3D-QSAR, Design, Synthesis and characterization of trisubstituted harmine derivatives with in vitro antiproliferative properties

ARTICLE *in* EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY · FEBRUARY 2015

Impact Factor: 3.45 · DOI: 10.1016/j.ejmech.2015.02.044

Accepted Manuscript

3D-QSAR, Design, Synthesis and characterization of trisubstituted harmine derivatives with *in vitro* antiproliferative properties

Céline Meinguet, Céline Bruyère, Raphaël Frédérick, Véronique Mathieu, Christelle Vancraeynest, Lionel Pochet, Julie Laloy, Jérémie Mortier, Gerhard Wolber, Robert Kiss, Bernard Masereel, Johan Wouters

PII: S0223-5234(15)00140-3

DOI: 10.1016/j.ejmech.2015.02.044

Reference: EJMECH 7726

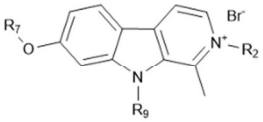
To appear in: *European Journal of Medicinal Chemistry*

Received Date: 4 March 2014

Revised Date: 20 February 2015

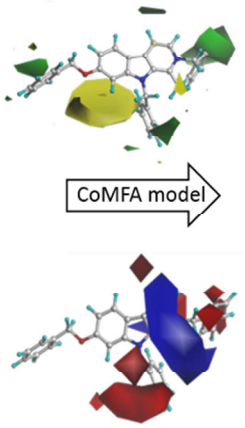
Accepted Date: 21 February 2015

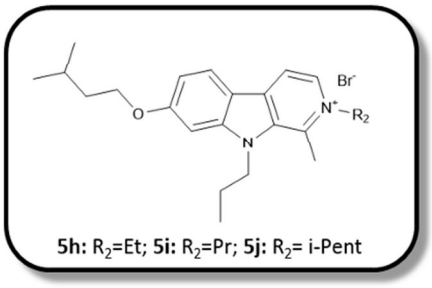
Please cite this article as: C. Meinguet, C. Bruyère, R. Frédérick, V. Mathieu, C. Vancraeynest, L. Pochet, J. Laloy, J. Mortier, G. Wolber, R. Kiss, B. Masereel, J. Wouters, 3D-QSAR, Design, Synthesis and characterization of trisubstituted harmine derivatives with *in vitro* antiproliferative properties, *European Journal of Medicinal Chemistry* (2015), doi: 10.1016/j.ejmech.2015.02.044.



2a: R_7=R_9=R_2=Bn
2b: R_7=R_9=3-FBn, R_2=4-FBn
2c: R_7=R_9=CH_2-cyclohexyl, R_2=Bn

Anticancer activity: IC_{50}≤2.7μM
Solubility: <72.5μg/ml





5h: R_2=Et; **5i:** R_2=Pr; **5j:** R_2= i-Pent

Anticancer activity: IC_{50}≤0.6μM
Solubility: >185μg/ml

3D-QSAR, Design, Synthesis and characterization of trisubstituted harmine derivatives with *in vitro* antiproliferative properties

Céline Meinguet[a*], Céline Bruyère[b], Raphaël Frédérick[c], Véronique Mathieu[b], Christelle Vancraeynest[a], Lionel Pochet[a], Julie laloy[a], Jérémie Mortier[d], Gerhard Wolber[d], Robert Kiss[b] , Bernard Masereel[a] and Johan Wouters[a]

(a) Namur Medicine & Drug Innovation Center (NAMEDIC-NARILIS), University of Namur (Unamur), 61, rue de Bruxelles, 5000 Namur ; (b) Laboratoire de Cancérologie et de Toxicologie Expérimentale, Faculté de Pharmacie, Université Libre de Bruxelles (ULB), Boulevard du Triomphe, 1050 Brussels, Belgium ; (c) Medicinal Chemistry Research Group (CMFA), University of Louvain (UCL), 73, avenue Mounier, 1200 Bruxelles (d) Institute of Pharmacy, Freie Universität Berlin , 2+4 Königin Luise Straβe, 14195 Berlin, Germany

*Corresponding author: Tel.: +32 81 724569; fax: +32 81 725440

Email address: celine.meinguet@unamur.be (Céline Meinguet)

Keywords:

Harmine derivatives; Antiproliferative activity; Cytostatic activity; Comparative Molecular Field Analysis.

Abstract:

Apolar trisubstituted derivatives of harmine show high antiproliferative activity on diverse cancer cell lines. However, these molecules present a poor solubility making these compounds poorly bioavailable. Here, new compounds were synthesized in order to improve solubility while retaining antiproliferative activity. First, polar substituents have shown a higher solubility but a loss of antiproliferative activity. Second, a Comparative Molecular Field Analysis (CoMFA) model was developed, guiding the design and synthesis of eight new compounds. Characterization has underlined the *in vitro* antiproliferative character of these compounds on five cancerous cell lines, combining with a high solubility at physiological pH, making these molecules druggable. Moreover, targeting glioma treatment, human intestinal

absorption and blood brain penetration have been calculated, showing high absorption and penetration properties.

1. Introduction

Harmine (**1**) is a natural β-carboline compound, which is a major alkaloid from *Peganum harmala* and *Banisteriopsis caapi,* presenting antiproliferative activity. This later property is attributed to several mechanisms of action such as antiangiogenesis activity on endothelial cells[1,2], apoptosis induction[3] or reverse drug resistance by inhibition of the Breast Cancer Resistance Protein-mediated drug efflux protein[4]. Based on these antiproliferative properties, our group previously synthesized about fifty harmine derivatives **2a-as** substituted on the 2, 7 or/and 9 position(s) (structure on supporting information)[5]. Their *in vitro* growth inhibitory activity (determination of the IC_{50} concentration) was evaluated on three glioma (U373, T98G, Hs683) and two oesophageal cancer cell lines (OE21, OE33) from human origin. This work highlighted that the 2,7,9-trisubstituted compounds were the most potent derivatives with some of them reaching a submicromolar antiproliferative activity (Figure 1)[5]. Moreover, the growth inhibitory potency of the best compounds was in the same range, both on cells resistant or not to apoptotic stimuli[6]. Quantitative videomicroscopy have shown a cytostatic behaviour on U373 glioma cell line[5].

To elucidate the mechanism of action of the **2a-c** antiproliferative molecules, anti-cancer profiles on the 60 cancer cell line panel of the National Cancer Institute (Bethesda, USA) were evaluated and compared to those of the 763,000 compounds of the NCI database[7]. The highest correlation was found with protein synthesis inhibitors, an hypothesis that was further confirmed by western blot on the eukaryotic initiation factor 2 (eiF2α) implicated in protein synthesis[5,8].

[Figure 1]

Despite a strong antiproliferative activity *in vitro*, these trisubstituted compounds possess a poor solubility at physiological pH, which is detrimental for their bioavailability and their development as drug[9]. In order to obtain novel druggable harmine-based antiproliferative molecules, and following the concept of early-ADME which leads to improve both biological and ADME properties, the goal of this work was to increase the solubility of the β-carboline derivatives preserving their antiproliferative activity on cancer cells[10]. On one hand, new β-carbolines were synthesized bearing polar substituents in order to *a priori* improve their solubility. On the other hand, a Comparative Molecular Field Analysis (CoMFA) model was generated to guide the synthesis of new molecules. The physicochemical properties (solubility, lipophilicity) and the antiproliferative activity (MTT colorimetric test, quantitative videomicroscopy) have been investigated for each newly synthesized compound. Finally, to mimic oral absorption and brain uptake which is required for glioma treatment, human intestinal absorption and blood brain barrier penetration have been calculated.

2. Results

2.1. Chemistry

Targeted 2,7,9-trisubstituted compounds (**4f-h** and **5c-j**) and intermediates (Table 1) discussed in this work were synthesized following the strategy depicted on Scheme 1[5]. Starting from **harmol**, the 7-desmethyl **harmine**, two routes have been adopted to obtain 7,9-disubstituted derivatives. First pathway, used when R7 is different from R9, is a two-step procedure involving O_7-alkylation of **harmol** by an alkyl-, a branched alkyl- or benzyl bromide in presence of cesium carbonate in DMF (**2j, 3**)

(a). Then, the O_7-substituted compound reacts with iodopropane and sodium hydride under argon in DMF to give the 7,9-disubstituted compounds **2w** and **5a** *(b).* A different strategy was used to obtain **4a.** In this case, **3** reacts with acrylonitrile and Triton B to add the N_9-propionitrile substituent *(c)*[11]. In the second pathway, used when R7 is identical to R9, **4b** and **5b** were synthesized by a simultaneous O_7- and N_9- alkylation of **harmol** using the required alkylbromide and potassium hydroxide in DMF *(d)*. 2,7,9-trisubstituted compounds **5c-j** were obtained by a N_2-alkylation of the corresponding 7,9-disubstituted compound in presence of the required alkylbromide in THF *(e)*. The nitrile derivative **4e**, obtained by the pathway *(e)*, was converted into the corresponding tetrazole (**4f**) by using sodium azide and ammonium chloride in DMF *(f)*[11]. Hydroxyl alkyl substituents, benzyl ethers **4c** and **4d**, obtained following *(e)* pathway, were reduced by Pd-catalysed hydrogenation using Pd/C affording **4g** and **4h** respectively *(g)*[12]. These last two reactions led to targeted trisubstituted compounds bearing charged (**4f**) or neutral (**4g-h**) polar substituent(s) unlike targeted compounds **5c-j** which only bear apolar substituents. The structure and purity of each final synthesized compound were analysed by ^{1}H-NMR, ^{13}C-NMR, LC, MS and elemental microanalyses.

[Scheme 1]

2.2. Physicochemical characterization

Experimental aqueous solubility at pH 7.4 and theoretical lipophilicity (Advanced Chemistry Development, Inc., ACD/Lab)[13] of compounds **4f-h** and **5c-j** were compared with the results obtained for the most active β-carboline of the first generation (**2a-c**) as shown in Table 1. Aryl and cycloalkyl substituents of **2a-c** are

responsible for their high molecular weight (MW>500 g/mol) and lipophilicity (logP>5) over Lipinski's rules[14]. Moreover, the poor solubility of **2a-c** is due to the presence of an aryl, an aryl halide or a cyclohexyl substituent. Addition of hydroxylated substituents (**4g-h**) or small branched/linear alkyl moieties (**5c-j**) strongly improves the solubility at physiological pH which become higher than 200 µg/ml and 160 µg/ml, respectively. Each newly synthesized compound (**4f-h** and **5c-j**) offers, as expected, a lower molecular weight (MW<500 g/mol) and a decreased lipophilicity (logP<5).

[Table 1]

2.3. Biological characterization

a) Determination of the *in vitro* growth inhibitory activity

Determination of *in vitro* growth inhibitory activity was performed on three glioma cell lines (U373, T98G, Hs683), one melanoma (SKMEL-28) and one lung cancer (A549) cell line using a MTT colorimetric assay[15]. Cells were cultured during three days in presence (test) or absence (control) of **4f-h** and **5c-j** compound at concentrations from 10nM to 100µM. For each β-carboline, the concentration required to reduce the global growth by 50% (the IC_{50} concentration) was determined on each cell line (Table 2). Compounds **4f-h** bearing polar substituents (tetrazole or hydroxyalkyl) are inactive (IC_{50}>100µM), independently of the presence of a charge at physiological pH (**4f**) or not (**4g-h**). On the contrary, molecules **5c-j** exhibit an IC_{50} concentration in the micromolar to submicromolar range which makes these compounds more active than harmine (IC_{50}=28µM)[5]. It is interesting to highlight that:

- For the same substituent on the N_2- and N_9-positions, the IC_{50} is 20-times lower when the O_7-propyl is replaced by an isopentyl moiety (**5c** vs **5h** and **5d** vs **5j**).
- Within the O_7-isopentyl derivative series, activity is improved when R2 is also a branched alkyl (**5h-5j**).

These observations underline the important effect of branched substituents of the harmine skeleton on antiproliferative effect.

[Table 2]

b) Cytotoxic and cytostatic effects of compounds on U373, Hs683 and SKMEL-28 cells

As **2a-c** display cytostatic activity on U373 glioma cell line at IC_{50} concentration[5], we examined the antiproliferative activity of the best inhibitors (**5f**, **5h, 5j**) on the same U373 glioma cell line as well as on Hs683 glioma and SKMEL-28 melanoma cell lines using quantitative videomicroscopy (Figure 2) (results on Hs683 and SKMEL-28 in supporting information). Cells were seeded and cultured 72h in the presence (test) or absence (control) of the β-carboline derivative, at a concentration corresponding to their IC_{50} concentration determined by the MTT colorimetric test. A picture of the bottom flask is registered each 4 min during the 72h of observation. The condensation of the 1080 images generated a 40s movie for each experiment operated in triplicate. Results on the three tested cell lines show a morphological cytostatic effect of the compounds **5f, 5h** and **5j** at their IC_{50} concentration, which are 1, 1 and 0.5µM respectively.

[Figure 2]

2.4. Computational chemistry

In order to generate a CoMFA model, 49 structures of β-carbolines (**1**, **2a-as** and **4f-g** compounds) were aligned on a pharmacophore to have alignment principally based on substituents. This alignment was performed in two steps in order to produce the best alignment of β-carboline compounds. The first step aimed to generate a starting pharmacophore with **2a**, **2ae** and **2ap** (see structures in supporting information) as training set with merged features. Based on this first pharmacophore, information about the harmine tricycle and the positive charge were removed in order to generate a bias pharmacophore including only information about the substituents. In the second step, a second pharmacophore was generated based on the bias pharmacophore with **2ac**, **2aq**, **2ae** and **2c** as training set. The final alignment of all compounds, performed with LigandScout 3.03[16], is depicted on Figure 3A. The CoMFA model was generated on Sybyl 8.1 software[17] on 80% of the molecules (training set). These molecules were chosen randomly by Discovery Studio 3.5[18]. The remaining 20% molecules were used as a test set. CoMFA model was elaborated using pIC_{50} activity on Hs683 cell line.

PLS statistics obtained during the development of the model are summarized in Table 3. The cross-validated correlation coefficient (q^2) represents the prediction capacity of the model and the conventional correlation coefficient (r^2) represents the capacity to fit the data with the 3D-QSAR model. The high value of r^2 indicates a good fitting of the derived model[19]. Moreover, the low standard error of estimate

(0.314) and the important value for Fisher test indicate that an important part of the data is explained by the model. Contributions of steric and electrostatic fields are similar with 46.1% and 53.9%, respectively. The experimental *versus* predicted pIC_{50} data are depicted on Figure 4, showing a good prediction of data for training and test sets (data are summarized in supporting information for **1**, **2a-as** and in Table 2 for **4f-h**).

[Figures 3 and 4]

[Table 3]

2.5. Human intestinal Absorption and blood brain barrier penetration calculation

Calculations have been performed with Discovery Studio software, using two developed models based on descriptors such as 2D polar surface area and AlogP98 calculations[18,20]. Models define four prediction levels for the intestinal absorption (from good to very poor) and blood brain barrier (from very high to low penetrants plus an undefined level) after oral administration. These different levels are defined by confidence ellipsoids at 95% and 99%. For human intestinal absorption, compounds depicted inside the ellipsoids are determined as well absorbed compounds (>90% absorbed) while compounds outside ellipsoids are predicted as poorly absorbed compounds (<30% absorbed). For blood brain barrier penetration, the definition is quite different: molecules outside the ellipsoids are undefined, while in the ellipsoids four regions are drawn corresponding to the four levels of BBB penetration. Figure 5 shows that **2a-c** compounds are predicted as very poorly absorbed by human intestines (A) and are undefined with respect to their blood brain penetration (B), while compounds **5c-j** are characterized by a good intestinal

absorption (A) and very high brain penetration (B), therefore making these compounds more druggable.

[Figure 5]

3. Discussion and conclusions

In order to improve the solubility of 2,7,9-trisubstituted harmine derivatives with antiproliferative activity and to expand the range of studied substituents, compounds bearing polar substituents (**4f-h**) have been synthesized and their physicochemical properties (Table 1) and antiproliferative efficacy (Table 2) were evaluated. Results indicate that polar substituents on β-carboline template increase solubility (> 200µg/ml) at physiological pH, as expected, but unfortunately, strongly reduce their antiproliferative activity with a mean IC_{50} higher than 100µM. Moreover, compared to the growth inhibitory activity of harmine (IC_{50}=28µM)[5], the IC_{50} value of **4f-h** show that polar substituents are not favourable for antiproliferative activity of trisubstituted β-carbolines.

As an alternative, a CoMFA model has been generated based on **1**, **2a-as** and **4f-h** biological activity in order to suggest molecules combining antiproliferative activity in the micromolar range and higher solubility than compounds **2a-c**. This model gives a good prediction of pIC_{50} values compared to experimental pIC_{50} values on Hs683 glioma cell line (Figure 4) and highlights steric and electrostatic contributions essential for antiproliferative activity. In order to visualize CoMFA model, contour maps were generated around compound **2a** (Figure 3B). Several conclusions can be

drawn based on these maps; firstly, the nature of O_7-substituent has little influence on the biological activity; secondly, N_9- and N_2-substituents must be sterically small; thirdly, the positive charge due to the presence of the third substituent is favourable to biological activity.

Based on these observations and in order to decrease the lipophilicity of initial molecules, diverse small substituents including propyl, isopentyl and benzyl were linked to the harmine ring (**5c-j**). Combination of these substituents leads to 8 new molecules whose calculated lipophilicity fits with Lipinski's rules (logP<3.4) (Table 1). Moreover, their biological activity on Hs683 glioma cell predicted by the CoMFA model suggest that those molecule present an antiproliferative effect in the micromolar range (Table 2). Among these 8 molecules, three of them, bearing an ethyl substituents on R2 position (**5c, 5e, 5h**) (not included in the training set) are predicted as less active than the others compounds. However, as these three molecules present a lower molecular weight and calculated lipophilicity compared to initial compounds, these molecules have been synthesized and included in our study. Physicochemical characterization underlined a higher solubility of these compounds **5c-j** compared to **2a-c**. Because the reference molecules (**2a-c**) and targeted molecules **5c-j** have the same β-carboline skeleton, we can relate the best solubility and lipophilicity to the decreased number of carbon atoms substituting the molecules, which make these compounds more "druggable" than **2a-c**. Biological characterization highlighted that these soluble compounds offer an antiproliferative activity in the micromolar to submicromolar range on five cancerous cell lines. **5f, 5h** and **5j** are the best antiproliferative compounds. Moreover, the experimental antiproliferative activity of compounds bearing an ethyl moiety (**5c, 5e** and **5h**) present a submicromolar IC_{50} on Hs683 glioma cells, despite a poor CoMFA

prediction (Table 2). This conclusion highlights the underestimated inhibitory potential of the ethyl substituent (not part of training set) by the generated model.

As the first generation of harmine substituted compounds (**2a-c**) has a cytostatic profile on U373 glioma cell line, quantitative videomicroscopy experiment was performed on the new molecules, highlighting a similar cytostatic behaviour of these compounds on three cancerous cell lines including two glioma cell lines.

With the aim to develop novel antiproliferative β-carboline which are orally bioavailable and focusing particularly on glioma treatment, human intestinal absorption and blood brain barrier penetration of newly synthesized compounds were calculated with Discovery Studio 3.5 software[18]. Results show better intestinal absorption and blood-brain barrier penetration for **5c-j** compounds compared to **2a-c**. Figure 5 shows that the different features of compounds **2a-c** and **5c-j** are due to their difference in lipophilicity rather than their 2D polar surface area, which is the same for these molecules characterized by the same skeleton bearing apolar substituents. Compounds **2a-c**, with aromatic substituents, are too lipophilic (logP>5) which is not propitious for a good oral bioavailability, as opposed to **5c-j** compounds substituted by small alkyl groups, which have a lower lipophilicity (logP<3.4) and are predicted to be very well absorbed.

In conclusion, in the continuation of our previous results showing that trisubstituted β-carbolines **2a-c** present an antiproliferative activity but a low solubility at pH 7.4 and using an early-ADME concept, new molecules combining antiproliferative activity and an improved solubility at pH 7.4 have been synthesized. After synthesis of these new harmine derivatives bearing polar substituents, a CoMFA model was generated with the β-carboline derivatives aligned on substituents. The good statistical values obtained for this CoMFA model (q^2=0.397, r^2=0.872) has guided synthesis to

sterically small substituents on R2 and R9 positions to increase biological activity. Based on this model, we thus synthesized eight new molecules (**5c-j**) with a good predicted antiproliferative activity and a decreased lipophilicity compared to the compounds of the first generation **2a-c**. Synthesis, physicochemical and biological characterization leads to high soluble original compounds with increased antiproliferative activity for **5f**, **5h** and **5j** compounds. Our results are in agreement with those recently presented by Cao and co-workers who have also presented good antiproliferative activity for trisubstituted harmine derivatives[21]. Quantitative videomicroscopy underlines a cytostatic effect of these three molecules on U373, Hs683 and SKMEL-28 cancer cell lines. Moreover, human intestinal absorption and blood brain barrier penetration has been calculated for **5c-j**, compared with initial compounds (**2a-c**) and suggests better druggability of the newly synthesized molecules mainly due to their decreased lipophilicity.

4. Experimental section

Chemistry

Melting points were measured on a Buchi Melting Point B540 apparatus in open capillaries with uncorrected values. All NMR (^{1}H and ^{13}C) were recorded on Jeol spectrometer (JNM EX-400) at 25°C. Chemical shifts are reported in parts per million (ppm) using the solvent residual peak as reference (DMSO-d_6: δ_H: 2.50 ppm, δ_C: 39.52 ppm; CD_3OD: δ_H: 3.31 ppm, δ_C: 49.00 ppm). Coupling constants (J) are reported in Hertz (Hz). The resonance multiplicity is described as s for singlet, d for doublet, t for triplet, q for quadruplet and m for multiplet. LC-MS analyses was realized on an Agilent 1100 series HPLC coupled with an MSD Trap SL system, using detection at 254nm equipped wih an Agilent Zorbax Eclipse XDB C8 4.6mm x

150mm, 5µm separation column in a 70:30 solution of 0.01M sodium 1-butanesulfonate in methanol/0.01M sodium 1-butanesulfonate in water as eluent. Mass determination was realized using electron spray ionization (ESI) in positive mode. High resolution mass spectrometry (HRMS) were obtained from ESI-Q-TOF, maXis Impact (Bruker). Elemental analyses were determined on a FlashEA 1112 series organic elemental analyzer. Thin-layer chromatography was realized on silica gel plates (silica gel GF254, VWR) visualized at 254nm. Column chromatography was realized in a BiotageSP1 25+M column equipped with UV spectrophotometer with a detector (wavelengths of 254 nm and 320 nm). Solvents from Biosolve were used without further purification. Microwave irradiation was realized on a Biotage initiator 2.0 apparatus in 20ml sealed tube. All new compounds were determined to be >95% pure by LC-MS.

General procedure for simultaneous O-7 and N-9 alkylation: The synthesis was performed as described elsewhere[5]

General procedure for microwave assisted synthesis of N-2 alkylation: The synthesis was performed as described elsewhere[5]

General procedure O-7 alkylation: The synthesis was performed as described elsewhere[22]

General procedure for N-9 alkylation: The 1-methyl-O7-substituted-7-hydroxy-β-carboline (1.25g) was dissolved in DMF. An amount of 1.5equiv of iodopropane and 5 equiv. of sodium hydride were added. The mixture was stirred o/n under Argon. The reaction was followed by TLC (85:15 dichloromethane: ethanol). At the end of the reaction, the mixture was extracted using dichloromethane. The organic layer was washed with water and brine and dried with $MgSO_4$, which was collected by

filtration. The organic layer was evaporated under vacuum and the crude product was purified via column chromatography with dichloromethane/ethanol (100:0 to 87:13) used as the eluent.

General procedure for simultaneous O-7 and N-9 alkylation (A): The synthesis was performed as described elsewhere[5] Briefly, harmol (1.50g, 5mmol) was dissolved in DMF (30ml). An amount of 5 equiv of potassium hydroxide was added and the mixture was stirred for 30 min under argon. Then, 2 equiv of alkyl bromide was added. The mixture was stirred at room temperature overnight under argon. The reaction was followed by TLC (85:15 dichloromethane: ethanol). At the end of the reaction, the mixture was extracted using dichloromethane. The organic layer was washed with water and brine and dried with $MgSO_4$, which was collected by filtration. The organic layer was evaporated under vacuum and the crude product was purified via column chromatography with dichloromethane/ethanol (100:0 to 70:30) used as the eluent.

General procedure O-7 alkylation (B): The synthesis was performed as described elsewhere[22]. Briefly, harmol (1.5g, 5mmol) was dissolved in DMF (30ml). An amount of 1.5 equiv of alkyl bromide and 3 equiv of cesium carbonate were added. The mixture was stirred during 24 hours at room temperature, under argon. The reaction was followed by TLC (85:15 dichloromethane: ethanol). At the end of the reaction, the mixture was extracted using dichloromethane. The organic layer was washed with water and brine and dried with $MgSO_4$, which was collected by filtration. The organic layer was evaporated under vacuum and the crude product was purified via column chromatography with dichloromethane/ethanol (100:0 to 80:20) used as the eluent.

General procedure for N-9 alkylation (C): The 1-methyl-O7-substituted-7-hydroxy-β-carboline (1.25g) was dissolved in DMF. An amount of 1.5equiv of iodopropane and 5 equiv. of sodium hydride were added. The mixture was stirred overnight under Argon. The reaction was followed by TLC (85:15 dichloromethane: ethanol). At the end of the reaction, the mixture was extracted using dichloromethane. The organic layer was washed with water and brine and dried with MgSO4, which was collected by filtration. The organic layer was evaporated under vacuum and the crude product was purified via column chromatography with dichloromethane/ethanol (100:0 to 87:13) used as the eluent.

General procedure for microwave assisted synthesis of N-2 alkylation (D): The synthesis was performed as described elsewhere[5]. Briefly, 1-methyl-O7-substituted-N9-substituted-7-hydroxy-β-carboline (0.300g) was dissolved in THF (10ml). An amount of 10 equiv of alkyl bromide was added. The mixture was placed on a microwave reactor at 140℃ during 4 hours. The reaction was followed by TLC (85:15 dichloromethane: ethanol). At the end of the reaction, precipitate was filtrate without any purification or the mixture was extracted using dichloromethane. The organic layer was washed with water and brine and dried with $MgSO_4$, which was collected by filtration. The organic layer was evaporated under vacuum and the crude product was purified via column chromatography with dichloromethane: ethanol (100:0 to 70:30) used as the eluent.

Synthesis. Synthesis of compounds 2a-2as has been previously reported [5,22].

7-(isopentyloxy)-1-methyl-β-carboline, 3:

The title compound was synthesized according to the general procedure B from 1-methyl-7-hydroxy-β-carboline (harmol) (1.98g, 7.4mmol) in the presence of cesium

carbonate (7.21g, 22.2mmol), 1-bromo-3-methylbutane (1.4ml, 11.1mmol) in 50ml of DMF. A yellow solid was obtained. Yield 65%, Mp 235-237°C, MS: $[M+H]^+$ 269.0, Rf=0.4 (85:15 dichloromethane: ethanol), ^{1}H NMR (DMSO-d_6) δ : 0.92 (d, J=6.64Hz, 6H, CH-*(CH$_3$)$_2$*), 1.64 (m, 2H, *CH$_2$*-CH-(CH$_3$)$_2$), 1.75-1.85 (m, 1H, *CH*-(CH$_3$)$_2$), 2.67 (s, 3H, CH$_3$), 4.06 (t, J=6.64Hz, 2H, O-CH$_2$), 6.79 (dd, J_{6-8}=2.06Hz, J_{5-6}=8.59Hz, 1H, H-6), 6.96 (d, J_{6-8}=2.06Hz, 1H, H-8), 7.75 (d, J_{3-4}=5.27Hz, 1H, H-4), 7.99 (d, J_{5-6}=8.47Hz, 1H, H-5), 8.10 (d, J_{3-4}=5.27Hz, 1H, H-3), 11.35 (bs, 1H, NH), ^{13}C NMR (DMSO-d_6) δ : 20.86, 23.02, 25.18, 37.98, 66.64, 95.76, 109.94, 112.43, 115.39, 123.12, 127.74, 135.05, 138.25, 141.761, 142.45, 159.96, Anal. Calcd for $C_{17}H_{20}N_2O$: C, 76.09%; H, 7.51%; N, 10.44%. Found: C, 76.08%; H, 7.56%; N, 10.17%.

7-(isopentyloxy)-1-methyl-9-propionitrile-β-carboline, 4a:

The title compound was synthesized according a method describe elsewhere[11]. **3** (1.289g, 4.5mmol) and acrylonitrile (3.0ml, 45mmol) were stirred. Addition dropwise of TritonB (0.28ml, 1.54mmol) leads to a red solution followed by TLC (85:15 dichloromethane: ethanol). After completion of the reaction, the mixture was extracted with dichloromethane (100ml) and organic layer was washed with water and brine. The organic layer was evaporated under vacuum and the crude product was used without any purification. An orange solid was obtained. Yield quant, Mp: 166-167°C, MS: $[M+H]^+$ 322.0, Rf=0.8 (85:15 dichloromethane: ethanol), ^{1}H NMR (Acetone-d_6) δ : 0.97 (d, J=6.64Hz, 6H, CH-*(CH$_3$)$_2$*), 1.68-1.74 (m, 2H, O-CH$_2$-*CH$_2$*), 1.82-1.92 (m, 1H, *CH*-(CH$_3$)$_2$), 3.01(s, 3H, CH$_3$), 3.10 (t, J=6.87Hz, 2H, N-CH$_2$-*CH$_2$*), 4.18 (t, J=6.64Hz, 2H, O-CH$_2$), 5.02 (t, J=6.87Hz, 2H, N-*CH$_2$*-CH$_2$), 6.90 (dd, J_{5-6}=8.59Hz, J_{6-8}=2.06Hz, 1H, H-6), 7.33 (d, J_{6-8}=2.06Hz, 1H, H-8), 7.81 (d, J_{3-4}=5.04Hz, 1H, H-4), 8.04 (d, J_{5-6}=8.04Hz, 1H, H-5), 8.20 (d, J_{3-4}=5.27Hz, 1H, H-3), ^{13}C NMR

(Acetone-d_6) δ : 18.07, 22.06, 23.05, 25.03, 38.01, 40.17, 66.64, 94.25, 110.36, 112.02, 115.13, 117.80, 122.24, 129.42, 134.92, 138.81, 140.84, 142.64, 160.78.

7-(2-benzyloxyethoxy)-9-(2-benzyloxyethyl)-1-methyl-β-carboline, 4b:

The title compound was synthesized according to the general procedure A from harmol (1.98g, 7.4mmol) in the presence of benzyl-2-bromoethylether (2.4ml, 14.8mmol) and potassium hydroxide (2.10g, 37mmol) in 40ml of DMF. A white solid was obtained after crystallization in EtOH. Yield 52%, Mp 82-83°C, MS: $[M+H]^+$ 467.0, Rf=0.9 (85:15 dichloromethane: ethanol), ^{1}H NMR (DMSO- d_6) δ : 2.92 (s, 3H, CH_3), 3.75-3.81 (m, 4H, N-CH_2-CH_2 + O-CH_2-CH_2), 4.20 (t, J=5.04Hz, 2H, O-CH_2), 4.32 (s, 2H, CH_2-Ar), 4.54 (s, 2H, CH_2-Ar), 4.75 (t, J=5.27Hz, 2H, N-CH_2), 6.86 (dd, J_{5-6}=8.59Hz, J_{6-8}=2.06Hz, 1H, H-6), 6.99-7.01 (m, 2H, Ar-H), 7.12-7.37 (m, 9H, Ar-H, Ar-H, H-8), 7.86 (d, J_{3-4}=5.27Hz, 1H, H-4), 8.05 (d, J_{5-6}=8.47Hz, 1H, H-5), 8.14 (d, J_{3-4}=5.04Hz, 1H, H-3), ^{13}C NMR (DMSO- d_6) δ : 23.85, 44.76, 68.08, 68.77, 69.42, 72.60, 72.68, 95.38, 110.27, 112.81, 114.89, 122.86, 127.45, 127.78, 127.99, 128.07, 128.59, 128.79, 129.04, 135.65, 137.88, 138.61, 138.86, 141.32, 143.54, 160.16.

7-(2-benzyloxyethoxy)-9-(2-benzyloxyethyl)-2-isopentyl-1-methyl-β-carbolin-2-ium bromide, 4c:

The title compound was synthesized according to the general procedure D from **4b** (0.307g, 0.6mmol) in the presence of 1-bromo-3-methylpropane (0.78ml, 6.4mmol) in 15ml of THF. A grey solid was obtained after crystallization in EtOH. Yield 33%, Mp 163-164°C, MS: $[M]^+$ 537.1, Rf=0.4 (85:15 dichloromethane: ethanol) , ^{1}H NMR (DMSO- d_6) δ : 0.93 (d, J=6.41Hz, 6H, CH-$(CH_3)_2$), 1.57-1.63 (m, 2H, N-CH_2-CH_2),

1.65-1.72 (m, 1H, *CH*-$(CH_3)_2$), 3.16 (s, 3H, CH_3), 3.80-3.85 (m, 4H, N-CH_2-*CH_2* + O-CH_2-*CH_2*), 4.28-4.32 (m, 4H, O-CH_2 + *CH_2*-Ar), 4.56 (s, 2H, *CH_2*-Ar), 4.63 (t, J=6.18Hz, 2H, N-CH_2), 4.95 (t, J=4.81Hz, 2H, N-CH_2), 6.88-6.90 (m, 2H, Ar-H), 7.07-7.14 (m, 4H, Ar-H), 7.25-7.33 (m, 5H, Ar-H), 7.43 (s, 1H, Ar-H), 8.93 (d, J=8.93Hz, 1H, Ar-H), 8.52 (d, 1H, J_{3-4}=6.41Hz, 1H, H-4), 8.62 (d, 1H, J_{3-4}=6.41Hz, 1H, H-3), ^{13}C NMR (DMSO- *d_6*) δ :16.74, 22.71, 26.04, 45.83, 56.29, 68.54, 69.06, 72.67, 72.71, 95.67, 113.34, 113.77, 114.87, 124.93, 127.47, 127.90, 128.04, 128.08, 128.60, 128.82, 113.12, 135.36, 136.09, 138.36, 138.78, 139.99, 147.73, 162.94.

7-(2-benzyloxyethoxy)-2,9-bis(2-benzyloxyethyl)-1-methyl-β-carbolin-2-ium bromide, 4d:

The title compound was synthesized according to the general procedure D from **4b** (0.394g, 0.84mmol) in the presence of benzyl-2-bromoethylether (2.0ml, 12.7mmol) in 15ml of THF. A green solid was obtained. Yield 54%, Mp 149-150℃, MS: $[M]^+$ 601.0, Rf=0.3 (85:15 dichloromethane: ethanol), ^{1}H NMR (DMSO- *d_6*) δ : 3.18 (s, 3H, CH_3), 3.81-3.87 (m, 6H, O-CH_2-*CH_2* + N-CH_2-*CH_2* + N-CH_2-*CH_2*), 4.30-4.34 (m, 4H, O-*CH_2* + *CH_2*-Ar) , 4.40 (s, 2H, *CH_2*-Ar), 4.55(s, 2H, *CH_2*-Ar), 4.92 (m, 4H, N-*CH_2* +N-*CH_2*), 6.91 (m, 2H, Ar-H), 7.10-7.17 (m, 9H, Ar-H), 7.25-7.33 (m, 5H, Ar-H), 7.41, (m, 1H, Ar-H), 8.32 (d, J=8.70Hz, 1H, Ar-H), 8.49 (d, J_{3-4}=6.41Hz, 1H, H-4), 8.55 (d, J_{3-4}=6.41Hz, 1H, H-4), ^{13}C NMR (DMSO- *d_6*) δ : 17.14, 45.87, 56.99, 68.53, 68.69, 69.02, 72.54, 72.64, 72.67, 95.66, 113.31, 113.81, 114.24, 124.95, 127.50, 127.80, 127.88, 128.03, 128.09, 128.60, 128.72, 128.81, 133.36, 135.77, 136.17, 138.20, 138.33, 138.79, 140.60, 147.94, 162.99.

2-isopentyl-7-(isopentyloxy)-1-methyl-9-propionitrile-β-carbolin-2-ium bromide, 4e:

The title compound was synthesized according to the general procedure D from **4a** (0.282g, 0.9mmol) in the presence of 1-bromo-3-methylpropane (1.7ml, 14mmol) in 15ml of THF. A yellow solid was obtained after crystallization in EtOH. Yield 15%, Mp 199-201°C, MS: $[M]^+$ 392.1, Rf=0.2 (85:15 dichloromethane: ethanol), ^{1}H NMR (DMSO- d_6) δ : 0.93-0.97 (m, 12H, CH-$(CH_3)_2$+ CH-$(CH_3)_2$), 1.66-1.74 (m, 5H, CH_2-CH-$(CH_3)_2$+ CH_2-CH-$(CH_3)_2$), 1.73-1.85 (m, 1H, CH_2-CH-$(CH_3)_2$), 3.14 (t, J=6.41Hz, 2H, CH_2-CN), 4.20 (t, J=6.64Hz, 2H, O-CH_2), 4.69 (t, J=7.10Hz, 2H, N-CH_2), 5.07 (t, J=6.64Hz, 2H, N-CH_2), 7.08 (dd, J_{6-8}=1.83Hz, J_{5-6}=8.93Hz, 1H, H-6), 7.55 (d, J_{6-8}=1.60Hz, 1H, H-8), 8.32 (d, J_{5-6}=8.93Hz, 1H, H-5), 8.53 (d, J_{3-4}=6.41Hz, 1H, H-4), 8.67 (d, J_{3-4}=6.64Hz, 1H, H-3), ^{13}C NMR (DMSO- d_6) δ : 16.52, 18.69, 22.74, 22.98, 25.15, 26.02, 29.56, 37.84, 41.06, 56.30, 67.50, 95.41, 113.14, 114.19, 114.87, 119.12, 124.96, 133.62, 135.11, 135.97, 139.92, 147.39, 163.41.

9-(2-[1*H*-tetrazol-5-yl]ethyl)-2-isopentyl-7-(isopentyloxy)-1-methyl-β-carbolin-2-ium bromide, 4f:

The title compound was synthesized according a procedure describe elsewhere[11]. **4e** (0.349g, 0.74mmol) in the presence of sodium azide (0.217g, 3.3mmol) and ammonium chloride (0.155g, 2.9mmol) in 4ml of DMF was refluxed 12h. The reaction was followed by TLC (85:15 dichloromethane: ethanol). After completion of the reaction, the mixture was extracted with dichloromethane and organic layer was washed with water and brine and dried with $MgSO_4$. The organic layer was evaporated under vacuum and the crude product was purified via column chromatography with dichloromethane/ethanol (100:0 to 50:50) used as the eluent. A

white solid was obtained after crystallization in EtOH. Yield 19%, HRMS Calcd for $C_{25}H_{35}N_6O^+$: 435.2867 $[M]^+$. Found: 435.2859, Rf=0.4 (CH_2Cl_2/ethanol 50/50), ^1H NMR (DMSO- *d_6*) δ : 0.95-0.97 (m, 12H, CH-*$(CH_3)_2$*+ CH-*$(CH_3)_2$*), 1.64-1.74 (m, 5H, *CH_2*-*CH*-$(CH_3)_2$+ *CH_2*-CH-$(CH_3)_2$), 1.75-1.87 (m, 1H, CH_2-*CH*-$(CH_3)_2$), 3.08 (s, 3H, CH_3) 3.19 (t, J=6.87Hz, 2H, *CH_2*-CN), 4.17 (t, J=6.41Hz, 2H, O-*CH_2*) , 4.60 (t, J=7.33Hz, 2H, N-*CH_2*), 4.96 (t, J=7.33Hz, 2H, N-*CH_2*), 7.00 (dd, $J_{6\text{-}8}$=1.83Hz, $J_{5\text{-}6}$=8.82Hz, 1H, H-6), 7.25 (d, $J_{6\text{-}8}$=1.83Hz, 1H, H-8), 8.28 (d, $J_{5\text{-}6}$=8.70Hz, 1H, H-5), 8.47 (d, $J_{3\text{-}4}$=6.41Hz, 1H, H-4), 8.57 (d, $J_{3\text{-}4}$=6.64Hz, 1H, H-3).

7-(2-hydroxyethoxy)-9-(2-hydroxyethyl)-2-isopentyl-1-methyl-β-carbolin-2-ium bromide, 4g:

The title compound was synthesized from **4c** (0.160g, 0.26mmol) in the presence of Pd/C 10% (41.6mg, 0.04mmol) and H_2 (1 bar) in 20ml of ethanol. The reaction was followed by TLC (85:15 dichloromethane: ethanol). After completion of the reaction, the mixture was filtered under celite and was evaporated under vacuum. The crude extract was crystallized with hot ethanol to give the title compound. A yellow solid was obtained after crystallization in EtOH. Yield 96%, Mp 232-234°C, HRMS Calcd for $C_{21}H_{29}N_2O_3^+$: 357.2173 $[M]^+$. Found: 357.2174, Rf=0.1 (85:15 dichloromethane: ethanol), ^1H NMR (DMSO- *d_6*) δ : 0.96 (d, J=5.72Hz, 6H, CH-*$(CH_3)_2$*), 1.65-1.73 (m, 3H, *CH_2*-*CH*-$(CH_3)_2$), 3.23 (s, 3H, CH_3), 3.76-3.83 (m, 4H, *CH_2*-OH + *CH_2*-OH), 4.19 (t, J=4.81Hz, 2H, O-*CH_2*), 4.69 (t, J=7.33Hz, 2H, N-*CH_2*), 4.77 (t, J=4.81Hz, 2H, N-*CH_2*), 4.95 (t, J=5.27Hz, 1H, OH), 5.03 (t, J= 5.27Hz, 1H, OH), 7.05 (dd, $J_{6\text{-}8}$=1.60Hz, $J_{5\text{-}6}$=8.82Hz, 1H, H-6), 7.35 (d, $J_{6\text{-}8}$=1.37Hz, 1H, H-8), 8.31 (d, $J_{5\text{-}6}$=8.93Hz, 1H, H-5), 8.51 (d, $J_{3\text{-}4}$=6.41Hz, 1H, H-4), 8.63 (d, $J_{3\text{-}4}$=6.64Hz, 1H, H-3), ^{13}C NMR (DMSO- *d_6*)

δ : 16.78, 22.73, 26.04, 39.52, 47.94, 56.30, 59.96, 60.33, 70.96, 95.33, 113.20, 113.72, 114.73, 124.85, 132.89, 135.12, 136.06, 139.94, 147.71, 163.15.

7-(2-hydroxyethoxy)-2,9-bis(2-hydroxyethyl)-1-methyl-β-carbolin-2-ium bromide, 4h:

The title compound was synthesized from **4d** (0.242g, 0.37mmol) in the presence of Pd/C 10% (0.02mg, 0.018mmol) and H_2 (1 bar) in 20ml of ethanol. The reaction was followed by TLC (85:15 dichloromethane: ethanol). After completion of the reaction, the mixture was filtered under celite and was evaporated under vacuum. The crude extract was crystallized with hot ethanol to give the title compound. A yellow solid was obtained after crystallization in EtOH. Yield 82%, Mp 192-194°C, MS: $[M]^+$ 331.0, Rf=0.1 (85:15 dichloromethane: ethanol), ^{1}H NMR (DMSO- d_6) δ : 3.25 (s, 3H, CH_3), 3.77-3.86 (m, 6H, CH_2-OH + CH_2-OH + CH_2-OH), 4.19 (t, J=4.58Hz, 2H, O-CH_2), 4.75-4.80 (m, 4H, N-CH_2 + N-CH_2), 4.95 (t, J=5.04Hz, 1H, CH_2-*OH*), 5.04 (bs, 1H, CH_2-*OH*), 5.17 (bs, 1H, CH_2-*OH*), 7.05 (dd, J_{6-8}=1.37Hz, J_{5-6}=8.70Hz, 1H, H-6), 7.53 (d, J_{6-8}=1.37Hz, 1H, H-8), 8.31 (d, J_{5-6}=8.70Hz, 1H, H-5), 8.48-8.53 (m, 2H, H-4, H-3), ^{13}C NMR (DMSO- d_6) δ : 17.14, 47.95, 59.41, 59.96, 60.36, 60.69, 70.94, 95.35, 113.19, 113.68, 114.11, 124.83, 133.02, 135.83, 135.96, 140.59, 147.81, 163.16, Anal. Calcd for $C_{18}H_{23}BrN_2O_4$: C, 52.56%; H, 5.64%; N, 6.81%. Found: C, 52.96%; H, 5.72%; N, 6.40%.

7-(isopentyloxy)-1-methyl-9-propyl-β-carboline, 5a:

The title compound was synthesized according to the general procedure C from **3** (1.265g, 4.7mmol) in the presence of iodopropane (1.198g, 7.05mmmol), sodium hydride (0.56g, 23.5mmol) in 180ml of DMF. A red solid was obtained. Yield 83%, Mp

82-83℃, MS: $[M+H]^+$ 311.1, Rf=0.8 (85:15 dichloromethane: ethanol), ^{1}H NMR (DMSO-d_6) δ : 0.88 (t, J=7.33Hz, 3H, CH_2-CH_3), 0.93 (d, J=6.64Hz, 6H, CH-$(CH_3)_2$), 1.61-1.87 (m, 5H, CH_2- CH-$(CH_3)_2$ + CH_2-CH_3), 2.89 (s, 3H, CH_3), 4.11 (t, J=6.41Hz, 2H, O-CH_2), 4.47 (t, J=7.33Hz, 2H, N-CH_2), 6.81 (dd, J_{5-6}=8.59Hz, J_{6-8}=1.60Hz, 1H, H-6), 7.16 (d, J_{6-8}=1.60Hz, 1H, H-8), 7.82 (d, J_{3-4}=5.04Hz, 1H, H-4), 8.02 (d, J_{5-6}=8.47Hz, 1H, H-5), 8.12 (d, J_{3-4}=5.04Hz, 1H, H-3), ^{13}C NMR (DMSO-d_6) δ : 11.47, 23.05, 23.13, 24.16, 38.10, 25.15, 45.88, 66.88, 94.91, 109.92, 112.72, 114.62, 122.85, 128.93, 135.14, 138.23, 140.99, 143.38, 160.43, Anal. Calcd for $C_{20}H_{26}N_2O$: C, 77.38%; H 8.44%; N, 9.02%. Found: C, 77.27%; H, 8.47%; N, 8.81%.

1-methyl-7-propoxy-9-propyl-β-carboline, 5b:

The title compound was synthesized according to the general procedure A from harmol (1.50g, 4.78mmol) in the presence of bromopropane (0.8ml, 9.55mmol) and potassium hydroxide in 30ml of DMF. A yellow solid was obtained. Yield: 52%, Mp: 73.1-75℃, MS: $[M+H]^+$ 283.2, Rf=0.40 (95:5 dichloromethane: ethanol) , ^{1}H NMR (DMSO- d_6) δ : 0.87 (t, J=7.33Hz, 3H, CH_2-CH_3), 0.99 (t, J=7.33Hz, 3H, CH_2-CH_3), 3.31 (s, 3H, CH_3), 4.04 (t, J=6.64Hz, 2H, O-CH_2-CH_2), 4.46 (t, J=7.56Hz, 2H, O-CH_2-CH_2), 6.82 (dd, J_{6-8}=2.06Hz, J_{5-6}=8.70, 1H, H-6), 7.15 (d, J_{6-8}=1.83Hz, 1H, H-8), 7.83(d, J_{3-4}=5.04Hz, 1H, H-4)), 8.03(d, J_{5-6}=8.47Hz, 1H, H-5), 8.46 (d, J_{3-4}=5.04Hz, 1H, H-3), ^{13}C NMR (DMSO- d_6) δ : 11.07, 11.47, 22.67, 23.57, 24.13, 45.88, 69.93, 94.85, 109.90, 112.74, 122.86, 128.92, 135.13, 138.21, 140.99, 143.37, 160.44.

2-ethyl-1-methyl-7-propoxy-9-propyl-β-carbolin-2-ium bromide, 5c:

The title compound was synthesized according to the general procedure D from **5b** (0.309g, 1.15mmol) in the presence of bromoethane (0.8ml, 10.5mmol) in 10ml of

THF. A white solid was obtained after crystallization in EtOH. Yield 75%, Mp 218℃, MS: $[M]^+$ 311.3, Rf=0.3 (85:15 dichloromethane: ethanol), ^{1}H NMR (CD_3OD) δ : 1.03 (t, J= 7.56Hz, 3H, CH_2-*CH_3*), 1.10 (t, J=7.33Hz, 3H, CH_2-*CH_3*), 1.62 (t, J=7.10, 3H, N-CH_2-*CH_3*), 1.85-2.00 (m, 4H, N-CH_2-*CH_2*+O-CH_2-*CH_2*), 3.24 (s, 3H), 4.15 (t, J=6.64Hz, 2H, O-*CH_2*), 4.64 (t, J=7.79, 2H, N-*CH_2*), 4.75 (q, J=7.10, 2H, N-*CH_2*), 7.05 (dd, J_{6-8}=2.06Hz, J_{5-6}=8.93, 1H, H-6), 7.20 (d, J_{6-8}=2.06Hz, 1H, H-8), 8.93(d, J_{5-6}=8.93Hz, 1H, H-5), 8.33(d, J_{3-4}=6.64Hz, 1H, H-4), 8.46 (d, J_{3-4}=6.64Hz, 1H, H-3), ^{13}C NMR (CD_3OD) δ : 9.56, 9.92, 14.84, 15.04, 22.27, 23.63, 46.80, 52.80, 70.13, 93.70, 112.90, 113.51, 114.09, 123.84, 133.69, 133.92, 135.09, 138.42, 147.99, 163.88, Anal. Calcd for $C_{17}H_{20}BrN_2O$: C, 61.38%; H, 6.95%; N, 7.16%. Found: C, 61.07%; H, 7.19%; N,7.32%.

2-isopentyl-1-methyl-7-propoxy-9-propyl-β-carbolin-2-ium bromide, 5d:

The title compound was synthesized according to the general procedure D from **5b** (0.513g, 1.8mmol) in the presence of 1-bromo-3-methylbutane (2.2ml, 18.3mmol) in 17ml of THF. A yellow solid was obtained after crystallization in EtOH. Yield 13%, Mp 227-228℃, MS: $[M]^+$ 353.4, Rf=0.3 (85:15 dichloromethane: ethanol), ^{1}H NMR (CD_3OD) δ : 1.00-1.16 (m, 12H, CH-*$(CH_3)_2$*+CH_2-*CH_3*+ CH_2-*CH_3*), 1.80-1.99 (m, 7H, *CH_2*-*CH*-$(CH_3)_2$+ *CH_2*-CH_3+ *CH_2*-CH_3), 3.23 (s, 3H, CH_3), 4.15 (t, J=6.41Hz, 2H, O-CH_2), 4.60-4.73 (m, 4H, N-CH_2, N-CH_2), 7.05 (dd, J_{5-6}=8.70Hz, J_{6-8}=2.06Hz, 1H, H-6), 7.20 (d, J_{6-8}=2.06Hz, 1H, H-8), 8.21 (d, J_{5-6}=8.93Hz, 1H, H-5), 8.33 (d, J_{3-4}=6.41Hz, 1H, H-4), 8.47 (d, J_{3-4}=6.64Hz, 1H, H-3), ^{13}C NMR (CD_3OD) δ : 9.56, 9.92, 21.33, 22.26, 23.60, 26.10, 39.35, 46.79, 56.18, 70.14, 93.70, 112.90, 113.53, 113.96, 123.85, 133.66, 134.32, 135.15, 138.26, 148.02, 163.91, Anal. Calcd for

$C_{23}H_{33}BrN_2O$: C, 63.74%; H, 7.67%; N, 6.46%. Found: C, 64.09%; H, 7.85%; N, 6.66%.

7-(benzyloxy)-2-ethyl-1-methyl-9-propyl-β-carbolin-2-ium bromide, 5e:

The title compound was synthesized according to the general procedure D from **2w** (0.305g, 0.9mmol) in the presence of bromoethane (0.7ml, 9mmol) in 15ml of THF. A yellow solid was obtained. Yield 85%, Mp 217℃, MS: $[M]^+$ 359.2, Rf=0.2 (85:15 dichloromethane: ethanol), ^{1}H NMR (CD_3OD) δ : 0.99 (t, J=7.33Hz, 3H, CH_2-*CH_3*), 1.61 (t, J=7.33Hz, 3H, N-CH_2-*CH_3*), 1.84-1.93 (m, 2H, N-CH_2-*CH_2*), 3.23 (s, 3H, CH_3), 4.62 (t, J=7.79Hz, 2H, N-*CH_2*-CH_2), 4.75 (q, J=7.33Hz, 2H, N-*CH_2*-CH_3), 5.32 (s, 2H, O-*CH_2*), 7.14 (dd, $J_{5\text{-}6}$=8.70Hz, $J_{6\text{-}8}$=2.06Hz, 1H, H-6), 7.28-7.51 (m, 6H, Ar-H + H-8), 8.23 (d, $J_{5\text{-}6}$=8.93Hz, 1H, H-5), 8.35 (d, $J_{3\text{-}4}$=6.64Hz, 1H, H-4), 8.47 (d, $J_{3\text{-}4}$=6.64Hz, 1H, H-3), ^{13}C NMR (CD_3OD) δ : 9.90, 14.99, 23.52, 29.34, 46.83, 52.81, 70.40, 94.55, 113.15, 113.81, 114.24, 123.93, 127.44, 127.89, 128.35, 133.64, 133.90, 135.19, 136.65, 138.46, 147.81, 163.26, Anal. Calcd for $C_{24}H_{27}BrN_2O$: C, 65.60%; H, 6.19%; N, 6.38%. Found: C, 65.94%; H, 6.35%; N, 6.27%.

7-(benzyloxy)-2-isopentyl-1-methyl-9-propyl-β-carbolin-2-ium bromide, 5f:

The title compound was synthesized according to the general procedure D from **2w** (0.399g, 1.2mmol) in the presence of 1-bromo-3-methylbutane (2.2ml, 18mmol) in 15ml of THF. A white solid was obtained. Yield 45%, Mp 222-224℃, MS: $[M]^+$ 401.3, Rf=0.4 (85:15 dichloromethane: ethanol), ^{1}H NMR (CD_3OD) δ : 0.99 (t, J=7.33Hz, 3H, CH_2-*CH_3*), 1.07 (d, J=6.41Hz, 6H, CH-*$(CH_3)_2$*), 1.81-1.93 (m, 5H, *CH_2*-CH_3 + *CH_2*- *CH*-$(CH_3)_2$), 3.22 (s, 3H, CH_3), 4.62 (t, J=7.56Hz, 2H, N-*CH_2*), 4.69 (t, J=7.79Hz, 2H, N-*CH_2*), 5.32 (s, 2H, O-*CH_2*), 7.14 (dd, $J_{5\text{-}6}$=8.93Hz, $J_{6\text{-}8}$=2.06Hz, 1H,

H-6), 7.28-7.52 (m, 6H, Ar-H + H-8), 8.23 (d, J_{5-6}=8.93Hz, 1H, H-5), 8.34 (d, J_{3-4}=6.64Hz, 1H, H-4), 8.46 (d, J_{3-4}=6.64Hz, 1H, H-3), ^{13}C NMR (CD_3OD) δ : 9.90, 14.97, 21.31, 23.54, 26.10, 39.32, 46.85, 56.20, 70.40, 94.56, 113.15, 113.82, 114.07, 123.92, 127.43, 127.88, 128.35, 133.62, 134.32, 135.19, 136.65, 138.52, 147.82, 163.27, Anal. Calcd for $C_{27}H_{33}BrN_2O$: C, 67.35%; H, 6.91%; N, 5.82%. Found: C, 67.10%; H, 6.77%; N, 5.93%.

7-(benzyloxy)-2-isobutyl-1-methyl-9-propyl-β-carbolin-2-ium bromide, 5g:

The title compound was synthesized according to the general procedure D from **2w** (0.41g, 1.2mmol) in the presence of 1-bromo-3-methylpropane (2.0ml, 18mmol) in 15ml of THF. A yellow solid was obtained. Yield 8%, Mp 237℃, MS: $[M]^+$ 387.3, Rf=0.4 (85:15 dichloromethane: ethanol), ^{1}H NMR (CD_3OD) δ : 0.99(t, J=7.56Hz, 3H, CH_2-*CH_3*), 1.03 (d, J=6.64Hz, 6H, CH-*$(CH_3)_2$*), 1.83-1.93 (m, 2H, *CH_2*-CH_3),2.25-2.36 (m, 1H, *CH*-$(CH_3)_2$), 3.23 (s, 3H, CH_3),4.55 (d, J=7.56Hz, 2H, N-CH_2), 4.63 (t, J=7.56Hz, 2H, N-CH_2), 5.32 (s, 2H, O-CH_2), 7.15 (dd, J_{5-6}=8.82Hz, J_{6-8}=2.06Hz, 1H, H-6), 7.28-7.52 (m, 6H, Ar-H + H-8), 8.25 (d, J_{5-6}=8.93Hz, 1H, H-5), 8.34 (d, J_{3-4}=6.64Hz, 1H, H-4), 8.42 (d, J_{3-4}=6.64Hz, 1H, H-3), ^{13}C NMR (CD_3OD) δ : 9.89, 15.21, 18.35, 23.57, 29.54, 46.87, 63.95, 70.41, 94.58, 113.17, 113.50, 113.87, 123.99, 127.44, 127.89, 128.35, 133.69, 135.12, 135.35, 136.64, 138.83, 147.91, 163.35, Anal. Calcd for $C_{26}H_{31}BrN_2O$: C, 66.81%; H, 6.68%; N, 5.99 %. Found: C, 66.61%; H, 6.81%; N, 5.75 %.

2-ethyl-7-(isopentyloxy)-1-methyl-9-propyl-β-carbolin-2-ium bromide, 5h:

The title compound was synthesized according to the general procedure D from **5a** (0.309g, 0.99mmol) in the presence of bromoethane (0.8ml, 10.7mmol) in 15ml of

THF. A yellow solid was obtained after crystallization in EtOH. Yield 42%, Mp 215℃, MS: $[M]^+$ 339.3, Rf=0.2 (85:15 dichloromethane: ethanol), ^{1}H NMR (CD_3OD) δ : 0.98-1.08 (m, 9H, CH-*$(CH_3)_2$* + CH_2-*CH_3*), 1.61 (t, J=7.33Hz, 3H, N-*CH_2*-CH_3), 1.76 (m, 2H, O-CH_2-*CH_2*), 1.85-1.97 (m, 3H, *CH_2*-CH_3 + *CH*-$(CH_3)_2$), 3.24 (s, 3H, CH_3), 4.23 (t, J=6.41Hz, 2H, O-CH_2), 4.66 (t, J=7.79Hz, 2H, N-CH_2), 4.75 (q, J=7.33Hz, 2H, N-CH_2), 7.06 (dd, J_{5-6}=8.70Hz, J_{6-8}=2.06Hz, 1H, H-6), 7.22 (d, J_{6-8}=1.83Hz, 1H, H-8), 8.22 (d, J_{5-6}=8.70Hz, 1H, H-5), 8.34 (d, J_{3-4}=6.64Hz, 1H, H-4), 8.46 (d, J_{3-4}=6.64Hz, 1H, H-3), ^{13}C NMR (CD_3OD) δ : 9.92, 14.81, 15.02, 21.63, 23.63, 24.96, 37.73, 46.80, 52.79, 67.03, 93.71, 112.92, 113.54, 114.09, 123.84, 133.71, 133.90, 135.10, 138.42, 148.03, 163.86, Anal. Calcd for $C_{22}H_{31}BrN_2O$: C, 63.00%; H, 7.45%; N, 6.68%. Found: C, 62.85%; H, 7.38%; N, 6.71%.

7-(isopentyloxy)-1-methyl-2,9-dipropyl-β-carbolin-2-ium bromide, 5i:

The title compound was synthesized according to the general procedure D from **5a** (0.311g, 1.00mmol) in the presence of bromopropane (0.9ml, 10.0mmol) in 15ml of THF. A yellow solid was obtained after crystallization in EtOH. Yield 29%, Mp 206-208℃, MS: $[M]^+$ 353.3, Rf=0.2 (85:15 dichloromethane: ethanol), ^{1}H NMR (CD_3OD) δ : 1.00-1.10 (m, 12H, CH-*$(CH_3)_2$* + CH_2-*CH_3* + CH_2-*CH_3*), 1.73-1.78 (m, 2H, O-CH_2-*CH_2*), 1.83-2.06 (m, 5H, *CH_2*-CH_3+ *CH_2*-CH_3+ *CH*-$(CH_3)_2$), 3.23 (s, 3H, CH_3), 4.23 (t, J=6.64Hz, 2H, O-CH_2), 4.66 (t, J=7.79Hz, 4H, N-CH_2 + N-CH_2), 7.06 (dd, J_{5-6}=8.93Hz, J_{6-8}=2.06Hz, 1H, H-6), 7.22 (d, J_{6-8}=2.06Hz, 1H, H-8), 8.22 (d, J_{5-6}=8.93Hz, 1H, H-5), 8.33 (d, J_{3-4}=6.64Hz, 1H, H-4), 8.46 (d, J_{3-4}=6.64Hz, 1H, H-3), ^{13}C NMR (CD_3OD) δ : 9.66, 9.92, 14.96, 21.64, 23.64, 23.90, 24.96, 37.73, 46.81, 58.76, 67.05, 93.73, 112.92, 113.55, 113.76, 123.87, 133.71, 134.47, 135.14, 138.50,

148.05, 163.88, , Anal. Calcd for $C_{23}H_{33}BrN_2O$: C, 63.74%; H, 7.67%; N, 6.46%. Found: C, 63.37%; H, 7.74%; N, 6.65%.

2-isopentyl-7-(isopentyloxy)-1-methyl-9-propyl-β-carbolin-2-ium bromide, 5j:

The title compound was synthesized according to the general procedure D from **5a** (0.499g, 1.6mmol) in the presence of 1-bromo-3-methylpropane (2.0ml, 16mmol) in 17ml of THF. A yellow solid was obtained after crystallization in EtOH. Yield 18 %, Mp 220-222°C, MS: $[M]^+$ 381.4, Rf=0.3 (85:15 dichloromethane: ethanol) , ^{1}H NMR (CD_3OD) δ : 0.99-1.08 (m, 15H, CH-*$(CH_3)_2$* + CH_2-*CH_3* + CH-*$(CH_3)_2$*), 1.73-1.79 (m, 2H, O-CH_2-*CH_2*), 1.84-1.99 (m, 6H, *CH*-$(CH_3)_2$ + *CH*-$(CH_3)_2$ + N-CH_2-*CH_2* + N-CH_2-*CH_2*), 3.23 (s, 3H, CH_3), 4.23 (t, J=6.41Hz, 2H, O-CH_2), 4.64-4.72 (m, 4H, N-CH_2 + N-CH_2), 7.06 (dd, J_{5-6}=8.70Hz, J_{6-8}=2.06Hz, 1H, H-6), 7.22 (d, J_{6-8}=2.06Hz, 1H, H-8), 8.22 (d, J_{5-6}=8.93Hz, 1H, H-5), 8.33 (d, J_{3-4}=6.41Hz, 1H, H-4), 8.46 (d, J_{3-4}=6.41Hz, 1H, H-3), ^{13}C NMR (CD_3OD) δ : 9.91, 14.96, 21.32, 21.63, 23.62, 24.96, 26.11, 37.73, 39.34, 46.79, 56.16, 67.04, 93.72, 112.92, 113.56, 113.94, 123.85, 133.70, 134.31, 135.15, 138.36, 148.05, 163.90, , Anal. Calcd for $C_{25}H_{37}BrN_2O$: C, 65.07%; H, 8.08%; N, 6.07%. Found: C, 65.12%; H, 8.23%; N, 6.18%.

CoMFA

Data set

In vitro antiproliferative activity against Hs683 glioma cells was chosen to construct a 3D-QSAR model on 49 β-carbolines compounds (**1**, **2a-as** and **4f-h** compounds). The IC_{50} (M) obtained on this cell line was transformed into pIC_{50} ($-\log IC_{50}$). Data set was divided randomly into a training set (80%: 39 compounds) and a test set (20%: 10 compounds) using Discovery Studio 3.5.

Alignment

Alignment was generated using LigandScout 3.03 software. This alignment was elaborated in two steps: first step was to generate a pharmacophore using **2a**, **2ad** and **2ap** as training set, using merged features. Based on this pharmacophore, features from β-carboline tricycle and positive charge were removed in order to give a bias pharmacophore. Based on this bias pharmacophore, the second step was to generate a second pharmacophore using **2c**, **2ac**, **2an** and **2aq** as training set. This combination of steps induces the best alignment for the whole β-carboline discussed in this work.

CoMFA analyse

CoMFA model was generated using Sybyl 8.1 software (TRIPOS Associates Inc.). Charges were calculated by the MMFF94s method in the software. CoMFA calculations were performed using default values. Steric and electrostatics fields were calculated at each grid points using a sp3 carbon atom probe with 1.52Å Van der Waals radius for steric field and a +1.0 charge for electrostatic filed, with an energy cut-off value of 30 kcal/mol.

Partial Least Square (PLS) Analyses

In order to evaluate statistically the 3D-QSAR model, PLS regression was used. A Leave-One-Out method was performed in order to obtain a crossvalidated coefficient correlation and the optimal number of components used in the final analysis. A column filtering of 2.0 kcal/mol was set. The non-crossvalidation analysis was performed and leads to a conventional correlation coefficient, the standard error of estimate and the Fisher-test value which determine the goodness-of-fit.

In vitro growth inhibitory activity

MTT colorimetric assay was performed on different cancerous cells (U373, T98G, Hs683, SKMEL-28, A549, MCF-7) using a protocol described previously[15]. The histological types and origins of the six cancer cell lines that were used for the MTT colorimetric assay are as follows. The cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA), the European Collection of Cell Culture (ECACC, Salisbury, UK) and the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany). The analysed cell lines include the U373 (ECACC code 08061901), Hs683 (ATCC code HTB-138) and T98G (ATCC code CRL-1690) gliomas; the SKMEL-28 melanoma (ATCC code HTB-72); the MCF-7 breast (DSMZ code ACC115) and the A549 non-small-cell lung (NSCLC; DSMZ code ACC107) carcinoma models. All cell lines are from human origin.

Quantitative videomicroscopy was performed on U373, Hs683 and SKMEL28 cancer cells at the IC_{50} determined by the MTT colorimetric test for each compound of interest, using the same protocol as previously[5].

Solubility

Solubility experiment was performed using a protocol described previously[23].

ADME Prediction

Human intestinal absorption and blood brain barrier penetration after oral administration were calculated using Discovery Studio 3.5 software[18]. For human intestinal absorption, a model was developed using 182 compounds in the training set with descriptors such as AlogP98 and 2D polar surface area[20]. Well absorbed compounds (>90% absorbed) are situated in an ellipse of 95% and 99% of confidence. Blood brain barrier model was generated using 102 compounds in a

training set and 86 compounds in a test set. This model was after validated twice using 881 compounds known as CNS compounds from the CMC database and using 124 compounds with known logBB value[18]. Prediction of BBB penetration is divided into 4 levels (very high to low) included onto 95% and 99% confidence ellipse and an undefined level excluded from the confidence ellipses.

Acknowledgement:

This project was funded by the FNRS (Télévie Grant 7.4.529.13.F. and FRSM Grant 4525.11). We are grateful to Marc Dieu from the Plateforme Technologique Spectrométrie de Masse – MaSUN (University of Namur) for the high resolution mass spectrometry analyses.

[1] T.P. Hamsa, G. Kuttan, Harmine inhibits tumour specific neo-vessel formation by regulating VEGF, MMP, TIMP and pro-inflammatory mediators both in vivo and in vitro, Eur. J. Pharmacol. 649 (2010) 64–73. doi:10.1016/j.ejphar.2010.09.010.

[2] F. Dai, Y. Chen, Y. Song, L. Huang, D. Zhai, Y. Dong, L. Lai, T. Zhang, D. Li, X. Pang, M. Liu, Z. Yi, A natural small molecule harmine inhibits angiogenesis and suppresses tumour growth through activation of p53 in endothelial cells, PLoS One. 7 (2012) e52162. doi:10.1371/journal.pone.0052162.

[3] M.-R. Cao, Q. Li, Z.-L. Liu, H.-H. Liu, W. Wang, X.-L. Liao, Y.-L. Pan, J.-W. Jiang, Harmine induces apoptosis in HepG2 cells via mitochondrial signaling pathway, Hepatobiliary Pancreat. Dis. Int. 10 (2011) 599–604. doi:10.1016/S1499-3872(11)60102-1.

[4] Y. Ma, M. Wink, The beta-carboline alkaloid harmine inhibits BCRP and can reverse resistance to the anticancer drugs Mitoxantrone and Camptothecin in breast cancer cells, Phyther. Res. 149 (2010) 146–149. doi:10.1002/ptr.

[5] R. Frédérick, C. Bruyère, C. Vancraeynest, J. Reniers, C. Meinguet, L. Pochet, A. Backlund, B. Masereel, R. Kiss, J. Wouters, Novel trisubstituted harmine derivatives with original in vitro anticancer activity, J. Med. Chem. 55 (2012) 6489–501. doi:10.1021/jm300542e.

[6] L. Ingrassia, F. Lefranc, J. Dewelle, L. Pottier, V. Mathieu, S. Spiegl-Kreinecker, S. Sauvage, M. El Yazidi, M. Dehoux, W. Berger, E. Van Quaquebeke, R. Kiss, Structure-activity relationship analysis of novel derivatives of narciclasine (an Amaryllidaceae isocarbostyril derivative) as potential anticancer agents, J. Med. Chem. 52 (2009) 1100–14. doi:10.1021/jm8013585.

[7] Developmental Therapeutic Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Rockville, MD, US. http://dtp.cancer.gov.

[8] S.R. Kimball, Eukaryotic initiation factor eIF2, Int. J. Biochem. Cell Biol. 31 (1999) 25–9.

[9] K.T. Savjani, A.K. Gajjar, J.K. Savjani, Drug solubility: importance and enhancement techniques, ISRN Pharm. 2012 (2012) 195727. doi:10.5402/2012/195727.

[10] C.A. Lipinski, Drug-like properties and the causes of poor solubility and poor permeability, J. Pharmacol. Toxicol. Methods. 44 (2000) 235–49.

[11] A. Rajasekaran, P.P. Thampi, CM21: Synthesis and antinociceptive activity of some substituted-5-[2-(1,2,3,4-tetrahydrocarbazol-9-yl)ethyl]tetrazol-1-ylalkanones, Eur. J. Med. Chem. 40 (2005) 1359–1364. doi:10.1016/j.ejmech.2005.07.013.

[12] C.H. Heathcock, R. Ratcliffe, A Stereoselective Total Synthesis, 2235 (1971) 1746–1757.

[13] ACD/logPDB, version 12.01, Advanced Chemistry Development, Inc., Toronto, ON, Canada, (2010). www.acdlabs.com.

[14] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug Deliv. Rev. 46 (1997) 3–26.

[15] P. Dumont, L. Ingrassia, S. Rouzeau, F. Ribaucour, S. Thomas, I. Roland, F. Darro, F. Lefranc, R. Kiss, The Amaryllidaceae Isocarbostyril Narciclasine induces apoptosis by activation of the death receptor and/or mitochondrial pathways in cancer cells but not in normal fibroblasts, Neoplasia. 9 (2007) 766–776. doi:10.1593/neo.07535.

[16] G. Wolber, A.A. Dornhofer, T. Langer, Efficient overlay of small organic molecules using 3D pharmacophores, J. Comput. Aided. Mol. Des. 20 (2006) 773–88. doi:10.1007/s10822-006-9078-7.

[17] Sybyl 8.1; Tripos Inc. South Hanley Rd, St-Louis, MO, 63144, U.S.

[18] Accelrys Software Inc., Discovery Studio Modeling Environment, Release 4.0, San Diego: Accelrys Software Inc., (2012). www.accelrys.com.

[19] R.D. Cramer, J.D. Bunce, D.E. Patterson, Crossvalidation, Bootstrapping, and Partial Least Squares Compared with Multiple Regression in Conventional, Quant. Struct. Relatsh. 25 (1988) 18–25.

[20] W.J. Egan, K.M. Merz, J.J. Baldwin, Prediction of drug absorption using multivariate statistics, J. Med. Chem. 43 (2000) 3867–77.

[21] R. Cao, W. Fan, L. Guo, Q. Ma, G. Zhang, J. Li, X. Chen, Z. Ren, L. Qiu, Synthesis and structure-activity relationships of harmine derivatives as potential antitumor agents, Eur. J. Med. Chem. 60 (2013) 135–43. doi:10.1016/j.ejmech.2012.11.045.

[22] J. Reniers, S. Robert, R. Frederick, B. Masereel, S. Vincent, J. Wouters, Synthesis and evaluation of β-carboline derivatives as potential monoamine oxidase inhibitors, Bioorg. Med. Chem. 19 (2011) 134–44. doi:10.1016/j.bmc.2010.11.041.

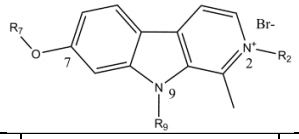
[23] E. Dolusic, P. Larrieu, L. Moineaux, V. Stroobant, L. Pilotte, D. Colau, L. Pochet, V. Den Eynde, B. Masereel, J. Wouters, 3-(2-(Pyridyl)ethenyl) indoles as potential anticancer immunomodulators, J. Med. Chem. 54 (2011) 5320–5334. doi:10.1021/jm2006782.

Table 1: Chemical structure, calculated lipophilicity (clogP), measured solubility (pH 7.4) of compounds **2a-c**, **3, 4a-h** and **5a-j.**

Table 2: *In Vitro* IC_{50} growth inhibitory potency after 3 days culture in presence of compounds **4f-h** or **5c-j** on various cancer cell lines, compared to predicted IC_{50} on Hs683 by CoMFA model.

Table 3: PLS statistics of CoMFA model.

Table 1

Cmpd	Chemical structure 			Calculated LogP	Measured solubility, pH 7.4, at rt (µg/ml)
	R7	R9	R2		
2a	CH_2-C_6H_5	CH_2-C_6H_5	CH_2-C_6H_5	5.27±1.51	72.5±3.8
2b	3'-fluorobenzyl	3'-fluorobenzyl	4'-fluorobenzyl	5.39±1.53	2.4±0.6
2c	CH_2-cyclohexyl	CH_2-cyclohexyl	CH_2-C_6H_5	6.73±1.51	<1.7
3	$(CH_2)_2$-$CH(CH_3)_2$	-	-	ND[a]	ND
4a	$(CH_2)_2$-$CH(CH_3)_2$	$(CH_2)_2$-CN	-	ND	ND
4b	$(CH_2)_2$-O-Bn	$(CH_2)_2$-O-Bn	-	ND	ND
4c	$(CH_2)_2$-O-Bn	$(CH_2)_2$-O-Bn	$(CH_2)_2$-$CH(CH_3)_2$	ND	ND
4d	$(CH_2)_2$-O-Bn	$(CH_2)_2$-O-Bn	$(CH_2)_2$-O-Bn	ND	ND
4e	$(CH_2)_2$-$CH(CH_3)_2$	$(CH_2)_2$-CN	$(CH_2)_2$-$CH(CH_3)_2$	ND	ND
4f	$(CH_2)_2$-$CH(CH_3)_2$	$(CH_2)_2$-tetrazole	$(CH_2)_2$-$CH(CH_3)_2$	1.86±1.54	ND
4g	$(CH_2)_2$-OH	$(CH_2)_2$-OH	$(CH_2)_2$-$CH(CH_3)_2$	-1.11±1.51	>218.7
4h	$(CH_2)_2$-OH	$(CH_2)_2$-OH	$(CH_2)_2$-OH	-2.6±1.52	>205.6
5a	$(CH_2)_2$-$CH(CH_3)_2$	$CH_2CH_2CH_3$	-	ND	ND
5b	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$	-	ND	ND
5c	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$	CH_2CH_3	2.09±1.51	173.8±5.5
5d	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$	$(CH_2)_2$-$CH(CH_3)_2$	2.46±1.51	195.9±5.2
5e	CH_2-C_6H_5	$CH_2CH_2CH_3$	CH_2CH_3	2.36±1.51	207.4±3.5
5f	CH_2-C_6H_5	$CH_2CH_2CH_3$	$(CH_2)_2$-$CH(CH_3)_2$	2.74±1.51	163.7±6.2
5g	CH_2-C_6H_5	$CH_2CH_2CH_3$	CH_2-$CH(CH_3)_2$	2.43±1.51	207.1±4.2
5h	$(CH_2)_2$-$CH(CH_3)_2$	$CH_2CH_2CH_3$	CH_2CH_3	2.95±1.51	186.2±4.6
5i	$(CH_2)_2$-$CH(CH_3)_2$	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$	3.06±1.51	185.1±2.2
5j	$(CH_2)_2$-$CH(CH_3)_2$	$CH_2CH_2CH_3$	CH_2-$CH(CH_3)_2$	3.32±1.51	189.2±13.4

[a]ND: Not determined

Table 2

Cmpd	IC_{50} (μM)						Predicted IC_{50} on Hs683 (μM)	Residuals[b]
	Mean + SEM	Lung cell line	Melanoma cell line	Glioma cell lines				
		A549	SKMEL-28	U373	T98G	Hs683		
4f	>100	>100	>100	ND[a]	ND	>100	43.6[c]	>56.4
4g	>100	>100	>100	>100	ND	>100	50.8[c]	>49.2
4h	>92	51± 6	51± 6	>100	ND	>100	103.0[c]	<-3
5c	13 ± 6	0.7	8	35	22	2	18.6[d]	-16.6
5d	6 ± 4	2	3	22	4	1	3.4[d]	-2.4
5e	8 ± 4	2	4	22	11	3	7.5[d]	-4.5
5f	1.0 ± 0.3	0.3	1	2	1.5	0.3	1.0[d]	-0.7
5g	1.3 ± 0.6	0.8	0.6	1.0	3.8	0.4	1.8[d]	-1.4
5h	0.6 ± 0.2	0.2	1.0	0.6	0.9	0.09	11.1[d]	-11.0
5i	0.6 ± 0.2	0.3	0.9	0.6	1.2	0.04	1.9[d]	-1.7
5j	<0.3	0.1	0.4	0.2	0.7	<0.01	1.6[d]	> -1.6

[a]ND: Not Determined

[b]Residuals define as difference between experimental IC_{50} and predicted IC_{50} on Hs683 cell line

[c]Included in CoMFA model training set

[d]Predicted based on CoMFA model results

Table 3

	q^{2a}	N[b]	r^{2c}	SEE[d]	F[e]	Fraction[f]	
						steric	electrostatic
CoMFA	0.397	3	0.872	0.314	73.220	46.1	53.9

[a]Crossvalidated correlation coefficient
[b]Optimum number of components obtained by PLS analysis and used in the non-validated analysis
[c]Conventional correlation coefficient
[d]Standard error of estimate
[e]Fisher-test value
[f]Contributions field

Figure 1: Structure and measured solubility (pH7.4, rt) for compounds **1**, **2a-c**.

Figure 2: Quantitative videomicroscopy analyses of the compounds **5f**, **5h** and **5j** on U373 glioma cell line. Compounds were tested at their IC50 value which was previously determined by the MTT colorimetric test.

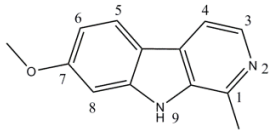
Figure 3: (A) Alignment of compounds **1**, **2a-as** and **4f-h** established with LigandScout 3.03. (B) CoMFA contour maps on **2a**. (B.1.) Steric field: green contour indicates a favourable group and yellow contour indicates an unfavourable group (B.2.) Electrostatic field: Blue contour indicates positively charge and H-donor favourable, red contour indicates an unfavourable positively charged and H-donor group.

Figure 4: Predicted *versus* experimental pIC50 values for training set (●) and test set (□).

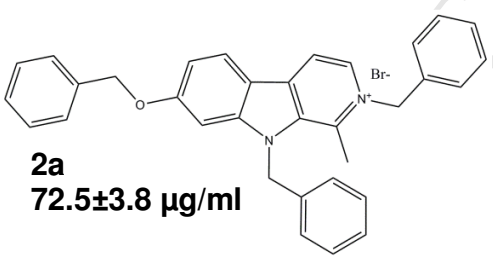
Figure 5: (A) human intestinal absorption and (B) blood brain barrier penetration prediction for compounds **2a-c** (■) and **5c-j** (●). Human intestinal absorption is defined by 99% (mauve line) and 95% (blue line) ellipsoids. Blood brain barrier penetration is defined by 99% (grey line) and 95% (yellow line) ellipsoids.

Scheme 1: Synthesis of β-carbolines derivatives **2j, 2w, 3, 4a-h, 5a-j.**

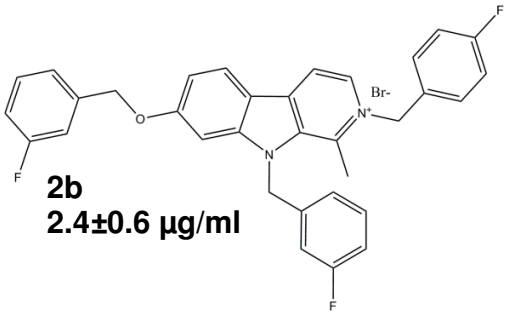
Figure 1



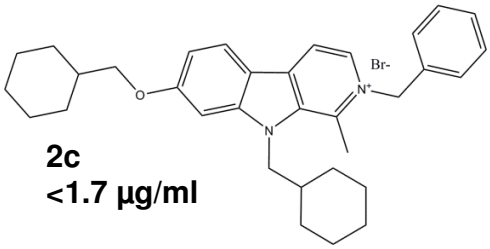
1 (Harmine)



2a
72.5±3.8 µg/ml



2b
2.4±0.6 µg/ml



2c
<1.7 µg/ml

Figure 2

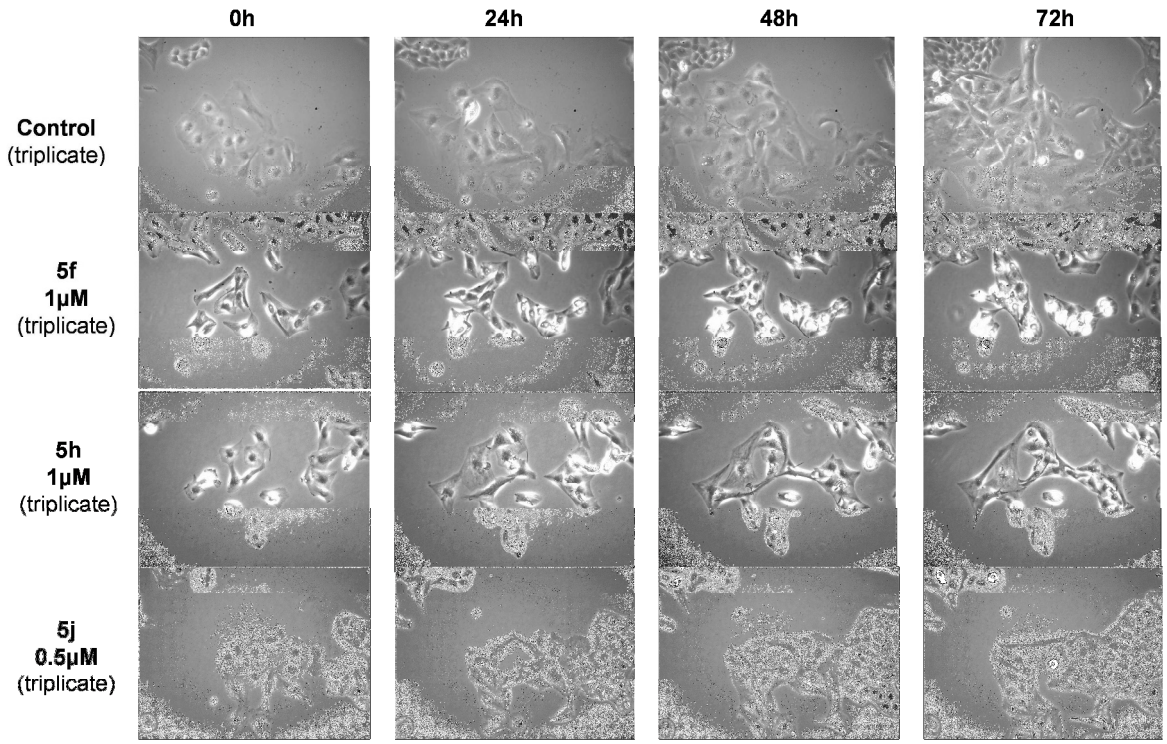
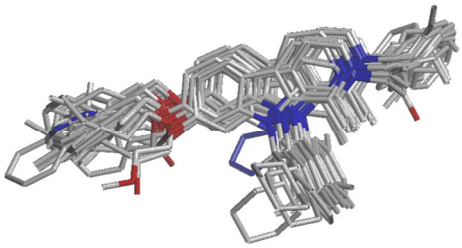
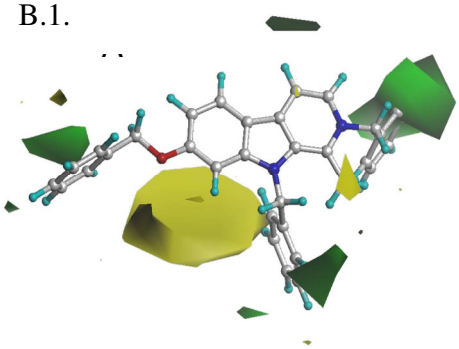


Figure 3

A.



B.1.



B.2.

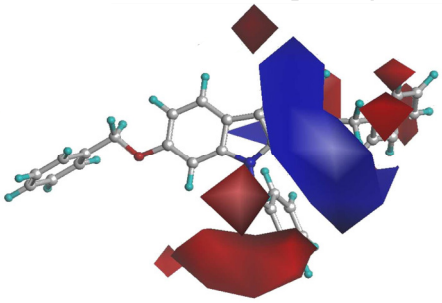


Figure 4

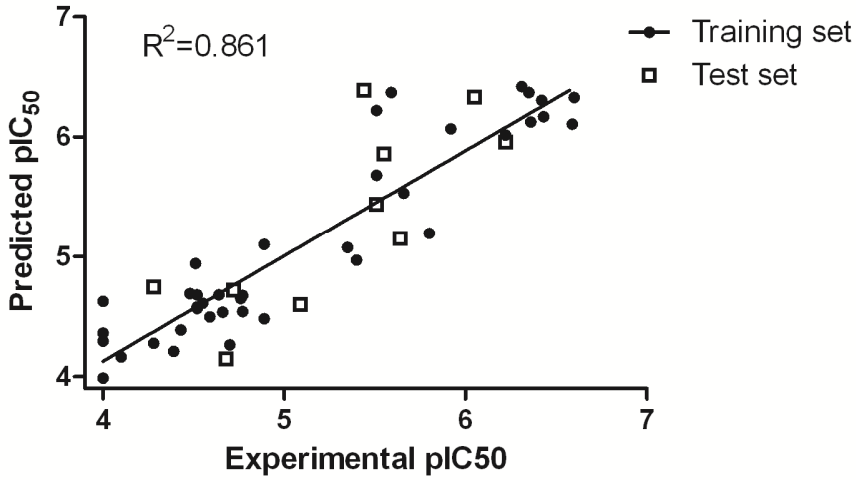
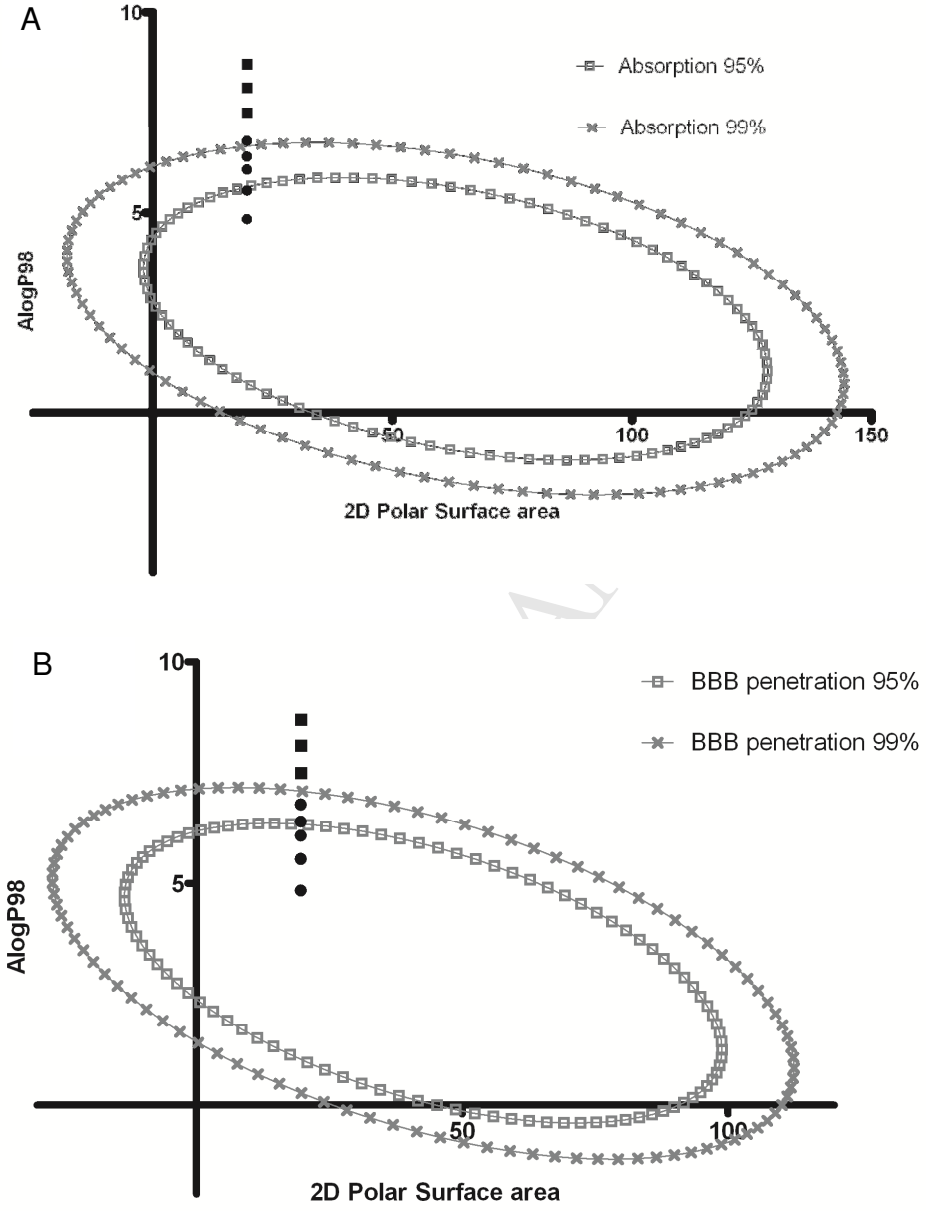
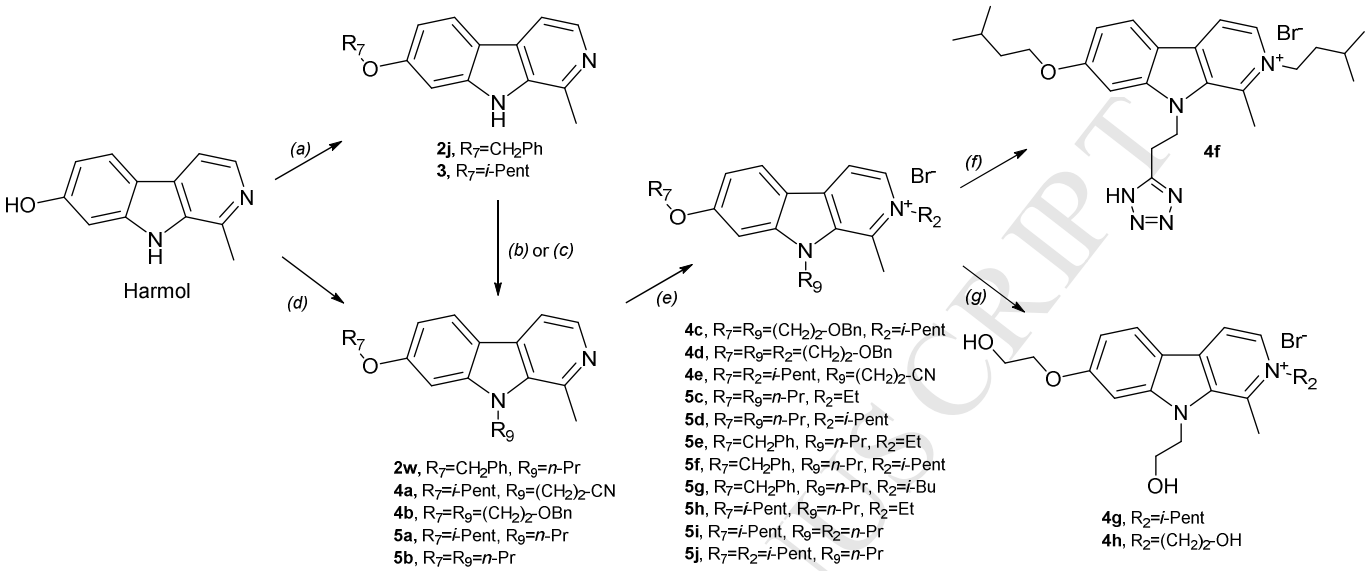


Figure 5


A
10
5
AlogP98
Absorption 95%
Absorption 99%
50
100
150
2D Polar Surface area
B
10
5
AlogP98
BBB penetration 95%
BBB penetration 99%
50
100
2D Polar Surface area

Scheme 1

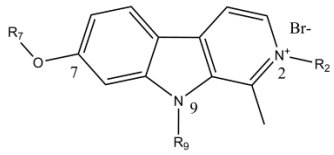


(a) R_7Br (1,5 equiv), Cs_2CO_3 (3 equiv), DMF, rt ; *(b)* iodopropane (1,5equiv), NaH (5 equiv), DMF, Ar, rt, 24h ; *(c)* acrylonitrile (7 equiv), Triton B, rt ; *(d)* $R_7(=R_9)Br$ (2 equiv), KOH (5 equiv), DMF, Ar, rt, 24h ; *(e)* R_2Br (10 equiv), THF, Δ, microwave, 140°C, 3h ; *(f)* NaN_3 (4.5 equiv), NH_4Cl (4 equiv), DMF, rt ; *(g)* H_2, Pd/C 10%, EtOH, rt.

- Harmine derivatives with polar groups were synthesized showing no *in vitro* activity
- Development of a CoMFA model has guided the synthesis of new harmine derivatives
- New compounds present high solubility and *in vitro* antiproliferative activity
- Predictions show interesting human intestinal absorption and BBB penetration

Supporting informations

1) *In Vitro* IC_{50} growth inhibitory potency after 3 days culture in presence of compounds **2a-as** on Hs683 cancer cell lines, compared to predicted pIC50 on Hs683 by CoMFA model.

Cmpd	Chemical structure			Experimental IC50 on Hs683 cell line	Predicted IC50 on Hs683 cell line	Residuals[a]
						
	R7	R9	R2			
1	CH_3	H	-	37	41[b]	0.04
2a	CH_2-C_6H_5	CH_2-C_6H_5	CH_2-C_6H_5	0.5	0.4[b]	-0.11
2b	3'-fluorobenzyl	3'-fluorobenzyl	4'-fluorobenzyl	4.5	8.5[b]	0.28
2c	CH_2-cyclohexyl	CH_2-cyclohexyl	CH_2-C_6H_5	0.4	0.5[b]	0.12
2d	CH_2-$CH=CH_2$	H	-	28	25[b]	-0.06
2e	$CH_2CH(CH_3)_2$	H	-	23	21[b]	-0.04
2f	$(CH_2)_2$-OCH_3	H	-	53	53[b]	0.00
2g	$(CH_2)_2$-OH	H	-	41	62[b]	0.18
2h	$(CH_2)_3$-CF_3	H	-	>100	23[b]	-0.63
2i	CH_2-cyclohexyl	H	-	22	29[b]	0.13
2j	CH_2-C_6H_5	H	-	17	21[b]	0.10
2k	$(CH_2)_2$-C_6H_5	H	-	21	71[c]	0.53
2l	CO-C_6H_5	H	-	53	18[c]	-0.46
2m	CH_2-2'-pyridyl	H	-	20	55[b]	0.44
2n	CH_2-3'-pyridyl	H	-	30	26[b]	-0.06
2o	CH_2-4'-pyridyl	H	-	8.1	25[c]	0.49
2p	CH_2-naphthyl	H	-	19	19[c]	0.00
2q	H	CH_2-C_6H_5	-	79	69[b]	-0.06
2r	CH_2-C_6H_5	CH_2-C_6H_5	-	33	20[b]	-0.21

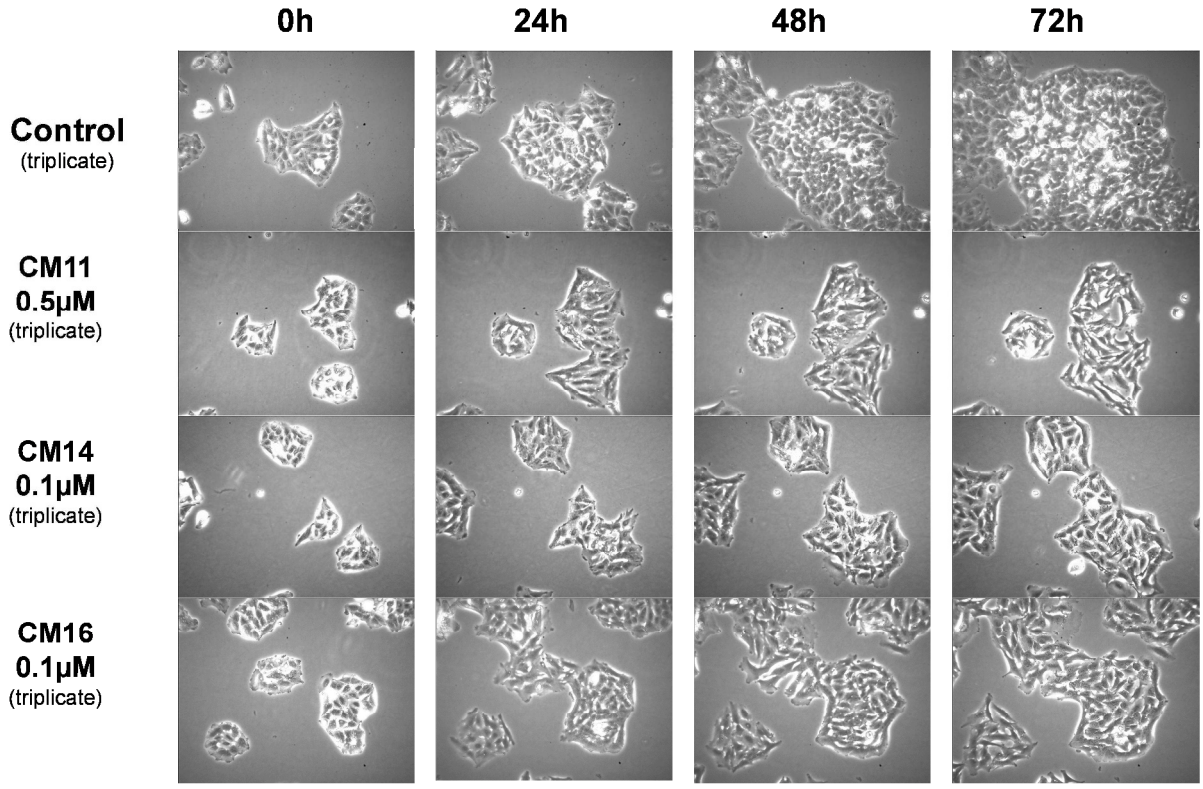
2s	3'-fluorobenzyl	3'-fluorobenzyl	-	17	22[b]	0.11
2t	4'-fluorobenzyl	4'-fluorobenzyl	-	17	29[b]	0.23
2u	CH_2-cyclohexyl	CH_2-cyclohexyl	-	30	27[b]	-0.05
2v	$(CH_2)_2$-$CH(CH_3)_2$	$(CH_2)_2$-$CH(CH_3)_2$	-	30	21[b]	-0.16
2w	CH_2-C_6H_5	$(CH_2)_2CH_3$	-	13	33[b]	0.41
2x	CH_2-C_6H_5	H	CH_2-C_6H_5	3.6	0.4[c]	-0.95
2y	CH_2-C_6H_5	CH_2-C_6H_5	2'-fluorobenzyl	0.4	0.8[b]	0.24
2z	CH_2-C_6H_5	CH_2-C_6H_5	4'-fluorobenzyl	1.6	6.5[b]	0.61
2aa	CH_2-C_6H_5	CH_2-C_6H_5	$(CH_2)_2$-C_6H_5	2.3	7.1[c]	0.49
2ab	CH_2-C_6H_5	CH_2-C_6H_5	$(CH_2)_2CH_3$	0.6	1.1[c]	0.27
2ac	CH_2-C_6H_5	CH_2-C_6H_5	$(CH_2)_5CH_3$	0.6	1.2[b]	0.29
2ad	CH_2-C_6H_5	CH_2-C_6H_5	$(CH_2)_2$-$CH(CH_3)_2$	0.4	0.7[b]	0.26
2ae	CH_2-C_6H_5	CH_2-C_6H_5	$(CH_2)_2OH$	31	11[b]	-0.43
2af	3'-fluorobenzyl	3'-fluorobenzyl	CH_2-C_6H_5	2.8	1.4[c]	-0.31
2ag	3'-fluorobenzyl	3'-fluorobenzyl	2'-fluorobenzyl	2.2	3.0[b]	0.13
2ah	4'-fluorobenzyl	4'-fluorobenzyl	CH_2-C_6H_5	0.9	0.5[c]	-0.28
2ai	4'-fluorobenzyl	4'-fluorobenzyl	2'-fluorobenzyl	1.2	0.9[b]	-0.15
2aj	4'-fluorobenzyl	4'-fluorobenzyl	4'-fluorobenzyl	13	7.9[b]	-0.21
2ak	CH_2-cyclohexyl	CH_2-cyclohexyl	2'-fluorobenzyl	0.3	0.5[b]	0.27
2al	CH_2-cyclohexyl	CH_2-cyclohexyl	4'-fluorobenzyl	3.1	0.6[b]	-0.71
2am	$(CH_2)_2$-$CH(CH_3)_2$	$(CH_2)_2$-$CH(CH_3)_2$	$(CH_2)_2OH$	4.0	11[b]	0.43
2an	$(CH_2)_2$-$CH(CH_3)_2$	$(CH_2)_2$-$CH(CH_3)_2$	$(CH_2)_2$-$CH(CH_3)_2$	0.3	0.8[b]	0.49
2ao	$(CH_2)_2$-$CH(CH_3)_2$	$(CH_2)_2$-$CH(CH_3)_2$	CH_2-C_6H_5	3.1	2.1[b]	-0.17
2ap	CH_2-C_6H_5	$(CH_2)_2CH_3$	CH_2-C_6H_5	2.6	0.4[b]	-0.78
2aq	CH_2-C_6H_5	$(CH_2)_2CH_3$	2'-fluorobenzyl	0.5	0.4[b]	-0.02
2ar	CH_2-C_6H_5	$(CH_2)_2CH_3$	4'-fluorobenzyl	3.1	3.6[c]	0.08
2as	CH_2-C_6H_5	CH_2-C_6H_5	CH_2- CO-C_6H_5	26	32[b]	0.09

[a]Residuals define as difference between experimental pIC50 and predicted pIC50 on Hs683 cell line

[b]Included in CoMFA model training set

[c]Included in CoMFA model test set

2) Quantitative videomicroscopy analyses of the compounds **5h** and **5j** on Hs683 glioma cell line. Compounds were tested at their IC_{50} value, determined by the MTT colorimetric test.



3) Quantitative videomicroscopy analyses of the compounds **5h** and **5j** on SKMEL-28 glioma cell line. Compounds were tested at their IC_{50} value, determined by the MTT colorimetric test.