity by modifying electronic density and interactions with amino acids or orientation of the molecule in the tubulin binding site.

4. Conclusion

We have developed a broad pharmacomodulation on the basis of the lead compounds **D-24241** and **D-24851**. SAR analysis highlighted two determinant structural requirements: (A) a (pyridin-4-yl)glyoxamide function at position 3 of the indole; (B) a 4-chloro- or 4-fluorobenzyl group at position 1 of the indole. In all

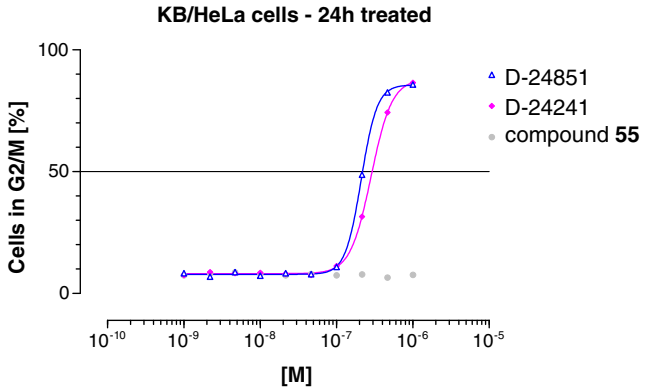


Figure 2. Effect on cell cycle progression for **D-24851**, **D-24241** and compound **55**.

cases, di or trisubstitution on the benzyl moiety was deleterious for cytotoxic activity except for 4-chloro-3-nitrobenzyl derivative **55**.

This compound is interestingly as active as the leads. It was selected for mode-of-action studies to elucidate the molecular target leading to cytotoxicity.

These SAR results are helpful for designing new potent compounds and extensive functionalization of the benzene ring of the indole scaffold on the lead **D-24241** will be reported in due time.

5. Experimental

5.1. General method: chemistry

All commercially available materials were used without further purification unless otherwise stated. Anhydrous solvents such as dimethylsulfoxide (DMSO) and acetone were obtained from Aldrich Chemical Co. Tetrahydrofuran (THF) and diethyl ether (Et_2O) were distilled from sodium benzophenone, dichloromethane (CH_2Cl_2) was distilled from P_2O_5. All reactions involving air- or moisture-sensitive compounds were performed under a N_2 atmosphere. Flash chromatography was performed using Merck Silica Gel 60 (70–230 mesh ASTM). Thin-layer chromatography (TLC) was performed with Merck silica gel TLC plates ($60F_{254}$). ^1H NMR spectra were determined with FT NMR Bruker spectrometer operating at 250 MHz. All NMR chemical shifts are reported as δ values, and coupling constants (J) are given in hertz (Hz). The splitting pattern abbreviations are as followed; s, singlet; d, doublet; t, triplet; q, quartet; m, unresolved multiplet; dd, doublet of doublet

and ddd, doublet of doublet of doublet. Melting points were measured with a Electrothermal IA9000 apparatus and are uncorrected. Mass spectra (ESI) were recorded on Bruker Esquire-LC ion trap mass spectrometer. Elemental analyses were found within ±0.4% of the theoretical values and are provided as Supplementary data.

D-24241[10] and **D-24851**[23] were obtained as previously described.

Two general synthetic methods (routes A–B) were employed to prepare the various benzyl-indole (**9–14**, **22–24**, **27**, **35–37**, **40–44**, **48–54**, **56**, **59**, **62–64** and **66**) analogues examined in this study. Three general synthetic methods (routes C–D) were employed to prepare the various glyoxamide analogues (**17–21**, **25**, **26**, **28–34**, **38**, **39**, **55**, **59**, **60** and **65**) examined in this study. A representative experimental procedure for each type of synthetic route employed is described below. All supporting characterization data are provided.

5.1.1. Route A: example: 1-(4-fluorobenzyl)-1*H*-indole (9)

To a solution of NaH (0.90 g, 37.6 mmol) in DMSO (100 mL) was added dropwise a solution of indole **8** (4.0 g, 34.1 mmol) in DMSO (25 mL). The reaction mixture was stirred at room temperature for 1 h. Then 4-fluorobenzyl bromide (4.5 mL, 37.6 mmol) was added and the reaction mixture was stirred for 1 h more. The reaction mixture was partitioned between H_2O and Et_2O. The organic layer was separated, washed three times with H_2O, dried over Na_2SO_4, and concentrated in vacuo to give an oil. The crude material was purified by silica gel column chromatography (CH_2Cl_2/petroleum ether = 7:3) to give **9** (6.8 g, 89%) as a white solid, mp 37–39 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 5.45 (s, 2H), 6.52 (d, J = 3.1 Hz, 1H), 7.05 (ddd, J = 8.0, 1.2 Hz, 1H), 7.13 (ddd, J = 8.0, 1.2 Hz, 1H), 7.17 (dd, J = 8.7 Hz, 2H), 7.29 (dd, J = 8.7, 5.6 Hz, 2H), 7.47–7.51 (m, 1H), 7.57–7.61 (m, 1H). ESMS m/z 226.3 (MH^+).

5.1.2. Route B: example: 2-[1-(4-fluorobenzyl)-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-3-yl)acetamide (22)

To a solution of 2-(1*H*-Indol-3-yl)-2-oxo-*N*-(pyridin-3-yl)acetamide **18** (0.80 g, 3 mmol) in anhydrous acetone (100 mL) was added K_2CO_3 (2.08 g, 15.1 mmol). The reaction mixture was stirred for 15 min. Then 4-fluorobenzyl chloride (0.43 g, 3 mmol) was added slowly. The reaction mixture was heated at reflux for 2 h. The resulting solution was filtrated and K_2CO_3 washed with acetone. The filtrate was concentrated in vacuo, the residue was dissolved in CH_2Cl_2 and washed with H_2O. The organic layer was extracted with CH_2Cl_2, dried over Na_2SO_4, filtered and evaporated in vacuo. This material was purified by trituration in diisopropylic ether to give **25** (0.58 g, 52%) of the title compound as a white crystalline solid, mp 172–174 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 5.64 (s, 2H), 7.21 (dd, J = 8.7 Hz, 2H), 7.34–7.48 (m, 5H), 7.68 (m, 1H), 8.28–8.40 (m, 3H), 9.08 (s, 2H), 11.01 (s, 1H). ESMS m/z 374.4 (MH^+). Anal. ($C_{22}H_{16}FN_3O_2$) C, H, N.

5.1.3. Route C: example: 2-(1*H*-indol-3-yl)-2-oxo-*N*-(pyridin-4-yl)acetamide (17)

To a solution of oxalyl chloride (2.43 mL, 25.3 mmol) in anhydrous Et_2O (50 mL) was added at 0 °C under N_2 a solution of indole **8** (3.0 g, 25.3 mmol) in Et_2O (100 mL). Upon heating to room temperature, the reaction mixture was heated at reflux for 2 h, then concentrated in vacuo to give a yellow solid. This crude product was dissolved in THF (100 mL), then cooled to 0 °C. A solution of 4-aminopyridine (5 g, 53.1 mmol) in THF (250 mL) was added dropwise under N_2. The reaction mixture was heated at reflux for 3 h, then allowed to cool to room temperature and stirred for other 4 h. The precipitate that formed was collected by filtration, washed with THF to give a yellow solid as crude product. The filtrate was concentrated in vacuo. The residue was dissolved in EtOAc, washed with H_2O. The organic layer was dried over Na_2SO_4, filtered and evaporated in vacuo to give more crude product. This material was purified by silica gel column chromatography (CH_2Cl_2/EtOH = 95:5) to afford **17** (5.4 g, 82%) as a yellow crystalline solid, mp >250 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 7.32–7.37 (m, 2H), 7.61 (m, 1H), 7.89 (d, J = 6.1 Hz, 2H), 8.31 (m, 1H), 8.56 (d, J = 6.1 Hz, 2H), 8.79 (s, 1H), 11.11 (s, 1H), 12.44 (s, 1H). ESMS m/z 266.3 (MH^+). Anal. ($C_{15}H_{11}N_3O_2$) C, H, N.

5.1.4. Route D: example: ethyl 4-({[1-(4-fluorobenzyl)-1*H*-indol-3-yl](oxo)acetyl}amino)benzoate (26)

To a solution of oxalyl chloride (1.62 mL, 17.1 mmol) in anhydrous Et_2O (40 mL) was added at 0 °C under N_2 a solution of indole **9** (3.85 g, 17.1 mmol) in Et_2O (80 mL). Upon heating to room temperature, the reaction mixture was heated at reflux for 2 h, then concentrated in vacuo to give a yellow solid. This crude product was dissolved in THF (50 mL); then triethylamine (2.9 mL, 20.5 mmol) was added under N_2. The reaction mixture was cooled to 0 °C. A solution of ethyl 4-aminobenzoate (3.39 g, 20.5 mmol) in THF (50 mL) was slowly added. The reaction mixture was heated at reflux for 5 h and then allowed to cool to room temperature. The precipitate that formed was collected by filtration, washed with THF to give a crude product. The filtrate was concentrated in vacuo. The residue was dissolved in EtOAc and washed with H_2O. The organic layer was extracted with EtOAc, dried over Na_2SO_4, filtered and evaporated in vacuo to give more crude product. This material was purified by silica gel column chromatography (CH_2Cl_2/EtOH = 95:5) to give the title compound (4.56 g, 60%) as a white crystalline solid, mp 141–143 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 1.36 (t, J = 7.1 Hz, 3H), 4.35 (q, J = 7.1 Hz, 2H), 5.65 (s, 2H), 7.22 (dd, J = 8.8 Hz, 2H), 7.34–7.42 (m, 2H), 7.45 (dd, J = 8.8, 5.5 Hz, 2H), 7.67–7.71 (m, 1H), 8.02 (d, J = 9.0 Hz, 2H), 8.07 (d, J = 9.0 Hz, 2H), 8.33–8.37 (m, 1H), 9.06 (s, 1H), 11.09 (s, 1H). ESMS m/z 445.5 (MH^+). Anal. ($C_{26}H_{21}FN_2O_4$) C, H, N.

5.1.5. 1-(3-Fluorobenzyl)-1*H*-indole (10)

It was synthesized via route A. Yield 84%, mp 57–58 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 5.49 (s, 2H), 6.54 (d, J = 3.1 Hz, 1H), 6.98–7.17 (m, 5H), 7.34–7.44 (m, 1H), 7.47–7.50 (m, 1H), 7.56 (d, J = 3.1, 1H), 7.59–7.62 (m, 1H). ESMS m/z 226.3 (MH^+).

5.1.6. 1-(2-Fluorobenzyl)-1*H*-indole (11)

It was synthesized via route A. Yield 99%, yellow oil. ^{1}H NMR (250 MHz, DMSO-d_6): δ 5.52 (s, 2H), 6.53 (dd, J = 3.1 Hz, J = 0.8 Hz, 1H), 7.02–7.18 (m, 4H), 7.22–7.30 (m, 1H), 7.31–7.41 (m, 1H), 7.48–7.52 (m, 2H), 7.58–7.61 (m, 1H). ESMS m/z 226.3 (MH^+).

5.1.7. 1-(4-Chloro-3-methoxybenzyl)-1*H*-indole (12)

4-Chloro-3-methoxybenzyl bromide was synthesized from 2-chloro-5-methylphenol in two steps. NaH (3.0 g, 77 mmol) was added slowly to a solution of 2-chloro-5-methylphenol (10.0 g, 70 mmol) in DMF (200 mL). The reaction mixture was stirred at room temperature for 1 h, then MeI (4.8 mL, 77 mmol) was slowly added. The reaction mixture was stirred during 1 h. After quenching on crushed ice, the mixture was extracted with CH_2Cl_2. The organic layer was separated and dried on Na_2SO_4. The solvent was evaporated to afford 2-chloro-5-methylanisole (5.6 g, 51%) as an orange oil. A mixture of 2-chloro-5-methylanisole (5.0 g, 31.9 mmol), NBS (6.2 g, 35.1 mmol) and AIBN (270 mg, 1.6 mmol), in CCl_4 (150 mL) was heated at reflux during 24 h. After cooling, the reaction mixture was filtered. The filtrate was evaporated to give a residue which was purified by silica gel column chromatography (petroleum ether/EtOAc = 9:1) to give 4-chloro-3-methoxybenzyl bromide. Yield 82%. 1-(4-Chloro-3-methoxybenzyl)-1*H*-indole **12** was synthesized via route A by reaction of indole **8** with 4-chloro-3-methoxybenzyl bromide. Yield 55%, mp 138–139 °C. ^{1}H

NMR (250 MHz, DMSO-d_6): δ 3.38 (s, 3H), 5.44 (s, 2H), 6.53 (dd, J = 3.3, 0.9 Hz, 1H), 6.67 (dd, J = 8.2, 1.8 Hz, 1H), 7.02–7.11 (m, 2H), 7.19 (d, J = 1.8 Hz, 1H), 7.35 (d, J = 8.2 Hz, 1H), 7.47–7.51 (m, 1H), 7.56 (d, J = 3.3 Hz, 1H), 7.57–7.61 (m, 1H). ESMS m/z 272.8 (MH^+).

5.1.8. 1-(4-Chloro-2-methoxybenzyl)-1*H*-indole (13)

(4-Chloro-2-methoxyphenyl)methanol was synthesized in two steps from 4-chloro-2-methoxybenzoic acid via method a or in one step via method b. (a) A solution of 4-chloro-2-methoxybenzoic acid (5.0 g, 26.8 mmol) in EtOH (60 mL) containing 5% of HCl was heated at reflux during 6 h. After cooling, the mixture was evaporated in vacuo. The residue was dissolved in dichloromethane, washed with a $NaHCO_3$ solution. The organic layer was dried over Na_2SO_4, filtered and evaporated in vacuo to give ethyl 4-chloro-2-methoxybenzoate (4.0 g, 69%) as a yellow oil. $LiAlH_4$ (67 mg, 17.7 mmol) was added to a solution of ethyl 4-chloro-2-methoxybenzoate (3.8 g, 17.7 mmol) in THF (30 mL). The reaction mixture was heated at reflux during 3 h. After quenching on crushed ice, the mixture was extracted with EtOAc. The organic layer was separated and dried on Na_2SO_4. The solvent was evaporated to afford a residue which was purified by silica gel column chromatography (CH_2Cl_2/EtOH = 9:1) to give (4-chloro-2-methoxyphenyl)methanol. Yield 89%. (b) A solution 1 M of BH_3·THF complex in THF (80.4 mL, 80.4 mmol) was added to a solution of 4-chloro-2-methoxybenzoic acid (5.0 g, 26.8 mmol) in tetrahydrofuran (50 mL). The reaction was stirred at room temperature for 24 h and then the solvent was evaporated. The residue was dissolved in CH_2Cl_2, and silica was added. The mixture was stirred for 1 h and filtered. The solvent was evaporated to afford a residue which was purified by silica gel column chromatography (dichloromethane/ethanol = 9:1) to give (4-chloro-2-methoxyphenyl)methanol. Yield 93%. PBr_3 (0.6 mL, 5.8 mmol) was added at 0 °C to a solution of (4-chloro-2-methoxyphenyl)methanol (1.0 g, 5.8 mmol) in Et_2O (25 mL). The reaction mixture was stirred at 0 °C during 1 h. H_2O was added while stirring and the mixture was extracted with Et_2O. The organic layer was separated, washed with a $NaHCO_3$ solution and then with H_2O. The solvent was evaporated to give 4-chloro-2-methoxybenzyl bromide (1.0 g, 75%) as a white powder, mp 47–48 °C. 1-(4-Chloro-2-methoxybenzyl)-1*H*-indole **13** was synthesized via route A by reaction of indole **8** with 4-chloro-2-methoxybenzyl bromide. Yield 48%. ^{1}H NMR (250 MHz, DMSO-d_6): δ 3.92 (s, 3H), 5.36 (s, 2H), 6.51 (dd, J = 3.1, 0.6 Hz, 1H), 6.75 (d, J = 8.0 Hz, 1H), 6.93 (dd, J = 8.0, 2.1 Hz, 1H), 7.01–7.13 (m, 2H), 7.57–7.63 (m, 1H). ESMS m/z 272.8 (MH^+).

5.1.9. 1-(4-Chloro-3-nitrobenzyl)-1*H*-indole (14)

(4-Chloro-3-nitrophenyl)methanol was synthesized as previously described (see Section 5.1.8, compound **13**), by reduction of 4-chloro-3-nitrobenzoic acid with BH_3·THF complex. Yield 96%, mp 64–65 °C. Bromination with NBS led to 4-chloro-3-nitrobenzyl bromide. Yield 31%. 1-(4-chloro-3-nitrobenzyl)-1*H*-indole **14** was synthesized via route A by reaction of indole **8** with 4-chloro-3-nitrobenzyl bromide. Yield 59%. ^{1}H NMR (250 MHz, DMSO-d_6): δ 5.57 (s, 2H), 6.56 (d, J = 3.4 Hz, 1H), 7.04–7.18 (m, 2H), 7.44 (dd, J = 8.2, 2.1 Hz, 1H), 7.49–7.53 (m, 1H), 7.58–7.63 (m, 2H), 7.75 (d, J = 8.2 Hz, 1H), 8.01 (d, J = 2.1 Hz, 1H). ESMS m/z 287.7 (MH^+).

5.1.10. 1-(4-Fluorophenyl)-1*H*-indole (16)

To a solution of 2.0 g (17.1 mmol) of indole **8** in DMF (30 mL), 7.92 g (124.6 mmol) of Cu, 17.22 g (124.6 mmol) of K_2CO_3 and 4.5 g (20.5 mmol) of 4-fluoroiodobenzene were added. The resulting solution was heated at 130 °C during 24 h. The reaction mixture was filtered on Celite and the residue was washed with CH_2Cl_2. The filtrate was concentrated under vacuo. The resulting oil was purified by chromatography, eluting with a mixture CH_2Cl_2/petroleum ether (4:1). Yield 78%, yellow oil. 6.73 (d, J = 3.3, 1H), 7.13–7.27 (m, 2H), 7.45 (dd, J_{HF} = J_{HH} = 8.8 Hz, 2H), 7.51–7.55 (m, 1H), 7.63–7.67 (m, 3H), 7.68–7.71 (m, 1H). ESMS m/z 212.2 (MH^+).

5.1.11. 2-(1*H*-Indol-3-yl)-2-oxo-*N*-(pyridin-3-yl)acetamide (18)

It was synthesized via route C from indole **8** using 3-aminopyridine. Yield 85%, mp 234–236 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 7.31–7.37 (m, 2H), 7.45 (dd, J = 8.3, 4.7 Hz, 1H), 7.60 (m, 1H), 8.27–8.33 (m, 2H), 8.39 (m, 1H), 8.82 (d, J = 4.1 Hz, 1H), 9.10 (d, J = 1.9 Hz, 1H), 10.98 (s, 1H), 12.40 (m, 1H). ESMS m/z 266.3 (MH^+).

5.1.12. 2-(1*H*-Indol-3-yl)-2-oxo-*N*-(pyridin-2-yl)acetamide (19)

It was synthesized via route C from indole **8** using 2-aminopyridine. Yield 30%, mp 173–175 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 7.25–7.36 (m, 3H), 7.66 (m, 1H), 7.94 (dd, J = 7.7 Hz, 1H), 8.22 (d, J = 7.7 Hz, 1H), 8.32 (m, 1H), 8.44 (d, J = 4.7 Hz, 1H), 9.03 (s, 1H), 10.53 (s, 1H), 12.51 (m, 1H). ESMS m/z 266.3 (MH^+).

5.1.13. 2-(1*H*-Indol-3-yl)-*N*-(4-nitrophenyl)-2-oxoacetamide (20)

It was synthesized via route C from indole **8** using 4-nitroaniline. Yield 85%, mp >250 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 7.31–7.35 (m, 2H), 7.60 (m, 1H), 8.16 (d, J = 8.6 Hz, 2H), 8.29–8.32 (m, 3H), 8.79 (s, 1H), 11.31 (s, 1H), 12.44 (s, 1H). ESMS m/z 310.3 (MH^+).

5.1.14. *N*-(4-Fluorophenyl)-2-(1*H*-indol-3-yl)-2-oxoacetamide (21)

It was synthesized via route C from indole **8** using 4-fluoroaniline. Yield 50%, mp 232–234 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 7.26 (dd, J = 9.0 Hz, 2H), 7.30–7.34 (m, 2H), 7.60 (m, 1H), 7.92 (dd, J = 9.0, 5.0 Hz, 2H), 8.31 (m, 1H), 8.80 (d, J = 3.2 Hz, 1H), 10.80 (s, 1H), 12.37 (m, 1H). ESMS m/z 283.3 (MH^+).

5.1.15. 2-[1-(4-Fluorobenzyl)-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-2-yl)acetamide (23)

It was synthesized via route B from **19** using 4-fluorobenzyl chloride. Yield 47%, mp 140–142 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 5.64 (s, 2H), 7.21 (dd, J = 8.7 Hz, 2H), 7.28–7.46 (m, 5H), 7.69 (m, 1H), 7.93 (dd, J = 8.7 Hz, 2H), 8.22 (d, J = 7.7 Hz, 1H), 8.31 (m, 1H), 8.43 (d, J = 4.7 Hz, 1H), 9.03 (s, 1H), 10.53 (s, 1H). ESMS m/z 374.4 (MH^+). Anal. ($C_{22}H_{16}FN_3O_2$) C, H, N.

5.1.16. 2-[1-(4-Fluorobenzyl)-1*H*-indol-3-yl]-*N*-(4-fluorophenyl)-2-oxoacetamide (24)

It was synthesized via route B from **21** using 4-fluorobenzyl chloride. Yield 95%, mp 198–200 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 5.63 (s, 2H), 7.18–7.46 (m, 8H), 7.67 (m, 1H), 7.92 (dd, J = 8.8, 5.2 Hz, 2H), 8.33 (m, 1H), 9.05 (s, 1H), 10.83 (s, 1H). ESMS m/z 391.4 (MH^+). Anal. ($C_{23}H_{16}F_2N_2O_2$) C, H, N.

5.1.17. *N*-(4-Bromophenyl)-2-[1-(4-fluorobenzyl)-1*H*-indol-3-yl]-2-oxoacetamide (25)

It was synthesized via route C from **9** using 4-bromoaniline. Yield 76%, mp 187–189 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 5.54 (s, 2H), 7.22 (dd, J = 8.5 Hz, 2H), 7.33–7.38 (m, 2H), 7.45 (dd, J = 8.5, 5.5 Hz, 2H), 7.61 (d, J = 8.8 Hz, 2H), 7.66–7.71 (m, 1H), 7.90 (d, J = 8.8 Hz, 2H), 8.32–8.37 (m, 1H), 9.05 (s, 1H). ESMS m/z 452.3 (MH^+). Anal. ($C_{23}H_{16}BrFN_2O_2$) C, H, N.

5.1.18. 2-[1-(4-Fluorobenzyl)-1*H*-indol-3-yl]-*N*-(4-nitrophenyl)-2-oxoacetamide (27)

It was synthesized via route B from **20** using 4-fluorobenzyl chloride. Yield 40%, mp >250 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 5.64 (s, 2H), 7.22 (dd, J = 8.7 Hz, 2H), 7.34–7.38 (m, 2H), 7.45 (dd,

J = 8.7, 5.5 Hz, 2H), 7.69 (m, 1H), 8.18 (d, *J* = 9.3 Hz, 2H), 8.31–8.35 (m, 2H), 8.33 (m, 1H), 9.05 (s, 1H), 11.36 (s, 1H). ESMS *m/z* 418.4 (MH^+). Anal. ($C_{23}H_{16}FN_3O_4$) C, H, N.

5.1.19. *N*-(4-Cyanophenyl)-2-[1-(4-fluorobenzyl)-1*H*-indol-3-yl]-2-oxoacetamide (28)

It was synthesized via route C from **9** using 4-cyanoaniline. Yield 65%, mp 237–239 °C. ^1H NMR (250 MHz, DMSO-d_6): δ 5.65 (s, 2H), 7.22 (dd, *J* = 8.7 Hz, 2H), 7.34–7.39 (m, 2H), 7.45 (dd, *J* =8.7, 5.7 Hz, 2H), 7.67–7.71 (m, 1H), 7.90 (d, *J* = 8.6 Hz, 2H), 8.12 (d, *J* = 8.6 Hz, 2H), 8.32–8.37 (m, 1H), 9.05 (s, 1H), 11.20 (s, 1H). ESMS *m/z* 398.4 (MH^+). Anal. ($C_{24}H_{16}FN_3O_2$) C, H, N.

5.1.20. 1-[1-(4-Fluorobenzyl)-1*H*-indol-3-yl]-2-oxo-2-[(4-pyridin-4-yl)piperazin-1-yl]ethanone (29)

It was synthesized via route D from **9** using 1-(pyridin-4-yl)piperazine. Yield 68%, mp 159–161 °C. ^1H NMR (250 MHz, DMSO-d_6): δ 3.36–3.53 (m, 4H), 3.58–3.80 (m, 4H), 5.60 (s, 2H), 6.89 (d, *J* = 5.6, 1H), 6.90 (d, *J* = 8.6 Hz, 1H), 7.18 (dd, *J* = 8.7 Hz, 2H), 7.34–7.45 (m, 4H), 7.66 (m, 1H), 8.24–8.26 (m, 3H), 8.56 (s, 1H). ESMS *m/z* 443.4 (MH^+). Anal. ($C_{26}H_{23}FN_4O_2$) C, H, N.

5.1.21. 2-[1-(4-Fluorobenzyl)-1*H*-indol-3-yl]-*N*-(3,4,5-trimethoxyphenyl)-2-oxoacetamide (30)

It was synthesized via route D from **9** using 3,4,5-trimethoxyaniline. Yield 57%, mp 104–106 °C. ^1H NMR (250 MHz, DMSO-d_6): δ 3.68 (s, 3H), 3.81 (s, 6H), 5.65 (s, 2H), 7.21 (dd, *J* = 8.6 Hz, 2H), 7.33–7.37 (m, 2H), 7.39 (s, 2H), 7.43 (dd, *J* = 8.6, 5.6 Hz, 2H), 7.65–7.69 (m, 1H), 9.15 (s, 1H), 10.62 (s, 1H). ESMS *m/z* 463.5 (MH^+). Anal. ($C_{26}H_{23}FN_2O_5$) C, H, N.

5.1.22. 2-[1-(4-Fluorobenzyl)-1*H*-indol-3-yl]-*N*-(4-fluoro-2-nitrophenyl)-2-oxoacetamide (31)

It was synthesized via route D from **9** using 4-fluoro-2-nitroaniline. Yield 49%, mp 183–185 °C. ^1H NMR (250 MHz, DMSO-d_6): δ 5.66 (s, 2H), 7.22 (dd, *J* = 8.8 Hz, 2H), 7.33–7.40 (m, 2H), 7.44 (dd, *J* = 8.7, 5.5 Hz, 2H), 7.67–7.72 (m, 1H), 7.80 (ddd, *J* = 9.2, 8.7, 3.0 Hz, 1H), 8.12 (dd, *J* = 8.7, 3.0 Hz, 1H), 8.30 (dd, *J* = 9.2, 5.3 Hz, 1H), 8.34–8.38 (m, 1H), 9.13 (s, 1H), 11.51 (s, 1H). ESMS *m/z* 436.4 (MH^+). Anal. ($C_{23}H_{15}F_2N_3O_4$) C, H, N.

5.1.23. 2-[1-(4-Fluorobenzyl)-1*H*-indol-3-yl]-*N*-(4-fluoro-3-nitrophenyl)-2-oxoacetamide (32)

It was synthesized via route D from **9** using 4-fluoro-3-nitroaniline. Yield 29%, mp 202–204 °C. ^1H NMR (250 MHz, DMSO-d_6): δ 5.66 (s, 2H), 7.22 (dd, *J* = 8.8 Hz, 2H), 7.34–7.39 (m, 2H), 7.46 (dd, *J* = 8.5, 5.5 Hz, 2H), 7.64–7.73 (m, 2H), 8.21 (ddd, *J* = 9.2, 4.0, 2.7 Hz, 1H), 8.33–8.37 (m, 1H), 8.94 (dd, *J* = 6.9, 2.7 Hz, 1H), 9.14 (s, 1H), 11.29 (s, 1H). ESMS *m/z* 436.4 (MH^+). Anal. ($C_{23}H_{15}F_2N_3O_4$) C, H, N.

5.1.24. *N*-(4-Chloro-2-nitrophenyl)-2-[1-(4-fluorobenzyl)-1*H*-indol-3-yl]-2-oxoacetamide (33)

It was synthesized via route C from **9** using 4-chloro-2-nitroaniline. Yield 92%, mp 183–185 °C. ^1H NMR (250 MHz, DMSO-d_6): δ 5.66 (s, 2H), 7.22 (dd, *J* = 8.6 Hz, 2H), 7.35–7.39 (m, 2H), 7.44 (dd, *J* = 8.6, 5.5 Hz, 2H), 7.67–7.71 (m, 1H), 7.96 (dd, *J* = 8.9, 2.4 Hz, 1H), 8.27 (d, *J* = 2.4 Hz, 1H), 8.36 (d, *J* = 8.9 Hz, 1H), 9.15 (s, 1H), 11.62 (s, 1H). ESMS *m/z* 452.8 (MH^+). Anal. ($C_{23}H_{15}ClFN_3O_4$) C, H, N.

5.1.25. *N*-(2-Chloro-4-nitrophenyl)-2-[1-(4-fluorobenzyl)-1*H*-indol-3-yl]-2-oxoacetamide (34)

It was synthesized via route C from **9** using 2-chloro-4-nitroaniline. Yield 57%, mp 213–215 °C. ^1H NMR (250 MHz, DMSO-d_6): δ 5.23 (s, 2H), 6.98 (dd, *J* = 8.6 Hz, 2H), 7.15 (dd, *J* = 8.6, 5.4 Hz, 2H), 7.26–7.36 (m, 3H), 8.16 (dd, *J* = 9.2, 2.5 Hz, 1H), 8.29 (d, *J* = 2.5 Hz, 1H), 8.39–8.42 (m, 1H), 8.73 (d, *J* = 9.2 Hz, 1H), 8.99 (s, 1H), 10.32 (s, 1H). ESMS *m/z* 452.8 (MH^+). Anal. ($C_{23}H_{15}ClFN_3O_4$) C, H, N.

5.1.26. 2-(1-Benzyl-1*H*-indol-3-yl)-2-oxo-*N*-(pyridin-4-yl)acetamide (35)

It was synthesized via route B from **17** using benzyl chloride. Yield 61%, mp 177–179 °C. ^1H NMR (250 MHz, DMSO-d_6): δ 5.65 (s, 2H), 7.31–7.36 (m, 7H), 7.66 (m, 1H), 7.89 (d, *J* = 5.6 Hz, 2H), 8.34 (m, 1H), 8.55 (d, *J* = 5.6 Hz, 2H), 9.04 (s, 1H), 11.14 (s, 1H). ESMS *m/z* 356.4 (MH^+). Anal. ($C_{22}H_{17}N_3O_2$) C, H, N.

5.1.27. 2-[1-(3-Chlorobenzyl)-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-4-yl)acetamide (36)

It was synthesized via route B from **17** using 3-chlorobenzyl chloride. Yield 87%, mp 196–198 °C. ^1H NMR (250 MHz, DMSO-d_6): δ 5.70 (s, 2H), 7.33–7.51 (m, 6H), 7.68–7.72 (m, 1H), 7.93 (d, *J* = 6.2 Hz, 2H), 8.57–8.60 (m, 1H), 8.58 (d, *J* = 6.2 Hz, 2H), 9.10 (s, 1H), 11.18 (s, 1H). MS *m/z*: 390.8 (M+H). Anal. ($C_{22}H_{16}ClN_3O_2$) C, H, N.

5.1.28. 2-[1-(2-Chlorobenzyl)-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-4-yl)acetamide (37)

It was synthesized via route B from **17** using 2-chlorobenzyl chloride. Yield 45%, mp 183–185 °C. ^1H NMR (250 MHz, DMSO-d_6): δ 5.75 (s, 2H), 7.03 (dd, *J* = 7.3, 1.4 Hz, 1H), 7.30–7.42 (m, 5H), 7.59 (m, 1H), 7.88(d, *J* = 6.1 Hz, 2H), 8.37 (m, 1H), 8.54 (d, *J* = 6.1 Hz, 2H), 8.95 (s, 1H), 11.13 (s, 1H). ESMS *m/z* 390.8 (MH^+). Anal. ($C_{22}H_{16}ClN_3O_2$) C, H, N.

5.1.29. 2-[1-(3-Fluorobenzyl)-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-4-yl)acetamide (38)

It was synthesized via route C from **10** using 4-aminopyridine. Yield 64%, mp 203–205 °C. ^1H NMR (250 MHz, DMSO-d_6): δ 5.69 (s, 2H), 7.13–7.27 (m, 3H), 7.33–7.48 (m, 3H), 7.66–7.70 (m, 1H), 7.91 (d, *J* = 6.2 Hz, 2H), 8.33–8.38 (m, 1H), 8.57 (d, *J* = 6.2 Hz, 2H), 9.08 (s, 1H), 11.16 (s, 1H). ESMS *m/z* 374.4 (MH^+). Anal. ($C_{22}H_{16}FN_3O_2$) C, H, N.

5.1.30. 2-[1-(2-Fluorobenzyl)-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-4-yl)acetamide (39)

It was synthesized via route C from **11** using 4-aminopyridine. Yield 30%, mp 196–198 °C. ^1H NMR (250 MHz, DMSO-d_6): δ 5.73 (s, 2H), 7.18–7.47 (m, 6H), 7.66–7.71 (m, 1H), 7.90 (d, *J* = 6.2 Hz, 2H), 8.33–8.38 (m, 1H), 8.56 (d, *J* = 6.2 Hz, 2H), 9.00 (s, 1H), 11.15 (s, 1H). ESMS *m/z* 374.4 (MH^+). Anal. ($C_{22}H_{16}FN_3O_2$) C, H, N.

5.1.31. 2-[1-(4-Bromobenzyl)-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-4-yl)acetamide (40)

It was synthesized via route B from **17** using 4-bromobenzyl bromide. Yield 75%, mp 238–240 °C. ^1H NMR (250 MHz, DMSO-d_6): δ 5.66 (s, 2H), 7.33 (d, *J* = 8.6 Hz, 2H), 7.35–7.38 (m, 2H), 7.59 (d, *J* = 8.6 Hz, 2H), 7.63–7.66 (m, 1H), 7.91 (d, *J* = 6.0 Hz, 2H), 8.33–8.37 (m, 1H), 8.56 (d, *J* = 6.0 Hz, 2H), 9.06 (s, 1H), 11.16 (s, 1H). ESMS *m/z* 435.3 (MH^+). Anal. ($C_{22}H_{16}BrN_3O_2$) C, H, N.

5.1.32. 2-[1-(2-Bromobenzyl)-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-4-yl)acetamide (41)

It was synthesized via route B from **17** using 2-bromobenzyl chloride. Yield 73%, mp 192–194 °C. ^1H NMR (250 MHz, DMSO-d_6): δ 5.72 (s, 2H), 6.94 (dd, *J* = 7.3, 1.9 Hz, 1H), 7.29–7.43 (m, 4H), 7.56–7.60 (m, 1H), 7.76 (dd, *J* = 7.3, 1.7 Hz, 1H), 7.88 (d, *J* = 6.2 Hz, 2H), 8.36–8.40 (m, 1H), 8.55 (d, *J* = 6.2 Hz, 2H), 8.95 (s, 1H), 11.15 (s, 1H). ESMS *m/z* 435.3 (MH^+). Anal. ($C_{22}H_{16}BrN_3O_2$) C, H, N.

5.1.33. 2-[1-(4-Nitrobenzyl)-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-4-yl)acetamide (42)

It was synthesized via route B from **17** using 4-nitrobenzyl chloride. Yield 54%, mp >250 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 5.82 (s, 2H), 7.30–7.36 (m, 2H), 7.53 (d, J = 8.6 Hz, 2H), 7.58 (d, J = 8.0 Hz, 2H), 7.86 (d, J = 5.9 Hz, 2H), 8.21 (d, J = 8.6 Hz, 2H), 8.32 (d, J = 7.6 Hz, 1H), 8.52 (d, J = 5.9 Hz, 2H), 9.08 (s, 1H), 11.11 (s, 1H). ESMS m/z 401.4 (MH^+). Anal. ($C_{22}H_{16}N_4O_4$) C, H, N.

5.1.34. 2-[1-(4-(Trifluoromethyl)benzyl)-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-4-yl)acetamide (43)

It was synthesized via route B from **17** using 4-(trifluoromethyl)benzyl chloride. Yield 53%, mp >250 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 5.76 (s, 2H), 7.30–7.36 (m, 2H), 7.50 (d, J = 8.1 Hz, 2H), 7.60 (d, J = 7.9 Hz, 1H), 7.72 (d, J = 8.2 Hz, 2H), 7.86 (d, J = 6.0 Hz, 2H), 8.32 (d, J = 7.5 Hz, 1H), 8.52 (d, J = 6.0 Hz, 2H), 9.06 (s, 1H), 11.10 (s, 1H). ESMS m/z 424.4 (MH^+). Anal. ($C_{23}H_{16}F_3N_3O_2$) C, H, N.

5.1.35. 2-[1-(4-Methoxybenzyl)-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-4-yl)acetamide (44)

It was synthesized via route B from **17** using 4-methoxybenzyl chloride. Yield 75%, mp 173–174 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 3.71 (s, 3H), 5.53 (s, 2H), 6.91 (d, J = 8.6 Hz, 2H), 7.30–7.33 (m, 4H), 7.66–7.68 (m, 1H), 7.86 (d, J = 6.0 Hz, 2H), 8.28–8.30 (m, 1H), 8.52 (d, J = 6.0 Hz, 2H), 8.96 (s, 1H), 11.07 (s, 1H). ESMS m/z 386.4 (MH^+). Anal. ($C_{23}H_{19}N_3O_3$) C, H, N.

5.1.36. 2-[1-(4-Hydroxybenzyl)-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-4-yl)acetamide (45)

It was synthesized by demethylation with boron tribromide[17] in CH_2Cl_2 from **44**. Yield 73%, mp >250 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 5.46 (s, 2H), 6.73 (d, J = 8.5 Hz, 2H), 7.21 (d, J = 8.4 Hz, 2H), 7.30–7.34 (m, 2H), 7.66–7.68 (m, 1H), 7.86 (d, J = 6.0 Hz, 2H), 8.28–8.30 (m, 1H), 8.52 (d, J = 5.9 Hz, 2H), 8.92 (s, 1H), 9.46 (s, 1H), 11.07 (s, 1H). ESMS m/z 372.4 (MH^+). Anal. ($C_{22}H_{17}N_3O_3$) C, H, N.

5.1.37. 2-[1-(4-Aminobenzyl)-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-4-yl)acetamide (46)

It was synthesized by hydrogenation over Raney nickel[18] from **42**. Yield 71%, mp 229–233 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 5.16 (s, 2H), 5.36 (s, 2H), 6.51 (d, J = 8.2 Hz, 2H), 7.07 (d, J = 8.2 Hz, 2H), 7.31–7.33 (m, 2H), 7.67–7.69 (m, 1H), 7.86 (d, J = 5.5 Hz, 2H), 8.27–8.29 (m, 1H), 8.52 (d, J = 5.5 Hz, 2H), 8.87 (s, 1H), 11.07 (s, 1H). ESMS m/z 371.4 (MH^+). Anal. ($C_{22}H_{18}N_4O_2$) C, H, N.

5.1.38. 2-[1-(4-(Acetylamino)benzyl)-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-4-yl)acetamide (47)

It was synthesized using acetic anhydride[19] from **46**. Yield 79%, mp >250 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 2.00 (s, 3H), 5.54 (s, 2H), 7.28–7.34 (m, 4H), 7.53 (d, J = 8.3 Hz, 2H), 7.63 (d, J = 7.4 Hz, 1H), 7.86 (d, J = 5.9 Hz, 2H), 8.30 (d, J = 6.9 Hz, 1H), 8.52 (d, J = 5.7 Hz, 2H), 8.95 (s, 1H), 9.94 (s, 1H), 11.08 (s, 1H). ESMS m/z 413.4 (MH^+). Anal. ($C_{24}H_{20}N_4O_3$) C, H, N.

5.1.39. 2-[1-(4-Methylbenzyl)-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-4-yl)acetamide (48)

It was synthesized via route B from **17** using 4-methylbenzyl chloride. Yield 64%, mp 215–217 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 2.25 (s, 3H), 5.56 (s, 2H), 7.15 (d, J = 7.9 Hz, 2H), 7.24 (d, J = 7.9 Hz, 2H), 7.29–7.34 (m, 2H), 7.62 (d, J = 7.1 Hz, 1H), 7.86 (d, J = 6.0 Hz, 2H), 8.30 (d, J = 8.2 Hz, 1H), 8.52 (d, J = 6.0 Hz, 2H), 8.97 (s, 1H), 11.08 (s, 1H). ESMS m/z 370.4 (MH^+). Anal. ($C_{23}H_{19}N_3O_2$) C, H, N.

5.1.40. 2-[1-(1,1′-Biphenyl-4-ylmethyl)-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-4-yl)acetamide (49)

It was synthesized via route B from **17** using 4-(chloromethyl)-1,1′-biphenyl. Yield 96%, mp 235–237 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 5.71 (s, 2H), 7.35–7.39 (m, 3H), 7.43–7.50 (m, 4H), 7.63–7.74 (m, 5H), 7.92 (d, J = 6.2 Hz, 2H), 8.34–8.38 (m, 1H), 8.57 (d, J = 6.2 Hz, 2H), 9.10 (s, 1H), 11.18 (s, 1H). ESMS m/z 432.5 (MH^+). Anal. ($C_{28}H_{21}N_3O_2$) C, H, N.

5.1.41. 2–Oxo-*N*-(pyridin-4-yl)-2-[1-(pyridin-4-ylmethyl)-1*H*-indol-3-yl]acetamide (50)

It was synthesized via route B from **17** using 4-picolyl chloride hydrochloride. Yield 50%, mp 232–234 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 5.75 (s, 2H), 7.25 (d, J = 6.3 Hz, 2H), 7.33–7.41 (m, 2H), 7.57 (dd, J = 6.8, 1.2 Hz, 1H), 7.90 (d, J = 6.4 Hz, 2H), 8.35 (m, 1H), 8.55–8.57 (m, 4H), 9.08 (s, 1H), 11.16 (s, 1H). ESMS m/z 357.2 (MH^+). Anal. ($C_{21}H_{16}N_4O_2$) C, H, N.

5.1.42. 2-Oxo-*N*-(pyridin-4-yl)-2-[1-(pyridin-3-ylmethyl)-1*H*-indol-3-yl]acetamide (51)

It was synthesized via route B from **17** using 3-chloromethylpyridine. Yield 22%, mp 178–180 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 5.71 (s, 2H), 7.35–7.42 (m, 3H), 7.73–7.76 (m, 2H), 7.89 (d, J = 5.1 Hz, 2H), 8.33 (m, 1H), 8.52–8.56 (m, 3H), 8.70 (s, 1H), 9.08 (s, 1H), 11.14 (s, 1H). ESMS m/z 357.4 (MH^+). Anal. ($C_{21}H_{16}N_4O_2$) C, H, N.

5.1.43. 2–Oxo-*N*-(pyridin-4-yl)-2-[1-(pyridin-2-ylmethyl)-1*H*-indol-3-yl]acetamide (52)

It was synthesized via route B from **17** using 2-chloromethylpyridine. Yield 24%, mp 201–203 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 5.75 (s, 2H), 7.34–7.42 (m, 4H), 7.62 (m, 1H), 7.82 (ddd, J = 7.6, 1.8 Hz, 1H), 7.90 (d, J = 6.0 Hz, 2H), 8.33 (m, 1H), 8.54–8.57 (m, 3H), 9.04 (s, 1H), 11.15 (s, 1H). ESMS m/z 357.4 (MH^+). Anal. ($C_{21}H_{16}N_4O_2$) C, H, N.

5.1.44. 2-[1-(4,5-Dichlorobenzyl)-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-4-yl)acetamide (53)

It was synthesized via route B from **17** using 4,5-dichlorobenzyl chloride. Yield 48%, mp >250 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 5.64 (s, 2H), 7.28 (dd, J = 8.3, 1.3 Hz, 1H), 7.32–7.36 (m, 2H), 7.61 (d, J = 8.3 Hz, 1H), 7.64 (dd, J = 6.7, 2.9 Hz, 1H), 7.68 (d, J = 1.3 Hz, 1H), 7.86 (d, J = 6.0 Hz, 2H), 8.31 (dd, J = 5.5, 2.1 Hz, 1H), 8.52 (d, J = 6.0 Hz, 2H), 9.04 (s, 1H), 11.09 (s, 1H). ESMS m/z 425.3 (MH^+). Anal. ($C_{22}H_{15}Cl_2N_3O_2$) C, H, N.

5.1.45. 2-[1-(2,4-Dichlorobenzyl)-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-4-yl)acetamide (54)

It was synthesized via route B from **17** using 2,4-dichlorobenzyl chloride. Yield 45%, mp 241–242 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 5.71 (s, 2H), 6.98 (d, J = 8.4 Hz, 1H), 7.32–7.38 (m, 2H), 7.41 (d, J = 8.3 Hz, 1H), 7.55 (d, J = 7.9 Hz, 1H), 7.74 (d, J = 1.7 Hz, 1H), 7.85 (d, J = 6.0 Hz, 2H), 8.33 (d, J = 7.6 Hz, 1H), 8.51 (d, J = 6.0 Hz, 2H), 8.92 (s, 1H), 11.09 (s, 1H). ESMS m/z 425.3 (MH^+). Anal. ($C_{22}H_{15}Cl_2N_3O_2$) C, H, N.

5.1.46. 2-[1-(4-Chloro-3-nitrobenzyl)-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-4-yl)acetamide (55)

It was synthesized via route C from **14** using 4-aminopyridine. Yield 46%, mp 271–272 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 5.77 (s, 2H), 7.36–7.39 (m, 2H), 7.62 (dd, J = 8.5, 2.0 Hz, 1H), 7.66–7.71 (m, 1H), 7.79 (d, J = 8.5 Hz, 1H), 7.90 (d, J = 5.8 Hz, 2H), 8.19 (d, J = 2.0 Hz, 1H), 8.34–8.36 (m, 1H), 8.56 (d, J = 5.8 Hz, 2H), 9.12 (s, 1H), 11.15 (s, 1H). ESMS m/z 435.8 (MH^+). Anal. ($C_{22}H_{15}ClN_4O_4$) C, H, N.

5.1.47. 2-[1-(4-Chloro-2-nitrobenzyl)-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-4-yl)acetamide (56)

4-Chloro-2-nitrobenzyl bromide was synthesized by bromination of 4-chloro-2-nitrotoluene with NBS as previously described (see Section 5.1.7, compound **12**). Yield 93%. Reaction of **17** with 4-chloro-2-nitrobenzyl bromide via route B led to **56**. Yield 72%, mp 197–198 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 6.04 (s, 2H), 6.62 (d, J = 8.5 Hz, 1H), 7.32–7.45 (m, 2H), 7.64 (d, J = 7.3 Hz, 1H), 7.75 (dd, J = 8.5, 2.4 Hz, 1H), 7.88 (d, J = 4.9 Hz, 2H), 8.33 (d, J = 2.4 Hz, 1H), 8.39 (d, J = 7.9 Hz, 1H), 8.55 (d, J = 4.9 Hz, 2H), 9.02 (s, 1H), 11.16 (s, 1H). ESMS m/z 435.8 (MH^+). Anal. ($C_{22}H_{15}ClN_4O_4$) C, H, N.

5.1.48. 2-[1-(3-Amino-4-chlorobenzyl)-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-4-yl)acetamide (57)

It was synthesized by reduction of **55** with tin chloride[20] in concentrated HCl. Yield 47%, mp 252–253 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 5.43 (s, 2H), 5.52 (s, 2H), 6.53 (dd, J = 7.9, 1.8 Hz, 1H), 6.68 (d, J = 1.8 Hz, 1H), 7.19 (d, J = 7.9 Hz, 1H), 7.34–7.38 (m, 2H), 7.58–7.61 (m, 1H), 7.90 (d, J = 6.4 Hz, 2H), 8.34–8.36 (m, 1H), 8.56 (d, J = 6.4 Hz, 2H), 8.99 (s, 1H), 11.15 (s, 1H). ESMS m/z 405.8 (MH^+). Anal. ($C_{22}H_{17}ClN_4O_2$) C, H, N.

5.1.49. 2-[1-(2-Amino-4-chlorobenzyl)-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-4-yl)acetamide (58)

It was synthesized by reduction of **56** with tin chloride[20] in concentrated HCl. Yield 60%, mp 258–259 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 5.45 (s, 2H), 5.70 (s, 2H), 6.54 (dd, J = 8.2, 1.6 Hz, 1H), 6.79 (d, J = 1.6 Hz, 1H), 6.86 (dd, J = 8.2 Hz, 1H), 7.35–7.39 (m, 2H), 7.64–7.66 (m, 1H), 7.88 (d, J = 6.2 Hz, 2H), 8.33–8.35 (m, 1H), 8.55 (d, J = 6.2 Hz, 2H), 8.91 (s, 1H), 11.12 (s, 1H). ESMS m/z 405.8 (MH^+). Anal. ($C_{22}H_{17}ClN_4O_2$) C, H, N.

5.1.50. 2-[1-(4-Chloro-3-methoxybenzyl)-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-4-yl)acetamide (59)

It was synthesized via route C from **12** using 4-aminopyridine. Yield 61% or via route A from **17** using 4-chloro-3-methoxybenzyl bromide. Yield 65%, mp 207–208 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 3.88 (s, 3H), 5.64 (s, 2H), 6.81–6.83 (m, 1H), 7.35–7.39 (m, 4H), 7.68–7.70 (m, 1H), 7.90 (d, J = 6.0 Hz, 2H), 8.33–8.35 (m, 1H), 8.56 (d, J = 6.0 Hz, 2H), 9.06 (s, 1H), 11.15 (s, 1H). ESMS m/z 420.9 (MH^+). Anal. ($C_{23}H_{18}ClN_3O_3$) C, H, N.

5.1.51. 2-[1-(4-Chloro-2-methoxybenzyl)-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-4-yl)acetamide (60)

It was synthesized via route C from **13** using 4-aminopyridine. Yield 59%, mp 180–181 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 3.91 (s, 3H), 5.55 (s, 2H), 7.00 (dd, J = 8.2, 1.9 Hz, 1H), 7.16–7.20 (m, 2H), 7.34–7.39 (m, 4H), 7.65–7.67 (m, 1H), 7.89 (d, J = 6.4 Hz, 2H), 8.34–8.35 (m, 1H), 8.55 (d, J = 6.4 Hz, 2H), 8.92 (s, 1H), 11.13 (s, 1H). ESMS m/z 420.9 (MH^+). Anal. ($C_{23}H_{18}ClN_3O_3$) C, H, N.

5.1.52. 2-[1-(4-Chloro-3-hydroxybenzyl)-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-4-yl)acetamide (61)

It was synthesized by demethylation of **59** with boron tribromide[17] in CH_2Cl_2. Yield 56%, mp 282–283 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 5.60 (s, 2H), 6.83–6.84 (m, 1H), 7.32–7.39 (m, 4H), 7.60–7.62 (m, 1H), 7.90 (d, J = 6.1 Hz, 2H), 8.33–8.35 (m, 1H), 8.55 (d, J = 6.1 Hz, 2H), 9.03 (s, 1H), 11.15 (s, 1H). ESMS m/z 406.8 (MH^+). Anal. ($C_{22}H_{16}ClN_3O_3$) C, H, N.

5.1.53. 2-[1-(3,4,5-Trimethoxybenzyl)-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-4-yl)acetamide (62)

It was synthesized via route A from **17** using 3,4,5-trimethoxybenzyl chloride. Yield 83%, mp 181–183 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 3.65 (s, 3H), 3.75 (s, 6H), 5.50 (s, 2H), 6.75 (s, 2H), 7.33–7.35 (m, 2H), 7.73–7.75 (m, 1H), 7.86 (d, J = 5.8 Hz, 2H), 8.29–8.31 (m, 2H), 8.52 (d, J = 5.8 Hz, 2H), 8.99 (s, 1H), 11.08 (s, 1H). ESMS m/z 446.5 (MH^+). Anal. ($C_{25}H_{23}N_3O_5$) C, H, N.

5.1.54. 2-[1-(3,5-Dimethoxybenzyl)-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-4-yl)acetamide (63)

It was synthesized via route A from **17** using 3,5-dimethoxybenzyl chloride. Yield 72%, mp 178–180 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 3.75 (s, 3H), 5.53 (s, 2H), 6.43 (d, J = 1.8 Hz, 1H), 6.47 (d, J = 1.9 Hz, 2H), 7.31–7.35 (m, 2H), 7.63–7.65 (m, 1H), 7.86 (d, J = 6.1 Hz, 2H), 8.29–8.31 (m, 1H), 8.52 (d, J = 6.1 Hz, 2H), 8.97 (s, 1H), 11.08 (s, 1H). ESMS m/z 416.4 (MH^+). Anal. ($C_{24}H_{21}N_3O_4$) C, H, N.

5.1.55. 2-[1-(6-Chloro-1,3-benzodioxol-5-yl)methyl-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-4-yl)acetamide (64)

It was synthesized via route A from **17** using 6-chloro-5-chloromethyl-1,3-benzodioxole. Yield 61%, mp 242–244 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 5.57 (s, 2H), 6.07 (s, 2H), 6.81 (s, 1H), 7.19 (s, 1H), 7.34–7.36 (m, 2H), 7.62–7.64 (m, 1H), 7.85 (d, J = 5.9 Hz, 2H), 8.31–8.33 (m, 1H), 8.51 (d, J = 5.9 Hz, 2H), 8.86 (s, 1H), 11.07 (s, 1H). ESMS m/z 434.8 (MH^+). Anal. ($C_{23}H_{16}ClN_3O_4$) C, H, N.

5.1.56. 2-[1-(4-Fluorophenyl)-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-4-yl)acetamide (65)

It was synthesized via route C from **16** using 4-aminopyridine. Yield 58%, mp 219–221 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 7.42–7.49 (m, 2H), 7.53 (dd, J = 8.9 Hz, 2H), 7.82 (dd, J = 8.9, 4.8 Hz, 2H), 7.90 (d, J = 6.2 Hz, 2H), 8.42–8.45 (m, 1H), 8.54–8.58 (m, 3H), 8.93 (s, 1H), 11.21 (s, 1H). ESMS m/z 360.4 (MH^+). Anal. ($C_{21}H_{14}FN_3O_2$) C, H, N.

5.1.57. 2-[1-(4-Fluorobenzoyl)-1*H*-indol-3-yl]-2-oxo-*N*-(pyridin-4-yl)acetamide (66)

It was synthesized via route B from **17** using 4-fluorobenzoyl chloride. Yield 33%, mp 204–206 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 7.53–7.60 (m, 4H), 7.85 (d, J = 5.9 Hz, 2H), 8.04 (dd, J = 8.7, 5.4 Hz, 2H), 8.32–8.45 (m, 2H), 8.54 (d, J = 5.9 Hz, 2H), 8.89 (s, 1H), 11.21 (s, 1H). ESMS m/z 388.4 (MH^+). Anal. ($C_{22}H_{14}FN_3O_3$) C, H, N.

5.2. General method: pharmacology

5.2.1. Cytotoxicity assays

The XTT assay quantifies cellular metabolic activity which correlates with cellular viability and/or cell number.[21] This cytotoxicity assessment has been conducted with three diverse tumor cell lines, namely Hela/KB (ATCC CCL17, human cervix carcinoma), L1210 (murine leukemia) and SKOV3 (ATCC HTB-77, human ovarian carcinoma).

Test compounds in 100% DMSO at 1 mg/mL are added to the tumor cell lines to final concentrations of 3.16 μg/mL of compound and 0.3% DMSO. After 45 h of incubation at 37 °C/5%CO_2 1 mg/mL XTT (Serva, cat. no 38450) and 76.6 μg/mL PMS (Sigma, cat. no P9625) are incubated with the cells for additional 3 h. After 48 h of total compound incubation, cellular metabolic activity is quantified by single point measurement of absorbance at 490 nm. Non-treated cells and blank controls w/o cells are set as reference values of 0% and 100% inhibition, respectively. Compounds which show an inhibition of cellular viability of 50% in at least one of the three cell lines analyzed are subjected subsequently to IC_{50} determination in the same panel of cell lines. The study was performed at least in duplicate to obtain the IC_{50} values expressed in nM units ± standard deviation.

5.2.2. Flow cytometry

For cell cycle analysis the CycleTEST PLUS DNA Reagent Kit (BD Bioscience) was used. The Kit provides a set of reagents for isolating and staining cell nuclei from surplus fresh or frozen solid tissue specimens or cell suspensions. Flow cytometric analysis of differentially stained normal and tumor cells is used in research to identify abnormal DNA stemlines and to estimate the DNA index (DI) and cell cycle phase distributions of these stemlines.

The method involves dissolving the cell membrane lipids with a nonionic detergent, eliminating the cellcytoskeleton and nuclear proteins with trypsin, digesting the cellular RNA with an enzyme, and stabilizing the nuclear chromatin with spermine.[24,25] Propidium iodide (PI) is stoichiometrically bound to the clean, isolated nuclei which are then run on a flow cytometer equipped with electronic doublet-discrimination capability.[26,27]

Propidium iodide-stained nuclei emit fluorescent light primarily at wavelengths between 580 and 650 nm. The FACS Calibur™ fluorescence 2 (FL2) detector is used to analyze light emitted between 564 nm and 606 nm by the stained cells. The resulting fluorescence histograms may be analyzed by Mod Fit LT™ cell cycle analysis software. Data points (number of cells in G2/M) were connected and EC_{50}-values calculated by nonlinear regression analysis using GraphPad Prism™ Software.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.07.048.

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