Bioorganic & Medicinal Chemistry 18 (2010) 4509–4515

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and cytotoxicity of novel indirubin-5-carboxamides

Xinlai Cheng, Paul Rasqué, Sandra Vatter, Karl-Heinz Merz *, Gerhard Eisenbrand

Department of Chemistry, Division of Food Chemistry and Toxicology, University of Kaiserslautern, Erwin-Schrödinger-Str. 52, PO Box 3049, 67663 Kaiserslautern, Germany

ARTICLE INFO

Article history:
Received 12 November 2009
Revised 19 April 2010
Accepted 21 April 2010
Available online 28 April 2010

Keywords:
Indirubin
Chemical synthesis
Antitumor agents
Protein kinase inhibitors

ABSTRACT

Indirubins have been reported to act as potent inhibitors of protein kinases relevant to tumorigenesis and of tumor cell growth, but their development to antitumor drugs suffer from their poor water solubility. We synthesized a novel class of indirubin derivatives, indirubin-5-carboxamides, carrying amide substituents with basic centers. Quaternization or protonation of these alkylamino substituents provided indirubins with significantly improved solubility without loss of bioactivity.

1. Introduction

Dangui Luhui Wan (or named *Danggui Longhui Wan*) is a recipe found by Zhengheng Zhu in 1481[1] that has been used in Traditional Chinese Medicine (TCM) for treatment of various diseases including Chronic Myelogenous Leukemia (CML).[2a] During the 7th decade of the last century, indirubin (**1**, Fig. 1) has been shown to act as an active ingredient thereof.[2b] In 1999, indirubins were found to inhibit cyclin dependent kinases (CDKs) binding to the ATP pocket.[3] Indirubin became a new lead structure for targeting CDKs,[3] GSK-3β,[4] and several tyrosine kinases including c-Src.[5] However, due to strong intermolecular and intramolecular forces in their crystal lattice,[6] indirubins are extremely poorly soluble in nearly all solvents resulting in low bioavailability.[7] For improvement of their aqueous solubility some pharmaceutical formulations[8] and chemical modifications[9] have been explored.

Here we focus on chemical modifications of substituents. Structure–activity studies suggest that substituents in 5- and/or 3′-position, which face the ribose/triphosphate canal (Fig. 1), are eligible to enhance solubility and bioactivity. Thus, we succeeded in synthesizing indirubin-3′-oxime ethers with potent inhibitory activity and improved water solubility that carry a sugar moiety linked by a C_2-spacer to the oxime.[10a] Previously, several 6-substituted indirubin-3′-oxime ethers with basic substituents targeting GSK-3β have been synthesized.[10b,c] We introduced amide substituents in 5-position with basic functional groups for improvement of solubility and bioactivity.

2. Results and discussion

The general synthesis of indirubin has already been developed in 1881, based on the basic condensation of isatin and indoxyl.[11a] It was modified later in 1969 by replacing indoxyl with the more stable 3-indoxyl acetate (**3**).[11b]

For the synthesis of indirubin-5-carboxamides, we first had to prepare 5-carboxyisatin (**2**) serving as a coupling partner for 3-indoxyl acetate (Scheme 1). The synthesis of **2** started by converting *p*-aminobenzoic acid (**10**) into 4-carboxyisonitrosoacetanilide (**11**) using the Sandmeyer method.[12] Cyclization of **11** with concentrated sulfuric acid afforded a mixture of **2** and 5-carboxyisatoxime (**12**),[13] that was difficult to separate. To obtain pure **2** we completely converted this mixture with hydroxylamin hydrochloride into **12**. Reduction of 5-carboxyisatoxime with Sn/HCl as reducing

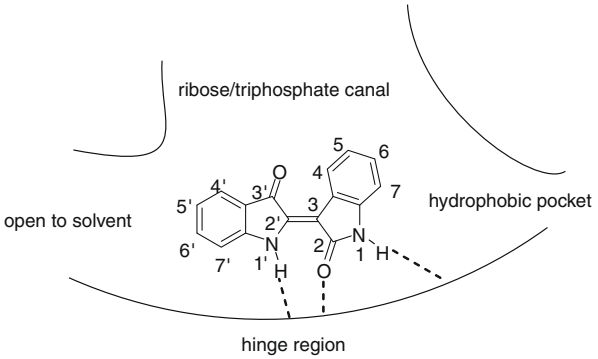
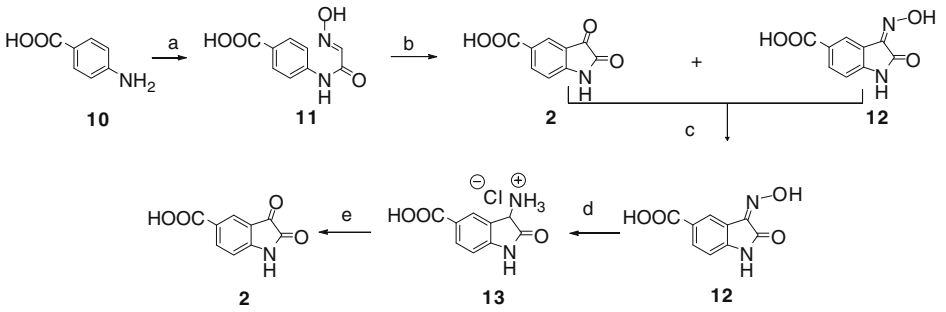


Figure 1. Outline of indirubin (**1**) bound to the ATP-binding pocket (based on the crystal structure of CDK2 in complex with indirubin-5-sulfonic acid).[3]

* Corresponding author. Tel.: +49 631 205 2976; fax: +49 631 205 3085.
E-mail address: khmerz@rhrk.uni-kl.de (K.-H. Merz).

0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2010.04.066



Scheme 1. Synthesis of 5-carboxyisatin (**2**). Reactants and conditions: (a) chloral hydrate, $H_2NOH{\cdot}HCl$, Na_2SO_4; (b) H_2SO_4 concd., ΔT; (c) $H_2NOH{\cdot}HCl$; (d) Sn/HCl, H_2S; (e) $FeCl_3$.

agent provided 3-amino oxindole hydrochloride (**13**), which was quantitatively oxidized by $FeCl_3$ to give **2**.[14]

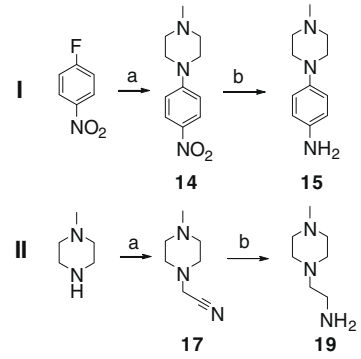
For esterifications and amidations the 5-carboxyl group of **2** was activated by pentafluorophenyl ester formation. Two ways proved practicable: Activation of **2** before coupling with 3-indoxyl acetate (Scheme 2, d–f) or activating 5-carboxyindirubin (**4**, Scheme 2, a–c). Activating 5-carboxyindirubin yielded the more stable pentafluorophenyl indirubin-5-carboxylate (**6**), directly enabling amidations with different amino compounds.

Synthesis of 1-(4-aminophenyl)-4-methylpiperazine (**15**) was achieved by nucleophilic aromatic substitution of 1-fluoro-4-nitrobenzol with 1-methylpiperazine and subsequent reduction with Pd/C (Scheme 3, **I**).[15] 1-(2-aminoethyl)-4-methyl-piperazine **19** was prepared by hydrogenation of 2-(4-methylpiperazino) acetonitrile (**17**) with lithium aluminium hydride. Compound **17** was obtained by nucleophilic substitution of chloroacetonitrile with 1-methylpiperazine (Scheme 3, **II**).[16]

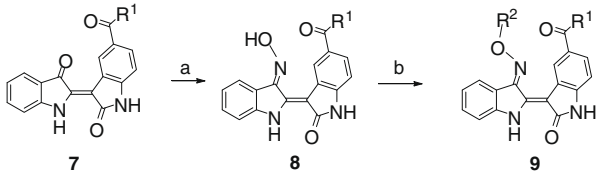
To further improve solubility, selected indirubin-5-carboxamides were converted into the corresponding 3′-oximes using hydroxylamine hydrochloride in pyridine. Two of the oximes were alkylated with substituted alkyl halogenides to produce 5-carboxamido-indirubin-3′-oxime ethers (**9**, Scheme 4). Tetra-*o*-acetyl-(2-bromoethyl)-β-D-glucopyranose, used for preparing **9b**, was synthesized according to the Königs–Knorr method.[17]

Some indirubin-5-carboxamides with basic centre placed in the amide substituent were transformed into their quarternary alkyl ammonium salts or hydrochlorides (**18**) (Scheme 5).

Inhibition of tumor cell proliferation by indirubin compounds was evaluated in human large cell lung cancer cells (LXFL529L cells) using the SRB assay.[18] Water solubility was determined spectrophotometrically as reported previously.[19] Chemical structures, IC_{50} values of tumor cell growth inhibition and solubility data are listed in Tables 1–3.

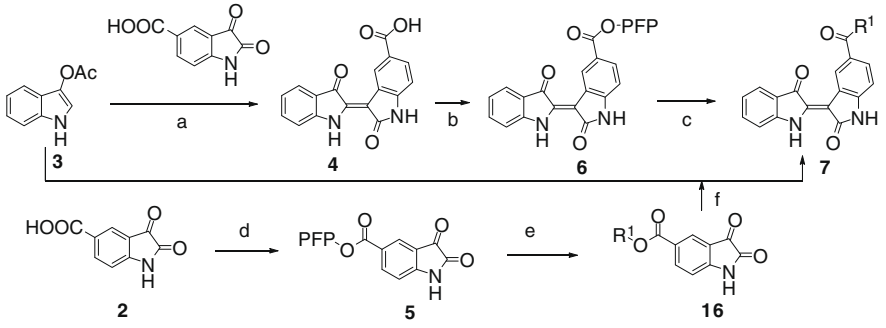


Scheme 3. I: Synthesis of **15**. Reactants and conditions: (a) NaOAc, EtOH, 1-methylpiperazine; (b) H_2, Pd/C, MeOH; **II**: Synthesis of **19**. Reactants and conditions: (a) chloroacetonitrile, K_2CO_3, acetonitrile; (b) $LiAlH_4$, dry Et_2O.

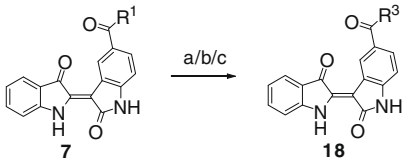


Scheme 4. Synthesis of 3′-hydroxyimino-indirubin-5-carboxamides (**8**) and 5-carboxamido-indirubin-3′-oxime ethers (**9**) (R^1 and R^2 see Table 2). Reactants and conditions: (a) $H_2NOH{\cdot}HCl$, pyridine; (b) 1,1,3,3-tetramethylguanidine, R^2–Cl or R^2–Br.

More than 50% of the newly synthesized indirubin-5-carboxamides **7a**–**7k** effected potent tumor cell growth inhibition (IC_{50} <5 μM, Table 1). From these, 50% show adequate to excellent (>500 mg/L) water solubility. Within that group, compound **7a**,

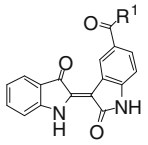


Scheme 2. Synthesis of indirubin-5-carboxamides (**7**). Reactants and conditions: (a) Na_2CO_3, MeOH; (b) pentafluorophenyl trifluoroacetate (PFP-trifluoroacetate), DMAP, pyridine, DMF; (c) R^1–H (R^1 see Table 1), DMAP, dioxane; (d) PFP-trifluoroacetate, DMAP, pyridine, DMF; (e) R^1–H (R^1 see Table 1), DMAP, DMF; (f) Na_2CO_3, MeOH.



Scheme 5. Conversion of indirubin-5-carboxylic acid alkylaminoalkyl-amides (**7**) into corresponding ammonium salts **18**. Reactants and conditions: (a) HCl/THF; (b) MeI/MeOH; (c) dimethyl sulfate, xylene, nitrobenzene. R^3 see Table 3.

Table 1
Structure, water solubility and inhibitory activity on tumor cell proliferation of indirubin-5-carboxamides (**7**)



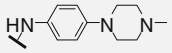
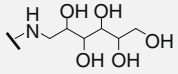
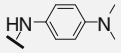
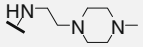
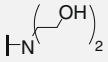
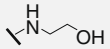
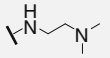
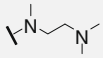
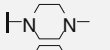
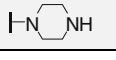
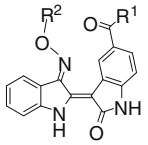
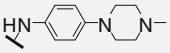
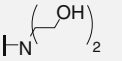
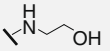
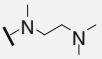
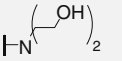
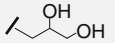
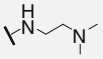
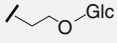
Compds	R^1	Solubility mg/L	IC_{50}, μM
4	OH	41	7
7a		520	0.54
7b		21.6	> 30
7c		55.5	> 10
7d		580	1.2
7e		700	7.8
7f		6.8	4.7
7g		9.5	9.2
7h		13	2.3
7i		35.4	5.4
7j		1000	3.9
7k		4.2	3.2

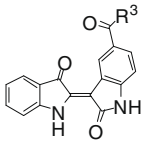
Table 2
Structure, water solubility and inhibitory activity on tumor cell proliferation of 3′-hydroxyimino-indirubin-5-carboxamides (**8**) and 3′-(subst)-alkoxyimino-indirubin-5-carboxamides (**9**)

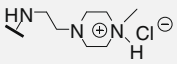
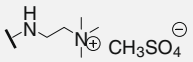
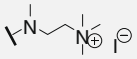
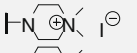
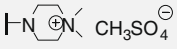


Compds	R^1	R^2	Solubility mg/L	IC_{50}, μM
8a		H	1600	4.2
8b		H	523	5.9
8c		H	179	4.5
8d		H	6400	2.2
9a			21	43
9b			>380	>10

Glc: β-D-glucopyranoside.

Table 3
Water solubility and inhibitory activity on tumor cell proliferation of alkylammonium indirubin-5-carboxamide salts (**18**)



Compds	R^3	Solubility mg/L	IC_{50}, μM
18a		>2000	6
18b		53,000	8.6
18c		1600	5.7
18d		2450	2.5
18e		60,000	3.5

carrying a methylpiperazino-phenylamino substituent attached to 5-carboxyindirubin, with a water solubility of 520 mg/L, was the most potent with an IC_{50} value of 0.54 μM. Replacing the methylpiperazine moiety by dimethylamine (**7d**) resulted in a slight reduction of inhibitory activity as well as a slight increase of solubility. Replacement of the phenyl moiety of **7a** by an C_2-spacer (**7e**) resulted in moderately enhanced solubility but markedly attenuated antitumor activity. The attachment of 3′-aminopyridine (**7c**) or aminosorbitol (**7b**) to 5-carboxyindirubin resulted in abrogation of in vitro cell growth. Indirubins with hydroxylated alkylamines (**7f** and **7g**) linked to the 5-carboxy group display decreased inhibitory activity and water solubility as compared to alkylaminoalkylamines (**7h** and **7i**). The methylpiperazino substituent (**7j**) led to a greater increase in water solubility as compared to the piperazino substituent (**7k**).

Transformation of the indirubin-5-carboxamides into the corresponding 3′-oximes generally brought about elevation of water solubility, and maintaining antiproliferative activity (Table 2). However, reaction of the oximes to oxime ethers abrogates growth inhibitory activity (**9a** and **9b**), presumably due to the additional steric crowding, counteracting binding affinity into ATP-binding sites of kinases driving tumor cell proliferation. Analogous effects have been noticed for indirubin-5-sulfonamides.[20]

Quaternization of amino substituents (Scheme 5) caused a drastic enhancement of water solubility, while inhibitory activity on cell proliferation, as expressed by IC_{50} values, mostly was preserved (Table 3).

To gain insight into the mechanism of action of indirubin-5-carboxamides, inhibitory activity of **7a** was tested with 30 different protein kinases at two concentrations (ProQinase, Freiburg, Germany). The table of inhibition data is given in supporting information. At 10 μM, the residual activity of 16 kinases was <50%, including ABL, CDKs (CDK1/CyclinB1, CDK2/CyclinA/E, CDK4/CyclinD1 and CDK5/p35), GSK-3β as well as several receptor tyrosine kinases, for example, FGR, EGFR and FLT3 (listed in

supporting information). At 1 μM, only the residual activity of GSK3-β was found to be <50%. Compared to indirubins with potent antiproliferative activity and moderate water solubility reported earlier,[10a] such as 5-methoxyindirubin-3′-(2-β-D-glucopyranosylethyl)-oxime ether (E729) with a water solubility of 42 mg/L and an IC_{50} value of 0.5 μM for growth inhibition of LXFL529L tumor cells), the overall kinase inhibitory activity of **7a** was found inferior, especially with respect to inhibition of CDKs and receptor (VEGFRs) and nonreceptor (Src) tyrosine kinases, whereas GSK-3β inhibitory activity (IC_{50}: 0.1 ± 0.01 μM, unpublished result) was retained. Still the antiproliferative activity was quite similar, possibly a reflection of retained GSK-3β inhibition or of other as yet undiscovered mechanisms accounting for antiproliferative activity of **7a**. Although the role of GSK-3β inhibition for antiproliferative activity is not yet clear, potent and selective GSK-3β inhibitors have been found to have moderately antiproliferative activity.[10c] Of note, the parent compound, indirubin (E211), was markedly inferior not only with respect to its extremely poor water solubility but also showing only moderate antiproliferative effectiveness (IC_{50}: 10 μM, LXFL529L) and low CDK inhibitory potential (>2 μM).[10a]

3. Conclusions

The synthesis of novel indirubin derivatives aimed at improving water solubility and antitumor activity is described. Indirubin-5-carboxamides are potent inhibitors of tumor cell proliferation with IC_{50}-values within or beneath the low μM range. Quaternization or protonation of amides bearing an alkyl amino group in the amide substituent provides better water soluble indirubin compounds with potent antiproliferative activity on human tumor cells.

4. Materials and methods

4.1. Cell culture

LXFL529L cells were grown at 37 °C in RPMI 1640 (Invitrogen, Karlsruhe, Germany), supplemented with 10% heat-inactivated fetal bovine serum, penicillin (100 units/mL), streptomycin (100 mg/mL), in a humidified atmosphere of 5% CO_2. The cells were tested routinely for absence of mycoplasm contamination.[16]

4.2. Sulforhodamine B (SRB) assay

Effects on cell growth were determined according to the method of Skehan et al. with slight modifications. Briefly, cells were seeded into 24-well plates and allowed to grow for 24 h before treatment. Thereafter, cells were incubated with the respective drug for 3 days in serum containing medium. Incubation was stopped by addition of trichloroacetic acid (50% solution). After 1 h at 4 °C, plates were washed four times with water. The dried plates were stained with a 0.4% solution of sulforhodamine B. The dye was eluted with Tris-buffer (10 mM, pH 10.5) and quantified photometrically at 570 nm. Cytotoxicity was determined as percent survival, determined by the number of treated over control cells ×100 (% T/C).[18,19]

4.3. Solubility in water

Standard solutions of the indirubin compounds were prepared in ethanol and maxima of absorbance were defined. Solubility was determined spectrophotometrically at maximum of absorbance by calibration method.[19]

5. Experimental

5.1. Reagents

Solvents and reagents obtained from commercial suppliers were at least of reagent grade and were distilled or dried according to prevailing methods prior to use, if necessary. The syntheses were done under argon atmosphere, when required. Argon 4.8 was purchased from Air Liquide and was dried over phosphorus pentoxide. For monitoring the reactions, Alugram SIL G/UV_{254} sheets for TLC (Macherey & Nagel) were used. Column chromatography was accomplished using Silica Gel 60 (Macherey & Nagel, 0.063–0.200 mm), for flash chromatography Silica Gel 60 (Macherey & Nagel, 0.040–0.063 mm) was used.

5.2. Analytical methods

^1H and ^{13}C NMR spectra were recorded on a Bruker AMX-400 (^1H NMR: 400 MHz, ^{13}C NMR: 100 MHz) or on a Bruker AMX-600 (^1H NMR: 600 MHz, ^{13}C NMR: 150 MHz). Chemical shifts are reported in ppm from tetramethylsilane with solvent as the internal standard (^1H $CDCl_3$: δ 7.26; ^{13}C $CDCl_3$: δ 77.0; ^1H DMSO-d_6: δ 2.49; ^{13}C DMSO-d_6: δ 39.5).

Mass spectra (MS) were recorded on a Bruker ApexQe hybrid 9.4 T FT-ICR (ESI) or JEOL JMS-700 sector field (EI, FAB). MALDI-TOF mass spectra were recorded on a Bruker BIFLEX III.

Elemental analyses were performed on an Element Analyzer Perkin–Elmer EA 240 or 2400 CHN at the University of Kaiserslautern, Department of Chemistry.

5.3. Intermediates

As yet unpublished NMR data of the intermediates **2**, **11**–**15**, **17** and **19**, synthesized following literature methods, are given in Supplementary data.

5.4. Pentafluorophenyl isatin-5-carboxylate (5)

Under argon atmosphere, pentafluorophenyl trifluoroacetate (1.08 g, 3.86 mmol) and pyridine (324 mg, 4.10 mmol) were added to a solution of **2** (350 mg, 1.83 mmol) in dry DMF. The mixture was stirred for 5.5 h at room temperature, diluted with ethyl acetate and washed with brine. After removal of the solvent the residue was dried in vacuo to yield **5** (635 mg, 97%). ^1H NMR (400 MHz; DMSO-d_6): 7.13 (d, 1H, ^3J = 8.2 Hz), 8.14 (d, 1H, ^4J = 1.6 Hz), 8.35 (dd, 1H, ^3J = 8.2 Hz, ^4J = 1.6 Hz), 11.61 (s, 1H); ^{13}C-{^1H} NMR (150 MHz; DMSO-d_6): 112.8, 119.6, 126.0, 136.2, 138.7, 139.5, 140.0, 141.94, 155.6, 159.6, 161.2, 182.6. Anal. Calcd for $C_{15}H_4F_5NO_4$: C, 50.44; H, 1.13; N, 3.92. Found: C, 50.00; H, 1.30; N, 3.74.

5.5. Isatin-5-carboxylic acid diethanolamide (16)

Under argon atmosphere a mixture of **5** (300 mg, 0.84 mmol), diethanolamine (170 mg, 1.24 mmol) pyridine (10 mL) and acetonitrile (20 mL) was stirred at room temperature for 2.5 h. After removal of the solvents the residue was purified by column chromatography on silica gel using ethyl acetate/methanol (4:1) as eluent to provide **16** as a orange solid (66.2 mg, 0.25 mmol, 30%). ^1H NMR (400 MHz; DMSO-d_6): 3.32–3.58 (m, 8H), 4.81 (s, 2H), 6.93 (d, 1H, ^3J = 7.8 Hz), 7.57 (s, 1H), 7.62 (d, 1H, ^3J = 7.8 Hz), 11.17 (s, 1H); ^{13}C-{^1H} NMR (150 MHz; DMSO-d_6): 47.4, 51.7, 111.9, 117.3, 123.4, 131.6, 137.2, 150.9, 159.4, 169.7, 184.0. Anal. Calcd for $C_{13}H_{14}N_2O_5$: C, 56.11; H, 5.07; N, 10.07. Found: C, 55.98; H, 4.86; N, 10.00.

5.6. Indirubin-5-carboxylic acid (4)

Under argon atmosphere a suspension of 3-indoxyl acetate (1.34 g, 7.67 mmol), 5-carboxyisatin (1.53 g, 8.03 mmol) and sodium carbonate (1.73 g, 16.32 mmol) in degassed methanol (80 mL) was stirred at room temperature for 20 h, diluted with water and filtered to get a reddish solid (2.26 g, 7.40 mmol, 92.0%). ^{1}H NMR (400 MHz; DMSO-d_6): 6.97 (d, 1H, ^3J = 8.2 Hz), 7.04 (t, 1H, ^3J = 7.4 Hz), 7.42 (d, 1H, ^3J = 8.1 Hz), 7.58 (t, 1H, ^3J = 7.7 Hz), 7.66 (d, 1H, ^3J = 7.5 Hz), 7.87 (dd, 1H, ^3J = 8.2 Hz, ^4J = 1.6 Hz), 9.43 (d, 1H, ^4J = 1.2 Hz), 11.08 (s, 1H), 11.22 (s, 1H), 12.63 (s, 1H); ^{13}C–{^{1}H} NMR (150 MHz; DMSO-d_6): 105.3, 109.1, 113.5, 119.0, 121.3, 121.5, 123.8, 124.4, 126.1, 130.8, 137.1, 139.0, 144.2, 152.4, 167.5, 171.2, 188.6. Anal. Calcd for $C_{17}H_{10}N_2O_4$: C, 66.67; H, 3.29; N, 9.15. Found: C, 66.68; H, 3.20; N, 9.05.

5.7. Pentafluorophenyl indirubin-5-carboxylate (6)

Under argon atmosphere a suspension of **4** (1.53 g, 5.0 mmol), pentafluorophenyl trifluoroacetate (2.42 g, 8.7 mmol), pyridine (643 mg, 8.14 mmol) and catalytic amount of dimethylaminopyridine (DMAP) in dry DMF (40 mL) was stirred at room temperature for 4 h, diluted with ethyl acetate and washed with 0.1 N HCl (200 mL). After removal of the solvent, the residue was dried in vacuo to yield a reddish solid (2.22 g, 4.7 mmol, 94.0%). ^{1}H NMR (400 MHz; DMSO-d_6): 7.04 (t, ^3J = 7.5 Hz), 7.12 (d, 1H, ^3J = 8.3 Hz), 7.43 (d, 1H, ^3J = 8.1 Hz), 7.59 (t, 1H, ^3J = 7.7 Hz), 7.67 (d, 1H, ^3J = 7.5 Hz), 8.08 (dd, 1H, ^3J = 8.3, ^4J = 1.8 Hz), 9.63 (d,1H, ^4J = 1.7 Hz), 11.18 (s, 1H), 11.54 (s, 1H); ^{13}C–{^{1}H} NMR (150 MHz; DMSO-d_6): 104.1, 110.1, 113.7, 118.2, 119.0, 121.9, 122.0, 124.7, 124.9, 126.6, 132.0, 136.8, 137.1, 137.4, 139.8, 140.7, 146.2, 152.6, 162.3, 171.1, 188.9. Anal. Calcd for $C_{23}H_9F_5N_2O_4$: C, 58.49; H, 1.92; N, 5.93. Found: C, 58.38; H, 1.76; N, 5.84.

5.8. General procedure for the synthesis of indirubin-5-carboxamides (7)

Under argon atmosphere a suspension of **5** (1.0 mmol), amino compound (1.8 mmol) and DMAP (1.2 mmol) in dry dioxane (40 mL) was refluxed for several hours till no educt could be detected by TCL. After cooling the mixture was diluted with 0.1 N HCl (150 mL). The precipitate was filtered and dried in vacuo to afford indirubin-5-carboxamides (**7a**, 92.0%; **7b**, 87.0%; **7c**, 99.9%; **7d**, 14.7%; **7e**, 84.0%; **7f**, 60.2%; **7g**, 69.0%; **7h**, 88.0%; **7i**, 64.0%, **7j**, 73.0%; **7k**, 47.0%).

5.8.1. 5-{*N*-[4-(4-Methylpiperazino)-phenyl]-aminocarbonyl}-indirubin (7a)

^{1}H NMR (400 MHz; DMSO-d_6): 2.21 (s, 3H), 2.44–2.45 (m, 4H), 3.08–3.10 (m, 4H), 6.92 (d, 2H, ^3J = 6.0 Hz), 6.98 (d, 1H, ^3J = 5.6 Hz), 7.03 (t, 1H, ^3J = 5.2 Hz), 7.43 (d, 1H, ^3J = 5.6 Hz), 7.58 (t, 1H, ^3J = 5.2 Hz), 7.62 (d, 2H, ^3J = 6.0 Hz), 7.67 (d, 1H, ^3J = 5.2 Hz), 7.83 (1H, dd, ^3J = 5.6 Hz, ^4J = 0.8 Hz), 9.32 (d, 1H, ^4J = 0.8 Hz), 9.99 (s, 1H), 11.10 (s, 1H), 11.18 (s, 1H); ^{13}C–{^{1}H} NMR (150 MHz; DMSO-d_6): 45.8, 48.6, 54.7, 105.7, 108.8, 113.6, 115.5, 119.0, 121.3, 121.6, 121.3, 124.5, 124.7, 128.6, 128.9, 131.5, 137.3, 138.9, 143.0, 147.3, 152.5, 165.5, 171.2, 188.6. Anal. Calcd for $C_{28}H_{25}N_5O_3{\cdot}0.5\ H_2O$: C, 68.84; H, 5.36; N, 14.34. Found: C, 69.16; H, 5.18; N, 14.12.

5.8.2. 1-(Indirubin-5-carbonyl)-amino-1-deoxy-glucitol (7b)

^{1}H NMR (400 MHz; DMSO-d_6): 3.25–3.81 (m, 8H), 4.32 (d, 1H, ^3J = 5.6 Hz), 4.37 (d, 1H, ^3J = 6.4 Hz), 4.44 (d, 1H, ^3J = 5.2 Hz), 4.47 (d, 1H, ^3J = 4.8 Hz), 4.92 (d, 1H, ^3J = 4.4 Hz), 6.93 (d, 1H, ^3J = 8.0 Hz), 7.04 (t, 1H, ^3J = 7.2 Hz), 7.42 (d, 1H, ^3J = 8.0 Hz), 7.58 (t, 1H, ^3J = 8.0 Hz), 7.67 (d, 1H, ^3J = 7.2 Hz), 7.75 (d, 1H, ^3J = 8.4 Hz), 8.11 (t, 1H, ^3J = 5.6 Hz), 9.26 (s, 1H), 11.07 (s, 1H), 11.12 (s, 1H); ^{13}C–{^{1}H} NMR (150 MHz; DMSO-d_6): 42.8, 63.3, 69.4, 71.5, 71.9, 72.3, 105.7, 108.8, 113.6, 119.6, 121.3, 121.6 (C5′), 124.4, 124.5, 128.1, 128.2, 137.2, 138.9, 142.9, 152.5, 167.0, 171.2, 188.6. Anal. Calcd for $C_{23}H_{23}N_3O_8{\cdot}H_2O$: C, 56.67; H, 5.17; N, 8.62. Found: C, 56.69; H, 5.08; N, 8.60.

5.8.3. 5-[*N*-(Pyridin-3-yl)-aminocarbonyl]-indirubin (7c)

^{1}H NMR (400 MHz; DMSO-d_6): 7.03–7.07 (m, 2H), 7.42 (dd, 1H, ^1J = 8.4 Hz), 7.45 (d, 1H, ^3J = 8.4 Hz), 7.60 (t, 1H, ^3J = 8.4 Hz), 7.70 (d, ^3J = 7.8 Hz, 1H), 7.92 (dd, 1H, ^3J = 8.4 Hz, ^4J = 1.8 Hz), 8.23 (m, 1H), 8.32 (dd, 1H, ^3J = 4.8 Hz, ^4J = 1.2 Hz), 9.00 (d, 1H, ^4J = 2.4 Hz), 9.40 (d, 1H, ^4J = 1.8 Hz), 10.44 (s, 1H), 11.13 (s, 1H), 11.26 (s, 1H); ^{13}C–{^{1}H} NMR (150 MHz; DMSO-d_6): 105.7, 108.8, 113.6, 119.6, 121.4, 121.7, 123.5, 124.6, 124.9, 127.1, 127.9, 128.8, 136.2, 137.2, 139.1, 141.7, 143.2, 144.2, 152.6, 166.4, 171.2, 188.6. Anal. Calcd for $C_{22}H_{14}N_4O_3{\cdot}H_2O$: C, 66.00; H, 4.03; N, 13.99. Found: C, 65.80; H, 3.92; N, 13.95.

5.8.4. 5-[*N*-(4-Dimethylaminophenyl)-aminocarbonyl]-indirubin (7d)

^{1}H NMR (400 MHz; DMSO-d_6): 3.10 (s, 6H), 6.78 (s, 2H), 6.97 (d, 1H, ^3J = 5.6 Hz), 7.04 (t, 1H, ^3J = 5.2 Hz), 7.43 (d, 1H, ^3J = 5.2 Hz), 7.57–7.61 (m, 3H), 7.67 (d, 1H, ^3J = 5.2 Hz), 7.84 (1H, dd, ^3J = 5.6 Hz, ^4J = 1.2 Hz), 9.36 (d, 1H, ^4J = 1.2 Hz), 10.42 (s, 1H), 11.10 (s, 1H), 11.26 (s, 1H); ^{13}C–{^{1}H} NMR (150 MHz; DMSO-d_6): 105.7, 108.8, 113.6, 119.0, 121.3, 121.6, 124.5, 124.7, 128.5, 128.9, 137.3, 138.9, 143.0, 152.5, 165.4, 171.2, 188.6. Anal. Calcd for $C_{25}H_{20}N_4O_3$: C, 70.74; H, 4.75; N, 13.20. Found: C, 70.70; H, 5.13; N, 12.80.

5.8.5. 5-{*N*-[2-(4-Methylpiperazino)-ethyl]-aminocarbonyl}-indirubin (7e)

^{1}H NMR (400 MHz; DMSO-d_6): 2.12–2.60 (m, 15H), 6.93 (d, 1H, ^3J = 5.5 Hz), 7.04 (dd, 1H, ^3J = 9.4 Hz, ^4J = 4.5 Hz), 7.42 (d, 1H, ^3J = 5.5 Hz), 7.58 (m, 1H), 7.61 (d, 1H, ^3J = 4.9 Hz), 7.70 (1H, dd, ^3J = 5.4 Hz, ^4J = 1.2 Hz), 8.16 (s, 1H), 9.23 (s, 1H), 11.07 (s, 1H), 11.14 (s, 1H). Anal. Calcd for $C_{24}H_{25}N_5O_3$: C, 66.81; H, 5.84; N, 16.23. Found: C, 66.62; H, 5.63; N, 16.13.

5.8.6. 5-[*N*,*N*-Bis(2-hydroxyethyl)-aminocarbonyl]-indirubin (7f)

^{1}H NMR (400 MHz; DMSO-d_6): 3.35–3.70 (m, 8H), 4.65–4.90 (s, 2H), 6.91 (d, 1H, ^3J = 8.0 Hz), 7.02 (t, 1H, ^3J = 7.4 Hz), 7.30 (d, 1H, ^3J = 8.0 Hz), 7.42 (d, 1H, ^3J = 8.0 Hz), 7.58 (t, 1H, ^3J = 7,7 Hz), 7.65 (d, 1H, ^3J = 7.5 Hz), 8.79 (d, 1H, ^4J = 1.3 Hz), 11.03 (s, 1H), 11.07 (s, 1H); ^{13}C–{^{1}H} NMR (150 MHz; DMSO-d_6): 51.9, 58.9, 105.8), 109.0, 113.5, 119.0, 121.0, 121.5, 123.3, 124.5, 128.1, 130.2, 137.3, 138.9, 141.2, 152.5, 170.9, 171.3, 188.7. Anal. Calcd for $C_{21}H_{19}N_3O_7{\cdot}2H_2O$: C, 58.74; H, 5.40; N, 9.79. Found: C,58.60; H, 5.67; N, 9.40.

5.8.7. 5-[*N*-(2-Hydroxyethyl)-aminocarbonyl]-indirubin (7g)

^{1}H NMR (400 MHz; DMSO-d_6): 3.35 (q, 2H, ^3J = 5.9 Hz), 3.52 (q, 2H, ^3J = 5.5 Hz), 4.73 (t, 1H, ^3J = 5.0 Hz), 6.92 (d, 1H, ^3J = 8.1 Hz), 7.03 (t, 1H, ^3J = 7.4 Hz), 7.42 (d, 1H, ^3J = 8.0 Hz), 7.58 (t, 1H, ^3J = 7.7 Hz), 7.67 (d, 1H, ^3J = 7.5 Hz), 7.73 (dd, 1H, ^3J = 8.2 Hz, ^4J = 1.5 Hz), 8.21 (t, 1H, ^3J = 5.4 Hz), 9.25 (d, 1H, ^4J = 1.3 Hz), 11.07 (s, 1H), 11.11 (s, 1H); ^{13}C–{^{1}H} NMR (150 MHz; DMSO-d_6): 42.2, 59.9, 105.8, 108.7, 113.6, 119.0, 121.2, 121.5, 124.4, 124.5, 128.2, 128.6, 128.3, 138.8, 142.9, 152.5, 166.8, 171.2, 188.5. Anal. Calcd for $C_{19}H_{15}N_3O_4{\cdot}0.25H_2O$: C, 64.49; H, 4.42; N, 11.88. Found: C, 64.77; H, 4.50; N, 11.87.

5.8.8. 5-[*N*-(2-Dimethylaminoethyl)-aminocarbonyl]-indirubin (7h)

^{1}H NMR (400 MHz; DMSO-d_6): 2.43 (s, 6H), 2.72 (t, 2H, ^3J = 6.5 Hz), 3.46 (q, 2H, ^3J = 6.2 Hz), 6.92 (d, 1H, ^3J = 8.1 Hz), 7.03

(t, 1H, ^3J = 7.4 Hz), 7.42 (d, 1H, ^3J = 8.1 Hz), 7.58 (t, 1H, ^3J = 7.7), 7.66 (d, 1H, ^3J = 7.5 Hz), 7.71 (dd, 1H, ^3J = 8.6 Hz, ^4J = 1.2 Hz), 8.32 (t, 1H, ^3J = 5.5 Hz), 9.25 (d, 1H, ^4J = 1.3 Hz), 11.08 (s, 1H), 11.16 (s, 1H); ^{13}C–{^{1}H} NMR (150 MHz; DMSO-d_6): 36.2, 44.1, 57.4, 105.7, 108.7, 113.6, 119.0, 121.2, 121.5, 124.4, 124.4, 127.9, 128.1, 137.2, 138.8, 143.0, 152.5, 166.9, 171.2, 188.5. MS (EI) *m*/*z*: 376.1 [M]$^+$; calcd for $C_{21}H_{20}N_4O_3$: 376.154.

5.8.9. 5-[*N*-(2-Dimethylaminoethyl)-*N*-methyl-amino-carbonyl]-indirubin (7i)

^{1}H NMR (400 MHz; DMSO-d_6): 2.15 (s, 6 H), 2.52 (s, 2H), 3.02 (s, 3H), 3.48 (t, 1H, ^3J = 6.4 Hz), 6.95 (d, 1H, ^3J = 7.9 Hz), 7.04 (t, 1H, ^3J = 7.3 Hz), 7.29 (d, ^3J = 7.8 Hz), 7.38 (d, ^3J = 8.1 Hz), 7.56 (t, ^3J = 7.5 Hz), 7.65 (d, ^3J = 7.5 Hz), 8.83 (s, 1H), 10.72 (s, 1H), 10.86 (s, 1H); ^{13}C–{^{1}H} NMR (150 MHz; DMSO-d_6): 37.9, 45.2, 48.7, 56.5, 100.7, 109.1, 113.5, 118.9, 120.8, 121.5, 123.1, 124.3, 128.3, 129.5, 137.2, 138.8, 141.4, 152.5, 170.1, 171.0, 188.7. Anal. Calcd for $C_{22}H_{22}N_4O_3$: C, 67.68; H, 5.68; N, 14.35. Found: C, 67.29; H, 5.87; N, 13.95.

5.8.10. 5-(4-Methylpiperazinocarbonyl)-indirubin (7j)

^{1}H NMR (400 MHz; DMSO-d_6,): 2.25 (s, 3H), 2.39 (t, 4H, ^3J = 4.9 Hz), 3.55 (t, 4H, ^3J = 4.9 Hz), 6.96 (d, 1H, ^3J = 7.9 Hz), 7.04 (t, 1H, ^3J = 7.4 Hz), 7.30 (dd, 1H, ^3J = 9.3 Hz, ^4J = 1.4 Hz), 7.38 (d, 1H, ^3J = 7,9 Hz), 7.57 (t, 1H, ^3J = 7.6 Hz), 7.66 (d, 1H, ^3J = 7.5 Hz), 8.8 (s, 1H), 10.72 (s, 1H), 10.83 (s, 1H); ^{13}C-{^{1}H} NMR (150 MHz; DMSO-d_6): 45,4, 54,4, 105.6, 109.0, 113.4, 118.9, 120.9, 121.4, 123.5, 124.3, 128.4, 128.6, 137.1, 138.8, 141.6, 152.4, 169.2, 170.0, 188.5. Anal. Calcd for $C_{22}H_{20}N_4O_3$·HCl·0.33 H_2O: C, 61.32; H, 5.07; N, 13.00. Found: C, 61.79; H, 5.47; N, 12.36.

5.8.11. 5-(Piperazinocarbonyl)-indirubin (7k)

^{1}H NMR (400 MHz; DMSO-d_6): 2.07 (s, 1H), 2.72 (s, 4H), 3.46 (s, 4H), 6.94 (d, 1H, ^3J = 7.9 Hz), 7.02 (t, 1H, ^3J = 7.3 Hz), 7.29 (dd, 1H, ^3J = 9.1 Hz, ^3J = 1.2 Hz), 7.41 (d, 1H, ^3J = 8.0 Hz), 7.57 (t, 1H, ^3J = 7.6 Hz), 7.66 (d, 1H, ^3J = 7.8 Hz), 8.82 (s, 1H), 11.08 (m, 2H); ^{13}C–{^{1}H} NMR (150 MHz; DMSO-d_6): 30.7, 45.8, 105.7, 109.3, 113.6, 119.0, 121.0, 121.5, 123.6, 124.5, 128.6, 128.8, 137.2, 138.9, 141.8, 152.6, 169.3, 171.8, 188.8. Anal. Calcd for $C_{21}H_{18}N_4O_3$·0.5 HCl·1.5 H_2O: C, 60.10; H, 5.16; N, 13.35. Found: C, 60.48; H, 5.02; N, 13.05.

5.9. General procedure for the synthesis of indirubin-3′-oximes (8)

A mixture of indirubins (1.1 mmol) and hydroxylamine hydrochloride (24.4 mmol) in pyridine (7 mL) was refluxed for 4 h. The mixture was diluted with 2 N HCl (50 mL) to yield a reddish solid **8** (**8a**, 67.9%; .**8b**, 49.0%; **8c**, 90.0%; **8d**, 82.0%).

5.9.1. 3′-Hydroxyimino-5-{*N*-[4-(4-methylpiperazino)-phenyl]-aminocarbonyl}-indirubin (8a)

^{1}H NMR (400 MHz; DMSO-d_6): 2.21 (s, 3H), 2.44–2.45 (m, 4H), 3.08–3.10 (m, 4H), 6.89–7.01 (m, 4H), 7.32–7.37 (m, 2H), 7.62–7.63 (m, 3H), 8.28 (d, 1H, ^3J = 6.6 Hz), 9.09 (s, 1H), 9.79 (s, 1H), 10.86 (s, 1H), 11.98 (s, 1H). Anal. Calcd for $C_{28}H_{26}N_6O_3$·0.5 H_2O: C, 66.79; H, 5.40; N, 16.69. Found: C, 66.79; H, 5.18; N, 16.79.

5.9.2. 3′-Hydroxyimino-5-[*N*,*N*-bis(2-hydroxyethyl)-aminocarbonyl]-indirubin (8b)

^{1}H NMR (400 MHz; DMSO-d_6): 3.36–3.63 (m, 10 H), 6.90 (d, 1H, ^3J = 7.9 Hz), 7.03 (m, 1H), 7.15 (dd, 1H, ^3J = 7.9 Hz, ^4J = 1.4 Hz), 7.40–7.41 (m, 2H), 8.23 (d, 1H, ^3J = 7.6 Hz), 8.59 (s, 1H), 10.86 (s, 1H), 11.80 (s, 1H), 13.54 (s, 1H); ^{13}C–{^{1}H} NMR (150 MHz; DMSO-d_6): 51.9 58.8, 98.3, 108.1, 111.5, 116.5, 121.0, 121.6, 122.2, 124.7, 128.0, 129.4, 132.0, 138.5, 144.6, 145.8, 151.4, 170.9, 171.8. Anal. Calcd for $C_{21}H_{20}N_4O_5$·H_2O: C, 59.15; H, 5.20; N, 13.14. Found: C, 59.37; H, 4.80; N, 12.85.

5.9.3. 3′-Hydroxyimino-5-[*N*-(2-hydroxyethyl)-aminocarbonyl]-indirubin (8c)

^{1}H NMR (400 MHz; DMSO-d_6): 3.37 (q, 2H, ^3J = 5.9 Hz), 3.52 (q, 2H, ^3J = 6.1 Hz), 6.91 (d, 1H, ^3J = 8.3 Hz), 7.02 (m, 1H), 7.38–7.42 (m, 2H), 7.64 (dd, 1H, ^3J = 7.8 Hz, ^4J = 1.5 Hz), 7.97 (s, 1H), 8.25 (t, 1H, ^3J = 7.8 Hz), 9.01 (d, 1H, ^3J = 1.5 Hz), 10.94 (s, 1H), 11.76 (s, 1H); ^{13}C–{^{1}H} NMR (150 MHz; DMSO-d_6): 43.2, 61.0, 99.2, 108.9, 112.5, 117.5, 122.6, 122.9, 123.3, 126.3, 128.3, 128.9, 132.9, 141.3, 145.6, 146.9, 152.3, 168.2, 172.1. Anal. Calcd for $C_{19}H_{16}N_4O_4$·0.5H_2O: C, 61.12; H, 4.59; N, 15.01. Found: C, 61.05; H, 4.45; N, 14.88.

5.9.4. 3′-Hydroxyimino-5-[*N*-(2-dimethylaminoethyl)-*N*-methyl-aminocarbonyl]-indirubin (8d)

^{1}H NMR (400 MHz; DMSO-d_6): 2.77 (s, 6 H), 3.07 (s, 3 H), 3,31 (s, 2H), 3.77 (t, 2H, ^3J = 6.3 Hz), 6.96 (d, 1H, ^3J = 7.9 Hz), 7,04 (t, 1H, ^3J = 7.4 Hz), 7.23 (dd, 1H, ^3J = 6.5 Hz, ^4J = 1.3 Hz), 7.33 (d, 1H, ^3J = 8.0 Hz), 7.40 (t, 1H, ^3J = 7.6 Hz), 8.27 (d, 1H, ^3J = 7.5 Hz), 8,70 (s, 1H) 10,56 (s, 1H), 11.71 (s, 1H), 13.60 (s, 1H); ^{13}C–{^{1}H} NMR (150 MHz; DMSO-d_6): 37.8, 40.0, 42.5, 53.4, 98.2, 108.4, 111.7, 116.5, 121.8, 122.0, 126.0, 127.9, 128.0, 132.1, 139.2, 144.7, 146.0, 151.4, 171.0, 171.7, 188.9. Anal. Calcd for $C_{22}H_{23}N_5O_3$·1.5 HCl·2.5 H_2O: C, 52.31; H, 5.89; N, 13.86. Found: C, 52.59; H, 5.68; N, 13.51.

5.10. General procedure for the synthesis of indirubin-3′-oxime ethers (9)

1,1,3,3-Tetramethylguanidine (582 μL) and alkyl-halogenide (5.4 mmol) were added to indirubin-3′-oxime (0.5 mmol) in ethanol (7 mL) with stirring. The mixture was refluxed for 2 h, cooled to 0 °C and diluted with water (20 mL) and 1 N HCl (20 mL). The precipitate was filtered, washed with water and dried to afford **9** (**9a**, 63.9%, **9b**, 17.0%).

5.10.1. 3′-(2,3-Dihydroxypropyloxyimino)-5-[*N*,*N*-bis(2-hydroxyethyl)-aminocarbonyl]-indirubin (9a)

^{1}H NMR (400 MHz; DMSO-d_6): 3.35–3.70 (m, 8H), 3.51 (d, 2H, ^3J = 5.4 Hz), 3.97 (m, 1H), 4.50 (dd, 1H, ^3J = 10.7 Hz, ^3J = 6.3 Hz), 4.59 (dd, 1H, ^3J = 10.8 Hz, ^3J = 3.8 Hz), 4.75 (m, 3H), 5.09 (s, H), 6.91 (d, 1H, ^3J = 7.9 Hz), 7.05 (m, 1H), 7.21 (dd, 1H, ^3J = 7,9 Hz, ^4J = 1.0 Hz), 7.41–7.45 (m, 2H), 8.17 (d, 1H, ^3J = 7.6 Hz), 8.71 (s, 1H), 10.91 (s, 1H), 11.69 (s, 1H); ^{13}C–{^{1}H} NMR (150 MHz; DMSO-d_6): 36.2, 62.8), 69.9, 78.6, 98.4, 108.3, 111.8, 116.2, 121.6, 121.8, 122.0, 125.2, 128.8, 129.4, 132.8, 138.9, 144.5, 145.3, 151.1, 170.9, 171.0. Anal. Calcd for $C_{24}H_{26}N_4O_7$·0.5H_2O: C, 58.65; H, 5.54; N, 11.40. Found: C, 58.81; H, 5.65; N, 11.58.

5.10.2. 3′-[(2-β-D-Glucopyranosylethyl)-oxyimino]-5-[*N*-(2-dimethylaminoethyl)-*N*-methyl-aminocarbonyl]-indirubin (9b)

^{1}H NMR (400 MHz; DMSO-d_6): 2.17 (s, 6H), 2.39 (m, 2H), 3.11 (m, 2H), 3.00–5.02 (m, 6.91 (s, 1H), 7.03 s, 1H), 7.41 (m, 1H), 7.62 (s, 1H), 8.01 (s, 1H), 8.21 (s, 1H), 9.17 (s, 1H), 11.65 (m, 4H); ^{13}C-{^{1}H} NMR (150 MHz; DMSO-d_6,): 30.5, 37.3, 45.1–76.8, 99.6, 103.0, 108.0 (C7), 111.7, 116.2, 121.5, 122.2, 122.9, 125.2, 125.8, 127.2, 128.5, 132.7, 145.6, 151.2, 166.9, 171.3. Anal. Calcd for $C_{29}H_{35}N_5O_9$·H_2O: C, 56.58; H, 6.06; N, 11.38. Found: C, 56.38; H, 6.01; N, 11.18.

5.11. 5-{*N*-[2-(4-Methylpiperazino)-ethyl]-aminocarbonyl}-indirubin hydrochloride 18a

To a solution of **7f** (100 mg, 0.23 mmol) in dried THF was added HCl saturated THF. The suspension was stirred for 2 h at room tem-

perature. The precipitate was filtered, washed with THF and dried in vacuo to yield **18a** (86 mg, 18.4 mmol, 80.0%). ^{1}H NMR (400 MHz; D_2O/DMSO-d_6): 2.86–3.68 (m, 15H), 6.96 (d, 1H, ^3J = 7.9 Hz), 7.04 (t, 1H, ^3J = 7.5 Hz), 7.41 (d, 1H, ^3J = 7.9 Hz), 7.57 (t, 1H, ^3J = 7.5 Hz), 7.67 (d, 1H, ^3J = 7.4 Hz), 7.81 (1H, dd, ^3J = 8.3 Hz, ^4J = 1.8 Hz), 9.29 (d, 1H, ^4J = 1.3 Hz). Anal. Calcd for $C_{24}H_{25}N_5O_3$. HCl: C, 61.60; H, 5.60; N, 14.97. Found: C, 61.43; H, 5.77; N, 14.58.

5.12. Procedures for the Quaternization of indirubin-5-carboxamides (18b–e)

Methode 1: To a suspension of indirubin-5-carboxamide (0.1 mmol) in methanol (10 mL) was added methyl iodide (5 mmol). The mixture was stirred for 24 h at room temperature. The solvent and excess MeI were eliminated. The residue was dissolved in methanol. After dilution with ether the precipitate was filtered and dried to afford **18c** (62.0%) or **18d** (82.0%).

Methode 2: To a suspension of indirubin-5-carboxamide (0.1 mmol) in xylene (1 mL) and nitrobenzene (2 mL) was added dimethylsulfate (1 mmol). The mixture was heated for 45 min and cooled. After addition of ether the precipitate was filtered and dried in vacuo to yield **18b** (82.0%) or **18e** (74.0%).

5.12.1. 2-(Indirubin-5-carbonylamino)-ethyl-trimethyl-ammonium methosulfate (18b)

^{1}H NMR (400 MHz; DMSO-d_6): 3.16 (s, 9H), 3.36 (s, 3H), 3.52 (t, 2H, ^3J = 6.4 Hz), 3.70 (q, 2H, ^3J = 5.7 Hz), 6.97 (d, 1H, ^3J = 8.2 Hz), 7.04 (t, 1H, ^3J = 7.4 Hz), 7.43 (d, 1H, ^3J = 8.0 Hz), 7.59 (t, 1H, ^3J = 7.4 Hz), 7.66 (d, 1H, ^3J = 7.5 Hz), 7.73 (dd, 1H, ^3J = 8.2 Hz, ^4J = 1.4 Hz), 8.63 (t, 1H, ^3J = 5.4 Hz), 9.28 (s, 1H), 11.10 (s, 1H), 11.17 (s, 1H). Anal. Calcd for $C_{23}H_{26}N_4O_7S{\cdot}1.5\ H_2O$: C, 52.16; H, 5.52; N, 10.58. Found: C, 52.06; H, 5.32; N, 10.88. MS (MALDI-TOF) m/z: 391.0 $[M]^+$; calcd for $C_{22}H_{23}N_4O_3^+$: 391,177.

5.12.2. 2-[*N*-(5-Indirubincarbonyl)-*N*-methyl]-aminoethyl-trimethyl-ammonium iodide (18c)

^{1}H NMR (400 MHz; DMSO-d_6): 3.82 (s, 3H), 3.16 (s, 6H), 3.65 (s, 2H), 3.89 (s, 2H), 6.96 (d, 1H, ^3J = 8.0 Hz), 7.04 (t, 1H, ^3J = 7.5 Hz), 7.37 (dd, 1H, ^3J = 9.5 Hz, ^4J = 1.5 Hz), 7.43 (d, 1H, ^3J = 8.1 Hz), 7.59 (t, 1H, ^3J = 7.2 Hz), 7.64 (d, 1H, ^3J = 7.5 Hz), 8.88 (s, 1H), 11.09 (s, 1H), 11.12 (s, 1H); ^{13}C–{^{1}H} NMR (150 MHz; DMSO-d_6): 40.1, 48.6, 52.5, 61.1, 105.5, 109.2, 113.6, 118.9, 121.1, 121.6, 123.7, 124.5, 128.2, 128.5, 137.4, 139.1, 142.0, 152.6, 170.9, 171.1, 188.9. Anal. Calcd for $C_{23}H_{25}IN_4O_3{\cdot}H_2O$: C, 50.19; H, 4.94; N, 10.18. Found: C, 50.03; H, 4.84; N, 10.40.

5.12.3. 4-(5-Indirubincarbonyl)-1,1-dimethylpiperazinium iodide (18d)

^{1}H NMR (400 MHz; DMSO-d_6): 3.19 (s, 9H), 3.47 (s, 4H), 3,94 (s, 4H), 6.98 (d, 1H, ^3J = 8.0 Hz), 7.04 (t, 1H, ^3J = 7.4 Hz), 7.30 (d, 1H, ^3J = 8.0 Hz), 7.43 (d, 1H, ^3J = 8.0 Hz), 7.6 (m, 2H), 8.9 (s, 1H), 11.11 (s, 1H), 11.15 (s, 1H); ^{13}C–{^{1}H} NMR (150 MHz; DMSO-d_6): 48.4, 50.6, 60.1, 105.4, 109.2, 113.4, 118.9, 121.1, 121.5, 123.8, 124.3, 126.9, 128.6, 137.2, 138.9, 142.2, 152.4, 169.4, 170.8, 188.6. Anal. Calcd for $C_{23}H_{23}IN_4O_3{\cdot}1.5\ H_2O$: C, 49.56; H, 4.70; N, 10.05. Found: C, 49.41; H, 4.45; N, 9.62.

5.12.4. 4-(5-Indirubincarbonyl)-1,1-dimethylpiperazinium methosulfate (18e)

^{1}H NMR (400 MHz; DMSO-d_6): 3.19 (s, 9H), 3.36 (s, 3H), 3.47 (s, 4H), 3,94 (s, 4H), 6.98 (d, 1H, ^3J = 8.0 Hz), 7.04 (t, 1H, ^3J = 7.4 Hz), 7.38 (d, 1H, ^3J = 8.0 Hz), 7.43 (d, 1H, ^3J = 8.0 Hz), 7.57–7.64 (m, 2H), 8.91 (s, 1H), 11.11 (s, 1H), 11.14 (s, 1H); ^{13}C–{^{1}H} NMR (150 MHz; DMSO-d_6): 40.1, 50.5, 60.1, 105.4, 109.3, 113.7, 118.9, 121.3, 121.6, 124.1, 124.5, 127.0, 128.8, 137.4, 139.1, 142.3, 152.6, 169.5, 170.9, 188.8. Anal. Calcd for $C_{24}H_{26}N_4O_7S{\cdot}1.5\ H_2O$: C, 53.23; H, 5.40; N, 10.35. Found: C, 53.00; H, 4.90; N, 10.60.

Acknowledgements

We are grateful to Mrs. C. Müller for recording the NMR spectra, and Mrs. E. Biel and Mrs. B. Dusch for performing CHN analyses.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.04.066.

References and notes

1. Zhu, Z. H. In *Dan Xi Xin Fa*; Wang, Y., Zhu, J. P., Jiang, L. Z., Eds.; People's medical publishing, 2005, ISBN 7-117-06711-X/R·6712.
2. (a) Tang, W.; Eisenbrand, G. *Chinese drugs of plant origin: Chemistry, Pharmacology, and Use in Traditional and Modern Medicine*; Springer: Berlin, Heidelberg, New York, 1990; (b) Institute of Hematology; Chinese Academy of Medical Sciences; Chengdu traditional Chinese Medical College; Sichuang Institute of Traditional Medicine. *Transf. Hematol.* **1978**, *2*, 17–20.
3. Hössel, R.; Leclerc, S.; Endicott, J. A.; Nobel, M.; Lawrie, A.; Tunnah, P.; Leost, M.; Damiens, E.; Marie, D.; Marko, D.; Niederberger, E.; Tang, W. C.; Eisenbrand, G.; Meijer, L. *Nat. Cell Biol.* **1999**, *1*, 60.
4. Meijer, L.; Skaltsounis, A. L.; Magiatis, P.; Polychronopoulos, P.; Knockaert, M.; Leost, M.; Ryan, X. P.; Vonica, C. A.; Brivanlou, A.; Dajani, R.; Crovace, C.; Tarricone, C.; Musacchio, A.; Roe, S. M.; Pearl, L.; Greengard, P. *Chem. Biol.* **2003**, *10*, 1255.
5. Nam, S.; Buettner, R.; Turkon, J.; Kim, D.; Cheng, J. Q.; Muehlbeyer, S.; Hippe, F.; Vatter, S.; Merz, K.-H.; Eisenbrand, G.; Jove, R. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 5998.
6. Pandraud, P. H. *Acta Crystallogr.* **1961**, *14*, 901.
7. Eisenbrand, G.; Hippe, F.; Jakobs, S.; Muehlbeyer, S. *J. Cancer Res. Clin. Oncol.* **2004**, *130*, 627.
8. Fiebig, H.-H.; Schüler, J. B. In vivo Antitumor Activity of 5-Methylindirubin. In *Indirubin, the Red Shade of Indigo*; Meijer, L., Guyard, N., Skaltsounis, L., Eisenbrand, G., Eds.; Life in Progress Editions; Roscoff: France, 2006; pp 209–213.
9. Jautelat, R.; Brumby, T.; Schäfer, M.; Briem, H.; Eisenbrand, G.; Schwahn, S.; Krüger, M.; Lücking, U.; Prien, O.; Siemeister, G. *ChemBioChem* **2005**, *6*, 531.
10. (a) Merz, K.-H.; Eisenbrand, G. Chemistry and Structure-Activity of Indirubins. In *Indirubin, the red shade of indigo*; Meijer, L., Guyard, N., Skaltsounis, L., Eisenbrand, G., Eds; Life in Progress Editions; Roscoff: France, 2006; pp 135–145; (b) Polychronopoulos, P.; Magiatis, P.; Skaltsounis, A. L.; Myrianthopoulos, V.; Mikros, E.; Tarricone, A.; Musacchio, A.; Roe, S. M.; Pearl, L.; Leost, M.; Greengard, P.; Meijer, L. *J. Med. Chem.* **2004**, *47*, 935; (c) Vougogiannopoulou, K.; Ferandin, Y.; Bettayeb, K.; Myrianthopoulos, V.; Lozach, O.; Fan, Y.; Johnson, C. H.; Magiatis, P.; Skaltsounis, A. L.; Mikros, E.; Meijer, L. *J. Med. Chem.* **2008**, *51*, 6421.
11. (a) Baeyer, A. *Chem. Ber.* **1881**, *14*, 1741; (b) Russell, G. A.; Kaupp, G. *J. Am. Chem. Soc.* **1969**, *91*, 3851.
12. Sandmeyer, T. *Helv. Chim. Acta.* **1919**, *2*, 234.
13. Waldmann, H. *J. Prakt. Chem.* **1937**, *147*, 338.
14. Giovaninni, E.; Portmann, P. *Helv. Chim. Acta* **1948**, *31*, 1392.
15. Romanelli, M. N.; Manetti, D.; Scapecchi, S.; Borea, P. A.; Dei, S.; Bartolini, A.; Ghelardini, C.; Gualtieri, F.; Guandalini, L.; Varani, K. *J. Med. Chem.* **2001**, *44*, 3946.
16. Tapia, I.; Alono-Cires, L.; Lopez-Tudconca, P. L.; Mosquera, R.; Labeaga, L.; Innerarity, A.; Orjales, A. *J. Med. Chem.* **1999**, *42*, 2870.
17. Königs, W.; Knorr, E. *Chem. Ber.* **1901**, *34*, 957.
18. Marko, D.; Schätzle, S.; Friedel, A.; Genzlinger, A.; Zankl, H.; Meijer, L.; Eisenbrand, G. *Br. J. Cancer* **2001**, *84*, 283.
19. Eisenbrand, G.; Cheng, X. L.; Vatter, S.; Merz, K.-H.; AACR 100th Annual Meeting, 2009; Denver, CO, AACR Abstr. #1808.
20. Merz, K.-H.; Schwahn, S.; Hippe, F.; Muehlbeyer, S.; Jakobs, S.; Eisenbrand, G. *Int. J. Clin. Pharmacol.Ther.* **2004**, *42*, 656.