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Synthesis and investigation of tetrahydro-β-carboline derivatives as inhibitors of the Breast Cancer Resistance Protein (ABCG2)

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Abstract

The breast cancer resistance protein (ABCG2) transports chemotherapeutic drugs out of cells which makes it a major player in mediating multidrug resistance (MDR) of cancer cells. To overcome this mechanism, inhibitors of ABCG2 can be used. Only a few potent and selective ABCG2 inhibitors have been discovered i.e. fumitremorgin C (FTC), Ko143, and the alkaloid harmine, which contain a tetrahydro-β-carboline or β-carboline backbone, respectively. However, toxicity and or instability prevent their use *in vivo*. Therefore there is a need for further potent inhibitors. We synthesized and pharmacologically investigated 37 tetrahydro-β-carboline derivatives. The inhibitory activity of two compounds (**51**, **52**) is comparable to Ko143, and they are selective for ABCG2 over ABCB1. Furthermore they are able to reverse the ABCG2-mediated resistance toward SN-38 and inhibit the ATPase activity. The cytotoxicity data show that their inhibitory effect is substantially higher than their toxicity.

Introduction

The ATP binding cassette (ABC) transporters represent a superfamily of membrane-transport proteins and are found ubiquitously in all organisms (eukaryotes and prokaryotes). While in prokaryotes importers and exporters are present, in eukaryotes only exporters are found. Using the energy obtained from the hydrolysis of ATP the exporters actively transport their substrates out of cells.[1,2] There are 48 known human ABC transporters which are divided into seven subfamilies ABCA – ABCG based on phylogenetic similarity. The typical structure of an ABC transporter consists of two transmembrane domains (TMD) which are composed of six α-helices and two nucleotide binding domains (NBD), respectively. Some ABC transporters are not specific but recognise a vast variety of structurally unrelated substrates, and have a protective function in tissue homeostasis because they can transport amongst other things toxins and xenobiotics out of the cell.[3,4,5] On the other hand, they have found to be frequently expressed in tumor tissue and can thus, increase the transport of chemotherapeutics out of the tumor cells. An overexpression of these transporters in tumors can cause MDR which can lead to the failure of cancer therapy.[5,6,7] The breast cancer resistant protein, now termed ABCG2 according to the gene nomenclature was first described in 1998.[7] It consists of 655 amino acids and has a molecular weight of 72 kDa. It contains one TMD and one NBD only and thus represents a half-transporter, which forms the functional transporter most probably by tetramerization.[8]

Compared to ABCB1 the number of known ABCG2 inhibitors is much less. The neurotoxic fungal toxin FTC is a potent and specific inhibitor of ABCG2.[9] Ko143 is a nontoxic analogue of FTC and the most potent inhibitor of ABCG2.[10] For a long time Ko143 was claimed to be a specific inhibitor for ABCG2, however, it was recently reported to inhibit also ABCB1 and ABCC1 at low micromolar concentrations.[11] Tetracyclic desmethoxy FTC- and Ko-derivatives with different substitution patterns (aliphatic, cyclic/aromatic) have been

investigated for their inhibitory activity toward ABCG2.[12,13] In this case the isobutyl substitution as present in Ko143 gave the best results.

The alkaloid harmine was also reported to be an ABCG2 inhibitor.[14] All three compounds, Ko143, FTC and harmine contain the same substructure, the tetrahydro-β-carboline or β-carboline moiety, see Figure 1.

On this basis, it was interesting to synthesize new derivatives with a tricyclic common scaffold and different substituents and to investigate them for their inhibitory properties toward ABCG2 in comparison to known inhibitors. Furthermore we examined the selectivity of the compounds toward ABCG2. For selected derivatives also cell toxicity, their ability to reverse MDR and their influence on the ATPase activity were determined.

Results and Discussion

Chemistry The investigated tetrahydro-β-carbolines with different modifications at position 1, 2 and 6 were synthesized by the procedure depicted in Scheme 1. In general, the compounds were synthesized by a Pictet-Spengler reaction using tryptamine or 5-methoxytrypamine, an appropriate aldehyde in presence of trifluoroacetic acid (TFA) in dichloromethane (DCM) to form the 1-substituted-tetrahydro-β-carboline (**1-19**) or the 1,6-disubtituted-tetrahydro-β-carboline (**20**, Scheme 1)[15] The intermediates were further reacted with benzoylchloride or differently substituted benzoylchlorides in tetrahydrofuran (THF) with triethylamine (TEA) to obtain the *N*2-acyl-1-substituted-tetrahydro-β-carbolines (**21-52**) and the *N*2-acyl-1,6-substituted-tetrahydro-β-carboline (**53**, Scheme 1). Two intermediates (**2, 19**) were reacted with benzyl bromide in dimethylformamide (DMF) and TEA to obtain the *N*2-aryl-1-substituted-tetrahydro-β-carbolines (**56, 57**).[16,17]

The target compounds (1-(3,4-dichlorophenyl)-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)(3-methoxyphenyl)methanone (**52)** and phenyl(1-phenyl-3,4-dihydro-1H-pyrido[3,4-

b]indol-2(9H)-yl)methanone **(22)** were further reacted with iodoethane in presence of sodium hydride in DMF to form the *N*2-acyl-*N*9-ethyl-1-substituted-tetrahydro-β-carbolines (**54, 55**).[18]

All test compounds were characterized by ^{1}H NMR and ^{13}C NMR and their purity was confirmed by elemental analysis. All synthesized compounds contain a chiral centre at C1. For biological investigation, the racemic mixtures were used.

Investigation of the ABCG2-inhibitory activity in Hoechst 33342 and pheophorbide A assays. All synthesized compounds were tested for their inhibitory potency using the Hoechst 33342 accumulation assay with Madin Darby Canine Kidney II (MDCK II) cells overexpressing ABCG2.

Because of the high autofluorescence of harmine in the Hoechst 33342 assay it was investigated with the pheophorbide A assay. Selected compounds were additionally tested in the pheophorbide A assay to demonstrate that the results of both assays are comparable. The comparison of the pIC_{50} values obtained in both assays shows a very high correlation of the results with a squared correlation coefficient (r^2) of 0.95 (Figure 2). This indicates that the inhibitory effect is not specific for the substrate. The determined resulting IC_{50} values are listed in Table 1. The 1-substituted-tetrahydro-β-carbolines derivatives (**2**, **3**) which are obtained by the Pictet-Spengler reaction had no inhibitory effect on ABCG2, neither in the Hoechst 33342 assay nor in the pheophorbide A assay. This result is in agreement with a previous study, where a number of tricyclic FTC analogs were found to be all inactive.[12] However among tetracyclic derivatives potent inhibitors were found. Two *N*2-acyl-1-substitituted-tetrahydro-β-carboline derivatives with a phenyl ring at R^3 and an isobutyl (**21:** IC_{50} = 2.33 μM) or phenyl (**22:** IC_{50} = 2.78 μM) substituent at R^1 showed moderate and comparable inhibitory activities. As variations on an aromatic ring system could be readily introduced, we continued with substituted phenyl derivatives. Various compounds with

variably substituted phenyl rings at R^1 were prepared. The selection of the substituents was based on availability and variations in physicochemical properties like sigma-Hammett and lipophilicity. Figure 3 shows the selected substituents in a sigma-pi scatter diagram. Linear regression yields a highly significant correlation with both variables (eq. 1).

$$pIC_{50} = 0.458(\pm0.041)\,\pi + 0.425(\pm0.092)\,\sigma + 5.639(\pm0.027) \quad (1)$$

$n = 13$ $R^2 = 0.954$ $R^2_{adj} = 0.945$ $s = 0.083$ $F = 104.5$

All *meta* and/or *para*-substituted derivatives were included in the correlation (e.g. compounds 22-27, 29, 32, 33, 35, 37-39). *Ortho*-substituted derivatives had to be omitted due to lack of sigma values. In case of disubstituted compounds, the sigma values were either used as sum of both positions or separately in the regression. If the sigma-Hammett values for the *meta* and *para* position are considered separately, the correlation is not improved and the same squared correlation coefficient is obtained.

According to the equation lipophilicity has a positive effect on inhibitory potency. This relationship has been reported several times for homologous series of ABCB1 inhibitors. And has been explained by the assumption that binding to the protein takes place from within the membrane. Thus higher lipophilicity leads to higher membrane partitioning and to higher local concentration. Electron withdrawing substituents favour the inhibition according to eq. 1. It can be speculated that their presence at the phenyl ring would favour π-π interactions with electron rich aromatic systems of aromatic amino acids of the protein.

Two compounds deviate from the relationship, namely the 3-nitro- and 4-(trifluoromethyl)phenyl substituted derivatives (**28, 30**) that should be highly active. We wanted to investigate whether these compounds are true outliers or probable solubility problems could explain their deviation. To do this, we measured their solubility over the time of the assay. For both compounds solutions of 10 μM, the highest concentration used, were prepared and the absorption was followed over time. It was found that they steadily precipitated and after 2 h the absorbance had decreased to 20 percent of its initial value,

meaning that at least 80 percent of the compound had been precipitated during the assay time. Thus the apparent inactivity could be due to fast precipitation, decreasing their concentration in the assay. Several other derivatives showed no or very low activity in the Hoechst 33342 assay. To exclude that their apparent low potency was due to low solubility, we determined the absorption versus time profiles for them and for selected active compounds as controls (nos. **22**, **29**, **34**, **36**, **48**, **51**, **54**-**57**). Only for (1-(2-bromophenyl)-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)(phenyl)methanone (**34)** and 1-(2-chlorophenyl)-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)(phenyl)methanone (**36)** a strong precipitation with absorbance values down to 40 percent of the initial absorbance was found, while the other investigated compounds remained almost quantitatively in solution, disproving the assumption of low potency due to low solubility. It should be noted that in no case a visible clouding was observed and the solutions appeared clear.

With regard to the structure-activity relationship (SAR) of the effects of the various substituents for a chloro and bromo substituent the position dependence of the substituent influence on activity can be estimated: 3-chloro-, 4-chloro- and 4-bromophenyl substitution increases the inhibitory activity to a sub-micromolar range (**37**:0.869 μM; **38**:0.741 μM; **35**:0.687 μM), while the 2-chloro- (**36**) and 2-bromophenyl (**34**) analogous have very low potency, also when taking into account their solubility problem. The same impact can be observed for the smaller fluorine substitution in *ortho* (**31**) or *para* (**32**) position to a lesser extent. A highly potent compound with an IC_{50} of 0.328 was obtained by a 3,4-dichlorophenyl substitution at R^1 (**39**). A substitution with 3,4-difluorophenyl (**33**) shows also a slightly increasing effect on the inhibitory activity in comparison to the 4-fluorophenyl (**32).** The variation at R^3 leads to different results. The substitution of the phenyl ring with a methoxy group in position 4 has no or just a slight increasing effect on the inhibitory activity. An exception to this is compound **48**, which get just because of the 4-methoxyphenyl substitution at R^3 an inhibitory activity with an IC_{50} of 1.23 μM (in comparison to **30** = no inhibitory

effect up to 10 μM). This effect may be due to the increased solubility of **48** in comparison to **30**, as described above. For all compounds with the 3,4-difluorophenyl at R^1 no distinct effects of different substituents on R^3 can be found (compare **33**:1.10 μM; **40**:1.26 μM; **44**:1.10 μM; **49**:0.805 μM). For other compounds, especially for the highly potent compounds with 3,4-dichlorophenyl at R^1 the substitution pattern at R^3 has a higher influence on the inhibitory activity. In this case a 4-chlorophenyl (**42**: 1.03 μM), 1-naphtyl (**45**: 1.32 μM) and 3,4-dimethoxyphenyl (**43**: 0.698 μM) at R^3 decrease, while a 4-methoxy- (**51**: 0.233 μM) or 3-methoxyphenyl (**52**: 0.238 μM) increase the inhibitory activity in comparison to a unsubstituted phenyl ring (**39**: 0.328 μM). The additional methoxy group (R^2) in position 6 **(53**, IC_{50}: 0.382 μM**)** has no enhancing effect on the inhibitory activity. The substitution at *N*2 with a benzyl (**56, 57**) instead of a benzoyl group has a strongly decreasing effect for the inhibitory efficacy toward ABCG2. The same effect is observed for the compounds with an additional alkyl substitution at *N*9 (**54, 55**).

Based on this results it can be assumed, that a chlorine or bromine substitution in *meta* or *para* position at the phenyl ring in R^1 gave highly potent compounds with a inhibitory activity in sub-micromolar range. By substitution with a 3,4-dichlorophenyl ring this effect could be increased. Other substituents have just minor (except difluorophenyl) or strong decreasing impacts on the inhibitory activity toward ABCG2 dependent on their lipophilic and electronic properties. The variation at R^3 influences the inhibitory activity toward ABCG2, but this effect is depending on the substitution pattern in R^1. The hydrogen bond donor-acceptor system of the hydrogen at *N9* and the acyl group at *N2* seems to be necessary for the inhibitory activity. In general, all active compounds show a better inhibitory activity than the alkaloid harmine (IC_{50}: 5.08 μM). The inhibitory activity of compound **51** (IC_{50}: 0.233 μM) and **52** (IC_{50}: 0.238 μM) is comparable to Ko143 (IC_{50}: 0.225 μM).

Investigation of the inhibitory activity on ABCB1 with the calcein-acetoxymethyl ester (calcein AM) assay. All compounds were screened for ABCB1 inhibition to investigate their selectivity toward ABCG2, see Figure 4. For this determination the calcein AM assay was used with the A2780 adr cell line. The concentration of the test compounds and the reference compound cyclosporine A (CsA) amounted to 10 µM. The most potent compounds on ABCG2 (**39**, **51**, **52**, **53**) are selective toward ABCG2 over ABCB1, because they show just a slight effect on ABCB1 of less than 25% at 10 µM (Figure 4). All compounds with a response of more than 25% were further investigated to determine their IC_{50} values, see Table 2. The *para*-acetamido substituted compound **29** shows similar inhibitory effects toward ABCB1 (7.40 µM) and ABCG2 (7.82 µM). All other compounds have a higher inhibitory activity against ABCG2 than ABCB1. The potent compounds (with the exception of **49**) with an IC_{50} < 1 µM on ABCG2 show only minor effects with IC_{50} 10 >.µM on ABCB1.

Investigation of cell toxicity and the ability to reverse MDR with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) Assay

The MTT assay was used to determine the intrinsic cytotoxicity of the most active and selective compounds **35**, **38**, **39**, **51**, **52** and **53** in comparison to the unsubstituted analog **22** and the known inhibitors harmine and Ko143. The MDCK II BCRP and wild type cells were incubated for 72 h with different concentrations of the compounds up to 100 µM. Compounds **39**, **51**, **52** and **53** show a similar toxic effect on the viability of the cells (Table 3). But, the effect on the cell viability is up to 20 fold weaker than their inhibitory efficacy against ABCG2. Compound **22** shows a lower toxicity and is comparable to Ko143. Compound **35** and **38** possess the lowest toxicity of the tested compounds and their inhibitory efficacy is 20-30 fold higher than their toxic effect. All tested compounds show similar toxicities to the MDCK II BCRP and MDCK wild-type cells, which suggests that they are no substrates of

ABCG2. The alkaloid harmine exhibits the highest toxicity and its toxic effect on cell viability is about 2 times stronger than the inhibitory potency.

Furthermore, the ability to reverse the multidrug resistance in ABCG2 overexpressing cells was investigated with selected compounds and compared to the known inhibitor Ko143. The cytotoxic agent SN-38 is the active metabolite of irinotecan and a substrate of ABCG2. Therefore, the cytotoxicity effect of SN-38 in MDCK II BCRP cells was measured in absence and presence of different concentrations of selected compounds. All tested compounds and Ko143 are able to reverse the transport of SN-38 out of the cells in a concentration dependent manner (Table 4). The shift in the dose–response curves of SN-38 in MDCK II BCRP cells in presence of an ABCG2 inhibitor in different concentrations are shown in Figure 5. The most potent compounds **39**, **51**, **52** and **53** show a 10fold weaker ability (1 μM vs. 0.1 μM) to reverse MDR in comparison to Ko143, despite similar IC_{50}-values. This effect is more pronounced for compounds (**22, 35, 38** and harmine) with higher IC_{50}-values in ABCG2 inhibition assays. This consistent difference between IC_{50}-values and MDR reversal for Ko143 and our compounds points to the possibility of substrate specific interactions.

ATPase assay. The results of the investigation of selected compounds in relation to their properties toward vanadate sensitive ATPase activity are shown in Figure 6.

For comparison, quercetin a known activator and Ko143 a known inhibitor of the ATPase activity were included. Compounds **22**, **35, 38**, **39, 51**, **52** and **53** inhibit the ATPase activity at a concentration of 1 μM, to the level of inhibition by 1 μM of Ko143 (Figure 6). Contrary, the alkaloid harmine shows no inhibitory effect on the ATPase activity. Furthermore, we determined dose-response curves of the inhibitory effect on basal and quercetin stimulated ATPase activity (Table 5). Figure 7 shows example curves for compound **53** and Ko143. The most active compounds (**39, 51, 52, and 53**) possess IC_{50}-values one magnitude larger than Ko143. Interestingly, there is a good linear relationship between pIC_{50}-values in the Hoechst

33342 and ATPase assays for all derivatives, except Ko143 (R^2=0.85 (basal), R^2=0.89 (for quercetin stimulated activity). This points to the involvement of ATPase activity inhibition.in the mode of action of the compounds. Stimulation with quercetin shifts all IC_{50}-values to higher values, by an approximately constant factor of nine, indicating a competitive interaction with ATPase stimulation by quercetin.

Conclusion

In this study we show, that tricyclic tetrahydro-β-carbolines are good inhibitors of ABCG2. We have found that the substitution pattern at R^1 and the acyl substitution at *N*2 are essential for the selectivity and inhibitory activity toward ABCG2. The most potent analogs **51** and **52** are selective for ABCG2 and their inhibitory properties are similar to those of Ko143 and much better than those of harmine. An advantage to Ko143 is that just a two-step synthesis is required to obtain quickly and cost effectively a wide range of good inhibitors. The cytotoxicity data of test compounds shows that the cell viability decrease after 72 h but the inhibitory potency is still up to 20-30 fold higher. We showed that the compounds are able to reverse the ABCG2-mediated SN-38 resistance, but this effect is less pronounced than for Ko143. Furthermore, the compounds inhibit the basal ATPase activity in isolated ABCG2 containing recombinant baculovirus-infected Spodoptera frugiperda ovarian cells (Sf9) membranes. For the quercetin stimulated ATPase activity a competitive interaction is observed. The alkaloid harmine did not prove to be a potent inhibitor of ABCG2 and shows a high cytotoxicity.

Experimental section

Chemistry.

All chemicals were purchased from Sigma Aldrich, Alfa Aeser, Acros and Merck. The reactions were monitored by thin layer chromatography (TLC) with silica gel plates (Silica Gel 60 F_{254}, Merck). The structure and the purity of all compounds were confirmed by ^{1}H and ^{13}C NMR and elemental analysis. The NMR spectra were recorded on a Bruker Avance 500, 500 MHz or 600, 600 MHz for ^{1}H NMR and Bruker Avance 500, 126 MHz or 600, 151 MHz for ^{13}C NMR. The spectra were all recorded in dimethyl sulfoxide-d_6 (DMSO-d_6) or chloroform-d ($CDCl_3$).

The chemical shifts are indicate in δ values (ppm) with the solvent peak as an internal standard. The resonance peaks multiplicity is displayed as singlet (s), doublet (d), triplet (t), quartet (q) and multiplet (m). The ^{13}C signals (coupling constant J are in hertz) were performed with the help of distortionless enhancement by polarization transfer (DEPT) and attached proton test (APT). The purity of all biologically evaluated compounds was determined to be > 95% by elemental analysis with Vario EL of Elementar. The results were all within ± 0.4% of the theoretical values, if not indicated otherwise. Melting points (mp) were determined with SMP10 (Stuart Scientific)

General procedure for the synthesis of 1-substituted-tetrahydro-β-carbolines and 1,6-substituted-tetrahydro-β-carbolines (**1-20**). The tryptamine (1.0 eqiv) derivatives and the appropriate aldehyde (1.2 eqiv) were dissolved in DCM (2mL/mmol).

TFA was added to the solution and the reaction mixture was stirred for 24 h at room temperature (rt). After completion of the reaction as indicated by TLC the solution was evaporated under reduced pressure. The residual was mixed with a 5% K_2CO_3 aqueous solution (3 mL/mmol) and extracted with DCM. The organic layer was dried over magnesium sulfate and evaporated under reduced pressure to get the crude product that was recrystallized from ethanol.

When the product was already precipitating from DCM, the suspension was filtered off and washed 5% K_2CO_3 aqueous solution and DCM. This product was used without further purification.

1-Isobutyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole **(1)**

The title compound was synthesized from tryptamine (7.5 mmol) and isovaleraldehyde (9 mmol), yield 92%, white powder. ^{1}H NMR (500 MHz, DMSO-d6) δ 11.10 (s, 1H), 9.25 (s, 1H), 7.51 – 7.32 (m, 2H), 7.15 – 6.97 (m, 2H), 4.70 (d, J = 8.6 Hz, 1H), 3.59 (dt, J = 12.3, 4.6 Hz, 1H), 3.40 – 3.32 (m, 1H), 3.00 – 2.86 (m, 2H), 2.05 – 1.89 (m, 2H), 1.75 (t, J = 10.2 Hz, 1H), 1.00 (dd, J = 25.3, 6.0 Hz, 6H).^{13}C NMR (126 MHz, DMSO-d6) δ 136.39, 130.72, 126.00, 121.86, 119.16, 118.12, 111.51, 105.76, 50.79, 41.08, 41.02, 23.53(2C), 21.40, 18.34.

1-Phenyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole **(2)**[15,19]

The title compound was synthesized from tryptamine (5 mmol) and benzaldehyde (6 mmol), yield 48%, pale beige powder. ^{1}H NMR (500 MHz, DMSO-d6) δ 10.38 (s, 1H), 7.40 (d, J = 7.7 Hz, 1H), 7.34 – 7.30 (m, 2H), 7.29 – 7.25 (m, 3H), 7.22 (dt, J = 8.0, 0.9 Hz, 1H), 7.03 – 6.96 (m, 1H), 6.98 – 6.90 (m, 1H), 5.08 (s, 1H), 3.06 (dt, J = 12.1, 5.3 Hz, 1H), 2.97 – 2.88 (m, 1H), 2.77 – 2.61 (m, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 143.39, 136.08, 135.50, 128.54 (2C), 128.20 (2C), 127.25, 127.01, 120.58, 118.27, 117.61, 111.16, 108.41, 56.71, 41.32, 22.40. Anal. Calcd for $C_{17}H_{16}N_2$: C, 82.22; H, 6.49; N, 11.28. Found: C, 82.01; H, 6.54; N, 11.06.

1-(3-Methoxyphenyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole **(3)** [15]

The title compound was synthesized from tryptamine (10 mmol) and 3-methoxybenzaldehyde (12 mmol), yield 72%, pale yellow powder. ^{1}H NMR (500 MHz, DMSO-d6) δ 10.36 (s, 1H), 7.40 (d, J = 7.6 Hz, 1H), 7.26 – 7.20 (m, 2H), 7.02 – 6.91 (m, 2H), 6.89 – 6.82 (m, 3H), 5.05 (s, 1H), 3.72 (d, J = 4.7 Hz, 3H), 3.11 – 3.04 (m, 1H), 2.97 – 2.89 (m, 1H), 2.77 – 2.61 (m,

3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 159.33, 144.91, 136.06, 135.47, 129.21, 126.99, 120.75, 120.59, 118.28, 117.62, 114.34, 112.59, 111.17, 108.27, 56.75, 55.12, 41.47, 22.35. Anal. Calcd for $C_{18}H_{18}N_2O$: C, 77.67; H, 6.52; N, 10.06. Found: C, 77.96; H, 6.59; N, 10.02.

1-(4-Methoxyphenyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole **(4)**

The title compound was synthesized from tryptamine (5 mmol) and 4-methoxybenzaldehyde (6 mmol), yield: 45%, pale yellow powder. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.86 (s, 1H), 9.48 (s, 1H), 7.52 (d, J = 7.9 Hz, 1H), 7.33 – 7.24 (m, 3H), 7.14 – 7.07 (m, 1H), 7.06 – 7.01 (m, 3H), 5.89 (s, 1H), 3.48 – 3.39 (m, 2H), 3.30 (s, 3H), 3.14 – 3.04 (m, 1H), 3.04 – 2.89 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.48, 136.65, 131.36 (2C), 128.87, 126.72, 125.83, 122.08, 119.17, 118.30, 114.37 (2C), 111.66, 107.39, 55.50, 55.32, 18.37, one peak is missing. ^{13}C NMR (151 MHz, CDCl3) δ 161.04, 136.43, 131.22(2C), 127.35, 126.03, 125.10, 123.24, 120.28, 118.66, 114.62(2C), 111.35, 108.98, 56.15, 55.38, 40.38, 18.46.

1-(3,4-Dimethoxyphenyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole **(5)**

The title compound was synthesized from tryptamine (5 mmol) and 3,4-dimethoxybenzaldehyde (6 mmol), yield 84%, pale yellow powder. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.82 (s, 1H), 9.36 (s, 1H), 7.51 (d, J = 7.9 Hz, 1H), 7.29 (dt, J = 8.1, 0.9 Hz, 1H), 7.14 – 6.99 (m, 4H), 6.82 (dd, J = 8.3, 2.1 Hz, 1H), 5.82 (s, 1H), 3.77 (s, 3H), 3.73 (s, 3H), 3.46 (dt, J = 12.5, 5.5 Hz, 1H), 3.44 – 3.34 (m, 1H), 3.13 – 3.03 (m, 1H), 3.03 – 2.93 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 150.01, 148.96, 136.63, 129.27, 127.38, 125.92, 122.42, 121.99, 119.11, 118.28, 113.38, 111.88, 111.66, 107.38, 55.86, 55.82, 55.73, 40.37, 18.60.

1-(p-Tolyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole **(6)**

The title compound was synthesized from tryptamine (5 mmol) and 4-methylbenzaldehyde (6 mmol), yield 43%, beige powder. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.32 (s, 1H), 7.42 – 7.37 (m, 1H), 7.21 (dd, J = 7.9, 1.1 Hz, 1H), 7.14 (q, J = 8.0 Hz, 4H), 7.01 – 6.91 (m, 2H), 5.03 (s, 1H), 3.06 (dt, J = 12.2, 5.2 Hz, 1H), 2.96 – 2.86 (m, 1H), 2.75 – 2.58 (m, 3H), 2.28 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 140.38, 136.33, 136.06, 135.75, 128.76 (2C), 128.45 (2C), 127.03, 120.53, 118.25, 117.58, 111.15, 108.28, 56.52, 41.44, 22.42, 20.83.

3-(2,3,4,9-Tetrahydro-1H-pyrido[3,4-b]indol-1-yl)benzonitrile **(7)**

The title compound was synthesized from tryptamine (5 mmol) and 3-formylbenzonitrile (6 mmol), yield 18%, pale yellow powder. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.43 (s, 1H), 7.77 – 7.70 (m, 2H), 7.63 (dt, J = 7.9, 1.5 Hz, 1H), 7.53 (t, J = 7.7 Hz, 1H), 7.42 (dd, J = 7.8, 1.1 Hz, 1H), 7.22 (dt, J = 8.0, 1.0 Hz, 1H), 7.05 – 6.97 (m, 1H), 6.99 – 6.92 (m, 1H), 5.16 (d, J = 1.7 Hz, 1H), 3.07 – 2.91 (m, 3H), 2.79 – 2.69 (m, 1H), 2.70 – 2.61 (m, 1H) ^{13}C NMR (126 MHz, DMSO-d_6) δ 145.01, 136.13, 134.51, 133.42, 132.08, 131.13, 129.52, 126.91, 120.88, 119.07, 118.45, 117.80, 111.19, 111.16, 108.74, 55.92, 41.36, 22.21.

1-(3-Nitrophenyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole **(8)**

The title compound was synthesized from tryptamine (5 mmol) and 3-nitrobenzaldehyde (6 mmol), yield 60%, yellow powder. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.94 (s, 1H), 9.85 (s, 1H), 8.37 – 8.34 (m, 1H), 8.33 – 8.29 (m, 1H), 7.83 – 7.75 (m, 2H), 7.56 (d, J = 7.9 Hz, 1H), 7.32 – 7.27 (m, 1H), 7.17 – 7.10 (m, 1H), 7.09 – 7.04 (m, 1H), 6.18 (s, 1H), 3.55 – 3.40 (m, 2H), 3.18 – 3.09 (m, 1H), 3.04 (dt, J = 10.3, 5.5 Hz, 1H) ^{13}C NMR (126 MHz, DMSO-d_6) δ 147.96, 136.91, 136.73, 136.70, 130.65, 127.74, 125.79, 124.97, 124.77, 122.39, 119.37, 118.51, 111.75, 108.00, 54.86, 40.41, 18.36.

N-(4-(2,3,4,9-Tetrahydro-1H-pyrido[3,4-b]indol-1-yl)phenyl)acetamide **(9)**

The title compound was synthesized from tryptamine (5 mmol) and 4-acetamidobenzaldehyde (6 mmol), yield 82%, beige powder. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.78 (s, 1H), 10.10 (s, 1H), 7.66 – 7.61 (m, 2H), 7.52 – 7.48 (m, 1H), 7.30 – 7.24 (m, 3H), 7.13 – 7.05 (m, 1H), 7.06 – 6.99 (m, 1H), 5.74 (d, J = 2.0 Hz, 1H), 3.41 – 3.29 (m, 3H), 3.08 – 2.99 (m, 1H), 2.98 – 2.92 (m, 1H), 2.04 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.65, 140.42, 136.58, 130.40, 130.19 (2C), 129.78, 126.02, 121.86, 119.19 (2C), 119.04, 118.21, 111.60, 107.56, 55.56, 40.25, 24.13, 18.99.

1-(4-(Trifluoromethyl)phenyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole **(10)**

The title compound was synthesized from tryptamine (5 mmol) and 4-(trifluoromethyl)benzaldehyde (6 mmol), yield 97%, white powder. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.90 (s, 1H), 9.73 (s, 1H), 7.87 (d, J = 8.1 Hz, 2H), 7.62 (d, J = 8.0 Hz, 2H), 7.55 (d, J = 7.9 Hz, 1H), 7.30 (d, J = 8.1 Hz, 1H), 7.13 (t, J = 7.6 Hz, 1H), 7.06 (t, J = 7.4 Hz, 1H), 6.08 (s, 1H), 3.53 – 3.40 (m, 2H), 3.12 (dt, J = 13.0, 6.3 Hz, 1H), 3.03 (dt, J = 16.1, 5.6 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 139.1, 136.6, 130.9 (2C), 130.12 (q, J = 32.1 Hz), 127.7, 125.71 (d, J = 3.4 Hz, 2C), 125.6, 123.95 (q, J = 272.4 Hz), 122.1, 119.1, 118.3, 111.5, 107.6, 54.9, 40.2, 18.2.

1-(2-Fluorophenyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole **(11)**

The title compound was synthesized from tryptamine (5 mmol) and 2-fluorobenzaldehyde (6 mmol), yield 32%, light beige powder. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.94 (s, 1H), 9.72 (s, 1H), 7.60 – 7.51 (m, 2H), 7.45 – 7.37 (m, 1H), 7.32 – 7.23 (m, 2H), 7.16 – 7.10 (m, 2H), 7.10 – 7.02 (m, 1H), 6.16 (s, 1H), 3.58 – 3.49 (m, 1H), 3.48 – 3.40 (m, 1H), 3.17 – 3.07 (m, 1H), 3.07 – 2.97 (m, 1H). ^{13}C NMR (126 MHz, DMSO- d_6) δ 160.77 (d, J = 249.1 Hz), 136.64, 132.38 (d, J = 8.5 Hz), 131.46, 127.48, 125.74, 125.06, 122.25, 121.89 (d, J = 13.2 Hz), 119.25, 118.37, 116.12 (d, J = 21.2 Hz), 111.67, 107.82, 48.88, 40.17, 18.30.

1-(4-Fluorophenyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole **(12)**

The title compound was synthesized from tryptamine (5 mmol) and 4-fluorobenzaldehyde (6 mmol), yield 76%, white powder. ^{1}H NMR (500 MHz, DMSO- d_6) δ 10.89 (s, 1H), 9.62 (s, 1H), 7.56 – 7.49 (m, 1H), 7.48 – 7.39 (m, 2H), 7.37 – 7.26 (m, 3H), 7.16 – 7.08 (m, 1H), 7.09 – 7.01 (m, 1H), 5.98 (s, 1H), 3.49 – 3.39 (m, 2H), 3.16 – 3.05 (m, 1H), 3.06 – 2.96 (m, 1H). ^{13}C NMR (126 MHz, DMSO- d_6) δ 163.00 (d, J = 246.6 Hz), 136.68, 132.37 (d, J = 8.7 Hz, 2C), 131.06 (d, J = 2.7 Hz), 128.36, 125.77, 122.20, 119.24, 118.37, 115.89 (d, J = 21.8 Hz, 2C), 111.68, 107.57, 54.94, 40.11, 18.30.

1-(3,4-Difluorophenyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole **(13)**

The title compound was synthesized from tryptamine (10 mmol) and 3,4-difluorobenzaldehyde (12 mmol), yield 91%, pale yellow powder. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.92 (s, 1H), 9.75 (s, 1H), 7.62 – 7.51 (m, 2H), 7.52 – 7.44 (m, 1H), 7.30 (d, J = 8.1 Hz, 1H), 7.26 – 7.21 (m, 1H), 7.17 – 7.09 (m, 1H), 7.08 – 7.02 (m, 1H), 5.99 (s, 1H), 3.52 – 3.40 (m, 2H), 3.10 (dt, J = 11.9, 5.9 Hz, 1H), 3.01 (dt, J = 16.0, 5.6 Hz, 1H) ^{13}C NMR (126 MHz, DMSO-d_6) δ 149.39 (dd, J = 249.2, 12.2 Hz), 148.45 (dd, J = 240.2, 12.8 Hz) , 136.72, 132.36, 127.91, 127.39, 125.76, 122.33, 119.31, 119.31 (d, J = 18.0 Hz) 118.47, 111.72, 118.11 (d, J = 17.4 Hz), 107.77, 54.63, 40.19, 18.29.

1-(2-Bromophenyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole **(14)**

The title compound was synthesized from tryptamine (6.25 mmol) and 2-bromobenzaldehyde (7.5 mmol), yield 56%, white crystals. ^{1}H NMR (500 MHz, DMSO- d_6) δ 10.95 (s, 1H), 9.65 (s, 1H), 7.89 – 7.79 (m, 1H), 7.55 (dd, J = 7.7, 1.0 Hz, 1H), 7.47 – 7.38 (m, 2H), 7.29 (dt, J = 8.1, 1.0 Hz, 1H), 7.19 – 6.99 (m, 3H), 6.15 (s, 1H), 3.60 – 3.51 (m, 1H), 3.43 – 3.34 (m, 1H), 3.18 – 3.09 (m, 1H), 3.05 – 2.96 (m, 1H). ^{13}C NMR (126 MHz, DMSO- d_6) δ 136.71, 133.89,

133.50, 132.05, 131.77, 128.47, 127.62, 125.65, 125.18, 122.36, 119.29, 118.44, 111.69, 108.06, 54.82, 39.78, 18.33.

1-(4-Bromophenyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole **(15)**

The title compound was synthesized from tryptamine (7.5 mmol) and 4-bromobenzaldehyde (9 mmol), yield 33%, white powder. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.88 (s, 1H), 9.64 (s, 1H), 7.73 – 7.67 (m, 2H), 7.53 (dd, J = 7.7, 1.0 Hz, 1H), 7.36 – 7.31 (m, 2H), 7.29 (dt, J = 8.1, 1.0 Hz, 1H), 7.16 – 7.09 (m, 1H), 7.08 – 7.01 (m, 1H), 5.96 (s, 1H), 3.48 – 3.39 (m, 2H), 3.14 – 3.06 (m, 1H), 3.05 – 2.97 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 136.70, 134.11, 132.21 (2C), 131.96 (2C), 128.10, 125.76, 123.45, 122.25, 119.27, 118.39, 111.69, 107.63, 55.07, 40.21, 18.31.

1-(2-Chlorophenyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole **(16)**

The title compound was synthesized from tryptamine (5 mmol) and 2-chlorobenzaldehyde (6 mmol), yield 56%, pale beige powder. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.95 (s, 1H), 9.67 (s, 1H), 7.68 (dd, J = 8.1, 1.2 Hz, 1H), 7.58 – 7.49 (m, 2H), 7.38 (td, J = 7.6, 1.3 Hz, 1H), 7.29 (dt, J = 8.1, 1.0 Hz, 1H), 7.16 – 7.07 (m, 2H), 7.09 – 7.02 (m, 1H), 6.21 (d, J = 1.5 Hz, 1H), 3.55 (dt, J = 12.1, 5.8 Hz, 1H), 3.44 – 3.35 (m, 1H), 3.17 – 3.09 (m, 1H), 3.07 – 2.97 (m, 1H) ^{13}C NMR (126 MHz, DMSO-d_6) δ 136.70, 134.34, 132.37, 131.80, 131.67, 130.17, 127.92, 127.64, 125.70, 122.33, 119.28, 118.43, 111.70, 108.11, 52.18, 39.92, 18.37.

1-(3-Chlorophenyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole **(17)**

The title compound was synthesized from tryptamine (5 mmol) and 3-chlorobenzaldehyde (6 mmol), yield 80%, pale yellow powder. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.92 (s, 1H), 9.66 (s, 1H), 7.59 – 7.47 (m, 4H), 7.34 – 7.28 (m, 2H), 7.16 – 7.09 (m, 1H), 7.09 – 7.02 (m, 1H), 5.98 (s, 1H), 3.52 – 3.37 (m, 2H), 3.16 – 3.06 (m, 1H), 3.01 (dt, J = 10.3, 5.6 Hz, 1H). ^{13}C

NMR (126 MHz, DMSO-d_6) δ 137.22, 136.70, 133.53, 130.92, 129.90, 129.84, 128.74, 127.97, 125.77, 122.29, 119.29, 118.44, 111.72, 107.77, 55.08, 40.30, 18.34.

1-(4-Chlorophenyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole **(18)**

The title compound was synthesized from tryptamine (5 mmol) and 4-chlorobenzaldehyde(6 mmol), yield 37%, yellow powder. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.89 (s, 1H), 9.64 (s, 1H), 7.59 – 7.51 (m, 3H), 7.42 – 7.38 (m, 2H), 7.29 (dt, J = 8.1, 1.0 Hz, 1H), 7.16– 7.08 (m, 1H), 7.08 – 7,01 (m, 1H), 5.98 (s, 1H), 3.44 (td, J = 7.0, 6.4, 4.3 Hz, 2H), 3.15 – 3.05 (m, 1H), 3.06 – 2.97 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 136.70, 134.75, 133.71, 131.95 (2C), 129.02 (2C), 128.15, 125.76, 122.25, 119.28, 118.40, 111.69, 107.63, 54.99, 40.21, 18.31.

1-(3,4-Dichlorophenyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole **(19)**

The title compound was synthesized from tryptamine (15 mmol) and 3,4-dichlorobenzaldehyde (18 mmol), yield 51%, beige powder. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.90 (s, 1H), 9.71 (s, 1H), 7.77 (d, J = 8.4 Hz, 1H), 7.69 (d, J = 2.1 Hz, 1H), 7.54 (d, J = 7.8 Hz, 1H), 7.36 – 7.27 (m, 2H), 7.13 (td, J = 8.2, 7.6, 1.3 Hz, 1H), 7.09 – 7.03 (m, 1H), 6.00 (s, 1H), 3.54 – 3.39 (m, 2H), 3.15 – 3.06 (m, 1H), 3.06 – 2.95 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 136.70, 135.63, 132.76, 132.11, 131.55, 131.18, 130.33, 127.69, 125.73, 122.38, 119.35, 118.48, 111.72, 107.83, 54.57, 40.42, 18.29.

1-(3,4-Dichlorophenyl)-6-methoxy-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole **(20)**

The title compound was synthesized from 5-methoxytryptamine (1.8 mmol) and 3,4-dichlorobenzaldehyde (2.16 mmol), yield 45%, white powder. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.72 (s, 1H), 9.68 (s, 1H), 7.76 (d, J = 8.3 Hz, 1H), 7.67 (d, J = 2.1 Hz, 1H), 7.32 (dd, J = 8.3, 2.2 Hz, 1H), 7.19 (d, J = 8.7 Hz, 1H), 7.04 (d, J = 2.5 Hz, 1H), 6.77 (dd, J = 8.8, 2.5 Hz, 1H), 5.97 (s, 1H), 3.77 (s, 3H), 3.52 – 3.38 (m, 2H), 3.07 (dt, J = 13.3, 6.3 Hz, 1H), 3.02 –

2.93 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 153.73, 135.70, 132.73, 132.07, 131.74, 131.54, 131.17, 130.30, 128.22, 126.09, 112.43 (2C), 107.60, 100.47, 55.60, 54.64, 40.46, 18.36.

General procedure for the synthesis of N2-acyl-1-substituted-tetrahydro-β-carbolines and N2-acyl-1,6-substituted-tetrahydro-β-carbolines **(21-53)** Under stirring at 0° the desired benzoyl chloride (1.1 equiv) was added slowly to a mixture of the appropriate compound (**1**-**20**) (1 equiv) and TEA (1.2 equiv) in dry THF (10 ml/mmol). The mixture was stirred for 1 h at 0° and then 12 h at rt. After completion of the reaction as indicated by TLC the precipated TEA-hydrochloride was removed by filtration. The clear solution was evaporated under reduce pressure to get the crude product, that was recrystallized from ethanol or ethanol/THF (9:1).

(1-Isobutyl-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)(phenyl)methanone **(21)**

The title compound was synthesized from **1** (2.19 mmol) and benzoylchloride (2.41 mmol), yield 21%, white crystals, mp 188-189 °C. ^{1}H NMR (500 MHz, DMSO- d_6) δ 10.93 (s, 1H), 7.50 – 7.45 (m, 3H), 7.40 – 7.36 (m, 2H), 7.33 (dd, J = 14.3, 7.9 Hz, 2H), 7.08 – 7.01 (m, 1H), 6.98 – 6.91 (m, 1H), 5.85 (dd, J = 10.5, 3.7 Hz, 1H), 3.70 (dd, J = 13.6, 4.6 Hz, 1H), 3.53 – 3.41 (m, 1H), 2.67 – 2.54 (m, 2H), 1.90 – 1.77 (m, 2H), 1.74 – 1.69 (m, 1H), 1.10 (d, J = 6.3 Hz, 3H), 0.99 (d, J = 6.4 Hz, 3H). ^{13}C NMR (126 MHz, DMSO d_6) δ 170.30, 137.08, 136.05, 135.23, 129.37, 128.70(2C), 126.48, 126.29(2C), 120.97, 118.61, 117.69, 111.20, 105.86, 46.94, 43.43, 41.07, 24.94, 23.69, 22.46, 22.04.

Anal. Calcd for $C_{22}H_{24}N_2O$: C, 79.48; H, 7.28; N, 8.43. Found: C, 79.17; H, 7.67; N, 8.02.

Phenyl(1-phenyl-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)methanone **(22)**[19]

The title compound was synthesized from **2** (1 mmol) and benzoylchloride (1.1 mmol), yield 63%, white-beige crystals, mp 238-239 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.05 (s, 1H),

7.47 (d, *J* = 5.9 Hz, 4H), 7.42 – 7.28 (m, 8H), 7.09 (t, *J* = 7.0 Hz, 1H), 7.01 (t, *J* = 7.2 Hz, 1H), 6.96 (s, 1H), 3.64 (d, *J* = 8.4 Hz, 1H), 3.26 (d, *J* = 11.7 Hz, 1H), 2.86 (t, *J* = 10.8 Hz, 1H), 2.74 (d, *J* = 15.2 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.71, 140.42, 136.39 (2C), 131.58, 129.65, 128.71 (2C), 128.66 (2C), 128.14, 127.96 (2C), 126.43 (2C), 126.32, 121.47, 118.79, 118.05, 111.38, 108.12, 51.65, 41.30, 21.83. Anal. Calcd for $C_{24}H_{20}N_2O$: C, 81.79; H, 5.72; N, 7.95. Found: C, 81.69; H, 5.48; N, 7.58.

(1-(3-Methoxyphenyl)-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)(phenyl)methanone **(23)**

The title compound was synthesized from **3** (1.08 mmol) and benzoylchloride (1.18 mmol), yield 77%, pale beige powder, mp 222-223 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.04 (s, 1H), 7.54 – 7.36 (m, 7H), 7.34 – 7.24 (m, 2H), 7.09 (t, *J* = 7.7 Hz, 1H), 7.01 (t, *J* = 7.4 Hz, 1H), 6.90 (d, *J* = 14.5 Hz, 3H), 3.72 (s, 3H), 3.65 (d, *J* = 11.7 Hz, 1H), One signal is missing (CH_2) under the water peak, 2.84 (d, *J* = 13.0 Hz, 1H), 2.74 (d, *J* = 15.5 Hz, 1H). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.11 (s, 1H), 7.51 (d, *J* = 7.8 Hz, 1H), 7.44 – 7.35 (m, 5H), 7.28 (d, *J* = 8.1 Hz, 1H), 7.23 – 7.15 (m, 2H), 7.15 – 7.10 (m, 1H), 7.08 (s, 1H), 7.01 (s, 1H), 6.95 (d, *J* = 7.6 Hz, 1H), 6.83 (d, *J* = 8.2 Hz, 1H), 3.78 (d, *J* = 13.3 Hz, 1H), 3.74 (s, 3H), 3.40 (t, *J* = 12.5 Hz, 1H), 2.97 – 2.86 (m, 1H), 2.77 (d, *J* = 15.3 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.77, 159.55, 141.97, 136.42 (2C), 131.56, 129.77, 129.70, 128.76 (2C), 126.47 (2C), 126.32, 121.52, 120.31, 118.83, 118.11, 114.36, 112.96, 111.41, 108.13, 55.22, 51.63, 41.48, 21.85. Anal. Calcd for $C_{25}H_{22}N_2O_2$: C, 78.51; H, 5.80; N, 7.32. Found: C, 78.48; H, 5.92; N, 7.34.

(1-(4-Methoxyphenyl)-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)(phenyl)methanone **(24)**[19]

The title compound was synthesized from **4** (1.0 mmol) and benzoylchloride (1.1 mmol), yield 29%, white crystals, mp 182-183 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.00 (s, 1H), 7.50 – 7.44 (m, 4H), 7.40 – 7.35 (m, 2H), 7.27 (dd, *J* = 46.7, 7.3 Hz, 3H), 7.09 (t, *J* = 7.3 Hz,

1H), 7.03 – 6.99 (m, 1H), 6.96 – 6.87 (m, 3H), 3.73 (s, 3H), 3.62 (d, *J* = 9.1 Hz, 1H), 3.27 (s, 1H), 2.85 (s, 1H), 2.74 (d, *J* = 14.8 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.60, 159.05, 136.53, 136.41, 132.55, 131.97, 129.64, 129.47 (2C), 128.73 (2C), 126.44 (2C), 126.37, 121.44, 118.78, 118.05, 114.03 (2C), 111.37, 108.06, 55.28, 51.18, 41.11, 21.89. Anal. Calcd for $C_{25}H_{22}N_2O_2$: C, 78.51; H, 5.80; N, 7.32. Found: C, 78.62; H, 5.82; N, 7.29.

(1-(3,4-Dimethoxyphenyl)-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)(phenyl)methanone **(25)**[20]

The title compound was synthesized from **5** (1.3 mmol) and benzoylchloride (1.43 mmol), yield 30%, white crystals, mp 238-239 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.01 (s, 1H), 7.47 (dd, *J* = 7.2, 4.1 Hz, 4H), 7.39 (dd, *J* = 6.4, 3.2 Hz, 2H), 7.32 (d, *J* = 7.7 Hz, 1H), 7.11 – 7.03 (m, 2H), 7.01 (td, *J* = 7.5, 1.0 Hz, 1H), 6.91 (d, *J* = 8.3 Hz, 2H), 6.68 (s, 1H), 3.72 (d, *J* = 8.2 Hz, 6H), 3.66 – 3.57 (m, 1H), one signal is missing (CH_2) under the water peak, 2.85 (s, 1H), 2.74 (d, *J* = 13.4 Hz, 1H). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.27 (s, 1H), 7.52 (d, *J* = 7.8 Hz, 1H), 7.45 – 7.36 (m, 5H), 7.29 (d, *J* = 8.1 Hz, 1H), 7.21 – 7.15 (m, 2H), 7.15 – 7.10 (m, 1H), 7.03 (s, 1H), 6.70 (t, *J* = 7.2 Hz, 2H), 3.82 (s, 3H), 3.78 (s, 4H), 3.38 (t, *J* = 12.0 Hz, 1H), 2.92 (d, *J* = 10.6 Hz, 1H), 2.78 (d, *J* = 14.7 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.67, 148.97, 148.79, 136.55, 136.42, 132.97, 131.86, 129.62, 128.73 (2C), 126.44 (2C) 126.32, 121.44, 120.57, 118.76, 118.06, 112.10, 111.70, 111.36, 108.11, 55.71 (2C), 51.47, 41.25, 21.91. Anal. Calcd for $C_{26}H_{24}N_2O_3$: C, 75.71; H, 5.86; N, 6.79. Found: C, 75.96; H, 5.95; N, 6.35.

Phenyl(1-(p-tolyl)-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)methanone **(26)**[19]

The title compound was synthesized from **6** (1.14 mmol) and benzoylchloride (1.25 mmol), yield 34%, white powder, mp 207-208 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.00 (s, 1H), 7.46 (dt, *J* = 7.4, 3.0 Hz, 4H), 7.40 – 7.36 (m, 2H), 7.32 (d, *J* = 7.9 Hz, 1H), 7.19 (t, *J* = 8.0

Hz, 4H), 7.11 – 7.06 (m, 1H), 7.03 – 6.98 (m, 1H), 6.92 (s, 1H), 3.62 (d, *J* = 9.9 Hz, 1H), 3.24 (d, *J* = 19.8 Hz, 1H), 2.85 (s, 1H), 2.73 (d, *J* = 14.1 Hz, 1H), 2.29 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.64, 137.53, 137.25, 136.50, 136.42, 131.83, 129.63, 129.19(2), 128.73(2), 128.14(2), 126.43(2), 126.37, 121.44, 118.79, 118.04, 111.38, 108.07, 51.43, 41.23, 21.88, 20.79.

Anal. Calcd for $C_{25}H_{22}N_2O$: C, 81.94; H, 6.05; N, 7.64. Found: C, 82.09; H, 5.94; N, 7.74.

3-(2-Benzoyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)benzonitrile **(27)**

The title compound was synthesized from **7** (0.84 mmol) and benzoylchloride (0.92 mmol), yield 73%, white powder, mp 231-234 °C (decomposition). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.00 (s, 1H), 7.83 (d, *J* = 7.5 Hz, 1H), 7.70 (d, *J* = 14.2 Hz, 2H), 7.62 (t, *J* = 7.7 Hz, 1H), 7.51 – 7.45 (m, 4H), 7.44 (s, 2H), 7.35 (d, *J* = 8.0 Hz, 1H), 7.15 – 7.08 (m, 1H), 7.06 – 6.99 (m, 1H), 6.93 (s, 1H), 3.69 (s, 1H), 3.32 (d, *J* = 9.9 Hz, 1H), 2.87 (d, *J* = 10.9 Hz, 1H), 2.78 (d, *J* = 12.5 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.12, 141.87, 136.50, 136.06, 132.92, 131.85, 131.54, 130.53, 130.09, 129.83, 128.71 (2C), 126.57 (2C), 126.24, 121.73, 118.94, 118.74, 118.21, 111.67, 111.51, 108.69, 51.41, 41.73, 21.63. Anal. Calcd for $C_{25}H_{19}N_3O$: C, 79.55; H, 5.07; N, 11.13. Found: C, 79.37; H, 5.19; N,11.03.

(1-(3-Nitrophenyl)-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)(phenyl)methanone **(28)**

The title compound was synthesized from **8** (1.02 mmol) and benzoylchloride (1.12 mmol), yield 39%, yellow powder, mp 284-286 °C (decomposition). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.05 (s, 1H), 8.22 (d, *J* = 7.8 Hz, 1H), 8.13 (s, 1H), 7.79 (s, 1H), 7.70 (t, *J* = 7.9 Hz, 1H), 7.53 – 7.45 (m, 4H), 7.43 (d, *J* = 6.2 Hz, 2H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.15 – 7.09 (m, 1H), 7.08 – 6.99 (m, 2H), 3.70 (s, 1H), One signal is missing (CH_2) under the water peak, 2.91 (t, *J* = 10.6 Hz, 1H), 2.80 (d, *J* = 14.0 Hz, 1H). No additional spectrum could be acquired as the compound is not soluble in $CDCl_3$. ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.2, 148.1, 142.5,

136.6, 136.0, 134.7, 130.5, 130.5, 129.9, 128.8 (2C), 126.6 (2C), 126.3, 123.1, 122.7, 121.9, 119.0, 118.3, 111.6, 108.8, 51.4, 41.8, 21.7. Anal. Calcd for $C_{24}H_{19}N_3O_3$: C, 72.53; H, 4.82; N, 10.57. Found: C, 72.42; H, 4.97; N, 10.48.

N-(4-(2-Benzoyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)phenyl)acetamide **(29)**

The title compound was synthesized from **9** (1.31 mmol) and benzoylchloride (1.44 mmol), yield 45%, yellow-orange powder, mp from 190 °C (decomposition). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.01 (s, 1H), 9.96 (s, 1H), 7.56 (d, J = 8.0 Hz,2H), 7.49 – 7.43 (m, 4H), 7.39 (d, J = 3.3 Hz, 2H), 7.32 (d, J = 7.6 Hz, 1H), 7.24 (d, J = 7.0 Hz, 2H), 7.11 – 7.06 (m, 1H), 7.04 – 6.97 (m, 1H), 6.90 (s, 1H), 3.63 (d, J = 9.2 Hz, 1H), 3.28 (s, 1H), 2.85 (s, 1H), 2.74 (d, J = 13.4 Hz, 1H), 2.02 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.62, 168.37, 139.05, 136.48, 136.40, 134.91, 131.82, 129.60, 128.70 (2C), 128.51 (2C), 126.44 (2C), 126.35, 121.41, 119.18 (2C), 118.76, 118.03, 111.37, 108.04, 51.31, 41.21, 24.03, 21.86. Anal. Calcd for $C_{26}H_{23}N_3O$: C, 76.26; H, 5.66; N, 10.26. Found: C, 74.72; H, 5.66; N, 9.94.

Phenyl(1-(4-(trifluoromethyl)phenyl)-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)methanone **(30)**

The title compound was synthesized from **10** (0.95 mmol) and benzoylchloride (1.04 mmol), yield 58%, white powder, mp 260-261 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.03 (s, 1H), 7.76 (d, J = 8.1 Hz, 2H), 7.56 (d, J = 6.5 Hz, 2H), 7.51 – 7.44 (m, 4H), 7.42 (d, J = 5.7 Hz, 2H), 7.34 (d, J = 8.1 Hz, 1H), 7.15 – 7.07 (m, 1H), 7.06 – 6.99 (m, 1H), 7.00 (s, 1H), 3.69 (d, J = 10.5 Hz, 1H), 3.27 – 3.19 (m, 1H), 2.89 (s, 1H), 2.77 (d, J = 14.5 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.83, 144.74, 136.33, 135.97, 130.63, 129.64 (2C), 128.88, 128.58 (2C), 128.16 (d, J = 31.8 Hz), 126.36 (2C), 126.13, 125.49 (d, J = 3.9 Hz, 2C), 124.27 (q, J = 272.3Hz).121.53, 118.78, 118.03, 111.33, 108.38, 51.27, 41.48, 21.58. Anal. Calcd for $C_{25}H_{19}F_3N_2O$: C, 71.42; H, 4.56; N, 6.66. Found: C, 71.61; H, 4.57; N, 6.77.

(1-(2-Fluorophenyl)-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)(phenyl)methanone **(31)**

The title compound was synthesized from **11** (1.12 mmol) and benzoylchloride (1.24 mmol), yield 50 %, white crystals, mp 211-213 °C. ^{1}H NMR (500 MHz, DMSO- d_6) δ 10.92 (s, 1H), 7.51 – 6.96 (m, 14H), 3.72 (s, 1H), 3.37 (s, 1H), 2.74 (s, 2H). ^{13}C NMR (126 MHz, DMSO d_6) δ 169.73, 160.14 (d, J = 248.2 Hz), 136.20 (d, J = 7.8 Hz), 130.70, 130.36, 130.09 (d, J = 7.5 Hz), 129.51, 128.48(2C), 126.91 (d, J = 17.0 Hz), 126.43(3C), 126.15,124.19 (d, J = 3.0 Hz), 121.38, 118.67, 117.89, 115.68 (d, J = 21.4 Hz), 111.31, 108.19, 46.34, 41.68, 21.73. Anal. Calcd for $C_{24}H_{19}FN_2O$: C, 77.82; H, 5.17; N,7.56. Found: C, 77.66; H, 5.37; N, 7.50

(1-(4-Fluorophenyl)-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)(phenyl)methanone **(32)**

The title compound was synthesized from **12** (1.88 mmol) and benzoylchloride (2.07 mmol), yield 54%, white crystals, mp 153-156 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.16 (s, 1H), 7.52 (d, J = 7.8 Hz, 1H), 7.45 – 7.34 (m, 7H), 7.28 (d, J = 8.1 Hz, 1H), 7.22 – 7.16 (m, 1H), 7.15 – 7.12 (m, 1H), 7.09 (s, 1H), 6.97 (t, J = 8.5 Hz, 2H), 3.82 – 3.73 (m, 1H), 3.39 – 3.27 (m, 1H), 2.93 (s, 1H), 2.84 – 2.73 (m, 1H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.66, 162.54 (d, J = 247.4 Hz), 136.34, 136.18, 135.68, 131.06, 130.61 (d, J = 7.0 Hz, 2C), 129.71(2C), 128.56(2C), 126.54, 126.48, 122.36, 119.76, 118.21, 115.42 (d, J = 21.4 Hz, 2C), 111.18, 109.74, 51.55, 41.34, 22.26. Anal. Calcd for $C_{24}H_{19}FN_2O$: C, 77.82; H,5.17; N,7.56. Found: C, 77.45; H, 5.44; N, 7.28

(1-(3,4-Difluorophenyl)-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)(phenyl)methanone **(33)**

The title compound was synthesized from **13** (1.05 mmol) and benzoylchloride (1.16 mmol), yield 44%, pale pink needles, mp 236-237 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.01 (s, 1H), 7.52 – 7.44 (m, 5H), 7.42 (d, J = 8.2 Hz, 2H), 7.34 (d, J = 8.1 Hz, 2H), 7.15 (s, 1H), 7.13 – 7.09 (m, 1H), 7.04 – 7.00 (m, 1H), 6.89 (s, 1H), 3.67 (s, 1H), One signal is missing (CH_2)

under the water peak, 2.87 (t, *J* = 10.6 Hz, 1H), 2.77 (d, *J* = 13.4 Hz, 1H). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.29 (s, 1H), 7.52 (d, *J* = 7.8 Hz, 1H), 7.46 – 7.39 (m, 3H), 7.39 – 7.34 (m, 2H), 7.28 (d, *J* = 8.2 Hz, 1H), 7.23 – 7.18 (m, 2H), 7.16 – 7.11 (m, 2H), 7.09 – 7.01 (m, 2H), 3.79 (dd, *J* = 14.1, 5.3 Hz, 1H), 3.37 – 3.26 (m, 1H), 2.99 – 2.88 (m, 1H), 2.79 (dd, *J* = 15.7, 4.1 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.01, 148.49 (dd, *J* = 246.0, 12.7 Hz), 148.19 (dd, *J* = 247.8, 12.4 Hz),, 138.08, 136.50, 136.13, 130.86, 129.84, 128.75 (2C), 126.58 (2C), 126.26, 125.00, 121.73, 118.95, 118.23,117.72 (d, *J* = 17.1 Hz), 117.15 (d, *J* = 16.6 Hz). , 111.52, 108.57, 51.05, 41.54, 21.70. Anal. Calcd for $C_{24}H_{18}F_2N_2O$: C, 74.21; H, 4.67; N, 7.21. Found: C, 73.95; H, 4.84; N, 7.09.

(1-(2-Bromophenyl)-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)(phenyl)methanone **(34)**

The title compound was synthesized from **14** (1.53 mmol) and benzoylchloride (1.68 mmol), yield 34%, white powder, mp 268-273 °C (decomposition). ^{1}H NMR (500 MHz, DMSO- d_6) δ 10.96 (s, 1H), 7.75 (d, J = 5.2 Hz, 1H), 7.51 – 7.38 (m, 6H), 7.34 – 7.27 (m, 3H), 7.13 – 7.06 (m, 1H), 7.06 – 6.97 (m, 2H), 6.82 – 6.78 (m, 1H), 3.68 (s, 1H), 3.27 – 3.19 (m, 1H), 2.70 (d, J = 15.2 Hz, 2H). ^{13}C NMR (126 MHz, DMSO- d_6) δ 171.12, 139.12, 136.29, 136.12, 133.45, 131.36, 131.31, 130.08, 130.01, 128.64(2C), 127.63, 127.02(2C), 126.34, 123.70, 121.58, 118.85, 118.05, 111.50, 108.51, 51.96, 41.79, 22.12. Anal. Calcd for $C_{24}H_{19}BrN_2O$: C, 66.83; H, 4.44; N, 6.49. Found: C, 66.85; H, 4.51; N, 6.47.

(1-(4-Bromophenyl)-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)(phenyl)methanone **(35)**

The title compound was synthesized from **15** (0.83 mmol) and benzoylchloride (0.92 mmol), yield 32%, white powder, mp 236-237 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.01 (s, 1H), 7.58 (d, *J* = 8.2 Hz, 2H), 7.50 – 7.44 (m, 4H), 7.40 (d, *J* = 5.4 Hz, 2H), 7.30 (dd, *J* = 25.7, 7.4 Hz, 3H), 7.12 – 7.07 (m, 1H), 7.05 – 6.98 (m, 1H), 6.90 (s, 1H), 3.66 (d, *J* = 9.6 Hz, 1H), 3.27 – 3.19 (m, 1H), 2.86 (s, 1H), 2.75 (d, *J* = 14.4 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ

169.87, 139.82, 136.47, 136.26, 131.63 (2C), 131.12, 130.43 (2C), 129.77, 128.76 (2C), 126.51 (2C), 126.32, 121.64, 121.26, 118.91, 118.16, 111.47, 108.41, 51.23, 41.41, 21.80. Anal. Calcd for $C_{24}H_{19}BrN_2O$: C, 66.83; H, 4.44; N, 6.49. Found: C, 66.76; H, 4.36; N, 6.09.

1-(2-Chlorophenyl)-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)(phenyl)methanone **(36)**

The title compound was synthesized from **16** (1.06 mmol) and benzoylchloride (1.16 mmol), yield 17%, white crystals, mp 276-278 °C (decomposition). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.96 (s, 1H), 7.57 (d, J = 7.1 Hz, 1H), 7.51 – 7.42 (m, 4H), 7.38 (t, J = 6.9 Hz, 3H), 7.32 (dt, J = 8.1, 0.8 Hz, 1H), 7.26 (td, J = 7.6, 1.1 Hz, 1H), 7.15 (s, 1H), 7.13 – 7.06 (m, 1H), 7.02 (dd, J = 11.0, 3.9 Hz, 1H), 6.82 (dd, J = 7.7, 1.6 Hz, 1H), 3.68 (s, 1H), 3.25 (s, 1H), 2.66 (d, J = 34.5 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.80, 137.51, 136.34, 136.22, 133.42, 131.19, 131.05, 130.08, 129.94, 129.89, 128.68, 127.14 (2C), 126.93 (2C), 126.38, 121.62, 118.89, 118.09, 111.53, 108.66, 49.66, 41.68, 22.10. Anal. Calcd for $C_{24}H_{19}ClN_2O$: C, 74.51; H, 4.95; N, 7.24. Found: C, 74.74; H, 5.16; N, 6.88.

(1-(3-Chlorophenyl)-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)(phenyl)methanone **(37)**

The title compound was synthesized from **17** (1.06 mmol) and benzoylchloride (1.17 mmol), yield 27%, pale yellow powder, mp 219-220 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.04 (s, 1H), 7.51 – 7.45 (m, 4H), 7.44 – 7.37 (m, 4H), 7.33 (t, J = 13.7 Hz, 3H), 7.13 – 7.09 (m, 1H), 7.04 – 7.00 (m, 1H), 6.91 (s, 1H), 3.68 (d, J = 10.2 Hz, 1H), One signal is missing (CH_2) under the water peak, 2.86 (d, J = 10.0 Hz, 1H), 2.77 (d, J = 14.2 Hz, 1H). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.21 (s, 1H), 7.52 (d, J = 7.8 Hz, 1H), 7.45 – 7.38 (m, 3H), 7.39 – 7.33 (m, 4H), 7.30 – 7.25 (m, 2H), 7.24 – 7.18 (m, 2H), 7.14 (td, J = 7.5, 1.0 Hz, 1H), 7.09 (s, 1H), 3.83 – 3.75 (m, 1H), 3.39 – 3.30 (m, 1H), 2.93 (t, J = 11.2 Hz, 1H), 2.82 – 2.74 (m, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.29, 143.16, 136.80, 136.49, 133.69, 131.20, 131.05 (2C), 130.16, 129.10, 128.41, 128.31, 127.19, 126.87 (2C), 126.61, 122.04, 119.28, 118.55, 111.85,

108.84, 51.74, 41.92, 22.06. Anal. Calcd for $C_{24}H_{19}ClN_2O_2$: C, 74.51; H, 4.95; N, 7.24. Found: C, 74.24; H, 4.96; N, 7.12.

(1-(4-Chlorophenyl)-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)(phenyl)methanone **(38)** [19]

The title compound was synthesized from **18** (1.06 mmol) and benzoylchloride (1.16 mmol), yield 16%, white powder, mp 223-224 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.02 (s, 1H), 7.51 – 7.42 (m, 6H), 7.36 (dd, J = 35.1, 6.8 Hz, 5H), 7.12 – 7.07 (m, 1H), 7.05 – 6.98 (m, 1H), 6.92 (s, 1H), 3.66 (d, J = 10.2 Hz, 1H), 3.26 – 3.19 (m, 1H), 2.87 (s, 1H), 2.75 (d, J = 14.7 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.80, 139.35, 136.41, 136.20, 132.62, 131.11, 130.01, 129.71 (2C), 128.70 (2C), 128.63 (2C), 126.45 (2C), 126.26, 121.57, 118.8, 118.09, 111.41, 108.33, 51.11, 41.34, 21.72. Anal. Calcd for $C_{24}H_{19}ClN_2O$: C, 74.51; H, 4.95; N, 7.24. Found: C, 74.75; H, 5.00; N, 7.40.

(1-(3,4-Dichlorophenyl)-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)(phenyl)methanone

(39)

The title compound was synthesized from **19** (0.95 mmol) and benzoylchloride (1.04 mmol), yield 40%, white crystals, mp 209-210 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.00 (s, 1H), 7.66 (d, J = 8.4 Hz, 1H), 7.53 – 7.45 (m, 5H), 7.42 (d, J = 5.7 Hz, 2H), 7.34 (t, J = 9.3 Hz, 2H), 7.15 – 7.08 (m, 1H), 7.06 – 6.99 (m, 1H), 6.88 (s, 1H), 3.68, One signal is missing (CH_2) under the water peak, (s, 1H), 2.92 – 2.83 (m, 1H), 2.78 (d, J = 13.1 Hz, 1H). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.27 (s, 1H), 7.47 (d, J = 7.8 Hz, 1H), 7.41 – 7.34 (m, 4H), 7.32 – 7.27 (m, 3H), 7.26 – 7.20 (m, 2H), 7.18 – 7.12 (m, 1H), 7.12 – 7.06 (m, 1H), 7.00 (s, 1H), 3.74 (d, J = 11.5 Hz, 1H), 3.25 (t, J = 12.5 Hz, 1H), 2.92 – 2.83 (m, 1H), 2.74 (d, J = 15.6 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.05, 141.38, 136.49, 136.04, 131.30, 130.99, 130.75, 130.54, 130.08, 129.85, 128.73 (2C), 128.48, 126.56 (2C), 126.22, 121.77, 118.97, 118.23,

111.52, 108.68, 51.04, 41.68, 21.63. Anal. Calcd for $C_{24}H_{18}Cl_2N_2O$: C, 68.42; H, 4.31; N, 6.65. Found: C, 68.22; H, 4.47; N, 6.45.

(4-Chlorophenyl)(1-(3,4-difluorophenyl)-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)methanone **(40)**

The title compound was synthesized from **13** (1.4 mmol) and 4-chlorobenzoylchloride (1.54 mmol), yield 15%, white crystals, mp 215-216 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.00 (s, 1H), 7.55 – 7.50 (m, 2H), 7.49 – 7.40 (m, 4H), 7.34 (d, J = 8.1 Hz, 2H), 7.15 (s, 1H), 7.10 (dd, J = 8.1, 1.1 Hz, 1H), 7.02 (td, J = 7.5, 1.0 Hz, 1H), 6.86 (s, 1H), 3.65 (s, 1H), One signal is missing (CH_2) under the water peak, 2.88 (t, J = 10.6 Hz, 1H), 2.77 (d, J = 12.5 Hz, 1H). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.02 (s, 1H), 7.53 (d, J = 7.8 Hz, 1H), 7.43 – 7.36 (m, 2H), 7.36 – 7.28 (m, 3H), one signal is missing under the chloroform peak,7.23 – 7.20 (m, 1H), 7.17 – 7.11 (m, 2H), 7.08 (dt, J = 9.9, 8.1 Hz, 1H), 7.02 (s, 1H), 3.76 (d, J = 11.8 Hz, 1H), 3.39 – 3.29 (m, 1H), 2.93 (t, J = 11.0 Hz, 1H), 2.82 (dd, J = 15.6, 4.2 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.02, 148.51 (dd, J = 245.3, 12.7 Hz), 148.22 (dd, J = 246.1, 12.7 Hz, 137.98, 136.51, 134.86, 134.57, 130.75, 128.85 (2C), 128.70 (2C), 126.25, 125.09, 121.76, 118.97, 118.23,117.72 (d, J = 17.0 Hz), 117.22 (d, J = 17.0 Hz). , 111.53, 108.56, 51.22, 41.63, 21.67. Anal. Calcd for $C_{24}H_{17}ClF_2N_2O$: C, 68.17; H, 4.05; N, 6.62. Found: C, 67.99; H, 3.99; N, 6.43.

(1-(4-Bromophenyl)-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)(4-chlorophenyl)methanone **(41)**

The title compound was synthesized from **15** (0.83 mmol) and 4-chlorobenzoylchloride (0.92 mmol), yield 41%, white needles, mp 236-237 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.01 (s, 1H), 7.57 (t, J = 8.4 Hz, 2H), 7.54 – 7.50 (m, 2H), 7.46 (dd, J = 13.2, 8.1 Hz, 3H), 7.32 (d, J =

8.0 Hz, 1H), 7.27 (d, *J* = 6.8 Hz, 2H), 7.12 – 7.08 (m, 1H), 7.05 – 6.98 (m, 1H), 6.88 (s, 1H), 3.64 (d, *J* = 10.5 Hz, 1H), One signal is missing (CH_2) under the water peak , 2.87 (s, 1H), 2.75 (d, *J* = 14.2 Hz, 1H). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.09 (s, 1H), 7.52 (d, J = 7.8 Hz, 1H), 7.43 – 7.37 (m, 4H), 7.32 – 7.24 (m, 5H), one signal is missing under the chloroform peak, 7.23 – 7.16 (m, 1H), 7.17 – 7.11 (m, 1H), 7.02 (s, 1H), 3.79 – 3.71 (m, 1H), 3.38 – 3.28 (m, 1H), 2.91 (t, J = 11.3 Hz, 1H), 2.80 (dd, J = 15.5, 4.1 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.86, 139.67, 136.47, 134.96, 134.49, 131.63 (2C), 130.99, 130.45 (2C), 128.86 (2C), 128.62 (2C), 126.29, 121.65, 121.31, 118.92, 118.15, 111.48, 108.39, 51.38, 41.49, 21.74. Anal. Calcd for $C_{24}H_{18}BrClN_2O$: C, 61.89; H, 3.90; N, 6.01. Found: C, 61.52; H, 4.07; N, 5.77.

(4-Chlorophenyl)(1-(3,4-dichlorophenyl)-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)methanone **(42)**

The title compound was synthesized from **19** (0.73 mmol) and 4-chlorobenzoylchloride (0.81 mmol), yield 42%, white crystals, mp 240-241 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.99 (s, 1H), 7.65 (d, *J* = 8.4 Hz, 1H), 7.57 – 7.42 (m, 6H), 7.32 (dd, *J* = 20.9, 7.9 Hz, 2H), 7.14 – 7.08 (m, 1H), 7.02 (td, *J* = 7.5, 0.9 Hz, 1H), 6.86 (s, 1H), 3.66 (s, 1H), One signal is missing (CH_2) under the water peak, 2.87 (dd, *J* = 18.7, 7.9 Hz, 1H), 2.78 (d, *J* = 12.5 Hz, 1H). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.16 (s, 1H), 7.53 (d, *J* = 7.8 Hz, 1H), 7.44 (s, 1H), 7.41 – 7.38 (m, 2H), 7.35 (d, *J* = 8.3 Hz, 1H), 7.34 – 7.25 (m, 4H), 7.25 – 7.19 (m, 1H), 7.18 – 7.12 (m, 1H), 7.01 (s, 1H), 3.76 (dd, *J* = 12.5, 4.6 Hz, 1H), 3.36 – 3.29 (m, 1H), 2.91 (d, *J* = 11.8 Hz, 1H), 2.82 (dd, *J* = 15.7, 4.1 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.08, 141.25, 136.51, 134.78, 134.61, 131.34, 131.25, 131.01, 130.83, 130.46, 130.14, 128.85 (2C), 128.71 (2C), 126.23, 121.81, 119.01, 118.26, 111.56, 108.69, 51.21, 41.78, 21.63. Anal. Calcd for $C_{24}H_{17}Cl_3N_2O$: C, 63.25; H, 3.76; N, 6.15. Found: C, 62.89; H, 4.06; N, 6.09.

(1-(3,4-Dichlorophenyl)-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)(3,4-dimethoxyphenyl)methanone **(43)**

The title compound was synthesized from **19** (0.44 mmol) and 3,4-dimethoxybenzoylchloride (0.49 mmol), yield 18%, light yellow powder, mp 131-134 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.20 (s, 1H), 7.53 (d, J = 7.8 Hz, 1H), 7.46 (s, 1H), 7.35 (d, J = 8.2 Hz, 1H), 7.29 (d, J = 8.1 Hz, 2H), 7.22 – 7.18 (m, 1H), 7.17 – 7.11 (m, 1H), 7.00 – 6.93 (m, 3H), 6.86 (d, J = 8.1 Hz, 1H), 3.97 – 3.84 (m, 7H), 3.32 (s, 1H), 2.96 (d, J = 12.5 Hz, 1H), 2.83 (dd, J = 15.4, 4.1 Hz, 1H). ^{13}C NMR (151 MHz, CDCl3) δ 170.61, 150.40, 149.01, 139.99, 136.41(2C), 132.75, 132.43, 130.53(2C), 130.23, 128.09, 126.45, 122.58, 119.88, 119.47, 118.31, 111.26, 110.59, 110.29(2C), 67.93, 56.01, 55.96, 55.93, 25.57. Anal. Calcd for $C_{26}H_{22}Cl_2N_2O_3$: C, 64.87; H, 4.61; N, 5.82. Found: C, 64.80; H, 5.04; N, 5,42.

(1-(3,4-Difluorophenyl)-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)(3,4-dimethoxyphenyl)methanone **(44)**

The title compound was synthesized from **13** (1.4 mmol) and 3,4-dimethoxybenzoylchloride (1.54 mmol), yield 49%, white powder, mp 192-193 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.97 (s, 1H), 7.49 (dd, *J* = 7.7, 1.1 Hz, 1H), 7.43 (dt, *J* = 10.6, 8.5 Hz, 1H), 7.36 – 7.30 (m, 2H), 7.14 (s, 1H), 7.14 – 7.07 (m, 1H), 7.04 – 6.96 (m, 4H), 6.82 (s, 1H), 3.78 (d, *J* = 14.4 Hz, 7H), One signal is missing (CH_2) under the water peak, 2.98 – 2.86 (m, 1H), 2.79 (dd, *J* = 15.8, 3.6 Hz, 1H). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.27 (s, 1H), 7.53 (d, J = 7.7 Hz, 1H), 7.31 – 7.25 (m, 1H), one signal is missing under the chloroform peak ,7.23 – 7.16 (m, 1H), 7.17 – 6.93 (m, 6H), 6.86 (d, J = 8.2 Hz, 1H), 3.97 – 3.81 (m, 7H), 3.38 – 3.26 (m, 1H), 2.98 (t, J = 13.2 Hz, 1H), 2.83 (dd, J = 15.4, 4.1 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.86, 150.15, 148.72,148.46 (dd, *J* = 246.2, 12.8 Hz), 148.14 (dd, *J* = 245.0, 12.6 Hz) , 138.10, 136.48, 131.10, 128.20, 126.29, 125.01, 121.70, 119.57, 118.92, 118.25,117.65 (d, *J* = 16.9

Hz), 117.24, 111.49, 110.73 (2C),108.61, 55.77, 55.73, 51.30, 41.77, 21.80. Anal. Calcd for $C_{26}H_{22}F_2N_2O_3$: C, 69.63; H, 4.94; N, 6.25. Found: C, 69.54; H, 4.80; N, 5.99.

(1-(3,4-Dichlorophenyl)-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)(naphthalen-1-yl)methanone **(45)**

The title compound was synthesized from **19** (0.73 mmol) and 1-naphthoylchloride (0.81 mmol), yield 44%, white powder, mp 264-265 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.05 (d, J = 13.1 Hz, 1H), 8.02 (t, J = 7.7 Hz, 2H), 7.79 – 7.66 (m, 1H), 7.61 – 7.51 (m, 4H), 7.51 – 7.40 (m, 3H), 7.36 (d, J = 8.1 Hz, 2H), 7.11 (dd, J = 13.8, 5.8 Hz, 2H), 7.06 – 6.98 (m, 1H), 3.32 (d, J = 7.8 Hz, 1H), 3.21 (dd, J = 15.2, 10.3 Hz, 1H), 2.81 (s, 1H), 2.69 (d, J = 13.5 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.16, 141.58, 136.58, 134.24, 133.76, 133.14, 131.46, 131.23, 130.97, 130.64, 130.07, 129.28, 128.60, 127.32, 126.69, 126.21, 125.62, 124.46, 123.70, 123.50, 121.83, 119.00, 118.27, 111.58, 108.96, 50.93, 41.30, 21.53. Anal. Calcd for $C_{28}H_{20}Cl_2N_2O$: C, 71.34; H, 4.28; N, 5.94. Found: C, 71.00; H, 4.20; N, 5.73.

(4-Methoxyphenyl)(1-phenyl-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)methanone **(46)** [19]

The title compound was synthesized from **2** (1.2 mmol) and 4-methoxybenzoylchloride (1.33 mmol), yield 48%, yellow beige powder, mp 190-191 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.01 (s, 1H), 7.47 (d, J = 7.8 Hz, 1H), 7.40 – 7.27 (m, 8H), 7.11 – 7.07 (m, 1H), 7.04 – 6.98 (m, 3H), 6.92 (s,1H), 3.77 (d, J = 19.9 Hz, 4H), One signal is missing (CH_2) under the water peak, 2.93 – 2.84 (m, 1H), 2.76 (dd, J = 15.3, 3.2 Hz, 1H). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.53 (d, J = 7.8 Hz, 1H), 7.43 – 7.33 (m, 4H), 7.32 – 7.26 (m, 4H), 7.21 – 7.15 (m, 1H), 7.16 – 7.10 (m, 1H), 7.08 (s, 1H), 6.92 – 6.88 (m, 2H), 3.82 (s, 4H), 3.39 (s, 1H), 2.96 (s, 1H), 2.81 (dd, J = 15.3, 4.0 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.66, 160.35, 140.46, 136.42, 131.84, 128.64 (2C), 128.57 (2C), 128.40, 128.21, 127.92 (2C),

126.38, 121.46, 118.81, 118.07, 113.99 (2C), 111.40, 108.17, 55.40, 51.83, 41.47, 21.94. Anal. Calcd for $C_{25}H_{22}N_2O_2$: C, 78.51; H, 5.80; N, 7.32. Found: C, 78.01; H, 5.97; N, 7.11.

(4-Methoxyphenyl)(1-(3-methoxyphenyl)-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)methanone **(47)**

The title compound was synthesized from **3** (1.08 mmol) and 4-methoxybenzoylchloride (1.18mmol), yield 45%, white-beige crystals, mp 219-220 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.01 (s, 1H), 7.48 – 7.45 (m, 1H), 7.38 (d, J = 8.4 Hz, 2H), 7.35 – 7.25 (m, 2H), 7.16 – 7.06 (m, 1H), 7.04 – 6.98 (m, 3H), 6.93 – 6.80 (m, 4H), 3.80 (s, 3H), 3.75 (d, J = 2.7 Hz, 1H), 3.71 (s, 3H), One signal is missing (CH_2) under the water peak, 2.88 (s, 1H), 2.81 – 2.72 (m, 1H). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.08 (s, 1H), 7.51 (d, J = 7.8 Hz, 1H), 7.39 – 7.34 (m, 2H), 7.28 (d, J = 8.1 Hz, 1H), 7.22 – 7.15 (m, 2H), 7.15 – 7.09 (m, 1H), 7.06 – 6.88 (m, 5H), 6.82 (dd, J = 8.3, 2.5 Hz, 1H), 3.88 (s, 1H), 3.82 (s, 3H), 3.73 (s, 3H), 3.45 – 3.36 (m, 1H), 3.00 – 2.88 (m, 1H), 2.84 – 2.75 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.67, 160.35, 159.50, 142.00, 136.38, 131.75, 129.70, 128.55 (2C), 128.37, 126.32, 121.47, 120.32, 118.79, 118.07, 113.98 (2C), 112.85, 111.37 (2C), 108.11, 55.38, 55.18, 51.77, 41.53, 21.86. Anal. Calcd for $C_{26}H_{24}N_2O_3$: C, 75.71; H, 5.86; N, 6.79. Found: C, 75.71; H, 6.06; N, 6.64.

(4-Methoxyphenyl)(1-(4-(trifluoromethyl)phenyl)-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)methanone **(48)**

The title compound was synthesized from **10** ((0.95 mmol) and 4-methoxybenzoylchloride (1.04 mmol), yield 56%, pale yellow powder, mp 231-232 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.01 (s, 1H), 7.75 (d, J = 8.2 Hz, 2H), 7.54 (s, 2H), 7.48 (d, J = 6.7 Hz, 1H), 7.40 (d, J = 8.7 Hz, 2H), 7.33 (d, J = 8.1 Hz, 1H), 7.14 – 7.07 (m, 1H), 7.05 – 6.97 (m, 3H), 6.96 (s, 1H), 3.80 (s, 4H), One signal is missing (CH_2) under the water peak, 2.91 (td, J = 13.8, 11.6, 5.7 Hz, 1H), 2.78 (dd, J = 15.4, 4.1 Hz, 1H). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.44 – 8.34 (m,

1H), 7.53 (d, *J* = 7.8 Hz, 1H), 7.50 (d, *J* = 7.6 Hz, 4H), 7.37 – 7.32 (m, 2H), 7.29 (d, *J* = 8.1 Hz, 1H), 7.22 – 7.18 (m, 1H), 7.16 – 7.10 (m, 2H), 6.93 – 6.88 (m, 2H), 3.89 (s, 1H), 3.83 (s, 3H), 3.35 – 3.25 (m, 1H), 3.01 – 2.92 (m, 1H), 2.81 (dd, *J* = 15.5, 4.1 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.7, 160.3, 144.8, 136.3, 130.8, 128.9 (2C), 128.5 (2C), 128.09 (d, *J* = 31.7 Hz), 127.9, 126.1, 125.42 (d, *J* = 3.5 Hz,2C), 124.14 (q, *J* = 272.1 Hz), 121.5, 118.8, 118.0, 113.8 (2C), 111.3, 108.4, 55.4, 51.5, 41.6, 21.6. Anal. Calcd for $C_{26}H_{21}F_3N_2O_2$: C, 69.33; H, 4.70; N, 6.22. Found: C, 69.14; H, 4.45; N, 5.87.

(1-(3,4-Difluorophenyl)-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)(4-methoxyphenyl)methanone **(49)**

The title compound was synthesized from **13** (1.4 mmol) and 4-methoxybenzoylchloride (1.54 mmol), yield 54%, white crystals, mp 222-223 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.24 (s, 1H), 7.52 (d, *J* = 7.8 Hz, 1H), 7.36 (d, *J* = 8.5 Hz, 2H), 7.28 (d, *J* = 8.0 Hz, 1H), 7.20 (t, *J* = 7.6 Hz, 2H), 7.14 (t, *J* = 7.4 Hz, 2H), 7.04 (q, *J* = 9.0, 8.4 Hz, 2H), 6.91 (d, *J* = 8.6 Hz, 2H), 3.89 (s, 1H), 3.83 (s, 3H), 3.31 (s, 1H), 2.97 (t, *J* = 10.6 Hz, 1H), 2.81 (dd, *J* = 15.4, 3.7 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.93, 160.50, 148.47 (dd, *J* = 246.7, 12.8 Hz), 148.15 (dd, *J* = 245.7, 12.5 Hz), 138.15, 136.49, 131.08, 128.71 (2C), 128.08, 126.29, 125.01, 121.70, 118.94, 118.22,117.66 (d, *J* = 17.1 Hz, 117.10, 113.99 (2C), 111.51, 108.57, 55.42, 51.35, 41.64, 21.71. Anal. Calcd for $C_{25}H_{20}F_2N_2O_2$: C, 71.76; H, 4.82; N, 6.69. Found: C, 71.89; H, 4.96; N, 6.60.

(*1-(3-Chlorophenyl)-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)(4-methoxyphenyl)methanone* **(50)**

The title compound was synthesized from **17** (1.06 mmol) and 4-methoxybenzoylchloride (1.17 mmol), yield 57%, pale beige powder, mp 225-226 °C (decomposition). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.00 (s, 1H), 7.48 (d, *J* = 7.9 Hz, 1H), 7.44 – 7.37 (m, 4H), 7.35 – 7.26

(m, 3H), 7.14 – 7.07 (m, 1H), 7.04 – 6.97 (m, 3H), 6.86 (s, 1H), 3.80 (s, 4H), One signal is missing (CH_2) under the water peak, 2.95 – 2.84 (m, 1H), 2.79 (dd, J = 15.4, 3.8 Hz, 1H). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.26 (s, 1H), 7.53 (d, J = 7.8 Hz, 1H), 7.38 – 7.25 (m, 6H), 7.22 – 7.17 (m, 2H), 7.17 – 7.11 (m, 1H), 7.04 (s, 1H), 6.93 – 6.89 (m, 2H), 3.89 (s, 1H), 3.82 (s, 3H), 3.34 (s, 1H), 3.00 – 2.91 (m, 1H), 2.81 (dd, J = 15.5, 4.1 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.88, 160.48, 142.87, 136.46, 133.32, 131.10, 130.66, 128.67(3C), 128.12, 128.00, 126.81, 126.31, 121.68, 118.93, 118.20, 114.02 (2C), 111.50, 108.51, 55.42, 51.66, 41.62, 21.78. Anal. Calcd for $C_{25}H_{21}ClN_2O_2$: C, 72.02; H, 5.08; N, 6.72. Found: C, 71.69; H, 4.91; N, 6.34.

(1-(3,4-Dichlorophenyl)-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)(4-methoxyphenyl)methanone **(51)**

The title compound was synthesized from **19** (0.73 mmol) and 4-methoxybenzoylchloride (0.81 mmol), yield 55%, white crystals, mp 232-233 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.99 (s, 1H), 7.67 – 7.63 (m, 1H), 7.49 (d, J = 7.8 Hz, 2H), 7.41 (d, J = 8.4 Hz, 2H), 7.35 (d, J = 8.1 Hz, 1H), 7.30 (d, J = 7.8 Hz, 1H), 7.16 – 7.08 (m, 1H), 7.06 – 6.99 (m, 3H), 6.84 (s, 1H), 3.81 (s, 4H), One signal is missing (CH_2) under the water peak, 2.96 – 2.86 (m, 1H), 2.80 (dd, J = 15.5, 3.6 Hz, 1H). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.19 (s, 1H), 7.53 (d, J = 7.8 Hz, 1H), 7.44 (s, 1H), 7.38 – 7.32 (m, 3H), 7.29 (d, J = 8.1 Hz, 2H), 7.24 – 7.17 (m, 1H), 7.17 – 7.11 (m, 1H), 7.00 (s, 1H), 6.93 – 6.89 (m, 2H), 3.90 (s, 1H), 3.83 (s, 3H), 3.36 – 3.26 (m, 1H), 2.96 (t, J = 12.0 Hz, 1H), 2.82 (dd, J = 15.5, 4.0 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.98, 160.53, 141.42, 136.50, 131.28, 130.96, 130.79, 130.71, 130.08, 128.73 (2C), 128.52, 128.00, 126.27, 121.76, 118.98, 118.25, 114.01 (2C), 111.54, 108.70, 55.42, 51.48, 41.87, 21.74. Anal. Calcd for $C_{25}H_{20}Cl_2N_2O_2$: C, 66.53; H, 4.47; N, 6.21. Found: C, 66.49; H, 4.37; N, 5.77.

(1-(3,4-Dichlorophenyl)-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)(3-methoxyphenyl)methanone **(52)**

The title compound was synthesized from **19** (0.63 mmol) and 3-methoxybenzoylchloride (0.69 mmol), yield 42%, pale yellow powder, mp 160-162 °C (decomposition). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.00 (s, 1H), 7.66 (d, J = 8.3 Hz, 1H), 7.48 (d, J = 7.9 Hz, 2H), 7.41 – 7.36 (m, 1H), 7.33 (dd, J = 16.6, 7.9 Hz, 2H), 7.11 (t, J = 7.2 Hz, 1H), 7.06 – 7.00 (m, 2H), 6.95 (d, J = 11.8 Hz, 2H), 6.86 (s, 1H), 3.78 (s, 3H), 3.70 (d, J = 10.4 Hz, 1H), One signal is missing (CH_2) under the water peak, 2.85 (d, J = 10.5 Hz, 1H), 2.77 (d, J = 14.1 Hz, 1H). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.26 (s, 1H), 7.52 (d, J = 7.8 Hz, 1H), 7.44 (s, 1H), 7.37 – 7.26 (m, 4H), 7.23 – 7.18 (m, 1H), 7.16 – 7.12 (m, 1H), 7.03 (s, 1H), 6.99 – 6.94 (m, 1H), 6.92 (dt, J = 7.5, 1.2 Hz, 1H), 6.89 (dd, J = 2.6, 1.4 Hz, 1H), 3.80 (s, 4H), 3.34 – 3.25 (m, 1H), 2.98 – 2.88 (m, 1H), 2.80 (dd, J = 15.2, 4.1 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.74, 159.40, 141.38, 137.42, 136.50, 131.31, 131.03, 130.77, 130.55, 130.13, 130.02, 128.56, 126.24, 121.79, 118.99, 118.52, 118.28, 115.67, 111.82, 111.54, 108.73, 55.44, 51.04, 41.65, 21.76. Anal. Calcd for $C_{25}H_{20}Cl_2N_2O_2$: C, 66.53; H, 4.47; N, 6.21. Found: C, 66.44; H, 4.54; N, 6.27.

(1-(3,4-Dichlorophenyl)-6-methoxy-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)(phenyl)methanone **(53)**

The title compound was synthesized from **20** (0.4 mmol) and benzoylchloride (0.44 mmol), yield 28%, pale beige powder, mp 262-263 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.83 (s, 1H), 7.66 (d, J = 8.3 Hz, 1H), 7.51 – 7.45 (m, 4H), 7.42 (s, 2H), 7.31 (s, 1H), 7.23 (d, J = 8.7 Hz, 1H), 6.98 (d, J = 2.3 Hz, 1H), 6.85 (s, 1H), 6.75 (dd, J = 8.7, 2.4 Hz, 1H), 3.76 (s, 3H), 3.66 (s, 1H), One signal is missing (CH_2) under the water peak, 2.84 (s, 1H), 2.74 (d, J = 13.2 Hz, 1H). No additional spectrum could be acquired as the compound is not soluble in $CDCl_3$. ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.21, 153.63, 141.55, 136.13, 131.63, 131.39, 131.27,

131.11, 130.84, 130.13, 129.99, 128.88, 128.53, 126.68 (2C), 126.65 (2C), 112.31, 111.79, 108.60, 100.56, 55.67, 51.20, 41.81, 21.80. Anal. Calcd for $C_{25}H_{20}Cl_2N_2O_2$: C, 66.53; H, 4.47; N, 6.21. Found: C, 66.24; H, 4.59; N, 5.90.

General procedure for the synthesis of N2-acyl-N9-ethyl-1-substituted-tetrahydro-β-carbolines

Compound **52** or **22** (1 eq.) was solved in dry DMF (5 ml/mmol). Sodium hydride (60% in mineral oil) (1.5 mmol/mmol) and iodoethane (3 eq.) were added and the mixture was stirred at room temperature for 30-60 min. The solution was evaporated under reduced pressure. To the residue water (15 ml) was added and the suspension extracted with ethyl acetate (3 x 15 ml). The organic phase was washed with water and brine and dryed over magnesium sulfate. The solution was evaporated under reduced pressure and the crude product was recrystallized from ethanol.

(1-(3,4-Dichlorophenyl)-9-ethyl-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)(3-methoxyphenyl)methanone **(54)**

The title compound was synthesized from 52 (0.89 mmol) and iodoethane (2.66 mmol) yield 62%, white powder, mp 165-167 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.66 (d, J = 8.3 Hz, 1H), 7.53 (d, J = 7.7 Hz, 2H), 7.44 (d, J = 8.2 Hz, 1H), 7.37 (t, J = 8.1 Hz, 1H), 7.28 (d, J = 8.3 Hz, 1H), 7.22 – 7.15 (m, 1H), 7.10 – 6.96 (m, 3H), 6.95 – 6.91 (m, 2H), 4.09 – 3.98 (m, 1H), 3.77 (s, 4H), 3.67 (d, J = 13.9 Hz, 1H), 3.27 (s, 1H), 2.94 – 2.75 (m, 2H), 1.00 (t, J = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.55, 159.39, 140.38, 137.30, 136.17, 131.52, 131.24, 131.11, 130.70, 130.51, 129.99, 128.97, 126.23, 121.95, 119.22, 118.60, 118.56, 115.79, 111.80, 109.96, 108.78, 55.45, 50.02, 40.88, 37.91, 21.56, 14.83. Anal. Calcd for $C_{27}H_{24}Cl_2N_2O_2$: C, 67.65; H, 5.05; N, 5.84. Found: C, 67.32; H, 5.33; N, 5.73.

(9-Ethyl-1-phenyl-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)(phenyl)methanone **(55)**

The title compound was synthesized from 22 (0.57mmol) and iodoethane (1.71 mmol), yield 67%, white powder, mp 118-120 °C. ^{1}H NMR (500 MHz, DMSO- d_6) δ 7.55 – 7.29 (m, 12H), 7.20 – 7.13 (m, 1H), 7.09 – 7.02 (m, 2H), 4.06 – 3.94 (m, 1H), 3.75 (td, J = 13.6, 6.4 Hz, 1H), 3.66 – 3.56 (m, 1H), 3.35 – 3.30 (m, 1H), 2.94 – 2.75 (m, 2H), 0.99 (t, J = 6.7 Hz, 3H).^{13}C NMR (126 MHz, DMSO-d_6) δ 169.57, 139.51, 136.27, 136.06, 131.81, 129.72, 128.86(2C), 128.71(2C), 128.60(2C), 128.41, 126.49, 126.31, 121.66, 119.08(2C), 118.39, 109.78, 108.19, 50.83, 40.73, 37.88, 21.72, 14.75. $C_{26}H_{24}N_2O$: C, 82.07; H, 6.36; N, 7.36. Found: C, 82.07; H, 6.79; N, 6.96.

General procedure for the synthesis of N2-aryl-1-substituted-tetrahydro-β-carbolines

To a solution of the 1-substituted-tetrahydro-β-carboline **19** or **2** (1 eq.) in dry DMF (1 ml/mmol) was added TEA (1 eq.) and benzylbromide (1 eq.). The mixture was stirred over night at room temperature. After completion of the reaction as indicated by TLC a 1M sodium hydroxide solution (12 ml) was added dropwise. The formed precipitate was filtered off and washed with water. The crude product was recrystallized from ethanol

2-Benzyl-1-(3,4-dichlorophenyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole **(56)**

The title compound was synthesized from **19** (1.58 mmol) and benzylbromide (1.58mmol), yield 48%, white powder, mp 175-176 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.40 (s, 1H), 7.61 (d, J = 8.3 Hz, 1H), 7.57 (d, J = 2.0 Hz, 1H), 7.42 (d, J = 7.6 Hz, 1H), 7.35 (dd, J = 8.4, 2.0 Hz, 1H), 7.33 – 7.29 (m, 4H), 7.26 – 7.23 (m, 1H), 7.22 (dt, J = 8.1, 0.9 Hz, 1H), 7.03 – 7.00 (m, 1H), 6.97 – 6.94 (m, 1H), 4.78 (s, 1H), 3.70 (d, J = 13.6 Hz, 1H), 3.51 (d, J = 13.7 Hz, 1H), 3.02 – 2.95 (m, 1H), 2.79 – 2.69 (m, 2H), 2.66 – 2.59 (m, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 143.26, 139.17, 136.56, 133.74, 130.92, 130.82, 130.62, 130.04, 129.06, 128.48 (2C), 128.37 (2C), 127.08, 126.44, 120.98, 118.53, 117.93, 111.22, 107.53, 61.68, 57.41,

46.59, 20.22. Anal. Calcd for $C_{24}H_{20}Cl_2N_2$: C, 70.77; H, 4.95; N, 6.88. Found: C, 70.44; H, 5.15; N, 6.84.

2-Benzyl-1-phenyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole **(57)**

The title compound was synthesized from **2** (1.24 mmol) and benzylbromide (1.24 mmol), yield 30%, white powder, mp 165-166 °C. ^{1}H NMR (500 MHz, DMSO- d_6) δ 10.30 (s, 1H), 7.41 (d, J = 7.8 Hz, 1H), 7.36 – 7.20 (m, 11H), 7.03 – 6.96 (m, 1H), 6.98 – 6.91 (m, 1H), 4.74 (s, 1H), 3.71 (d, J = 13.6 Hz, 1H), 3.50 (d, J = 13.6 Hz, 1H), 3.01 (dt, J = 11.9, 5.2 Hz, 1H), 2.82 – 2.66 (m, 2H), 2.67 – 2.58 (m, 1H). ^{13}C NMR (126 MHz, DMSO d_6) δ 141.95, 139.55, 136.51, 134.71, 128.97(2C), 128.52(2C), 128.35(2C), 128.33(2C), 127.53, 126.99, 126.61, 120.69, 118.38, 117.77, 111.21, 107.16, 62.70, 57.36, 46.49, 20.31. $C_{24}H_{22}N_2$: C, 85.17; H, 6.55; N, 8.28. Found: C, 84.80; H, 6.60; N, 8.25.

Biological Investigation

Materials. The reference compound XR9577 (N-(2-((4-(2-(3,4-dihydroisoquinolin-2(1H)-yl)ethyl)phenyl)carbamoyl)phenyl)-quinoline-3-carboxamide) was synthesized according to the literature.[21,22] Ko143 ((3S,6S,12aS)-1,2,3,4,6,7,12,12a-Octahydro-9-methoxy-6-(2-methylpropyl)-1,4-dioxopyrazino[1′,2′:1,6]pyrido[3,4- b]indole-3-propanoic acid 1,1-dimethylethyl ester) and CsA were purchased from Tocris Bioscience (Bristol, United Kingdom). Pheophorbide A was obtained from Fontier Scientific Inc. (Logan, UT, USA) and calcein AM from Merck KGaA (Darmstadt, Germany). Cell culture material was bought from Sarstedt (Newton, USA). The other chemicals were purchased from Sigma-Aldrich (Taufkirchen, Germany).

All assays were performed in Krebs-HEPES buffer, pH 7.4 (KHB). Stock solutions (10 mM) of the test compounds were prepared in DMSO. The dilution series (concentrations up to 10

μM) of the tested compound was prepared in KHB and contained up to max. 5% methanol in the highest concentration.

Cell culture. The cell lines were kept in an incubator at 37 °C and 5% CO_2. When the cell monolayer had reached a confluence of approximately 90%, the cells were subcultured by detaching them with 0.05% trypsin and 0.02% EDTA. The cell count was determined by CASY1 model TT cell counter with 150 μm capillary (Schaerfe System GmbH, Reutlingen, Germany).

The MDCK II BCRP cell line was generated by retroviral transfection of MDCK cells with the human ABCG2 cDNA. The ABCG2 gene is fused to the green fluorescent protein (GFP). These cells were made available kindly by Dr. A. Schinkel (The Netherlands Cancer Institute, Amsterdam, The Netherlands). The cells were cultivated in Dulbecco´s modified eagle medium (DMEM) with 2 mM L-glutamine, 10% fetal bovine serum, 50 U/ml penicillin G und 50 μg/ml streptomycin. The ABCB1 overexpressing, doxorubicin resistant human ovarian carcinoma cell line A2780 adr (No. 93112520) was purchased from the European Collection of Cell Cultures (ECACC, Salisbury, UK). These cells were cultivated in RPMI-1640 medium with 10% fetal bovine serum, 50 U/ml penicillin G und 50 μg/ml streptomycin,

Hoechst 33342 accumulation assay. The bisbenzimidazole derivative Hoechst 33342 is a substrate of ABCB1 and ABCG2. Hoechst 33342 shows only a very weak fluorescence in the aqueous environment. In contrast it intercalates intracellularly with lipophilic substances or attaches in the phospholipid bilayer of the cell membrane whereby the fluorescence intensity from Hoechst 33342 increases. In cells which overexpress these ABC-transporters Hoechst can effectively be transported out whereby the intracellular fluorescence decreases. In presence of an inhibitor the intracellular fluorescence increases in dependence of the inhibitor concentration. The assay was previously described and was used with slight modifications.[23, 39]

[24, 25, 26, 27, 28, 29, 30, 31, 32, 33 34, 35] When the cells reached a confluency of approximately 90% they were detached with trypsin 0.05% / EDTA 0.02%, fresh medium was added and the cell suspension was transferred to a 50 ml tube. After centrifugation (266 g, 4 °C, 4 min) the cell pellet was resuspended in fresh medium and the cell number was determined with the CASY1 model TT cell counter. The desired amount of the cell suspension was washed three times with KHB and resuspended in the required quantity of KHB. 160 µl (approximately 27.000 cells) of this cell suspension were added to each well of a black 96 well plates (Greiner, Frickenhausen, Germany). 20 µl of each test compound in different concentrations were added and the 96 well plate was incubated for 30 min at 37 °C and 5% CO_2. After 30 min 20 µl of a 6 µM Hoechst 33342 solution were added to each well and the fluorescence was measured immediately with a microplate reader. (BMG POLARstar microplate reader, BMGLabtech, Offenburg, Germany)

The increase of intracellular fluorescence (λext. = 355 nm, λem. = 460 nm) was measured continuously (60 s) over a period of 120 min at 37 °C. For analysis, the average of fluorescence values in the steady state, from 100 to 109 min, from each concentration were used.

Concentration-response curves were generated by nonlinear regression analysis using the four parameter logistic equation with variable Hillslope or alternatively, using the three parameter logistic equation in which the Hill coefficient is fixed at 1, whichever was statistically preferred (GraphPad Prism 5.0, San Diego, USA).

Pheophorbide A assay Pheophorbide A is a photoactive porphyrin derivative and a selective ABCG2 substrate. It passes through passive diffusion in the cell and can accumulate there. After incubation, a steady state reached between inflow and outflow. By overexpression of ABCG2, pheophorbide A is actively transported out of the cell. The presence of an ABCG2 inhibitor leads to a concentration-dependent increase of the fluorescence in cells, which can

be determined by flow cytometric examination. The assay was previously described and was used with slight modifications.[23, 30, 34, 36]

For cell preparation see description for Hoechst 33342 assay. 160 µl (approximately 36.000 cells) were added to each well of a clear 96 U-bottom well plates (Greiner, Frickenhausen, Germany). 20 µl of each test compound in different concentrations was added and the 96 well plate was pre-incubated for 30 min at 37 °C and 5% CO_2. After this 30 min 20 µl of a 5 µM pheophorbide A solution was added to each well and the plate was incubated for further 120 min at 37 °C and 5% CO_2 under light protection.

The fluorescence was measured by flow cytometry (FACScalibur, Becton Dickinson Biosciences, Heidelberg, Germany) at λext. = 488 nm, λem. ≥ 670 nm (FL3 channel)

The generation of the concentration-response curves were performed by nonlinear regression analysis using a four parameter logistic equation with variable Hillslope or alternatively, using the simplified 3-parameter logistic equation in which the Hill coefficient is assumed as 1.

Calcein AM assay The non-fluorescent calcein AM is a substrate of ABCB1, which can pass the cell membrane by passive diffusion. Intracellular calcein AM is cleaved by non-specific esterases to fluorescent calcein which cannot overcome the cell membrane due to its multiple negative charges and hence accumulates within the cell. In cells with high expression of ABCB1 calcein AM can be effectively transported out of the cell. Thus a lesser amount of the ester which can be converted to fluorescent calcein is located intracellularly. An ABCB1 inhibitor leads to a concentration-dependent increase of the intracellular fluorescence. The assay was previously described and was used with slight modifications.[23,24,25,26,30,31,33,34,35]

The preparation of the A2780 adr cells was the same like for the MDCK II BCRP cells, see Hoechst 33342 assay. 160 µl (approximately 27.000 cells) were added to each well of a clear 96 F-bottom well plates (Greiner, Frickenhausen, Germany). 20µl of each test compound in different concentrations were added and the 96 well plate were incubated for 30 min at 37 °C

and 5% CO_2. 30 min 20 µl of a 3.125 µM calcein AM solution were added to each well and the fluorescence were measured immediately with a microplate reader. (BMG POLARstar microplate reader, BMGLabtech, Offenburg, Germany)

The increase of intracellular fluorescence (λex. = 485 nm, λem. = 520 nm) was measured continuously (60 s) over a period of 60 min at 37 °C. For analysis, the first line part of the fluorescence time curves from each concentration were used to calculate the slope. With these slopes concentration-response curves were generated by nonlinear regression analysis using the four parameter logistic equation with variable Hillslope or alternatively, using the three parameter logistic equation in which the Hill coefficient is fixed at 1, whichever was statistically preferred (GraphPad Prism 5.0, San Diego, USA).

MTT-assay The toxicity of the compounds toward the MDCK II cell line was determined by using the MTT assay. This assay is based on that the water-soluble yellow MTT can be reduced by living cells to the slightly soluble blue dye formazan. The absorption of the dye is measured and is proportional to cell vitality. The assay was previously described and was used with slight modifications. [26,30,32,35, 37,38,39] MDCK II BCRP and MDCK II wild type cells were used.

The cells were trypsinized and seeded into 96-well tissue culture plates (Sarstedt, Newton, USA) with a cell density of 3 x 10^3 cells per well in 180 µl and allowed to attach for 6 hours at 37 °C and 5% CO_2. 20 µl of each compound were added. A mixture of 10% (v/v) DMSO and pure growth medium were used as positive and negative control, respectively. To reduce the evaporation of the solvents during the incubation of 72 h, the intermediate spaces of the plate were filled with PBS buffer. 40 µl of a solution of the MTT reagent (5 mg/ml) was added to each well and the plates were incubated for 1h. After incubation the reaction was stopped by removal of the supernatant and restocking with 100 µl DMSO per well. The

absorbance of the formed formazan was determine at 570 nm and background correction at 690 nm using a Multiscan Ex microplate photometer (Thermo Fisher Scientific, Waltham, MA, USA). The obtained data were normalised and GI_{50} values were calculated by nonlinear regression, assuming a sigmoidal concentration response curve with variable Hill slope.

To determine the ability of MDR reversal of the compounds in ABCG2 overexpressing (MDCK II BCRP) and wild-type cells the MTT assay was used with slight modifications. The cytotoxic effect of SN-38 was measured in presence and absence of the selected compound.

ATPase assay. The assay was performed as previously described with slight modifications.[40, 39] A membrane preparation of Sf9 cells showing a vanadate sensitive ATPase activity was used. The recombinant baculovirus with the human wild-type ABCG2 cDNA was generous gift from Dr. Özvegy-Laczka (Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary). The Sf9 cells were grown in protein-free insect medium (Spodopan, PAN-Biotech GmbH, Aidenbach, Germany) complemented with 50 µg/ml streptomycin, 50 units/ml penicillin G and 0.125 µg/ml Fungizone® antimycotic as adherent monolayer culture. The cell infection with the recombinant baculovirus for membrane preparation was performed as described in literature. The Sf9 cells were harvested and the membrane fraction was isolated including a cholesterol-loading step, see literature.[40, 41] The membrane protein was quantified by the Pierce™ BCA (bicinchoninic acid assay) protein assay kit (Thermo scientific, Rockford, USA). The reaction mixture contained a mix of 40 mM 3-(N-morpholino)propanesulfonic acid-Tris (pH 7.0), 50 mM KCl, 2 mM dithiothreitol, 0.5 mM EGTA-Tris (pH 7.0), 5 mM sodium azide, 1 mM oubain, 10 µg of the prepared membrane protein (1 mg/ml) and the tested compounds. The reaction was started by adding 3.3 mM MgATP and incubated for 20 min at 37 °C and was stopped by addition of 100 µl 5% sodium dodecyl sulfate (SDS). The basal activity was determined in the presence of 1 µM DMSO. The colorimetric detection was carried out by adding 300 µl P_i-reagent (2.5 mM

H_2SO_4, 1% ammonium molybdate, 0.014% antimony potassium tartrate), 750 µl 20% acetic acid and 150 µl 1% freshly prepared ascorbic acid. After a 20 min incubation the optical density was measured at a wavelength of 880 nm. K_2HPO_4 was used as standard for determination the amount of phosphate from the absorbance values.

Investigation of Precipitation Behavior during Fluorescence Accumulation Assays. Ten micromolar test solutions were prepared from the stock solutions as done for the Hoechst 33342 assay. Absorbance of the samples was measured at the maximum of an absorbance peak in constant time intervals (120 s) for a period of 2 h at room temperature using an Ultrospec 2001 Pro UV/visible spectrophotometer (Pharmacia Biotech Ltd., UK). The absorbance of the solution without compound was used as reference and subtracted automatically from the values of samples.

Ancillary Information

Supporting Information.

Molecular formula strings and the associated biological data are given.

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Abbreviations Used

ABC, ATP-binding cassette; AM, acetoxymethyl ester; APT, attached proton test; BCA, bicinchoninic acid assay; CsA, cyclosporine A; DMEM, Dulbecco´s modified eagle medium; FTC, fumitremorgin C; GI_{50}, half-maimal growth inhibition; KHB, Krebs HEPES buffer, MDCK, Madin Darby Canine Kidney; MDR, multidrug resistance; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NBD, nucleotide binding domain; Sf9 cells, recombinant baculovirus-infected Spodoptera frugiperda ovarian cells; TEA, triethylamine; TMD, transmembrane domain

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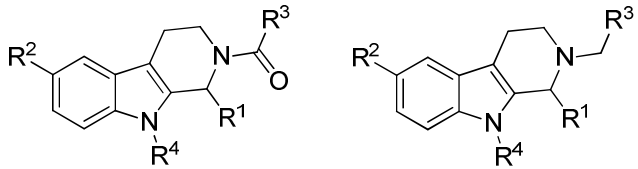
Table 1: Inhibitory effect on ABCG2 of synthesized compounds and harmine in Hoechst 33342 and pheophorbide A assays using the MDCK II BCRP cell line. XR9577 and Ko143 were used as standard inhibitors. Data shown is mean of IC_{50} ± SD with n ≥ 3.



21-55

56-57

Compound	R^1	R^2	R^3	R^4	Hoechst 33342 IC_{50} ± SD [μM]	Pheophorbide A IC_{50} ± SD [μM]
21	isobutyl	H	phenyl	H	2.33 ± 0.23	*n.t.*[a)]
22	phenyl	H	phenyl	H	2.78 ± 0.15	*n.t.*[)]
23	3-OCH_3-phenyl	H	phenyl	H	1.92 ± 0.24	*n.t.*
24	4-OCH_3-phenyl	H	phenyl	H	2.21 ± 0.33	*n.t.*
25	3,4-OCH_3-phenyl	H	phenyl	H	3.62 ± 0.41	*n.t.*
26	4-CH_3-phenyl	H	phenyl	H	1.43 ± 0.20	*n.t.*
27	3-CN-phenyl	H	phenyl	H	2.00 ± 0.18	*n.t.*
28	3-NO_2-phenyl	H	phenyl	H	>> 10	*n.t.*
29	4-acetamido-phenyl	H	phenyl	H	7.82 ± 1.32	*n.t.*
30	4-CF_3-phenyl	H	phenyl	H	>> 10	*n.t.*
31	2-F-phenyl	H	phenyl	H	4.49 ± 0.34	*n.t.*
32	4-F-phenyl	H	phenyl	H	1.89 ± 0.09	*n.t.*
33	3,4-F-phenyl	H	phenyl	H	1.10 ± 0.10	1.80 ± 0.18
34	2-Br-phenyl	H	phenyl	H	>> 10	*n.t.*
35	4-Br-phenyl	H	phenyl	H	0.687 ± 0.127	*n.t.*
36	2-Cl-phenyl	H	phenyl	H	>> 10	*n.t.*
37	3-Cl-phenyl	H	phenyl	H	0.869 ± 0.068	0.811 ± 0.140
38	4-Cl-phenyl	H	phenyl	H	0.741 ± 0.123	0.784 ± 0.195

39	3,4-Cl-phenyl	H	phenyl	H	0.328 ± 0.055	0.365 ± 0.036
40	3,4-F-phenyl	H	4-Cl-phenyl	H	1.26 ± 0.30	*n.t.*
41	4-Br-phenyl	H	4-Cl-phenyl	H	1.78 ± 0.19	*n.t.*
42	3,4-Cl-phenyl	H	4-Cl-phenyl	H	1.03 ± 0.10	*n.t.*
43	3,4-Cl-phenyl	H	3,4-OCH_3-phenyl	H	0.698 ± 0.116	*n.t.*
44	3,4-F-phenyl	H	3,4-OCH_3-phenyl	H	1.10 ± 0.20	*n.t.*
45	3,4-Cl-phenyl	H	1-naphthyl	H	1.32 ± 0.23	*n.t.*
46	phenyl	H	4-OCH_3-phenyl	H	1.75 ± 0.16	*n.t.*
47	3-OCH_3-phenyl	H	4-OCH_3-phenyl	H	1.45 ± 0.16	*n.t.*
48	4-CF_3-phenyl	H	4-OCH_3-phenyl	H	1.23 ± 0.10	*n.t.*
49	3,4-F-phenyl	H	4-OCH_3-phenyl	H	0.805 ± 0.076	*n.t.*
50	3-Cl-phenyl	H	4-OCH_3-phenyl	H	0.883 ± 0.093	*n.t.*
51	3,4-Cl-phenyl	H	4-OCH_3-phenyl	H	0.233 ± 0.044	0.237 ± 0.080
52	3,4-Cl-phenyl	H	3-OCH_3-phenyl	H	0.238 ± 0.044	0.206 ± 0.025
53	3,4-Cl-phenyl	OCH_3	phenyl	H	0.382 ± 0.077	*n.t.*
54	3,4-Cl-phenyl	H	3-OCH_3-phenyl	ethyl	>> 10	*n.t.*
55	phenyl	H	phenyl	ethyl	10.4 ± 1.8	*n.t.*
56	3,4-Cl-phenyl	H	phenyl	H	>> 10	*n.t.*
57	phenyl	H	phenyl	H	>> 10	*n.t.*
XR9577					0.704 ± 0.147	0.741 ± 0.146
harmine					*N/A* [b]	5.08 ± 0.35
Ko143					0.221 ± 0.024	*n.t.*

[a] *n.t* = not tested; [b] *N/A* = not applicable

Table 2: Inhibitory activity toward ABCB1, of the compounds showing a response of more than 25% in the screening, against ABCB1 overexpressing A2780adr cells

Compound	Calcein AM $IC_{50} \pm SD$ [μM] [a]
21	12.9 ± 0.6
22	7.94 ± 1.83
23	6.22 ± 0.38
24	12.0 ± 2.8
25	7.33 ± 0.85
27	6.55 ± 0.77
29	7.40 ± 0.94
31	11.3 ± 1.3
32	13.4 ± 0.5
33	8.76 ± 1.30
37	22.5 ± 4.8
40	23.4 ± 2.5
43	14.3 ± 1.5
44	2.86 ± 0.19
46	9.55 ± 2.32
47	3.38 ± 0.64
49	8.24 ± 1.03
50	19.2 ± 1.8
55	9.23 ± 0.88
Cyclosporine A	1.09 ± 0.02

[a] $n \geq 3$

Table 3: Intrinsic toxicity of selected compounds in MDCK II BCRP and MDCK wild-type cells as determined by the MTT cytotoxicity assay.

Compound	R^1	R^2	R^3	MDCK II BCRP $GI_{50} \pm SD$ [µM][a]	MDCK wild-type $GI_{50} \pm SD$ [µM][a]
Ko143				11.1 ± 0.75	10.9 ± 0.9
harmine				2.42 ± 0.72	2.30 ± 0.63
22	phenyl	H	phenyl	12.9 ± 1.1	12.4 ± 1.7
35	4-Br-phenyl	H	phenyl	20.6 ± 1.8	19.4 ± 1.0
38	4-Cl-phenyl	H	phenyl	18.1 ± 1.5	16.4 ± 0.4
39	3,4-Cl-phenyl	H	phenyl	6.95 ± 1.07	7.29 ± 0.52
51	3,4-Cl-phenyl	H	4-OCH_3-phenyl	4.67 ± 0.46	4.83 ± 0.57
52	3,4-Cl-phenyl	H	3-OCH_3-phenyl	5.82 ± 0.60	6.55 ± 0.15
53	3,4-Cl-phenyl	OCH_3	phenyl	4.17 ± 0.75	5.27 ± 1.44

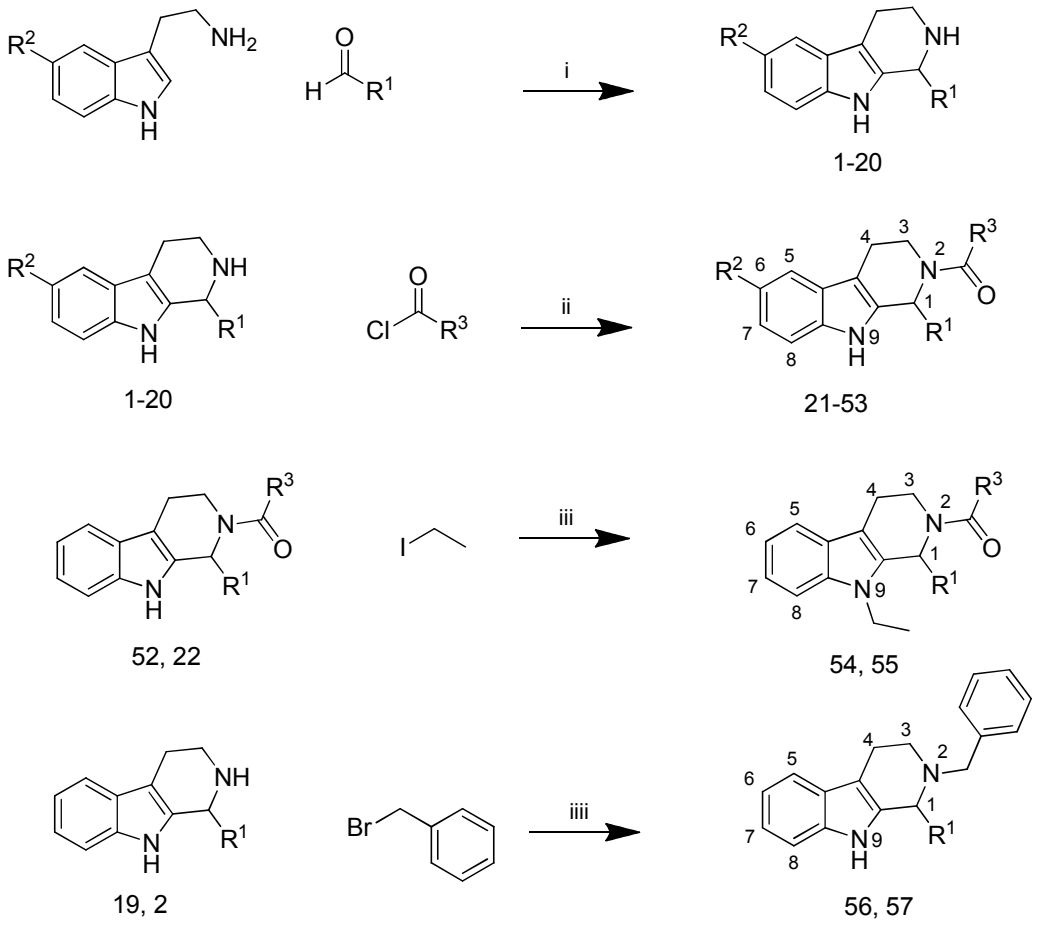
[a] n = 3

Table 4: The ability of Ko143 and selected compounds to reverse resistance toward SN-38 in MDCK II BCRP and MDCK wild-type cells.

Compound	MDCK II BCRP GI_{50} ± SD [μM][a]	MDCK II BCRP + 0.01 μM test compound GI_{50} ± SD [μM]	MDCK II BCRP + 0.1 μM test compound GI_{50} ± SD [μM]	MDCK II BCRP + 1 μM test compound GI_{50} ± SD [μM]	MDCK wild-type GI_{50} ± SD [μM][a]
Ko143	1.65 ± 0.04	1.05 ± 0.05	0.279 ± 0.026	0.292 ± 0.018	0.218 ± 0.082
harmine	2.12 ± 0.21	1.95 ± 0.19	1.84 ± 0.08	1.22 ± 0.11	0.300 ± 0.072
22	1.96 ± 0.39	1.75 ± 0.04	1.58 ± 0.12	0.865 ± 0.036	0.300 ± 0.052
35	3.19 ± 0.26	2.88 ± 0.14	2.21 ± 0.05	0.946 ± 0.063	0.313 ± 0.009
38	3.49 ± 0.37	3.52 ± 0.45	2.76 ± 0.01	1.11 ± 0.04	0.285 ± 0.007
39	2.22 ± 0.11	1.69 ± 0.02	1.17 ± 0.06	0.484 ± 0.006	0.311 ± 0.027
51	2.23 ± 0.11	1.62 ± 0.15	0.774 ± 0.103	0.438 ± 0.004	0.298 ± 0.076
52	2.00 ± 0.29	1.75 ± 0.19	0.868 ± 0.001	0.429 ± 0.003	0.288 ± 0.023
53	2.00 ± 0.12	1.44 ± 0.16	0.655 ± 0.071	0.410 ± 0.032	0.258 ± 0.053
average	2.28 ± 0.39				0.283 ± 0.047

Table 5: Inhibitory effect of selected compounds on vanadate sensitive ATPase activity of isolated ABCG2 containing Sf9 membranes for the basal activity and quercetin stimulated activity.

Compound	Basal activity IC_{50} ± SD [µM]	Quercetin stimulated activity IC_{50} ± SD [µM]	Shift factor
Ko143	0.004 ± 0.000	0.028 ± 0.001	7.2
22	0.196 ± 0.032	2.02 ± 0.37	10.3
35	0.112 ± 0.015	0.863 ± 0.028	7.7
38	0.060 ± 0.012	0.560 ± 0.106	9.3
39	0.031 ± 0.002	0.262 ± 0.036	8.4
51	0.026 ± 0.002	0.285 ± 0.004	11.0
52	0.038 ± 0.003	0.262 ± 0.030	7.0
53	0.029 ± 0.005	0.299 ± 0.023	10.4



Scheme 1: General synthesis of target compounds. Reagents and conditions: (i) DCM and TFA, rt, 24 h; (ii) THF, TEA, 1 h at 0°, 12 h at rt; (iii) DMF, NaH, 30-60 min. at rt; (iiii) DMF, TEA, over night at rt

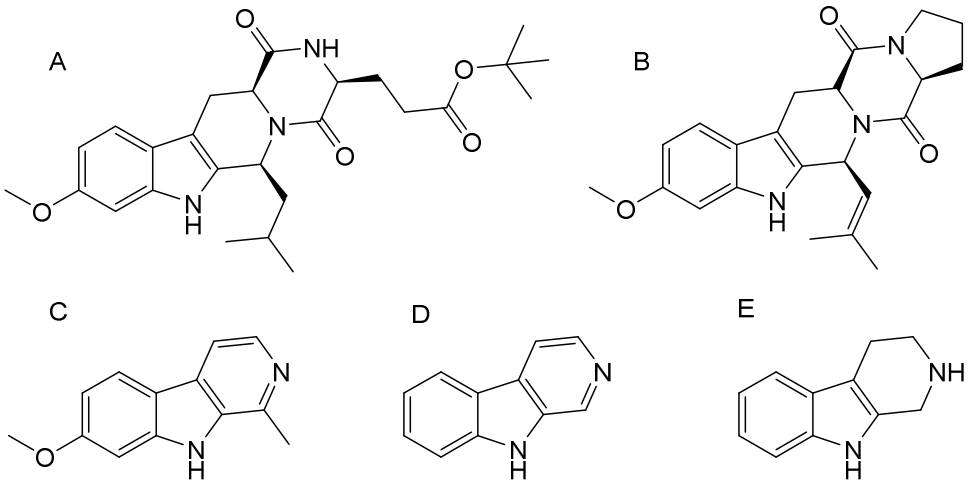


Figure 1: Structures of Ko143 (A), Fumitremorgin C (B), harmine (C), ß-carboline (D), tetrahydro-ß-carboline (E)

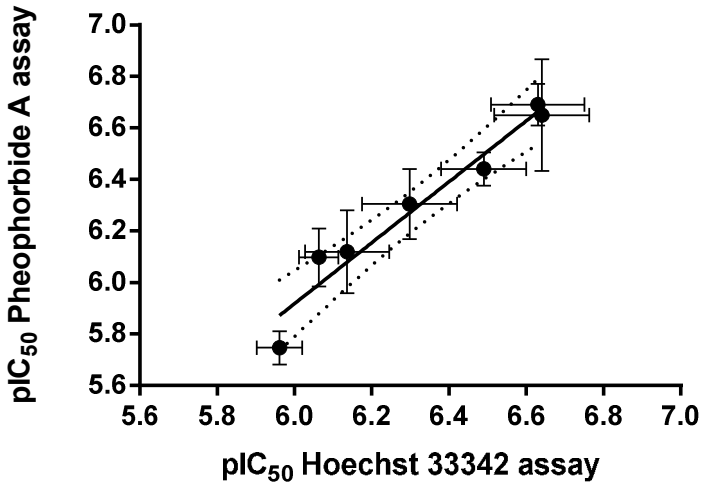


Figure 2: Scatterplot of pIC_{50} values of ABCG2 inhibitors tested in both, Hoechst 33342 and pheophorbide A assays (compounds 33, 37, 38, 39, 51, 52 and XR9577). Shown is mean of $pIC_{50} \pm SD$, ($n \geq 3$); squared correlation coefficient $r^2 = 0.95$

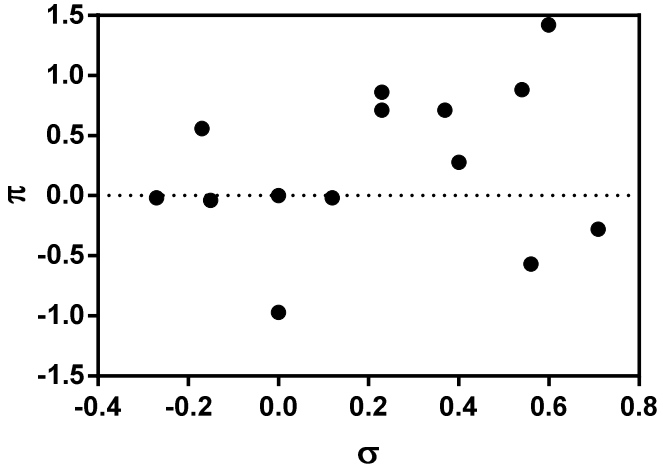


Figure 3: Distribution of substituents at the phenyl ring at position R^3 in a σ-π scatter diagram.

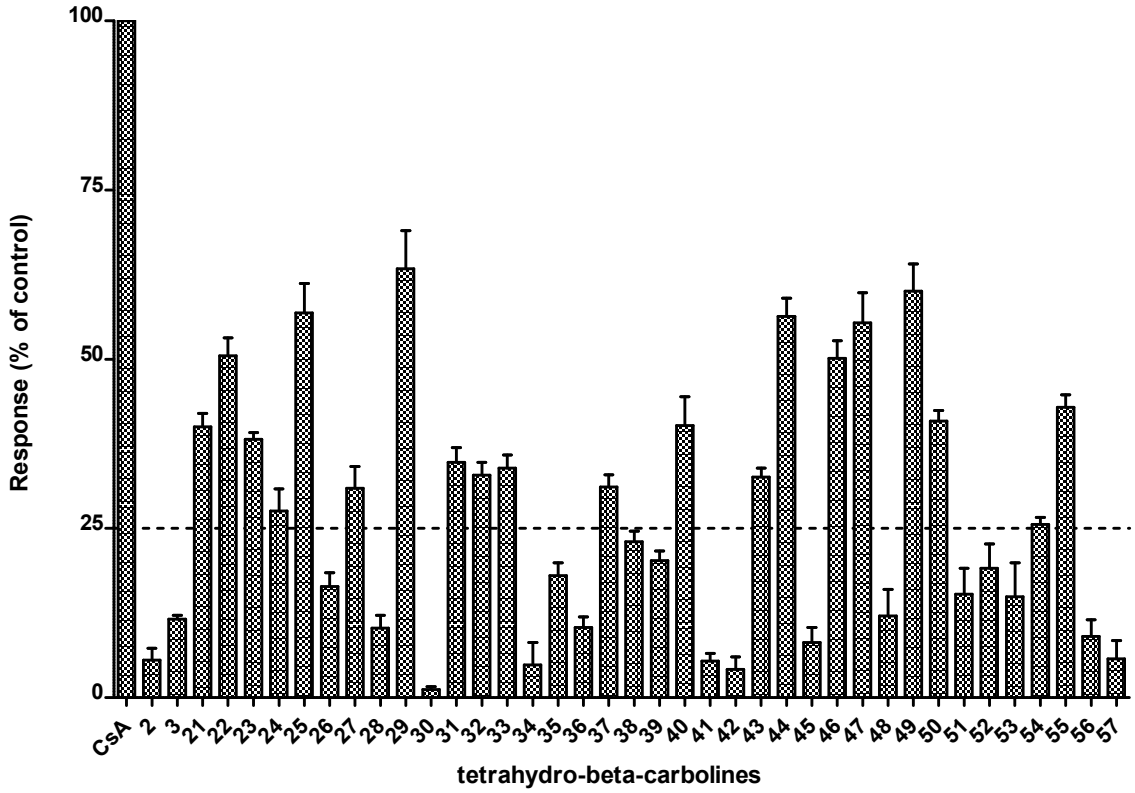


Figure 4: Screening of the inhibitory effect of compounds **2,3** and **21**-**57**, at a concentration of 10 μM, against ABCB1 overexpressing A2780adr cells using the calcein AM assay. Cyclosporine A (10 μM) was used as positive control. Data are expressed as response in percentage of the positive control ($n \geq 3$).

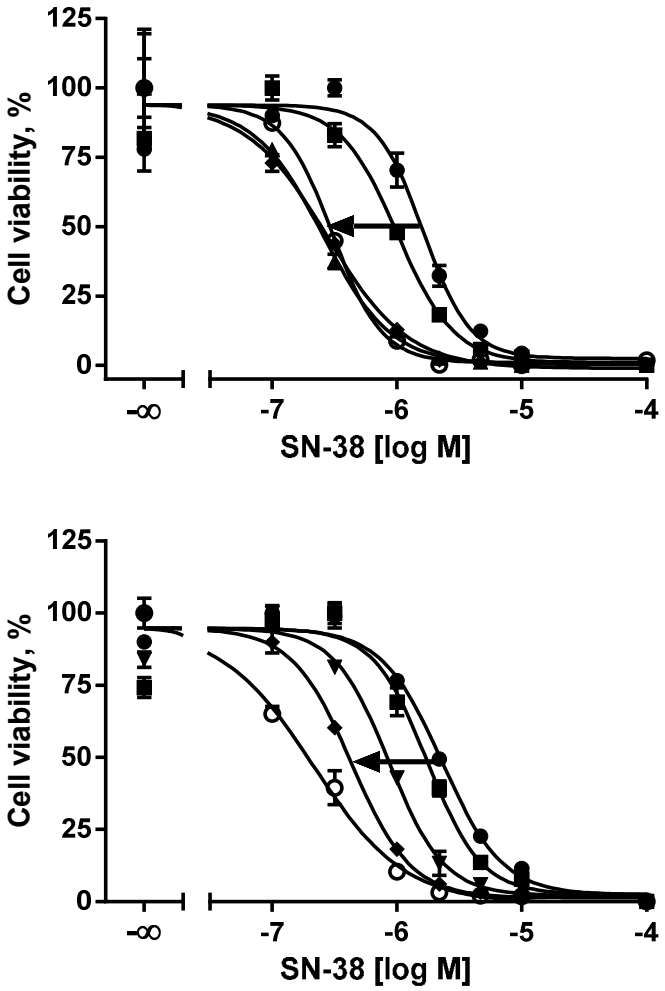


Figure 5: The ability of Ko143 (top) and compound **51** (bottom) to reverse resistance toward the cytotoxic SN-38 in MDCK II BCRP cells. Closed circles: control; closed squares: 0.01 μM; closed triangles: 0.1 μM; closed rhomb: 1 μM; opened circles: wild-type. The arrow indicates the shift in GI_{50}-values caused by presence of increasing concentrations of reverser..

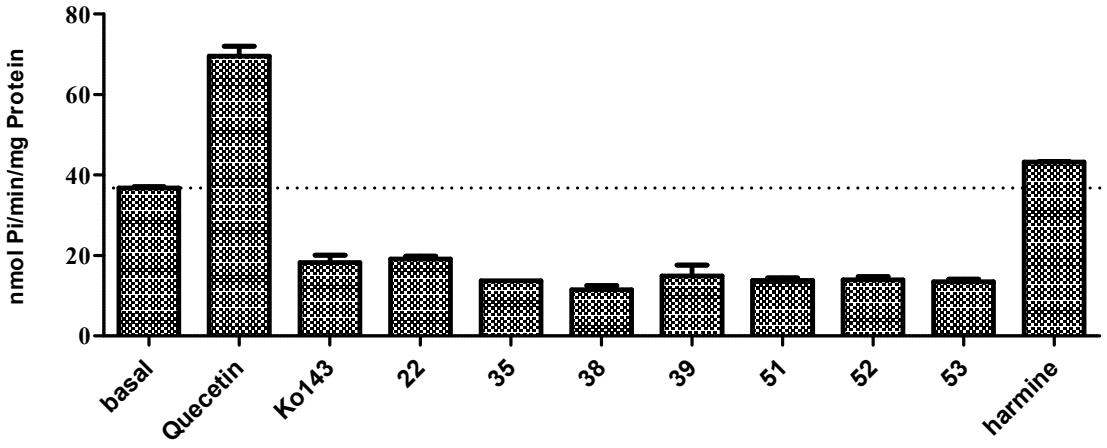


Figure 6: Effect of selected compounds and harmine on vanadate sensitive ATPase activity of isolated ABCG2 containing Sf9 membranes. The activator quercetin was used as positive control, while the inhibitor Ko143 was used as negative control. All compounds were investigated at a concentration of 1 μM.

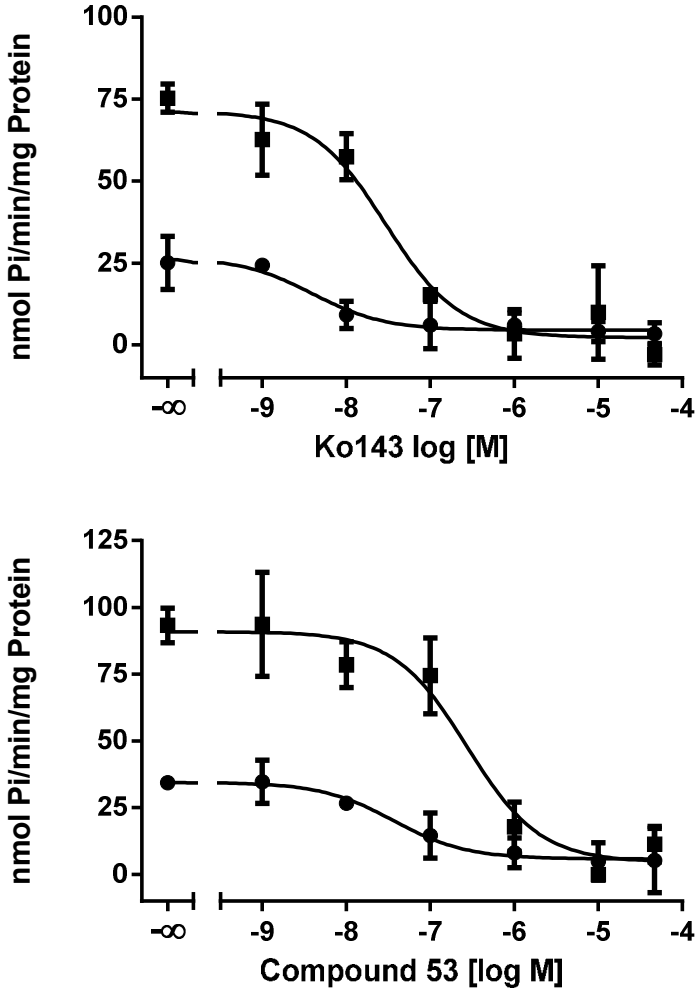


Figure 7: Inhibitory effect curves of Ko143 (top) and compound **53** (bottom) on vanadate sensitive ATPase activity of isolated ABCG2 containing Sf9 membranes for the basal activity (closed circles) and quercetin (1 μM) stimulated activity (closed squares).

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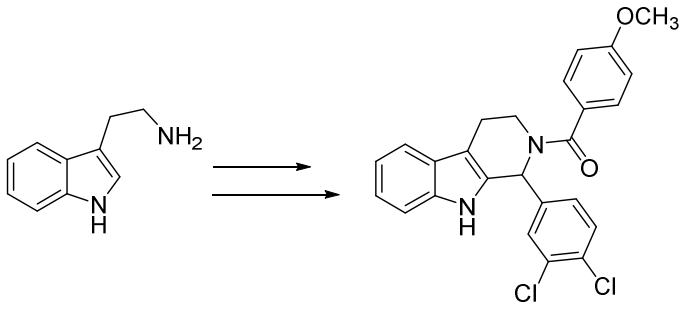

Inhibitory activity against ABCG2
IC_{50} = 0.2 μM