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Original article

Synthesis and biological evaluation of tetrahydro[1,4]diazepino[1,2-*a*]indol-1-ones as cyclin-dependent kinase inhibitors

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ABSTRACT

New series of 2,3,4,5-tetrahydro[1,4]diazepino[1,2-*a*]indol-1-ones and 3,4,5,10-tetrahydro-2*H*-diazepino[3,4-*b*]indol-1-ones have been synthesized through an iodolactonisation/lactone-to-lactam rearrangement sequence. These compounds were evaluated as potential protein kinase inhibitors (CDK1, CDK5 and GSK-3). 11-Iodo-2,3,4,5-tetrahydro[1,4]diazepino[1,2-*a*]indol-1-one derivatives exhibited submicromolar inhibitory activity against cyclin-dependent kinases. Docking studies were realized to determine the binding mode of the inhibitors into the ATP binding domain of the CDK5 catalytic site. Our result highlighted two weak Van-der-Waals bonding interactions established between the iodine atom and both phenyl group of Phe 80 and ammonium end of Lys 33.

1. Introduction

Numerous physiological processes and human diseases involve protein phosphorylation. The involvement of cyclin-dependent kinases (CDKs) in cell cycle control, apoptosis, pain signaling, transcription, RNA splicing and neuronal functions has stimulated considerable interest in this kinase family [1]. CDKs are deregulated in proliferative diseases such as cancers, kidney disease and viral infections [2,3]. CDK5 is abnormally up-regulated in non proliferative disease neurodegenerative disorders such as Alzheimer's and Parkinson's diseases, ischemia and traumatic brain injury [4,5]. CDKs are primordial targets in many therapeutic areas and constitute a strong support to the search and optimization of selective small molecular weight inhibitors. Currently, pan-CDK inhibitors such as alvocidib (flavopiridol), seliciclib ((R)-roscovitine), dinaciclib (SCH727965) and AT7519 and CDK4,6 specific inhibitors palbociclib (PD0332991), LY2835219 and LEE011 have reached clinical phase evaluation, mostly against cancer, but none have been marketed (Fig. 1) [6–8]. All CDK inhibitors identified so far act by competing with ATP for binding at the catalytic site of their kinase targets. Small molecular weight inhibitors have been co-crystallized with CDK2 or CDK5 confirming their binding in the ATP pocket of CDKs.

In the past years, we have conducted two optimization studies of promising CDK kinase inhibitors named meriolins and N-&-N1 (GP02010), bioisotere of (R)-roscovitine [9,10]. Now, we are focused on the identification of original scaffolds with an easy synthetic access and important structural modification possibilities. For that purpose, two series of seven-membered ring fused indole derivatives: 2,3,4,5-tetrahydro[1,4]diazepino[1,2-*a*]indol-1-ones **I** and 3,4,5,10-tetrahydro-2*H*-diazepino[3,4-*b*]indol-1-ones **II** were developed as potential CDK inhibitors (Fig. 2). Both scaffolds are quasi planar and formation of donor or acceptor hydrogen bond with the hinge region in the ATP site can be considered. Structure–activity relationship studies on **I** and **II** can be easy envisioned in order to improve the inhibition activity and selectivity over other protein kinases.

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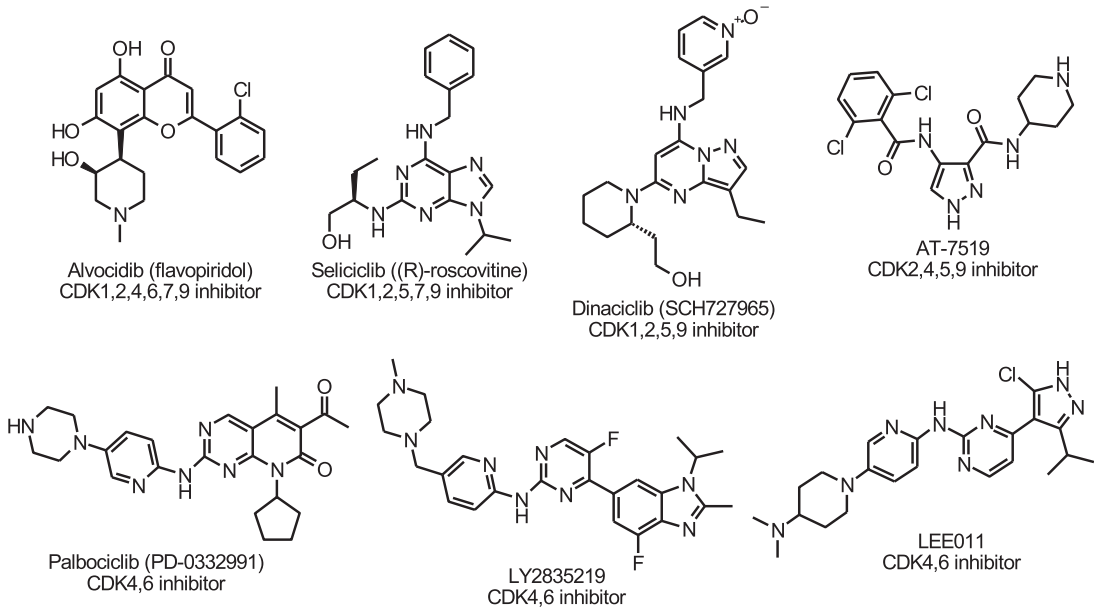


Fig. 1. Selected CDK inhibitors in clinical trials.

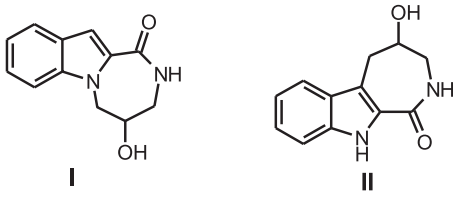


Fig. 2. Structure of **I** and **II**.

2. Results and discussion

2.1. Chemistry

Access of both scaffolds **I** and **II** is based on a unique and simple synthetic approach involving, first, an iodolactonisation of allylindole-2-carboxylic acids followed by a lactone-to-lactam rearrangement [11,12]. As a first application, the synthesis of 2,3,4,5-tetrahydro[1,4]diazepino[1,2-*a*]indol-1-ones **5** is reported in Scheme 1 (yields in Table 1). The starting esters **1** were prepared by esterification of commercially available acids (**1a** and **1c**), via Hemetsberger reaction from commercial aldehydes (**1b**, **1d** and **1e**) [13] or C3-bromation of esters **1a** and **1d** (**1f** and **1g**) [14]. *N*-Allylation of indoles **1** was performed in the presence of allylbromide and potassium carbonate and provided **2** in good yields (82–99%). Saponification of **2** led to acids **3** in quasi-quantitative yields. Lactonisation of **3** was carried out in the presence of *N*-iodosuccinimide and 2,6-lutidine in dichloromethane at −20 °C to give **4** in 33–96% yields. At the same time, iodination at the C11 position occurs when the C3 position of starting material (**3a**–**e**) is unsubstituted. Treatment of lactones **4** with ammonia in MeOH/THF (2:1) gave lactams **5** in 33–83% yields. Starting from substituted indoles, structural modulations have been achieved on benzenic part of the indole. Demethylation of **5d** was performed in the presence of boron tribromide to provide **5h** in 36% yield (Scheme 2).

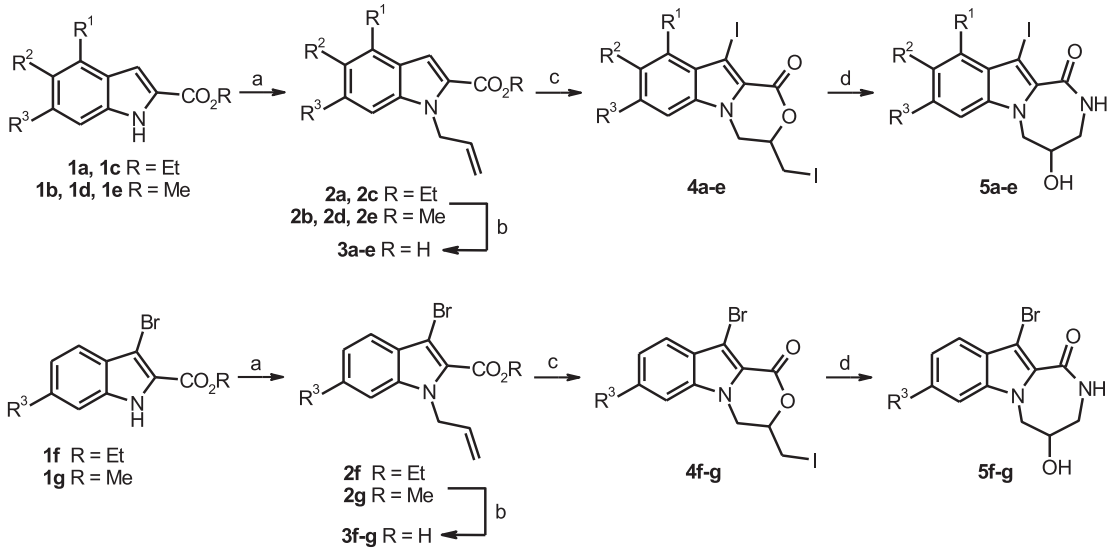
The presence of the iodine atom at C11 position opens the way to various functionalisations via palladium-mediated reaction. Catalytic hydrogenation of **5a** led to the C11 free derivative **6**. Methylation, cyanation, Suzuki-Myaura, Sonogashira and Heck palladium-catalyzed cross-coupling reactions were performed with **5a** to produce **7**–**11** in fair yields (Scheme 3) [15–19]. Finally, catalytic hydrogenation of **10** gave C11-pentyl compound **12** in 92% yield.

Based on the same synthetic approach, the second scaffold was prepared from ethyl 1-(*tert*-butoxycarbonyl)-3-iodo-1*H*-indole-2-carboxylate (**13**) [20] (Scheme 4). Stille reaction between **13** and tri-*n*-butylallylstannane afforded 3-allylindole **14** in 85% yield. Saponification of ester **14** gave **15** in quasi-quantitative yield. 3-Allyl-1-methylindole-2-carboxylic acid **17** was prepared from **15** in two steps (O- and N-methylation (**16**) then saponification). Both acids **15** and **17** were engaged in an iodocyclisation to provide compounds **18a**–**b** in 72–75% yields. The rearrangement of **18** in the presence of ammonia gave 3,4,5,10-tetrahydro-2*H*-diazepino [3,4-*b*]indol-1-ones **19** in 53–73% yields.

2.2. Biological evaluation

Compounds **5**–**12** and **19** were tested on three purified protein kinases, cyclin-dependent kinases CDK1/cyclin B and CDK5/p25 and glycogen synthase kinase-3 (GSK-3α/β). Catalytic activities were determined in the presence of a range of concentrations of new derivatives using an assay with [γ-^{33}P]-ATP as reported in the experimental part. IC_{50} values were calculated from dose–responses curves (Table 2). Among compounds tested, several tetrahydro[1,4]diazepino[1,2-*a*]indol-1-ones **5a**, **5d**, **5g** and **5h** exhibit sub-micromolar inhibitory activity against cyclin-dependent kinases or/and glycogen synthase kinase. The second scaffold (tetrahydro-2*H*-diazepino[3,4-*b*]indol-1-one) shows no inhibitory activity towards the same protein kinase panel.

For the first series, the presence of iodine atom at C11 is essential to observe the inhibitory activity against protein kinases (e.g. **5a** IC_{50} CDK5/p25 = 0.43 μM). The replacement of the iodine atom by hydrogen, methyl, cyano, pentyne, pentyl or phenyl substituent leads to inactive derivatives (**6**–**12** IC_{50} > 10 μM). The substitution of iodine by bromine at C11 (compare **5a** and **5f** IC_{50} CDK5/p25 = 5.5 μM) reduces the inhibitory activity. This likely results from the loss of a key interaction between the inhibitor and the ATP site.

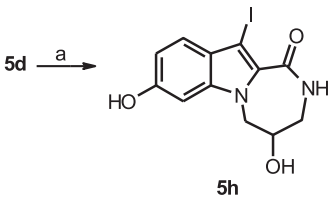


Scheme 1. Synthesis of tetrahydro[1,4]diazepino[1,2-*a*]indol-1-ones **5**. Reagents and conditions: (a) Allyl bromide, K_2CO_3, MeCN, rflx, 2.5 days; (b) NaOH, EtOH/H_2O, rflx; (c) NIS, 2,6-lutidine, CH_2Cl_2, −20 °C, 3.5 h; (d) NH_3(g), MeOH/THF 2:1, RT, 24 h–48 h.

Table 1
Yields of compounds **2**, **3**, **4** and **5**.

Cpd	R	R^1	R^2	R^3	Yield (%)			
					2	**3**	**4**	**5**
1a	Et	H	H	H	**2a** – 94	**3a** – 99	**4a** – 81	**5a** – 64
1b	Me	OMe	H	H	**2b** – 99	**3b** – 84	**4b** – 96	**5b** – 83
1c	Et	H	OMe	H	**2c** – 94	**3c** – 96	**4c** – 94	**5c** – 79
1d	Me	H	H	OMe	**2d** – 88	**3d** – 98	**4d** – 67	**5d** – 81
1e	Me	H	H	OBn	**2e** – 99	**3e** – 89	**4e** – 60	**5e** – 33
1f	Et	H	H	H	**2f** – 87	**3f** – 94	**4f** – 91	**5f** – 75
1g	Me	H	H	OMe	**2g** – 82	**3g** – 99	**4g** – 33	**5g** – 76

The nature and the position of substituent on the benzene ring of the indole moiety is a second parameter to retain the inhibitory activities. The presence of a methoxy group at C10 or C9 (**5b**, **5c**) leads to inactive derivatives. The same substituent at C8 is not detrimental in terms of inhibition as shown with **5d** (IC_{50} CDK5/p25 = 0.73 μM) which exhibits an IC_{50} value close to that **5a**. In the case of **5g** that bears a bromine atom at C11 (IC_{50} CDK5/p25 = 1 μM), the inhibitory activity is enhanced, compared to unsubstituted **5f** (IC_{50} CDK5/p25 = 5.5 μM). Contrary to **5d** and **5a**, compound **5g** displays also GSK-3 inhibitory activity (**5a**, IC_{50} GSK3 > 10 μM; **5d**, IC_{50} GSK3 = 2.2 μM; **5g**, IC_{50} GSK3 = 0.7 μM). The size of the substituent at C8 is clearly important to retain the inhibition kinase activity: small substituents such as hydroxy or methoxy (**5h** or **5d**) display sub-micromolar inhibitory activity (**5h** IC_{50} CDK5/p25 = 0.40 μM, **5d** IC_{50} CDK5/p25 = 0.73 μM); a larger substituent as benzyloxy (**5e**) leads to the loss of inhibitory activity (IC_{50} > 10 μM).



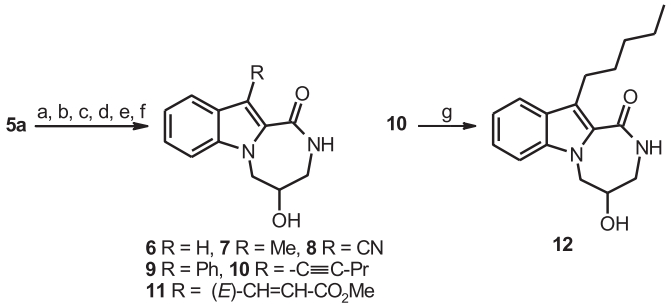
Scheme 2. Synthesis of **5h**. Reagents and conditions: (a) BBr_3, CH_2Cl_2, RT, 4 h, **5h**: 36%.

The analysis of biological results obtained from **5**–**12** allows to select 11-iodo-2,3,4,5-tetrahydro[1,4]diazepino[1,2-*a*]indol-1-one as a CDK inhibitor pharmacophore for this series.

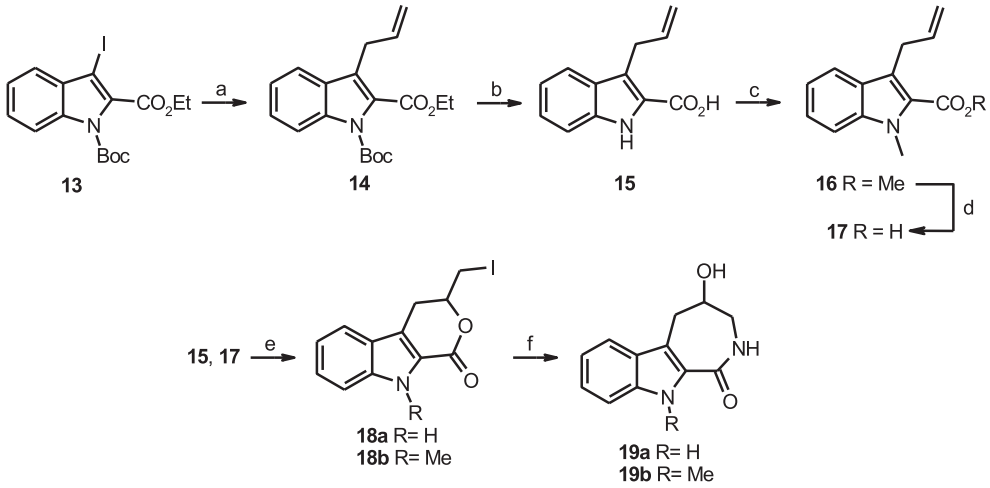
2.3. Molecular modelling

Molecular modelling was conducted on **5h**. The 7-membered ring of the molecule leads to two conformers in which this ring is off the plane defined by the indole motif and bend either towards the *re* or *si* face of the carbonyl group (Fig. 3). These two conformers were fully optimized at the DFT-B3LYP. The difference of stability is less than a kcal mol^{-1} in Gibbs energy, and hence has to be regarded as isoenergetic.

For each enantiomer, both conformers were considered for the molecular docking. One of the best positioning of **5h** in terms of binding energy and cluster size is obtained with the S enantiomer. The specific interactions between **5h** and CDK5-active site are illustrated on Fig. 4. The docking cluster is characterized by up to three hydrogen bonds that involve the protein backbone peptide links. Namely, the carbonyl of Gln 130 interacts with the phenol group of **5h**, the hydroxy function of the 7-membered ring binds CO or NH groups of Cys 83, and, the NH-group of the 7-membered ring



Scheme 3. Synthesis of tetrahydro[1,4]diazepino[1,2-*a*]indol-1-ones **6**–**12**. Reagents and conditions: (a) H_2 (10 bars), 10% Pd/C, MeOH, RT, 16 h, **6**: 93%; (b) $Pd(PPh_3)_4$, $AlMe_3$, THF, rflx, overnight, **7**: 68%; (c) $Pd(PPh_3)_4$, $Zn(CN)_2$, DMF, 150 °C, MW, 5 min, **8**: 82%; (d) 10% Pd/C, $PhB(OH)_2$, Na_2CO_3, DME/H_2O 1:1, 85 °C, 4 h, **9**: 88%; (e) $Pd(PPh_3)_2Cl_2$, CuI, pent-1-yne, Et_3N, DMF, 50 °C, 4 h, **10**: 63%; (f) $Pd(OAc)_2$, PPh_3, methyl acrylate, Et_3N, DMF, 90 °C, 5 h, **11**: 87%; (g) H_2 (20 bars), 10% Pd/C, MeOH/THF 5:1, RT, 18 h, **12**: 92%.



Scheme 4. Synthesis of tetrahydro-2*H*-diazepino[3,4-*b*]indol-1-ones **19**. Reagents and conditions: (a) $Pd(PPh_3)_4$, $(n\text{-}Bu)_3SnCH_2{-}CH{=}CH_2$, toluene, rflx, 20 h, **14**: 85%; (b) NaOH, $EtOH/H_2O$, rflx, 4 h, **15**: 92%; (c) NaH, MeI, DMF, 0 °C to RT, overnight, **16**: 96%; (d) $LiOH.H_2O$, $THF/MeOH/H_2O$ 6:3:1, rflx, 1 h, **17**: 78%; (e) NIS, 2,6-lutidine, CH_2Cl_2, -20 °C, 3.5 h, **18a** (R = H): 75%, **18b** (R = Me): 72%; (f) NH_3(g), MeOH/THF 3:1, 0 °C to RT, 4 days, **19a** (R = H): 73%, **19b** (R = Me): 53%.

peptide group is H-bonded to backbone CO-group of Glu 81. More interestingly, two weak Van-der-Waals bonding interactions are established between the iodo-group of **5h** and both the phenyl group of Phe 80 and the ammonium end of Lys 33. Distances between iodine atom and the centroid of the Phe 80 aromatic ring and the nitrogen atom of Lys 33 are respectively of 3.87 Å and 3.98 Å. These distances belong to the typical range observed in stacking and cation/π interactions [21–24].

3. Conclusion

11-Iodo-2,3,4,5-tetrahydro[1,4]diazepino[1,2-*a*]indol-1-ones **5a**, **5d** and **5h** are easily prepared from methyl or ethyl indole-2-carboxylates via an iodolactonisation/lactone-to-lactam rearrangement sequence. These compounds exhibit sub-micromolar inhibitory activity against CDK1 and/or CDK5 protein kinases. Docking studies on **5h** show two weak Van-der-Waals bonding interactions established between the iodine atom and amino acids (Phe 80 and Lys 33) of the ATP site which can explain the importance of this halogen at C11 for the biological activity of the series. The preparation of pure enantiomers of **5h** is in progress to determine the most potent stereoisomer and validate our docking results. Structure–activity relationship studies can be also envisioned on seven-membered ring to reach new potential CDK inhibitors.

4. Experimental section

4.1. Chemistry

4.1.1. General methods

Commercial reagents (Fluka, Aldrich) were used without purification. Solvents were distillated prior to use. Melting points were determined using a Büchi capillary instrument and are uncorrected. IR spectra were recorded on a Perkin–Elmer 681 infrared spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 300 or 500 MHz spectrometer. Chemical shifts are reported in ppm (δ) and were referenced to DMSO-d_6 (2.50 ppm) or $CDCl_3$ (7.26 ppm). Mass spectra were recorded with a Perkin–Elmer SCIEX API spectrometer. Elemental analyses were performed on a Thermoquest Flash 1112 series EA analyzer. Compounds **4a**, **4d**, **5**–**12** and **19** gave satisfactory microanalyses ±0.4 calculated values. Thin Layer Chromatography (TLC) analyses were conducted on aluminium sheets silica gel Merck $60F_{254}$. The spots were visualized using an ultraviolet light. Flash chromatography was carried out on silica gel 60 (40–63 μm, Merck) using the indicated solvents. The light petroleum ether refers to the fraction boiling at 40–60 °C. Microwave irradiations were carried out on a Biotage Initiator Eight in heavy wall 2–5 mL Biotage sealed tubes.

4.1.2. General procedure for preparation of compounds ***2a–g***

To a solution of methyl or ethyl indole-2-carboxylate **1** (5.34 mmol) and allylbromide (1.24 mL, 14.41 mmol) in anhydrous MeCN (15 mL) was added K_2CO_3 (1.70 g, 12.28 mmol). The reaction mixture was refluxed 2.5 days then concentrated in vacuo. The crude residue was diluted with water (20 mL) and extracted with EtOAc (3 × 15 mL). The organic extracts were combined, dried over Na_2SO_4, filtered and concentrated in vacuo. The crude residue was purified by flash column chromatography on silica gel (PE/EtOAc) to give **2**.

*4.1.2.1. Ethyl 1-allyl-1H-indole-2-carboxylate (**2a**) (CAS: 108797-23-5).* Starting ester = ethyl 1*H*-indole-2-carboxylate (**1a**). Chromatography eluent: PE/EtOAc 85:15; yield: 94%; oil; IR (neat, cm^{-1}): ν 3050, 2975, 1705, 1450, 1190, 1145, 920, 740; ^1H NMR (300 MHz, $CDCl_3$): δ 1.42 (t, 3H, J = 7.2 Hz, CH_3), 4.39 (q, 2H, J = 7.2 Hz, CH_2), 4.92 (d, 1H, J = 17.1 Hz, $=CH_2$), 5.12 (d, 1H, J = 10.4 Hz, $=CH_2$), 5.23–5.26 (m, 2H, CH_2), 5.97–6.09 (m, 1H, =CH), 7.14–7.20 (m, 1H, H_{Ar}), 7.32–7.39 (m, 3H, H_{Ar}), 7.70 (d, 1H, J = 8.1 Hz, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.5 (CH_3), 46.9 (CH_2), 60.7 (CH_2), 110.8 (CH), 110.8 (CH), 116.1 ($=CH_2$), 120.8 (CH), 122.7 (CH), 125.2 (CH), 126.2 (Cq), 127.6 (CH), 134.0 (Cq), 139.3 (Cq), 162.1 (CO); MS (ESI): *m/z* 230 $[M+H]^+$.

*4.1.2.2. Methyl 1-allyl-4-methoxy-1H-indole-2-carboxylate (**2b**) (CAS: 325149-41-5).* Starting ester = methyl 4-methoxy-1*H*-indole-2-carboxylate (**1b**). Chromatography eluent: PE/EtOAc 95:5; yield: 99%; oil; IR (neat, cm^{-1}): ν 3051, 2960, 2833, 1712, 1618, 1450, 1175, 930; ^1H NMR (300 MHz, $CDCl_3$): δ 3.89 (s, 3H, CH_3), 3.96 (s, 3H, CH_3), 4.88 (d, 1H, J = 17.5 Hz, $=CH_2$), 5.09 (d, 1H, J = 10.2 Hz, $=CH_2$), 5.20–5.21 (m, 2H, CH_2), 5.94–6.06 (m, 1H, =CH), 6.51 (d, 1H, J = 7.7 Hz, H_{Ar}), 6.96 (d, 1H, J = 8.5 Hz, H_{Ar}), 7.23–7.28 (m, 1H, H_{Ar}), 7.44 (s, 1H, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 47.0 (CH_2), 51.6 (CH_3), 55.3 (CH_3), 99.8 (CH), 103.6 (CH), 108.5 (CH), 115.9 ($=CH_2$), 117.4

Table 2
Effects of compounds **5**–**12** and **19** on the catalytic activity of three purified protein kinases.

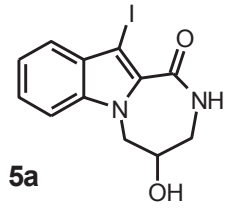
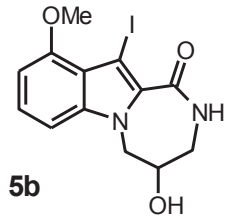
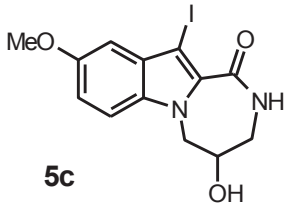
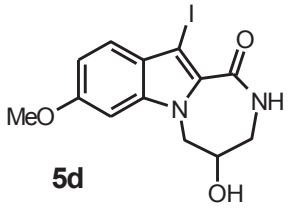
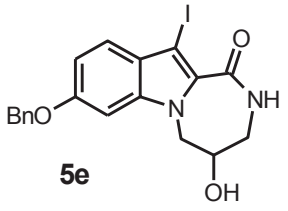
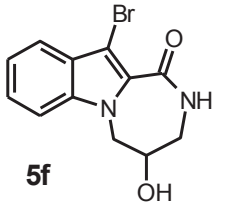
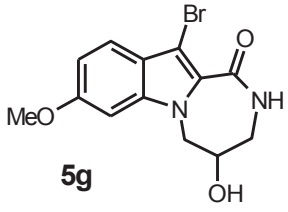
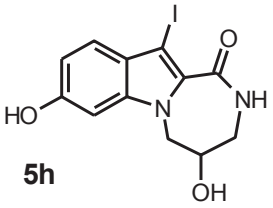
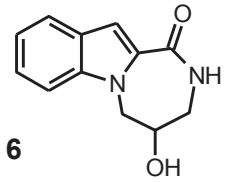
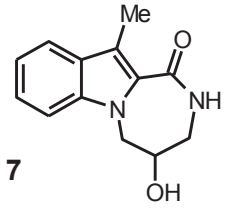
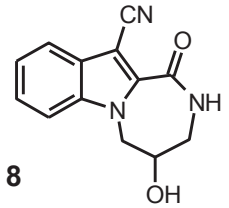
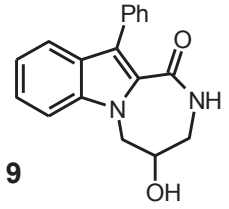
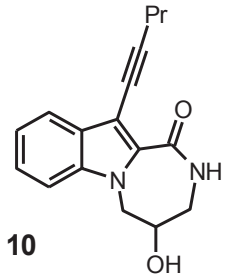
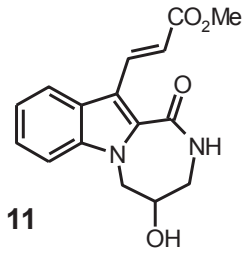
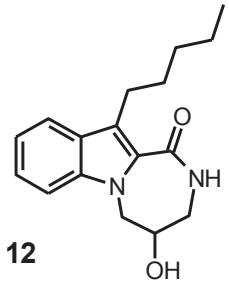
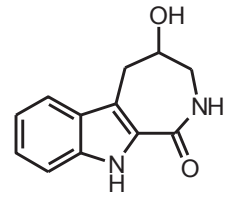
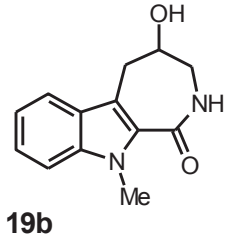
Cpd	IC_{50} (μM)		
	CDK1/cyclin B	CDK5/p25	GSK3-α/β
(R)-roscovitine	0.33[a]	0.28[a]	60[a]
 **5a**	3	0.43	>10
 **5b**	1.9	2.9	>10
 **5c**	5.4	23	>10
 **5d**	0.45	0.73	2.2
 **5e**	>10	>10	>10
 **5f**	4	5.5	>10
 **5g**	0.6	1	0.7

Table 2 (*continued*)

Cpd	IC_{50} (μM)		
	CDK1/cyclin B	CDK5/p25	GSK3-α/β
 **5h**	0.4	0.4	1.1
 **6**	>10	NT	>10
 **7**	>10	>10	>10
 **8**	>10	>10	>10
 **9**	>10	>10	>10
 **10**	>10	>10	>10
 **11**	>10	>10	>10

(*continued on next page*)

Table 2 (*continued*)

Cpd	IC_{50} (μM)		
	CDK1/cyclin B	CDK5/p25	GSK3-α/β
 **12**	>10	>10	>10
 **19a**	>10	>10	>10
 **19b**	>10	>10	>10

NT: not tested.
[a] Data from Ref. [25].

(Cq), 125.8 (Cq), 126.3 (CH), 133.9 (CH), 140.7 (Cq), 154.7 (Cq), 162.3 (CO); MS (ESI): *m/z* 246 $[M+H]^+$.

*4.1.2.3. Ethyl 1-allyl-5-methoxy-1H-indole-2-carboxylate (**2c**) (CAS: 918161-85-0).* Starting ester = methyl 5-methoxy-1*H*-indole-2-carboxylate (**1c**). Chromatography eluent: PE/EtOAc 9:1; yield: 94%; oil; IR (neat, cm^{-1}): *ν* 3054, 2922, 2829, 1709, 1620, 1463, 1172, 994, 912; ^1H NMR (300 MHz, $CDCl_3$): *δ* 1.40 (t, 3H, *J* = 7.1 Hz, CH_3), 3.85 (s, 3H, CH_3), 4.36 (q, 2H, *J* = 7.1 Hz, CH_2), 4.88 (dd, 1H, *J* = 1.3, 17.1 Hz, =CH_2), 5.09 (dd, 1H, *J* = 1.3, 10.4 Hz, =CH_2), 5.17–5.20 (m, 2H, CH_2), 5.92–6.05 (m, 1H, =CH), 7.01 (dd, 1H, *J* = 1.9, 8.9 Hz, H_{Ar}), 7.07 (d, 1H, *J* = 1.9 Hz, H_{Ar}), 7.24–7.28 (m, 2H, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 14.4 (CH_3), 46.9 (CH_2), 55.7 (CH_3), 60.5 (CH_2), 102.5 (CH), 110.0 (CH), 111.6 (CH), 115.9 (=CH_2), 116.7 (CH), 126.3 (Cq), 127.7 (Cq), 134.1 (CH), 134.7 (Cq), 154.8 (Cq), 161.9 (CO); MS (ESI): *m/z* 260 $[M+H]^+$.

*4.1.2.4. Methyl 1-allyl-6-methoxy-1H-indole-2-carboxylate (**2d**) (CAS: 918161-86-1).* Starting ester = methyl 6-methoxy-1*H*-indole-2-carboxylate (**1d**). Chromatography eluent: PE/EtOAc 95:5; yield: 88%; oil; IR (neat, cm^{-1}): *ν* 3056, 2953, 2844, 1714, 1619, 1436, 1176, 924; ^1H NMR (300 MHz, $CDCl_3$): *δ* 3.87 (s, 3H, CH_3), 3.88 (s, 3H, CH_3), 4.89 (dd, 1H, *J* = 1.3, 17.1 Hz, =CH_2), 5.11 (dd, 1H, *J* = 1.3, 10.4 Hz, =CH_2), 5.17–5.19 (m, 2H, CH_2), 5.94–6.07 (m, 1H, =CH), 6.73 (d, 1H, *J* = 2.1 Hz, H_{Ar}), 6.83 (dd, 1H, *J* = 2.1, 8.7 Hz, H_{Ar}), 7.27 (s, 1H, H_{Ar}), 7.54 (d, 1H, *J* = 8.7 Hz, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 46.7 (CH_2), 51.4 (CH_3), 55.5 (CH_3), 92.7 (CH), 111.3 (CH), 112.2 (CH), 115.9 (=CH_2), 120.4 (Cq), 123.5 (CH), 126.1 (Cq), 133.8 (CH), 140.4 (Cq), 158.9 (Cq), 162.3 (CO); MS (ESI): *m/z* 246 $[M+H]^+$.

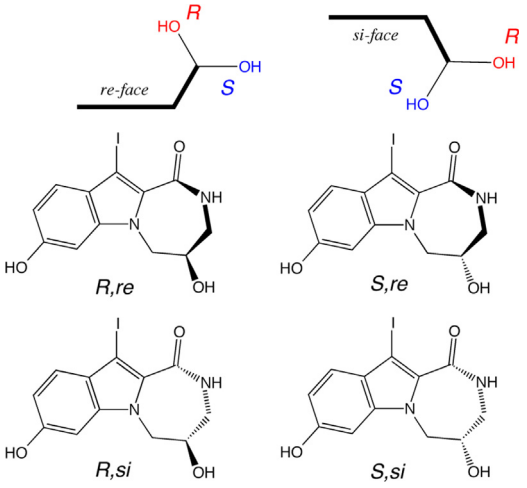


Fig. 3. Isomers and their simplified projection representation.

*4.1.2.5. Methyl 1-allyl-6-benzyloxy-1H-indole-2-carboxylate (**2e**).* Starting ester = methyl 6-benzyloxy-1*H*-indole-2-carboxylate (**1e**). Chromatography eluent: PE/EtOAc 95:5; yield: 99%; oil; IR (neat, cm^{-1}): *ν* 3065, 3034, 2920, 1709, 1619, 1454, 1385, 1254, 1177, 917; ^1H NMR (300 MHz, $CDCl_3$): *δ* 3.88 (s, 3H, CH_3), 4.87 (dd, 1H, *J* = 1.1, 17.0 Hz, =CH_2), 5.07–5.17 (m, 5H, 2 × CH_2+ =CH_2), 5.91–6.04 (m, 1H, =CH), 6.82 (d, 1H, *J* = 1.7 Hz, H_{Ar}), 6.91 (dd, 1H, *J* = 1.7, 8.8 Hz, H_{Ar}), 7.27–7.48 (m, 6H, H_{Ar}), 7.56 (d, 1H, *J* = 8.8 Hz, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 46.7 (CH_2), 51.4 (CH_3), 70.3 (CH_2), 94.1 (CH), 111.2 (CH), 112.5 (CH), 116.0 (=CH_2), 120.5 (Cq), 123.5 (CH), 126.1 (Cq), 127.5 (2 × CH), 127.9 (CH), 128.5 (2 × CH), 133.7 (CH), 136.9 (Cq), 140.2 (Cq), 157.9 (Cq), 162.2 (CO); MS (ESI): *m/z* 322 $[M+H]^+$.

*4.1.2.6. Ethyl 1-allyl-3-bromo-1H-indole-2-carboxylate (**2f**).* Starting ester = ethyl 3-bromo-1*H*-indole-2-carboxylate (**1f**). Chromatography eluent: PE/EtOAc 98:2; yield: 87%; oil; IR (neat,

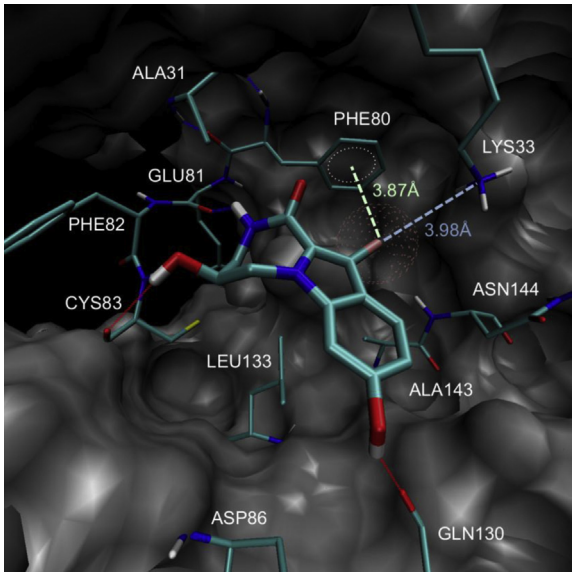


Fig. 4. Molecular docking (Autodock 4) of **5h** within the active site of CDK5 (PDB ID 1UNL). Closest residue to **5h** are explicitly represented and labelled.

cm^{-1}): ν 3054, 2983, 1722, 1623, 1455, 1401, 1193, 920, 741; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46 (t, 3H, J = 7.2 Hz, CH_3), 4.44 (q, 2H, J = 7.2 Hz, CH_2), 4.94 (dd, 1H, J = 1.1, 17.1 Hz, =CH_2), 5.12 (dd, 1H, J = 1.1, 10.4 Hz, =CH_2), 5.17–5.19 (m, 2H, CH_2), 5.91–6.04 (m, 1H, =CH), 7.21–7.24 (m, 1H, H_{Ar}), 7.34–7.42 (m, 2H, H_{Ar}), 7.69 (d, 1H, J = 8.1 Hz, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.3 (CH_3), 47.6 (CH_2), 61.2 (CH_2), 99.3 (Cq), 110.7 (CH), 116.4 (=CH_2), 121.5 (CH), 121.6 (CH), 125.1 (Cq), 126.2 (CH), 126.9 (Cq), 133.6 (CH), 137.7 (Cq), 161.2 (CO); MS (ESI): m/z 308 (^{79}Br), 310 (^{81}Br) [M+H]$^+$.

*4.1.2.7. Ethyl 1-allyl-3-bromo-6-methoxy-1H-indole-2-carboxylate (**2g**).* Starting ester = ethyl 3-bromo-6-methoxy-1*H*-indole-2-carboxylate (**1g**). Chromatography eluent: PE/EtOAc 95:5; yield: 82%; oil; IR (neat, cm^{-1}): ν 3085, 2950, 2822, 1704, 1624, 1436, 1170, 917; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.88 (s, 3H, CH_3), 3.95 (s, 3H, CH_3), 4.93 (d, 1H, J = 17.5 Hz, =CH_2), 5.11–5.16 (m, 3H, CH_2+ =CH_2), 5.91–6.03 (m, 1H, =CH), 6.71 (d, 1H, J = 1.9 Hz, H_{Ar}), 6.89 (dd, 1H, J = 1.9, 8.7 Hz, H_{Ar}), 7.55 (d, 1H, J = 8.7 Hz, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 47.5 (CH_2), 51.5 (CH_3), 55.3 (CH_3), 92.1 (CH), 99.7 (C), 112.9 (CH), 116.1 (=CH_2), 121.0 (C), 122.2 (CH), 123.4 (C), 133.4 (CH), 138.6 (C), 159.5 (C), 161.3 (CO); MS (ESI): m/z 338 (^{79}Br), 340 (^{81}Br) [M+H]$^+$.

*4.1.3. General procedure for preparation of compounds **3a–g***

To a solution of **2** (4.17 mmol) in EtOH (17 mL) was added NaOH (333 mg, 8.33 mmol) and H_2O (2 mL). The mixture was refluxed for 1 h, then concentrated in vacuo. The crude residue was acidified with 6 N HCl and extracted with EtOAc (3 × 20 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated in vacuo. The crude residue was purified by crystallization or trituration to provide compound **3**.

*4.1.3.1. 1-Allyl-1H-indole-2-carboxylic acid (**3a**) (CAS: 155193-48-9).* Starting ester = **2a**; yield: 99%; solid; mp 174–176 °C (EtOAc/PE); IR (KBr, cm^{-1}): ν 3200–2500, 1675, 1510, 1270, 1010, 920, 745; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.93 (d, 1H, J = 17.1 Hz, =CH_2), 5.13 (d, 1H, J = 10.4 Hz, =CH_2), 5.24–5.26 (m, 2H, CH_2), 5.98–6.07 (m, 1H, =CH), 7.15–7.21 (m, 1H, H_{Ar}), 7.37–7.39 (m, 2H, H_{Ar}), 7.51 (s, 1H, H_{Ar}), 7.72 (d, 1H, J = 7.5 Hz, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 47.0 (CH_2), 110.9 (CH), 113.2 (CH), 116.3 (=CH_2), 121.1 (CH), 123.1 (CH), 126.0 (CH), 126.1 (C), 126.3 (C), 133.9 (CH), 139.9 (C), 167.0 (CO); MS (ESI): m/z 202 [M+H]$^+$.

*4.1.3.2. 1-Allyl-4-Methoxy-1H-indole-2-carboxylic acid (**3b**) (CAS: 325149-42-6).* Starting ester = **2b**; yield: 84%; mp 185–186 °C (CH_2Cl_2/PE); IR (KBr, cm^{-1}): ν 3092–2436, 1674, 1517, 1259, 918; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.89 (s, 3H, CH_3), 4.76 (d, 1H, J = 17.1 Hz, =CH_2), 5.03 (d, 1H, J = 10.4 Hz, =CH_2), 5.19–5.21 (m, 2H, CH_2), 5.90–6.02 (m, 1H, =CH), 6.59 (d, 1H, J = 7.7 Hz, H_{Ar}), 7.09 (d, 1H, J = 8.5 Hz, H_{Ar}), 7.19–7.26 (m, 2H, H_{Ar}), 12.86 (br s, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 46.3 (CH_2), 55.2 (CH_3), 100.0 (CH), 104.0 (CH), 107.1 (CH), 115.6 (=CH_2), 116.5 (C), 126.0 (CH), 126.7 (C), 134.7 (CH), 140.1 (C), 153.9 (C), 162.6 (CO); MS (ESI): m/z 232 [M+H]$^+$.

*4.1.3.3. 1-Allyl-5-methoxy-1H-indole-2-carboxylic acid (**3c**) (CAS: 918161-87-2).* Starting ester = **2c**; yield: 96%; mp 156–158 °C (CH_2Cl_2/PE); IR (KBr, cm^{-1}): ν 3105–2510, 1663, 1521, 1270, 1030; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.77 (s, 3H, CH_3), 4.77 (d, 1H, J = 17.1 Hz, =CH_2), 5.03 (d, 1H, J = 10.2 Hz, =CH_2), 5.19–5.21 (m, 2H, CH_2), 5.89–6.01 (m, 1H, =CH), 6.95 (dd, 1H, J = 2.3, 9.0 Hz, H_{Ar}), 7.13–7.15 (m, 2H, H_{Ar}), 7.43 (d, 1H, J = 9.0 Hz, H_{Ar}), 12.85 (br s, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 46.2 (CH_2), 55.3 (CH_3), 102.4 (CH), 109.5 (CH), 112.0 (CH), 115.5 (=CH_2), 116.0 (CH), 125.8 (Cq), 128.1 (Cq), 134.2 (Cq), 134.9 (CH), 154.2 (Cq), 162.7 (CO); MS (ESI): m/z 232 [M+H]$^+$.

*4.1.3.4. 1-Allyl-6-methoxy-1H-indole-2-carboxylic acid (**3d**) (CAS: 918161-88-3).* Starting ester = **2d**; yield: 98%; mp 171–173 °C (CH_2Cl_2/PE); IR (KBr, cm^{-1}): ν 3120–2581, 3085, 2963, 2829, 1678, 1518, 1256, 1024, 914; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.81 (s, 3H, CH_3), 4.80 (dd, 1H, J = 1.4, 17.1 Hz, =CH_2), 5.04 (dd, 1H, J = 1.4, 10.2 Hz, =CH_2), 5.21 (d, 2H, J = 4.7 Hz, CH_2), 5.90–6.02 (m, 1H, =CH), 6.77 (dd, 1H, J = 2.1, 8.9 Hz, H_{Ar}), 6.99 (d, 1H, J = 2.1 Hz, H_{Ar}), 7.19 (s, 1H, H_{Ar}), 7.55 (d, 1H, J = 8.9 Hz, H_{Ar}), 12.67 (s, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 46.0 (CH_2), 55.4 (CH_3), 93.2 (CH), 110.6 (CH), 111.8 (CH), 115.6 (=CH_2), 119.7 (Cq), 123.1 (CH), 126.8 (Cq), 134.7 (CH), 139.9 (Cq), 158.2 (Cq), 162.6 (CO); MS (ESI): m/z 232 [M+H]$^+$.

*4.1.3.5. 1-Allyl-6-benzyloxy-1H-indole-2-carboxylic acid (**3e**).* Starting ester = **2e**; yield: 89%; mp 136–138 °C (EtOAc/Et_2O); IR (KBr, cm^{-1}): ν 3070–2517, 1667, 1521, 1251, 1028, 911 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.90 (dd, 1H, J = 1.1, 17.0 Hz, =CH_2), 5.08–5.17 (m, 5H, 2 × CH_2 + =CH_2), 5.92–6.04 (m, 1H, =CH), 6.82 (d, 1H, J = 1.7 Hz, H_{Ar}), 6.93 (dd, 1H, J = 1.7, 8.8 Hz, H_{Ar}), 7.34–7.49 (m, 6H, H_{Ar}), 7.59 (d, 1H, J = 8.8 Hz, H_{Ar}); ^{13}C NMR (75 MHz, DMSO-d_6): δ 46.0 (CH_2), 69.6 (CH_2), 94.6 (CH), 110.5 (CH), 112.2 (CH), 115.7 (=CH_2), 119.9 (Cq), 123.2 (CH), 127.0 (C), 127.8 (CH), 127.9 (2 × CH), 128.4 (2 × CH), 134.6 (CH), 137.1 (Cq), 139.8 (Cq), 157.1 (Cq), 162.7 (CO); MS (ESI): m/z 308 [M+H]$^+$.

*4.1.3.6. 1-Allyl-3-bromo-1H-indole-2-carboxylic acid (**3f**).* Starting ester = **2f**; yield: 94%; mp 163–165 °C (EtOAc/PE); IR (KBr, cm^{-1}): ν 3043–2585, 1675, 1504, 1274, 1011, 929, 735; ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.85 (d, 1H, J = 17.1 Hz, =CH_2), 5.07 (d, 1H, J = 10.2 Hz, =CH_2), 5.20–5.23 (m, 2H, CH_2), 5.90–6.02 (m, 1H, =CH), 7.22–7.62 (m, 4H, H_{Ar}); ^{13}C NMR (75 MHz, DMSO-d_6): δ 47.1 (CH_2), 97.1 (Cq), 111.5 (CH), 116.1 (=CH_2), 120.5 (CH), 121.5 (CH), 125.9 (CH), 126.0 (Cq), 126.0 (Cq), 134.4 (CH), 137.1 (Cq), 161.8 (CO). MS (ESI) m/z 280 (^{79}Br), 282 (^{81}Br) [M+H]$^+$.

*4.1.3.7. 1-Allyl-3-bromo-6-methoxy-1H-indole-2-carboxylic acid (**3g**).* Starting ester = **2g**; yield: 99%; mp 153 °C (dec.) (trituration with Et_2O); IR (KBr, cm^{-1}): ν 3078–2503, 1671, 1497, 1253, 1027, 903; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.83 (s, 3H, CH_3), 4.84 (d, 1H, J = 17.3 Hz, =CH_2), 5.06 (d, 1H, J = 10.4 Hz, =CH_2), 5.20 (d, 2H, J = 4.5 Hz, CH_2), 5.88–6.01 (m, 1H, =CH), 6,87 (dd, 1H, J = 1.9, 8.7 Hz, H_{Ar}), 7,08 (d, 1H, J = 1.9 Hz, H_{Ar}), 7.44 (d, 1H, J = 8.7 Hz, H_{Ar}), 13.38 (br s, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 47.0 (CH_2), 55.6 (CH_3), 93.4 (CH), 98.1 (Cq), 113.0 (CH), 116.0 (=CH_2), 120.3 (Cq), 121.5 (CH), 124.6 (Cq), 134.5 (CH), 138.4 (Cq), 159.1 (Cq), 161.9 (CO); MS (ESI) m/z 310 (^{79}Br), 312 (^{81}Br) [M+H]$^+$.

*4.1.4. General procedure for preparation of compounds **4a–g***

2,6-Lutidine (1.30 mL, 11.10 mmol) and *N*-iodosuccinimide (3.83 g, 17.0 mmol) were added successively at −20 °C under a nitrogen atmosphere to a solution of **3** (7.40 mmol) in anhydrous CH_2Cl_2 (90 mL) and the mixture was stirred at that temperature for 3.5 h. 6 N HCl was added and the mixture was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic layers were washed with 1 M HCl (10 mL), saturated aqueous $Na_2S_2O_3$ (2 × 30 mL), brine (2 × 30 mL), and saturated aqueous $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered and evaporated in vacuo. The crude solid was purified by recrystallization, trituration or by column chromatography to provide **4**.

*4.1.4.1. 10-Iodo-3-(iodomethyl)-3,4-dihydro-1H-[1,4]oxazino[4,3-a]indol-1-one (**4a**) (CAS: 918161-89-4).* Starting acid = **3a**.

Recrystallization from CH_2Cl_2; yield: 81%; solid; mp 169 °C (dec.); IR (KBr, cm^{-1}): ν 3020, 2950, 1710, 1100, 750; ^1H NMR (300 MHz, $CDCl_3$): δ 3.42 (dd, 1H, J = 8.7, 10.6 Hz, CH_2), 3.59 (dd, 1H, J = 4.4, 10.6 Hz, CH_2), 4.27 (dd, 1H, J = 9.0, 12.8 Hz, CH_2), 4.74 (dd, 1H, J = 3.3, 12.8 Hz, CH_2), 4.80–4.89 (m, 1H, CH), 7.31 (t, 1H, J = 8.0 Hz, H_{Ar}), 7.36 (d, 1H, J = 8.0 Hz, H_{Ar}), 7.50 (t, 1H, J = 8.0 Hz, H_{Ar}), 7.62 (d, 1H, J = 8.0 Hz, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 1.1 (CH_2), 44.9 (CH_2), 68.9 (Cq), 76.2 (CH), 110.4 (CH), 121.9 (Cq), 122.7 (CH), 124.2 (CH), 127.8 (CH), 131.1 (Cq), 136.8 (Cq), 157.4 (CO); MS (ESI): *m/z* 454 $[M+H]^+$.

*4.1.4.2. 10-Iodo-3-(iodomethyl)-9-methoxy-3,4-dihydro-1H-[1,4] oxazino[4,3-a]indol-1-one (**4b**) (CAS: 935280-31-2).* Starting acid = **3b**. Chromatography eluent: PE/EtOAc 3:2; yield: 96%; mp 150–151 °C; IR (KBr, cm^{-1}): ν 3000, 2956, 2835, 1710, 1614, 1511, 1455, 1263, 1129; ^1H NMR (300 MHz, $CDCl_3$): δ 3.39 (dd, 1H, J = 8.6, 10.6 Hz, CH_2), 3.57 (dd, 1H, J = 4.4, 10.6 Hz, CH_2), 3.97 (s, 3H, CH_3), 4.19 (dd, 1H, J = 9.0, 12.8 Hz, CH_2), 4.66 (dd, 1H, J = 4.7, 12.8 Hz, CH_2), 4.75–4.83 (m, 1H, CH), 6.58 (d, 1H, J = 7.9 Hz, H_{Ar}), 6.96 (d, 1H, J = 8.5 Hz, H_{Ar}), 7.35 (t, 1H, J = 8.3 Hz, H_{Ar}); ^{13}C NMR (75 MHz, DMSO-d_6): δ 1.3 (CH_2), 45.0 (CH_2), 55.4 (CH_3), 62.4 (C), 75.8 (CH), 101.5 (CH), 102.8 (CH), 119.9 (C), 120.9 (C), 128.2 (CH), 138.3 (C), 155.5 (C), 157.4 (C); MS (ESI): *m/z* 484 $[M+H]^+$.

*4.1.4.3. 10-Iodo-3-(iodomethyl)-8-methoxy-3,4-dihydro-1H-[1,4] oxazino[4,3-a]indol-1-one (**4c**) (CAS: 918161-90-7).* Starting acid = **3c**. Trituration with EtOAc/PE, yield: 94%. mp 145–147 °C; IR (KBr, cm^{-1}): ν 3032, 3003, 2941, 2829, 1724, 1619, 1407, 1092; ^1H NMR (300 MHz, $CDCl_3$): δ 3.41 (dd, 1H, J = 8.7, 10.7 Hz, CH_2), 3.58 (dd, 1H, J = 4.3, 10.7 Hz CH_2), 3.92 (s, 3H, CH_3), 4.23 (dd, 1H, J = 9.2, 12.3 Hz, CH_2), 4.68 (dd, 1H, J = 3.4, 12.3 Hz, CH_2), 4.79–4.87 (m, 1H, CH), 6.94 (d, 1H, J = 2.3 Hz, H_{Ar}), 7.14 (dd, 1H, J = 2.3, 8.9 Hz, H_{Ar}), 7.26 (d, 1H, J = 8.9 Hz, H_{Ar}); ^{13}C NMR (75 MHz, DMSO-d_6): δ 4.1 (CH_2), 44.8 (CH_2), 55.4 (CH_3), 66.6 (Cq), 75.7 (CH), 102.2 (CH), 112.8 (CH), 118.7 (CH), 122.2 (Cq), 130.5 (Cq), 131.6 (Cq), 155.5 (Cq), 157.5 (Cq); MS (ESI): *m/z* 484 $[M+H]^+$.

*4.1.4.4. 10-Iodo-3-(iodomethyl)-7-methoxy-3,4-dihydro-1H-[1,4] oxazino[4,3-a]indol-1-one (**4d**) (CAS: 918161-91-8).* Starting acid = **3d**. Trituration with $Et_2O/CHCl_3$; yield: 67%; mp 165–167 °C; IR (KBr, cm^{-1}): ν 3014, 2957, 1730, 1620, 1405, 1090; ^1H NMR (300 MHz, DMSO-d_6): δ 3.55–3.66 (m, 2H, CH_2), 3.86 (s, 3H, CH_3), 4.14 (dd, 1H, J = 10.2, 12.8 Hz, CH_2), 4.81 (dd, 1H, J = 3.3, 12.8 Hz, CH_2), 4.86–4.94 (m, 1H, CH), 6.90 (dd, 1H, J = 1.9, 8.9 Hz, H_{Ar}), 7.14 (d, 1H, J = 1.9 Hz, H_{Ar}), 7.35 (d, 1H, J = 8.9 Hz, H_{Ar}); ^{13}C NMR (75 MHz, DMSO-d_6): δ 4.2 (CH_2), 44.9 (CH_2), 55.7 (CH_3), 68.4 (C), 75.4 (CH), 92.8 (CH), 114.1 (CH), 121.1 (Cq), 123.7 (CH), 124.6 (Cq), 137.4 (Cq), 157.6 (Cq), 159.7 (Cq); MS (ESI): *m/z* 484 $[M+H]^+$.

*4.1.4.5. 7-Benzyloxy-10-Iodo-3-(iodomethyl)-3,4-dihydro-1H-[1,4] oxazino[4,3-a]indol-1-one (**4e**).* Starting acid = **3e**. Trituration with CH_2Cl_2; yield: 60%; mp 186–187 °C; IR (KBr, cm^{-1}): ν 3063, 3027, 2958, 1718, 1617, 1406, 1095, 741, 698; ^1H NMR (300 MHz, DMSO-d_6): δ 3.56–3.66 (m, 2H, CH_2), 4.14 (dd, 1H, J = 10.0, 11.5 Hz, CH_2), 4.79 (dd, 1H, J = 3.0, 13.0 Hz, CH_2), 4.89–4.94 (m, 1H, CH), 5.20 (s, 2H, CH_2), 6.99 (dd, 1H, J = 2.1, 8.9 Hz, H_{Ar}), 7.29–7.51 (m, 7H, H_{Ar}); ^{13}C NMR (75 MHz, DMSO-d_6): δ 4.1 (CH_2), 44.9 (CH_2), 68.4 (Cq), 69.8 (CH_2), 75.5 (CH), 94.1 (CH), 114.4 (CH), 121.2 (Cq), 123.8 (CH), 124.8 (Cq), 127.8 (2 × CH), 128.0 (CH), 128.5 (2 × CH), 136.7 (Cq), 137.3 (Cq), 157.6 (Cq), 158.7 (Cq); MS (ESI): *m/z* 560 $[M+H]^+$.

*4.1.4.6. 10-Bromo-3-(iodomethyl)-3,4-dihydro-1H-[1,4]oxazino[4,3-a]indol-1-one (**4f**).* Starting acid = **3f**. Trituration with $CHCl_3/Et_2O$; yield: 91%; mp 124–126 °C; IR (KBr, cm^{-1}): ν 3058, 3018, 2978, 1716, 1615, 1105, 743; ^1H NMR (300 MHz, DMSO-d_6): δ 3.33–3.68 (m, 2H, CH_2I), 4.20 (dd, 1H, J = 10.0, 12.8 Hz, NCH_2), 4.83 (dd, 1H, J = 3.4, 12.8 Hz, NCH_2), 4.94–5.02 (m, 1H, CH), 7.27–7.67 (m, 4H, H_{Ar}); ^{13}C NMR (75 MHz, DMSO-d_6): δ 3.9 (CH_2), 44.6 (CH_2), 76.0 (CH), 97.6 (Cq), 111.6 (CH), 119.5 (Cq), 120.5 (CH), 122.1 (CH), 126.3 (C), 126.9 (CH), 135.2 (Cq), 157.1 (CO); MS (ESI): *m/z* MS (ESI) *m/z* 406 (^{79}Br), 408 (^{81}Br) $[M+H]^+$.

*4.1.4.7. 10-Bromo-3-(iodomethyl)-7-methoxy-3,4-dihydro-1H-[1,4] oxazino[4,3-a]indol-1-one (**4g**).* Starting acid = **3g**. Trituration with Et_2O; yield: 33%; mp 167–169 °C; IR (KBr, cm^{-1}): ν 3001, 2914, 2836, 1718, 1624, 1407, 1099; ^1H NMR (300 MHz, DMSO-d_6): δ 3.56–3.66 (m, 2H, CH_2), 3.86 (s, 3H, CH_3), 4.12 (dd, 1H, J = 10.0, 12.8 Hz, CH_2), 4.80 (dd, 1H, J = 3.2, 12.8 Hz, CH_2), 4.88–4.96 (m, 1H, CH), 6.92 (dd, 1H, J = 1.9, 8.9 Hz, H_{Ar}), 7.18 (d, 1H, J = 1.9 Hz, H_{Ar}), 7.48 (d, 1H, J = 8.9 Hz, H_{Ar}); ^{13}C NMR (75 MHz, DMSO-d_6): δ 4.1 (CH_2), 44.8 (CH_2), 55.8 (CH_3), 75.7 (CH), 92.9 (CH), 98.4 (Cq), 114.3 (CH), 118.3 (Cq), 120.8 (Cq), 121.5 (CH), 136.5 (Cq), 157.1 (Cq), 159.7 (Cq); MS (ESI) *m/z* 436 (^{79}Br), 438 (^{81}Br) $[M+H]^+$.

*4.1.5. General procedure for preparation of compounds **5a–g***

A solution of **4** (7.95 mmol) in anhydrous MeOH/THF (2:1) (90 mL) was added dropwise over a period of 20 min to an ice-cold saturated ammonia solution in MeOH (30 mL). The reaction mixture was allowed to warm up to RT and stirred for an appropriate time (see reaction time below). The solvents were then removed by evaporation and the crude residue was purified by trituration or by column chromatography to give **5**.

*4.1.5.1. 4-Hydroxy-11-iodo-2,3,4,5-tetrahydro-[1,4]diazepino[1,2-a] indol-1-one (**5a**) (CAS: 918161-92-9).* Starting lactone = **4a**. Reaction time: 48 h; trituration with EtOAc/EtOH; yield: 64%; mp 199 °C (dec.); IR (KBr, cm^{-1}): ν 3410, 3320–3200, 3060, 2920, 1635, 1515, 1090, 745; ^1H NMR (300 MHz, DMSO-d_6): δ 2.73–2.80 (m, 1H, CH_2), 3.15–3.21 (m, 1H, CH_2), 4.18–4.29 (m, 2H, CH_2 + CH), 4.43–4.49 (m, 1H, CH_2), 5.38 (d, 1H, J = 3.4 Hz, OH), 7.19 (t, 1H, J = 7.9 Hz, H_{Ar}), 7.35 (t, 1H, J = 7.9 Hz, H_{Ar}), 7.39 (d, 1H, J = 7.9 Hz, H_{Ar}), 7.58 (d, 1H, J = 7.9 Hz, H_{Ar}), 8.30 (br t, 1H, J = 5.5 Hz, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 45.3 (CH_2), 48.7 (CH_2), 61.3 (Cq), 69.5 (CH), 111.0 (CH), 120.8 (CH), 121.7 (CH), 124.6 (CH), 129.2 (Cq), 133.4 (Cq), 137.2 (Cq), 164.0 (CO); MS (ESI): *m/z* 343 $[M+H]^+$.

*4.1.5.2. 4-Hydroxy-11-Iodo-10-methoxy-2,3,4,5-tetrahydro-[1,4]diazepino[1,2-a]indol-1-one (**5b**).* Starting lactone = **4b**. Reaction time: 24 h; trituration with CH_2Cl_2, yield: 83%; mp 139–141 °C; IR (KBr, cm^{-1}): ν 3475–3180, 3273, 3071, 2929, 2837, 1633, 1515, 1450, 1095, 927; ^1H NMR (300 MHz, DMSO-d_6): δ 2.66–2.75 (m, 1H, CH_2), 3.08–3.15 (m, 1H, CH_2), 3.86 (s, 3H, CH_3), 4.11–4.22 (m, 2H, CH_2 + CH), 4.35–4.42 (m, 1H, CH_2), 5.35 (d, 1H, J = 2.4 Hz, OH), 6.58 (dd, 1H, J = 2.3, 6.3 Hz, H_{Ar}), 7.16–7.23 (m, 2H, H_{Ar}), 8.25 (br t, 1H, J = 5.5 Hz, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 45.1 (CH_2), 48.5 (CH_2), 54.1 (C), 55.1 (CH_3), 69.3 (CH), 99.6 (CH), 102.4 (CH), 116.9 (C), 124.6 (CH), 132.7 (C), 138.5 (C), 153.5 (C), 164.0 (C); MS (ESI): *m/z* 373 $[M+H]^+$.

*4.1.5.3. 4-Hydroxy-11-Iodo-9-methoxy-2,3,4,5-tetrahydro-[1,4]diazepino[1,2-a]indol-1-one (**5c**) (CAS: 918161–93-0).* Starting lactone = **4c**. Reaction time: 24 h; chromatography eluent: CH_2Cl_2/MeOH 95:5; yield: 79%; mp > 210 °C (washing with CH_2Cl_2); IR (KBr, cm^{-1}): ν 3405–3200, 3242, 3073, 2929, 2847, 1636, 1518, 1457, 1092, 927; ^1H NMR (300 MHz, DMSO-d_6): δ 2.71–2.79 (m, 1H, CH_2), 3.12–3.20 (m, 1H, CH_2), 3.81 (s, 3H, CH_3), 4.16–4.23 (m, 2H, CH_2 + CH), 4.39–4.46 (m, 1H, CH_2), 5.37 (d, 1H, J = 3.0 Hz, OH), 6.79 (d, 1H, J = 2.3 Hz, H_{Ar}), 6.98 (dd, 1H, J = 2.3, 8.9 Hz, H_{Ar}), 7.50 (d, 1H, J = 8.9 Hz, H_{Ar}), 8.25 (br t, 1H, J = 5.5 Hz, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 45.4 (CH_2), 48.9 (CH_2), 55.4 (CH_3), 60.5 (Cq), 69.6 (CH),

102.1 (CH), 112.1 (CH), 115.7 (CH), 129.6 (Cq), 132.3 (Cq), 133.5 (Cq), 154.7 (Cq), 164.0 (Cq); MS (ESI): *m/z* 373 $[M+H]^+$.

*4.1.5.4. 4-Hydroxy-11-Iodo-8-methoxy-2,3,4,5-tetrahydro-[1,4]diazepino[1,2-a]indol-1-one (**5d**) (CAS: 918161–94-1).* Starting lactone = **4d**. Reaction time: 24 h; chromatography eluent: CH_2Cl_2/MeOH 95:5; yield: 81%; mp 194–196 °C (washing with CH_2Cl_2/EtOH/pentane); IR (KBr, cm^{-1}): ν 3410, 3376–3250, 3070, 2915, 2837, 1640, 1519, 1452, 1093, 925; ^1H NMR(300 MHz, DMSO-d_6): δ 2.75–2.84 (m, 1H, CH_2), 3.14–3.21 (m, 1H, CH_2), 3.83 (s, 3H, CH_3), 4.15–4.22 (m, 2H, CH_2 + CH), 4.44 (dd, 1H, J = 6.0, 15.8 Hz, CH_2), 5.34 (d, 1H, J = 3.0 Hz, OH), 6.83 (dd, 1H, J = 1.9, 8.9 Hz, H_{Ar}), 7.13 (d, 1H, J = 1.9 Hz, H_{Ar}), 7.25 (d, 1H, J = 8.9 Hz, H_{Ar}), 8.16 (br t, 1H, J = 5.5 Hz, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 45.5 (CH_2), 48.5 (CH_2), 55.5 (CH_3), 61.8 (Cq), 69.3 (CH), 93.2 (CH), 111.9 (CH), 122.5 (CH), 123.6 (Cq), 132.3 (Cq), 138.0 (Cq), 158.2 (Cq), 164.1 (CO); MS (ESI): *m/z* 373 $[M+H]^+$.

*4.1.5.5. 8-Benzyloxy-4-hydroxy-11-iodo-2,3,4,5-tetrahydro-[1,4]diazepino[1,2-a]indol-1-one (**5e**).* Starting lactone = **4e**. Reaction time: 24 h; chromatography eluent: CH_2Cl_2/MeOH 95:5; yield: 33%; mp 205–206 °C (washing with MeOH); IR (KBr, cm^{-1}): ν 3530, 3253, 2933, 1668, 1613, 1523, 1452, 1078, 743, 698; ^1H NMR (300 MHz, DMSO-d_6): δ 2.75–2.84 (m, 1H, CH_2), 3.14–3.19 (m, 1H, CH_2), 4.15–4.21 (m, 2H, CH_2 + CH), 4.42–4.47 (m, 1H, CH_2), 5.17 (s, 2H, CH_2), 5.34 (d, 1H, J = 3.5 Hz, OH), 6.91 (dd, 1H, J = 1.3, 8.8 Hz, H_{Ar}), 7.25–7.51 (m, 7H, H_{Ar}), 8.18 (br t, 1H, J = 5.3 Hz, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 45.5 (CH_2), 48.6 (CH_2), 61.9 (Cq), 69.4 (CH), 69.8 (CH_2), 94.6 (CH), 112.3 (CH), 122.6 (CH), 123.8 (Cq), 127.9 (3 × CH), 128.8 (2 × CH), 132.5 (Cq), 137.0 (Cq), 138.0 (Cq), 157.3 (Cq), 164.1 (Cq); MS (ESI): *m/z* 449 $[M+H]^+$.

*4.1.5.6. 11-Bromo-4-hydroxy-2,3,4,5-tetrahydro-[1,4]diazepino[1,2-a]indol-1-one (**5f**).* Starting lactone = **4f**. Reaction time: 48 h; chromatography eluent: CH_2Cl_2/MeOH 95:5; yield: 75%; mp > 210 °C (washing with EtOAc); IR (KBr, cm^{-1}): ν 3397, 3221–3100, 2944, 1658, 1537, 1084, 754, 555; ^1H NMR (300 MHz, DMSO-d_6): δ 2.74–2.83 (m, 1H, CH_2), 3.16–3.24 (m, 1H, CH_2), 4.21–4.28 (m, 2H, CH_2 + CH), 4.41–4.48 (m, 1H, CH_2), 5.38 (d, 1H, J = 3.0 Hz, OH), 7.20 (t, 1H, J = 7.2 Hz, H_{Ar}), 7.36 (t, 1H, J = 7.2 Hz, H_{Ar}), 7.52 (d, 1H, J = 8.0 Hz, H_{Ar}), 7.63 (d, 1H, J = 8.0 Hz, H_{Ar}), 8.32 (br t, 1H, J = 5.5 Hz, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 45.2 (CH_2), 48.7 (CH_2), 69.4 (CH), 92.2 (Cq), 110.9 (CH), 119.5 (CH), 120.9 (CH), 124.7 (CH), 125.7 (Cq), 130.3 (Cq), 136.0 (Cq), 163.4 (CO); MS (ESI) *m/z* 295 (^{79}Br), 297 (^{81}Br) $[M+H]^+$.

*4.1.5.7. 11-Bromo-4-hydroxy-8-methoxy-2,3,4,5-tetrahydro-[1,4]diazepino[1,2-a]indol-1-one (**5g**).* Starting lactone = **4g**. Reaction time: 24 h; chromatography eluent: CH_2Cl_2/MeOH 95:5, yield: 76%; mp > 210 °C (washing with MeOH); IR (KBr, cm^{-1}): ν 3457, 3330–3250, 3063, 2944, 2836, 1655, 1540, 1449, 1094; ^1H NMR (300 MHz, DMSO-d_6): δ 2.79–2.85 (m, 1H, CH_2), 3.16–3.23 (m, 1H, CH_2), 3.83 (s, 3H, CH_3), 4.15–4.21 (m, 2H, CH_2 + CH), 4.42–4.45 (m, 1H, CH_2), 5.35 (d, 1H, J = 3.0 Hz, OH), 6.84 (dd, 1H, J = 1.9, 8.9 Hz, H_{Ar}), 7.16 (d, 1H, J = 1.9 Hz, H_{Ar}), 7.37 (d, 1H, J = 8.9 Hz, H_{Ar}), 8.19 (br t, 1H, J = 5.5 Hz, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 45.5 (CH_2), 48.5 (CH_2), 55.6 (CH_3), 69.3 (CH), 92.9 (Cq), 93.3 (CH), 112.2 (CH), 120.1 (Cq), 120.4 (CH), 129.2 (Cq), 137.1 (Cq), 158.3 (Cq), 163.6 (CO); MS (ESI) *m/z* 325 (^{79}Br), 327 (^{81}Br) $[M+H]^+$.

*4.1.6. 4,8-Dihydroxy-11-iodo-2,3,4,5-tetrahydro-[1,4]diazepino[1,2-a]indol-1-one (**5h**)*

1 M BBr_3 solution in CH_2Cl_2 (0.322 mL, 0.322 mmol) was added at 0 °C to a suspension of **5d** (20 mg, 0.054 mmol) in anhydrous CH_2Cl_2 (0.3 mL). The reaction mixture was warmed to RT and stirred for 4 h. Ice was added and the resulting precipitate was collected to provide **5h** (7.0 mg, 36%) as a solid. Mp 140–142 °C (dec.) (washing with CH_2Cl_2); IR (KBr, cm^{-1}): ν 3600–3200, 3050, 2992, 1624, 1526, 1449, 1116, 1083; ^1H NMR (300 MHz, DMSO-d_6): δ 2.73–2.80 (m, 1H, CH_2), 3.13–3.21 (m, 1H, CH_2), 4.08 (dd, J = 3.4, 14.4 Hz, CH_2), 4.11–4.22 (m, 1H, CH), 4.29 (dd, 1H, J = 4.3, 14.4 Hz, CH_2), 5.35 (d, 1H, J = 3.9 Hz, OH), 6.72 (dd, 1H, J = 1.9, 8.6 Hz, H_{Ar}), 6.81 (d, 1H, J = 1.9 Hz, H_{Ar}), 7.17 (d, 1H, J = 8.6 Hz, H_{Ar}), 8.11 (br t, 1H, J = 5.5 Hz, NH), 9.52 (s, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 45.3 (CH_2), 48.9 (CH_2), 62.1 (Cq), 69.3 (CH), 95.0 (CH), 112.2 (CH), 122.6 (CH), 122.9 (Cq), 131.8 (Cq), 138.4 (Cq), 156.1 (Cq), 164.2 (Cq); MS (ESI): *m/z* 359 $[M+H]^+$.

*4.1.7. 4-Hydroxy-2,3,4,5-tetrahydro-[1,4]diazepino[1,2-a]indol-1-one (**6**)*

The compound **5a** (30 mg, 0.09 mmol) was hydrogenated at 10 bars over 10% Pd/C (10 mg) in MeOH (6 mL) at RT for 16 h. The catalyst was filtrated on Celite pad washed with MeOH. The filtrate was concentrated in vacuo and the residue purified by flash chromatography (CH_2Cl_2/MeOH 95:5) to give **6** (17.6 mg, 93%) as a solid. Mp > 210 °C (washing with CH_2Cl_2/MeOH); IR (KBr, cm^{-1}): ν 3315, 3188, 3047, 2923, 1640, 1458, 1380, 1130, 1087, 740; ^1H NMR (300 MHz, DMSO-d_6): δ 2.78–2.87 (m, 1H, CH_2), 3.18–3.23 (m, 1H, CH_2), 4.17–4.23 (m, 2H, CH_2 + CH), 4.39–4.44 (m, 1H, CH_2), 5.37 (d, 1H, J = 3.6 Hz, OH), 6.92 (s, 1H, H_{Ar}), 7.08 (t, 1H, J = 7.5 Hz, H_{Ar}), 7.27 (t, 1H, J = 8.3 Hz, H_{Ar}), 7.56 (d, 1H, J = 8.3 Hz, H_{Ar}), 7.63 (d, 1H, J = 7.5 Hz, H_{Ar}), 8.09 (br s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 45.6 (CH_2), 48.2 (CH_2), 69.5 (CH), 105.3 (CH), 110.4 (CH), 119.9 (CH), 121.6 (CH), 123.5 (CH), 126.0 (Cq), 134.7 (Cq), 137.5 (Cq), 165.5 (CO); MS (spray ionique): *m/z* 217 $[M+H]^+$.

*4.1.8. 4-Hydroxy-11-methyl-2,3,4,5-tetrahydro-[1,4]diazepino[1,2-a]indol-1-one (**7**)*

To a solution of **5a** (50 mg, 0.146 mmol) and $Pd(PPh_3)_4$ (8.4 mg, 0.007 mmol) in anhydrous THF (1.5 mL) was added 2 M $AlMe_3$ in toluene (0.22 mL, 0.44 mmol) at RT. The mixture was refluxed overnight, cooled to RT and a saturated aqueous of Rochelle salt solution was added. The mixture was extracted with CH_2Cl_2 (3 × 20 mL) and combined organic layers washed with brine (20 mL), dried over Na_2SO_4 and evaporated in vacuo. The crude solid was triturated with EtOAc then recrystallized from EtOH to give **7** (23 mg, 68%). Mp > 210 °C (EtOH); IR (KBr, cm^{-1}): ν 3400–3200, 3070, 2911, 1629, 1554, 1065, 731; ^1H NMR (300 MHz, DMSO-d_6): δ 2.40 (s, 3H, CH_3), 2.78–2.83 (m, 1H, CH_2), 3.14–3.19 (m, 1H, CH_2), 4.12–4.21 (m, 2H, CH_2 + CH), 4.36 (dd, 1H, J = 4.0, 13.6 Hz, CH_2), 5.30 (d, 1H, J = 3.8 Hz, OH), 7.06 (t, 1H, J = 7.5 Hz, H_{Ar}), 7.24 (t, 1H, J = 7.9 Hz, H_{Ar}), 7.50 (d, 1H, J = 8.3 Hz, H_{Ar}), 7.59 (d, 1H, J = 7.9 Hz, H_{Ar}), 8.01 (br t, 1H, J = 5.4 Hz, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 9.1 (CH_3), 45.6 (CH_2), 48.0 (CH_2), 69.4 (CH), 110.1 (CH), 114.9 (Cq), 119.1 (CH), 119.7 (CH), 123.7 (CH), 126.9 (Cq), 129.8 (Cq), 136.5 (Cq), 165.8 (CO); MS (ESI): *m/z* 231 $[M+H]^+$.

*4.1.9. 11-Cyano-4-hydroxy-2,3,4,5-tetrahydro-[1,4]diazepino[1,2-a]indol-1-one (**8**)*

A mixture of **5a** (200 mg, 0.58 mmol), $Pd(PPh_3)_4$ (34 mg, 0.03 mmol) and $Zn(CN)_2$ (68.6 mg, 0.58 mmol) in anhydrous DMF (2.9 mL) was heated under microwave irradiation at 150 °C for 5 min. After cooling, H_2O was added and the mixture extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, filtered and evaporated in vacuo. The crude solid was triturated with EtOAc, filtered off to give **8** (116 mg, 82%) as a solid. Mp > 210 °C (washing with EtOAc); IR (KBr, cm^{-1}): ν 3187, 3070, 2911, 2221, 1647, 1546, 1423, 1097, 749 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.79–2.86 (m, 1H, CH_2), 3.24–3.30

(m, 1H, CH_2), 4.30–4.37 (m, 2H, CH_2 + CH), 4.53 (dd, 1H, J = 4.5, 14.4 Hz, CH_2), 5.48 (d, 1H, J = 4.1 Hz, OH), 7.34 (t, 1H, J = 7.3 Hz, H_{Ar}), 7.46 (t, 1H, J = 7.1 Hz, H_{Ar}), 7.72 (d, 1H, J = 7.9 Hz, H_{Ar}), 7.78 (d, 1H, J = 8.5 Hz, H_{Ar}), 8.62 (br t, 1H, J = 5.5 Hz, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 45.0 (CH_2), 49.0 (CH_2), 69.5 (CH), 86.7 (CN), 112.0 (CH), 114.6 (Cq), 119.3 (CH), 122.8 (CH), 125.4 (CH), 126.2 (Cq), 136.2 (Cq), 140.0 (Cq), 162.5 (CO); MS (ESI): m/z 264 $(M + Na)^+$.

4.1.10. 4-Hydroxy-11-phenyl-2,3,4,5-tetrahydro-[1,4]diazepino[1,2-a]indol-1-one (**9**) (CAS: 918161-95-2)

To a solution of **5a** (100 mg, 0.29 mmol) in DME/H_2O (1:1) (5 mL) were added successively Na_2CO_3 (61 mg, 0.58 mmol), benzeneboronic acid (71 mg, 0.58 mmol) and 10% Pd/C (16 mg). The mixture was stirred at 85 °C for 4 h then cooled to RT and filtrated over a Celite pad washed with MeOH. The filtrate was concentrated in vacuo, H_2O was added (10 mL) and the mixture was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated in vacuo. The crude product was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH 95:5) to give **9** (75 mg, 88%) as a solid. Mp > 210 °C (washing with EtOAc); IR (KBr, cm^{-1}): ν 3420, 3310–3250, 3070, 2980, 1640, 1550, 1490, 1085, 750, 700; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.82–2.91 (m, 1H, CH_2), 3.20–3.30 (m, 1H, CH_2), 4.25–4.30 (m, 2H, CH_2 + CH), 4.42–4.48 (m, 1H, CH_2), 5.38 (d, 1H, J = 3.4 Hz, OH), 7.12 (t, 1H, J = 7.5 Hz, H_{Ar}), 7.29–7.64 (m, 8H, H_{Ar}), 8.25 (br t, 1H, J = 5.9 Hz, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 45.3 (CH_2), 47.9 (CH_2), 69.6 (CH), 110.5 (CH), 118.4 (C), 119.9 (CH), 120.3 (CH), 123.8 (CH), 125.4 (C), 126.4 (CH), 128.1 (2 CH), 129.7 (2 × CH), 129.9 (Cq), 133.7 (Cq), 136.3 (Cq), 164.8 (CO); MS (ESI): m/z 293 $[M+H]^+$.

4.1.11. 4-Hydroxy-11-(pent-1-ynyl)-2,3,4,5-tetrahydro-[1,4]diazepino[1,2-a]indol-1-one (**10**) (CAS: 918161-99-6)

To a solution of **5a** (100 mg, 0.29 mmol), CuI (5.6 mg, 0.03 mmol) and $Pd(PPh_3)_2Cl_2$ (20.5 mg, 0.03 mmol) in anhydrous DMF (3 mL) was added Et_3N (0.17 mL, 1.23 mmol) and pent-1-yne (0.20 mL, 2.03 mmol). The mixture was stirred at 50 °C for 4 h then concentrated in vacuo. H_2O (15 mL) was added and the mixture was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH 95:5) to give **10** (52 mg, 63%) as a solid. Mp 200–202 °C (washing with EtOAc/Et_2O); IR (KBr, cm^{-1}): ν 3388–3100, 3300, 3080, 2961, 1657, 1551, 1449, 1367, 1085, 745; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.05 (t, 3H, J = 7.4 Hz, CH_3), 1.53–1.65 (m, 2H, CH_2), 2.73–2.80 (m, 1H, CH_2), 3.14–3.20 (m, 3H, 2 × CH_2), 4.19–4.23 (m, 2H, CH_2 + CH), 4.36–4.44 (m, 1H, CH_2), 5.37 (d, 1H, J = 3.8 Hz, OH), 7.16 (t, 1H, J = 7.2 Hz, H_{Ar}), 7.32 (t, 1H, J = 7.7 Hz, H_{Ar}), 7.56–7.62 (m, 2H, H_{Ar}), 8.17 (br t, 1H, J = 5.9 Hz, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.3 (CH_3), 21.2 (CH_2), 21.9 (CH_2), 45.2 (CH_2), 48.3 (CH_2), 69.4 (CH), 73.2 (C), 94.2 (C), 100.2 (C), 110.8 (CH), 120.0 (CH), 120.6 (CH), 124.4 (CH), 127.7 (C), 134.9 (C), 135.9 (C), 163.8 (CO); MS (ESI): m/z 283 $[M+H]^+$.

4.1.12. Methyl (E)-3-(4-hydroxy-1-oxo-2,3,4,5-tetrahydro-[1,4]diazepino[1,2-a]indol-11-yl) acrylate (**11**) (CAS: 918161-85-0)

To a solution of **5a** (50 mg, 0.15 mmol) in DMF (1.7 mL) was successively added $Pd(OAc)_2$ (3.3 mg, 0.02 mmol), PPh_3 (7.7 mg, 0.03 mmol), Et_3N (35 μL, 1.23 mmol) and methyl acrylate (132 μL, 1.50 mmol). The mixture was stirred at 90 °C for 5 h, cooled and filtrated over a Celite pad washed with CH_2Cl_2. After removal of the solvent, the crude solid was triturated with CH_2Cl_2 then filtered off to give **11** (38 mg, 87%) as a solid. Mp > 210 °C (washing with CH_2Cl_2); IR (KBr, cm^{-1}): ν 3423, 3269–3195, 3061, 2944, 1685, 1665, 1623, 1383, 983, 731; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.74–2.80 (m, 1H, CH_2), 3.16–3.24 (m, 1H, CH_2), 3.72 (s, 3H, CH_3), 4.24–4.30 (m, 2H, CH_2 + CH), 4.43–4.50 (m, 1H, CH_2), 5.42 (d, 1H, J = 3.8 Hz, OH), 6.56 (d, 1H, J = 16.4 Hz, =CH), 7.26 (t, 1H, J = 7.6 Hz, H_{Ar}), 7.40 (t, 1H, J = 7.6 Hz, H_{Ar}), 7.69 (d, 1H, J = 8.3 Hz, H_{Ar}), 8.01 (d, 1H, J = 8.3 Hz, H_{Ar}), 8.20 (d, 1H, J = 16.4 Hz, =CH), 8.51 (br t, 1H, J = 5.5 Hz, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 45.2 (CH_2), 48.3 (CH_2), 51.2 (CH_3), 69.5 (CH), 111.3 (CH), 112.7 (C), 114.4 (CH), 121.3 (CH), 122.0 (CH), 124.0 (C), 124.6 (CH), 135.7 (C), 137.1 (C), 137.4 (CH), 164.3 (C), 167.5 (CO); MS (ESI): m/z 301 $[M+H]^+$.

4.1.13. 4-Hydroxy-11-Pentyl-2,3,4,5-tetrahydro-[1,4]diazepino[1,2-a]indol-1-one (**12**)

The compound **10** (30 mg, 0.11 mmol) was hydrogenated at 20 bars over 10% Pd/C (6 mg) in MeOH/THF (5:1) (6 mL) at RT for 18 h. The catalyst was removed on Celite pad washed with MeOH. After evaporation of the solvent, the crude product was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH 95:5) to give **12** (27.9 mg, 92%) as a solid. Mp 160–162 °C (washing with CH_2Cl_2); IR (KBr, cm^{-1}): ν 3300–3152, 3052, 2925, 1637, 1557, 1454, 1365, 1097, 736; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.83 (t, 3H, J = 6.8 Hz, CH_3), 1.24–1.27 (m, 4H, 2 × CH_2), 1.54–1.63 (m, 2H, CH_2), 2.73–2.81 (m, 1H, CH_2), 2.85–2.95 (m, 2H, CH_2), 3.12–3.18 (m, 1H, CH_2), 4.09–4.17 (m, 2H, CH_2 + CH), 4.33–4.39 (m, 1H, CH_2), 5.32 (d, 1H, J = 3.6 Hz, OH), 7.05 (t, 1H, J = 7.4 Hz, H_{Ar}), 7.24 (t, 1H, J = 7.4 Hz, H_{Ar}), 7.49 (d, 1H, J = 8.5 Hz, H_{Ar}), 7.61 (d, 1H, J = 8.1 Hz, H_{Ar}), 8.03 (br t, 1H, J = 5.6 Hz, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.0 (CH_3), 21.9 (CH_2), 23.3 (CH_2), 30.2 (CH_2), 31.0 (CH_2), 45.5 (CH_2), 47.9 (CH_2), 69.5 (CH), 110.2 (CH), 119.1 (CH), 119.7 (Cq), 119.8 (CH), 123.5 (CH), 126.2 (Cq), 129.8 (Cq), 136.4 (Cq), 165.7 (CO); MS (ESI): m/z 287 $[M+H]^+$.

4.1.14. Ethyl 3-allyl-1-(tert-butoxycarbonyl)-1H-indole-2-carboxylate (**14**)

To a solution of **13** (2.5 g, 6.0 mmol) and $Pd(PPh_3)_4$ (0.7 g, 0.6 mmol) in anhydrous toluene (60 mL) was added allyltributylstananne (2.3 mL, 7.2 mmol) at RT and the mixture was then stirred at reflux for 20 h. After cooling, the mixture was diluted with H_2O (50 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine and dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel (PE/Et_2O 95:5) to give **14** (1.7 g, 85%) as an oil. IR (neat, cm^{-1}): ν 3070, 2975, 2920, 1730, 1100, 990, 920, 745; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.39 (t, 3H, J = 7.2 Hz, CH_3), 1.63 (s, 9H, 3 × CH_3), 3.61 (dt, 2H, J = 1.5, 6.2 Hz, CH_2), 4.39 (q, 2H, J = 7.2 Hz, CH_2), 5.03–5.14 (m, 2H, =CH_2), 5.90–6.03 (m, 1H, =CH), 7.26 (dt, 1H, J = 0.8, 8.4 Hz, H_{Ar}), 7.40 (dt, 1H, J = 1.3, 7.7 Hz, H_{Ar}), 7.59 (d, 1H, J = 7.7 Hz, H_{Ar}), 8.07 (d, 1H, J = 8.4 Hz, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.3 (CH_3), 28.1 (3 × CH_3), 28.8 (CH_2), 61.5 (CH_2), 84.5 (Cq), 115.2 (CH), 116.1 (=CH_2), 120.6 (CH), 123.1 (CH), 124.3 (Cq), 126.7 (CH), 127.4 (Cq), 128.7 (Cq), 135.5 (CH), 136.8 (Cq), 149.5 (CO), 162.7 (CO); MS (ESI): m/z 330 $[M+H]^+$.

4.1.15. 3-Allyl-1H-indole-2-carboxylic acid (**15**) (CAS: 908105–30-6)

To a solution of **14** (1.5 g, 4.6 mmol) in EtOH (40 mL) was added NaOH (400 mg, 10.1 mmol) and H_2O (9 mL). The solution was stirred at reflux for 4 h and then concentrated in vacuo. The crude residue was acidified with 1 N HCl (pH 1) and extracted with EtOAc (3 × 30 mL). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The crude product was triturated with Et_2O/PE, sonicated and the resulting solid was filtered off to give **15** (0.84 g, 92%). Mp 119–121 °C (Et_2O/PE); IR (KBr, cm^{-1}): ν 3420, 3060–2500, 1680, 1550, 1090, 745; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.96 (dd, 2H, J = 1.4, 4.8 Hz, CH_2), 5.02–5.15 (m, 2H, =CH_2), 6.00–6.13 (m, 1H, =CH), 7.16 (dt, 1H, J = 1.4, 7.3 Hz, H_{Ar}), 7.36 (dt, 1H, J = 1.0, 8.3 Hz, H_{Ar}), 7.41 (d, 1H, J = 8.3 Hz, H_{Ar}), 7.73 (d, 1H, J = 7.3 Hz, H_{Ar}), 8,77 (s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 29.3

(CH_2), 112.0 (CH), 115.4 (=CH_2), 120.5 (CH), 121.5 (CH), 122.3 (Cq), 124.6 (Cq), 126.5 (CH), 128.0 (Cq), 136.6 (Cq), 136.6 (=CH), 167.8 (CO); MS (ESI): *m/z* 202 $[M+H]^+$.

4.1.16. Methyl 3-Allyl-1-methyl-indole-2-carboxylate (**16**)

To a solution of **15** (455 mg, 2.26 mmol) in DMF (11 mL) was added NaH (60% dispersion in oil, 271 mg, 6.8 mmol) portionwise at 0 °C. The mixture was stirred at 0 °C for 1 h, then MeI (0.56 ml, 9.0 mmol) was added and the reaction mixture was stirred overnight at RT. H_2O (10 mL) was carefully added and the mixture was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine and dried over Na_2SO_4, filtered and concentrated in vacuo. The crude residue was purified by flash chromatography on silica gel (PE/EtOAc 95:5) to give **16** (500 mg, 96%) as an oil. IR (neat, cm^{-1}): ν 3050, 2948, 1701, 1465, 1190, 1240, 1102, 910, 739; ^1H NMR (300 MHz, $CDCl_3$): δ 3.86 (d, 2H, J = 6.2 Hz, CH_2), 3.95 (s, 3H, CH_3), 4.00 (s, 3H, CH_3), 4.99 (dd, 1H, J = 1.7, 10.0 Hz, =CH_2), 5.06 (dd, 1H, J = 1.7, 17.1 Hz, =CH_2), 5.94–6.07 (m, 1H, =CH), 7.11–7.18 (m, 1H, H_{Ar}), 7.35–7.37 (m, 2H, H_{Ar}), 7.69 (d, 1H, J = 8.1 Hz, H_{Ar}); ^{13}C RMN (75 MHz, $CDCl_3$): δ 29.8 (CH_2), 32.0 (CH_3), 51.3 (CH_3), 110.1 (CH), 114.6 (=CH_2), 119.9 (CH), 120.8 (CH), 122.2 (Cq), 124.7 (Cq), 125.3 (CH), 126.6 (Cq), 137.3 (CH), 138.8 (Cq), 163.1 (CO); MS (ESI): *m/z* 230 $[M+H]^+$.

4.1.17. 3-Allyl-1-methyl-indole-2-carboxylic acid (**17**)

To a solution of **16** (518 mg, 2.26 mmol) in THF/MeOH/H_2O (6:3:1) (10 mL) was added LiOH.H_2O (190 mg, 5.52 mmol). The reaction mixture was stirred at reflux for 1 h and then concentrated in vacuo. The crude residue was acidified with 1 N HCl (pH 1) and extracted with EtOAc (3 × 30 mL). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The crude product was triturated with pentane, sonicated and the resulting solid was filtered off to give **17** (378 mg, 78%). Mp 134–136 °C (washing with pentane); IR (KBr, cm^{-1}): ν 3300–2500, 2926, 1657, 1520, 1271, 912, 606; ^1H NMR (300 MHz, $CDCl_3$): δ 4.02 (d, J = 6.2 Hz, CH_2), 4.09 (s, 3H, CH_3), 5.07 (dd, 1H, J = 1.6, 9.9 Hz, =CH_2), 5.16 (dd, 1H, J = 1.6, 17.1 Hz, =CH_2), 6.07–6.21 (m, 2H, =CH), 7.20 (dt, 1H, J = 1.4, 7.3 Hz, H_{Ar}), 7.39–7.47(m, 2H, H_{Ar}), 7.77 (d, 1H, J = 8.0 Hz, H_{Ar}), 12.79 (s, 1H, OH); ^{13}C RMN (75 MHz, $CDCl_3$): δ 30.0 (CH_2), 32.5 (CH_3), 110.4 (CH), 115.4 (=CH_2), 120.2 (CH), 121.3 (CH), 123.7 (Cq), 125.3 (Cq), 126.2 (CH), 126.6 (Cq), 137.1 (CH), 139.5 (CH), 168.6 (CO); MS (ESI): *m/z* 216 $[M+H]^+$.

4.1.18. 3-(Iodomethyl)-4,9-dihydro-3H-pyrano[3,4-b]indol-1-one (**18a**)

2,6-Lutidine (174 μL, 1.5 mmol) and *N*-iodosuccinimide (514 mg, 2.3 mmol) were added successively at −20 °C to a solution of **15** (200 mg, 1.0 mmol) in anhydrous CH_2Cl_2 (15 mL) The mixture was stirred at that temperature for 3.5 h. 6 N HCl (10 mL) was added and the mixture was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic layers were washed with saturated aqueous $Na_2S_2O_3$ (2 × 20 mL), brine (2 × 20 mL), dried over Na_2SO_4 and concentrated in vacuo. The crude solid was washed with EtOH to give **18a** (244 mg, 75%) as a solid. Mp 188–190 °C; IR (KBr, cm^{-1}): ν 3290, 3060, 2980, 1690, 1550, 1160, 1080, 740; ^1H NMR (300 MHz, DMSO-d_6): δ 3.04 (dd, 1H, J = 11.7, 16.8 Hz, CH_2), 3.34 (dd, 1H, J = 3.8, 16.8 Hz, CH_2), 3.63–3.74 (m, 2H, CH_2), 4.68–4.75 (m, 1H, CH), 7.13 (t, 1H, J = 7.5 Hz, H_{Ar}), 7.33 (t, 1H, J = 7.3 Hz, H_{Ar}), 7.44 (d, 1H, J = 8.3 Hz, H_{Ar}), 7.69 (d, 1H, J = 8.1 Hz, H_{Ar}), 11.98 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 8.9 (CH_2), 26.6 (CH_2), 78.7 (CH), 112.9 (CH), 120.2 (CH), 121.0 (CH), 121.1 (Cq), 121.8 (Cq), 124.0 (Cq), 126.1 (CH), 138.5 (Cq), 159.6 (CO); MS (ESI): *m/z* 328 $[M+H]^+$.

4.1.19. 3-(Iodomethyl)-9-methyl-3,4-dihydropyrano[3,4-b]indol-1-one (**18b**)

The compound **18b** was prepared from **17** according to the procedure described for the preparation of **18a**. The crude residue was purified by flash chromatography on silica gel (PE/EtOAc 95:5 to 7:3) to give **18b**; yield: 72%; mp 107–109 °C (washing with pentane); IR (KBr, cm^{-1}): ν 3060, 2980, 1700, 1080, 740 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.13 (dd, 1H, J = 11.3, 16.6 Hz, CH_2), 3.44 (dd, 1H, J = 4.0, 16.6 Hz, CH_2), 3.53 (dd, 1H, J = 7.1, 10.4 Hz, CH_2), 3.59 (dd, 1H, J = 4.7, 10.4 Hz, CH_2), 4.07 (s, 3H, CH_3), 4.66–4.75 (m, 1H, CH), 7.20 (t, 1H, J = 7.5 Hz, H_{Ar}), 7.38–7.47 (m, 2H, H_{Ar}), 7.64 (d, 1H, J = 8.1 Hz, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 5.9 (CH_2), 27.1 (CH_2), 31.3 (CH_3), 79.0 (CH), 110.7 (CH), 120.8 (CH), 121.0 (CH), 121.5 (Cq), 121.7 (Cq), 123.4 (Cq), 126.7 (CH), 140.2 (Cq), 159.6 (CO); MS (ESI): *m/z* 342 $[M+H]^+$.

4.1.20. 4-Hydroxy-3,4,5,10-tetrahydro-2H-azepino[3,4-b]indol-1-one (**19a**)

A solution of **18a** (220 mg, 0.67 mmol) in anhydrous MeOH/THF (3:1) (40 mL) was added dropwise over 40 min to a saturated ammonia solution in MeOH (5 mL). The mixture was stirred at 0 °C for 30 min then 4 days at RT and finally evaporated. The crude residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH 92:8) to give **19a** (106 mg, 73%) as a solid. Mp 188–190 °C (washing with CH_2Cl_2); IR (KBr, cm^{-1}): ν 3400–3100, 3040, 2905, 1640, 1545, 1330, 1070, 750; ^1H NMR (300 MHz, DMSO-d_6): δ 2.90 (dd, 1H, J = 8.9, 16.9 Hz, CH_2), 3.24–3.32 (m, 3H, 2 × CH_2), 3.95–4.10 (m, 1H, CH), 5.22 (d, 1H, J = 4.3 Hz, OH), 7,02 (t, 1H, J = 7.5 Hz, H_{Ar}), 7.20 (t, 1H, J = 7.3 Hz, H_{Ar}), 7.38 (d, 1H, J = 8.1 Hz, H_{Ar}), 7.55 (d, 1H, J = 8.1 Hz, H_{Ar}), 7.92 (br s, 1H, NH), 11.19 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 33.9 (CH_2), 48.3 (CH_2), 67.8 (CH), 112.0 (CH), 113.3 (Cq), 119.1 (CH), 119.9 (CH), 124.2 (CH), 126.6 (Cq), 127.4 (Cq), 135.9 (Cq), 164.0 (CO); MS (ESI): *m/z* 217 $[M+H]^+$.

4.1.21. 4-Hydroxy-10-methyl-3,4,5,10-tetrahydro-2H-azepino[3,4-b]indol-1-one (**19b**)

The compound **19b** was prepared from **18b** according to the procedure described for the preparation of **19a**. The crude residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH 95:5 to 9:1) to give **19b**; yield: 53%; mp 178–179 °C (washing with CH_2Cl_2); IR (KBr, cm^{-1}): ν 3400–3100, 3060, 2910, 1620, 1530, 1320, 1040, 730; ^1H NMR (300 MHz, DMSO-d_6): δ 2.81 (dd, 1H, J = 6.4, 15.5 Hz, CH_2), 3.00 (dt, 1H, J = 6.0, 14.2 Hz, CH_2), 3.14–3.23 (m, 2H, CH_2), 3.87 (s, 3H, CH_3), 4.10–4.20 (m, 1H, CH), 5.09 (d, 1H, J = 4.3 Hz, OH), 7.10 (t, 1H, J = 7.5 Hz, H_{Ar}), 7.28 (t, 1H, J = 7.3 Hz, H_{Ar}), 7.48 (d, 1H, J = 8.5 Hz, H_{Ar}), 7.63 (d, 1H, J = 8.0 Hz, H_{Ar}), 8.05 (br t, 1H, J = 5.6 Hz, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 30.7 (CH_2), 31.1 (C), 47.9 (CH_2), 71.5 (CH), 110.2 (CH), 115.8 (Cq), 119.5 (CH), 119.7 (CH), 123.9 (CH), 125.9 (Cq), 129.0 (Cq), 137.9 (Cq), 166.1 (CO); MS (ESI): *m/z* 231 $[M+H]^+$.

4.2. Biology

4.2.1. Kinase preparations and assays

Kinase activities were assayed in Buffer A (10 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT, 25 mM Tris–HCl pH 7.5, 50 μg heparin/mL) or C (60 mM β-glycerophosphate, 15 mM p-nitrophenylphosphate, 25 mM Mops (pH 7.2), 5 mM EGTA, 15 mM $MgCl_2$, 1 mM DTT, 1 mM sodium vanadate, 1 mM phenylphosphate), at 30 °C, at a final ATP concentration of 15 μM. Blank values were subtracted and activities expressed in % of the maximal activity, i.e. in the absence of inhibitors. Controls were performed with appropriate dilutions of DMSO. The GSK-3 peptide substrate was obtained from Millegen (Labège, France).

CDK1/cyclin B (M phase starfish oocytes, native), and *CDK5/p25* (human, recombinant) were prepared as previously described [25]. Their kinase activity was assayed in buffer C, with 1 mg histone H1/mL, in the presence of 15 μM [γ-^{33}P] ATP (3000 Ci/mmol; 10 mCi/mL) in a final volume of 30 μL. After 30 min incubation at 30 °C, the reaction was stopped by harvesting onto P81 phosphocellulose papers (Whatman) using a FilterMate harvester (Packard) and were washed in 1% phosphoric acid. Scintillation fluid was added and incorporated radioactivity measured in a Packard counter.

GSK-3α/β (porcine brain, native) was assayed, as described for CDK1 but in Buffer A and using a GSK-3 specific substrate (GS-1: YRRAAVPPSPSLSRHSSPHQSpEDEEE) (pS stands for phosphorylated serine) [26].

4.3. Molecular modelling

4.3.1. Quantum calculations

Geometry optimizations were performed at the density functional theory (DFT) level using the B3LYP [27,28] hybrid functional as implemented in the Gaussian program code [29]. Carbon, hydrogen, oxygen and nitrogen atoms were described by their 6-311G (d,p) all-electron polarized triple-zeta basis sets [30]. Iodine atoms were represented by means of a Stuttgart–Dresden effective core potentials in association with their basis sets [31], augmented by a d-polarization function ($\alpha = 0.730$) [32]. Optimizations were carried out without any symmetry restraints. The nature of optimized extrema (minimum or transition state) was verified by a frequency calculation. The two conformers were both considered in the docking procedure.

4.3.2. Molecular docking

The docking was performed on the 1UNL [33] Cdk5 protein structure. Protein was preprocessed in AutoDockTool. Ligands were built and optimized at the AM1 level, Gasteiger charges were assigned with AutoDockTool on ligand optimized geometry. In the docking procedure, all σ-bonds not involved in cycles were unconstrained, side chain of Gln 130, Lys 133 and Asn 144 were considered as flexible in the docking protocol. Lamarkian genetic algorithm with up to 2.5 10^6 evaluations per run were used. Analysis of the molecular docking and clustering were carried out with AutoDockTool. The 5 ligand-CDK5 co-crystals (PDB code 1UNG, 1UNH, 1UNL [33], 3O0G [34], 4AU8 [35]) were considered as a benchmark set for the docking protocol. Molecular docking was achieved with Autodock 4 [36] the following parameterization allows to reproduce the position of aloisine, indirubin, (R)-roscovitine, {4-amino-2-[(4-chlorophenyl)amino]-1,3-thiazol-5-yl}(3-nitrophenyl)methanone and 4-(1,3-benzothiazol-2-yl)thiophene-2-sulfonamide.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.06.063.

References

[1] S. Lim, P. Kaldis, Cdks, cyclins and CKIs: roles beyond cell cycle regulation, Development 140 (2013) 3079–3093.
[2] S. Wang, P.M. Fischer, Cyclin-dependent kinase 9: a key transcriptional regulator and potential drug target in oncology, virology and cardiology, Trends Pharmacol. Sci. 26 (2008) 302–313.
[3] M. Malumbres, M. Barbacid, Cell cycle, CDKs and cancer: a changing paradigm, Nat. Rev. Cancer 9 (2009) 153–166.
[4] Z.H. Cheung, N.Y. Ip, CDK5: a multifaceted kinase in neurodegenerative diseases, Trends. Cell. Biol. 22 (2012) 169–175.
[5] M. Slevin, J. Krupinski, Cyclin-dependent kinase-5 targeting for ischaemic stroke, Curr. Opin. Pharmacol. 9 (2009) 119–124.
[6] S. Lapenna, A. Giordano, Cell cycle kinases as therapeutic targets for cancer, Nat. Rev. Drug. Discov. 8 (2009) 548–566.
[7] J. Cicenas, M. Valius, The CDK inhibitors in cancer research and therapy, J. Cancer Res. Clin. Oncol. 137 (2011) 1409–1418.
[8] H. Galons, N. Oumata, O. Gloulou, L. Meijer, Cyclin-dependent kinases inhibitors closer to market launch? Expert. Opin. Ther. Pat. 23 (2013) 945–963.
[9] Echalier, K. Bettayeb, Y. Ferandin, O. Lozach, M. Clement, A. Valette, F. Liger, B. Marquet, J.C. Morris, J.A. Endicott, B. Joseph, L. Meijer, Meriolins (3-(pyrimidin-4-yl)-7-azaindoles): synthesis, kinase inhibitory activity, cellular effects and structure of a CDK2/cyclin A/meriolin complex, J. Med. Chem. 51 (2008) 737–751.
[10] F. Popowycz, G. Fournet, C. Schneider, K. Bettayeb, Y. Ferandin, C. Lamigeon, O.M. Tirado, S. Mateo-Lozano, V. Notario, P. Colas, P. Bernard, L. Meijer, B. Joseph, Pyrazolo[1,5-*a*]-1,3,5-triazine as a purine bioisotere: access to potent cyclin-dependent kinase inhibitor (*R*)-roscovitine analogue, J. Med. Chem. 52 (2009) 655–663.
[11] A. Putey, G. Fournet, B. Joseph, General and easy access to 11-substituted 4-hydroxy-2,3,4,5-tetrahydro[1,4]diazepino[1,2-*a*]indol-2-one derivatives, Synlett (2006) 2755–2757.
[12] A. Putey, F. Popowycz, B. Joseph, A non-classical route to 2,3-diiodoindoles from indole-2-carboxylic acids, Synlett (2007) 419–422.
[13] D.L. Boger, L.R. Cerbone, D. Yohannes, Oxidative coupling of methyl 6-hydroxyindole-2-carboxylate with primary amines: preparation of 2-substituted methyl pyrrolo[2,3-e]benzoxazole-5-carboxylates, J. Org. Chem. 53 (1988) 5163–5166.
[14] M. Tani, H. Ikegami, M. Tashiro, T. Hiura, H. Tsukioka, C. Kaneko, T. Notoya, M. Shimizu, M. Uchida, Y. Aida, Y. Yokoyama, Y. Murakami, Regioselective bromination of methoxy derivatives of ethyl indole-2-carboxylate, Heterocycles 34 (1992) 2349–2362.
[15] H. Kosaku, K. Yukio, K. Yoshitake, I. Yoshiaki, M. Yoshifumi, Facile synthesis of thymidine derivatives by cross-coupling of 5-halogenouridine derivatives with trimethylaluminium, Synthesis (1993) 213–215.
[16] M. Alterman, A. Hallberg, Fast microwave-assisted preparation of aryl and vinyl nitriles and the corresponding tetrazoles from organo-halides, J. Org. Chem. 65 (2000) 7984–7989.
[17] K. Sonogashira, Y. Tohda, N. Hagihara, A convenient synthesis of acetylenes: catalytic substitutions of acetylenic hydrogen with bromoalkenes, iodoarenes, and bromopyridines, Tetrahedron Lett. 16 (1975) 4467–4470.
[18] R.F. Heck, J.P. Nolley Jr., Palladium-catalyzed vinylic hydrogen substitution reactions with aryl, benzyl, and styryl halides, J. Org. Chem. 37 (1972) 2320–2322.
[19] N. Miyaura, A. Suzuki, Stereoselective of arylated (*E*)-alkenes by the reaction of alk-1-enylboranes with aryl halides in the presence of palladium catalyst, J. Chem. Soc. Chem. Commun. (1979) 866–867.
[20] J.T. Kuethe, K.M. Maloney, A concise synthesis of 3,4-fused spiro[isobenzofuran-3-ones], spiro[furo[3,4-*b*]pyridin-5(7*H*)-ones], 3-aryl, and alkylphthalides, Tetrahedron 69 (2013) 5248–5258.
[21] C.A. Hunter, K.R. Lawson, J. Perkins, C.J. Urch, Aromatic interactions, J. Chem. Soc. Perkin Transm. 2 (2001) 651–699.
[22] S.L. Cockroft, C.A. Hunter, Chemical double-mutant cycles: dissecting non-covalent interactions, Chem. Soc. Rev. 36 (2007) 172–188.
[23] S.E. Wheeler, K.N. Houk, Substituent effects in the benzene dimer are due to direct interactions, J. Am. Chem. Soc. 130 (2008) 10854–10855.
[24] J.C. Ma, D.A. Dougherty, The cation-π interaction, Chem. Rev. 97 (1997) 1303–1324.
[25] K. Bettayeb, N. Oumata, A. Echalier, Y. Ferandin, J.A. Endicott, H. Galons, L. Meijer, CR8, a potent and selective, roscovitine-derived inhibitor of cyclin-dependent kinase inhibitors, Oncogene 27 (2008) 5797–5807.
[26] S. Bach, M. Knockaert, J. Reinhardt, O. Lozach, S. Schmitt, B. Baratte, M. Koken, S.P. Coburn, L. Tang, T. Jiang, D.C. Liang, H. Galons, J.F. Dierick, L.A. Pinna, F. Meggio, F. Totzke, C. Schaechtele, A.S. Lerman, A. Camero, Y. Wan, N. Gray, L. Meijer, Roscovitine targets, protein kinases and pyridoxal kinase, J. Biological Chem. 280 (2005) 31208–31219.
[27] A.D. Becke, Density-functional thermochemistry. III. The role of exact exchange, J. Chem. Phys. 98 (1993) 5648–5652.
[28] C. Lee, W. Yang, R.G. Parr, Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density, Phys. Rev. B 37 (1988) 785–789.
[29] Gaussian 09, Revision D.01, M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G.A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H.P. Hratchian, A.F. Izmaylov,

J. Bloino, G. Zheng, J.L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J.A. Montgomery Jr., J.E. Peralta, F. Ogliaro, M. Bearpark, J.J. Heyd, E. Brothers, K.N. Kudin, V.N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J.M. Millam, M. Klene, J.E. Knox, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, R.L. Martin, K. Morokuma, V.G. Zakrzewski, G.A. Voth, P. Salvador, J.J. Dannenberg, S. Dapprich, A.D. Daniels, Ö. Farkas, J.B. Foresman, J.V. Ortiz, J. Cioslowski, D.J. Fox, Gaussian, Inc, Wallingford CT, 2009.

[30] K. Raghavachari, J.S. Binkley, R. Seeger, J.A. Pople, Self-consistent molecular orbital methods. 20. Basis set for correlated wave-functions, J. Chem. Phys. 72 (1980) 650–654.

[31] A. Bergner, M. Dolg, W. Kuechle, H. Stoll, H. Preuss, Ab initio energy-adjusted pseudopotentials for elements of groups 13–17, Mol. Phys. 80 (1993) 1431–1441.

[32] L. Maron, C. Teichteil, On the accuracy of averaged relativistic shape-consistent pseudopotentials, Chem. Phys. 237 (1998) 105–122.

[33] M. Mapelli, L. Massimiliano, C. Crovace, M.A. Seeliger, L.-H. Tsai, L. Meijer, A. Musacchio, Mechanism of CDK5/p25 binding by CDK inhibitors, J. Med. Chem. 48 (2005) 671–679.

[34] J.S. Ahn, M.L. Radhakrishnan, M. Mapelli, S. Choi, B. Tidor, G.D. Cuny, A. Musacchio, L.-A. Yeh, K.S. Kosik, Defining Cdk5 ligand chemical space with small molecule inhibitors of tau phosphorylation, Chem. Biol. 12 (2005) 811–823.

[35] J. Malmström, J. Viklund, C. Slivo, A. Costa, M. Maudet, C. Sandelin, G. Hiller, L.L. Olsson, A. Aagaard, S. Geschwindner, Y. Xue, M. Vasänge, Synthesis and structure-activity relationship of 4-(1,3-benzothiazol-2-yl)-thiophene-2-sulfonamides as cyclin-dependent kinase 5 (cdk5)/p25 inhibitors, Bioorg. Med. Chem. Lett. 22 (2012) 5919–5923.

[36] G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Goodsell, A.J. Olson, Autodock4 and autodocktools4: automated docking with selective receptor flexibility, J. Comput. Chem. 16 (2009) 2785–2791.