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Identification of azepinone fused tetracyclic heterocycles as new chemotypes with protein kinase inhibitory activities

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ABSTRACT

The design and synthesis of small tetracyclic heterocycles which bear two new regioisomeric 2-carboxyethyl-1*H*-pyrrole-annulated indoloazepinone scaffolds is described. An azepinone motif, which is inherent in the structures of many well studied protein kinase inhibitors, serves as prominent structural feature of the new compounds. Concise access to the new regioisomeric tetracyclic derivatives was accomplished through amide coupling of appropriate pyrrole and indole precursors followed by an intramolecular Heck coupling reaction of the intermediate amide conjugates. Preliminary evaluation of newly synthesized tetracyclic molecules against a panel of protein kinases indicated their inhibitory activities and revealed promising selectivity profiles. The new compounds displayed no significant antiproliferative activity against MCF-7 cancer cells. Interestingly, derivative **19a** exhibited selective TAK1 kinase inhibitory activity and figures as a promising chemotype for the discovery of new TAK1 inhibitors.

1. Introduction

Protein kinases are key components of many cellular signaling pathways and play a crucial role in vital cellular processes such as cell cycle regulation, DNA replication, transcription, metabolism, immune responses and nervous system functions.[1] Deregulation of protein kinase activity results in a wide range of pathological disorders among which cancer plays a prominent role. Over the past decades, a great deal of research, both in pharmaceutical industry and in academia, has been directed towards the discovery of new protein kinase inhibitors.[2] As a result, a number of small molecules have been licensed for the treatment of various types of cancer, while many others are under clinical trials.[3] Although, significant progress has been achieved in this field, a large fraction of the human kinome remains unexplored offering new opportunities for drug discovery efforts.[4]

The majority of protein kinase inhibitors reported in the literature are small heterocycles targeting the ATP-binding site of the enzymes. Despite their structural diversity, recent data of fragment-based approaches indicate that their structures cover a limited part of chemical space.[5] Consequently, the identification of new scaffolds which may be used as starting points for the discovery of novel lead compounds with potent and selective protein kinase inhibitory activities remains an open challenge.

Many bioactive protein kinase inhibitors contain an azepinone motif as part of their structure (Fig. 1). Amongst them, paullones (**1**), a class of synthetic benzazepinones, have been established as inhibitors of various kinase targets such as cyclin dependent kinases (CDKs)[6] and glycogen synthase kinase-3β (GSK-3β).[7] Structural modifications of the paullone basic scaffold switched the selectivity profile leading to dual polo-like kinase 1 (PLK1)/vascular endothelial growth factor-receptor 2 (VEGF-R2) inhibitors with in vitro antiproliferative and antiangiogenetic activities.[8] Furthermore, congener derivatives have been described with interesting sirtuin 1 (SIRT1) modulatory,[9] antitrypanosomal[10] and antileishmanial activity.[11] Hymenialdisine (**2**), a pyrroloazepinone natural product, has shown potent inhibitory activity against CDKs, GSK-3β, casein kinase 1 (CK1) and checkpoint kinase (Chk) 1 and 2,[12] as well as anti-inflammatory,[13] neuroprotective and antioxidant properties.[14] Importantly, structural data confirm that azepinone motif influences strongly the binding mode of inhibitors to the enzymes, acting as an anchor point and allowing the development of crucial hydrogen bonds in the hinge region of the ATP-binding site. Particularly, crystal structure analysis of alsterpaullone (**1a**)-GSK-3β complex demonstrated the formation of three hydrogen bonds between the lactam group and amino acid residues of the hinge region.[15] Similarly, the azepinone moiety of

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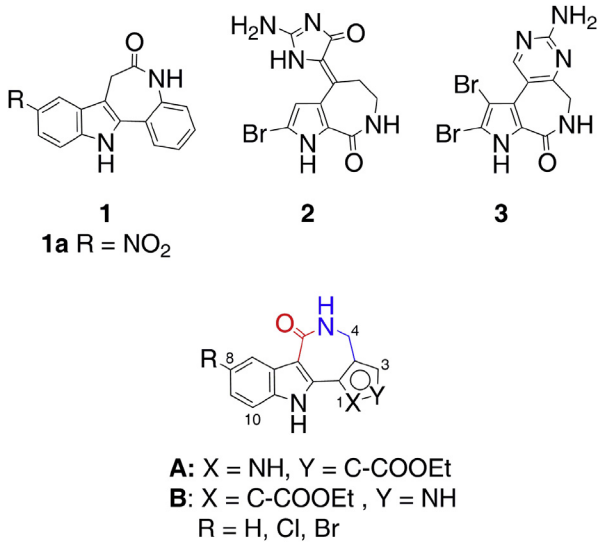


Fig. 1. Structures of bioactive azepinone heterocycles and target scaffolds **A** and **B**.

hymenialdisine participates on the formation of two directed hydrogen bonds in the ATP-binding pocket of CDK2.[12]

Moreover, a number of reported observations underline remarkable bioactivities of structurally related azepinone fused heterocycles. Latondunine A (**3**) corrected cystic fibrosis transmembrane conductance regulator (CFTR) trafficking to the cell membrane via poly(ADP-ribose) polymerase-3 (PARP-3) inhibition providing new therapeutic opportunities for cystic fibrosis.[16]

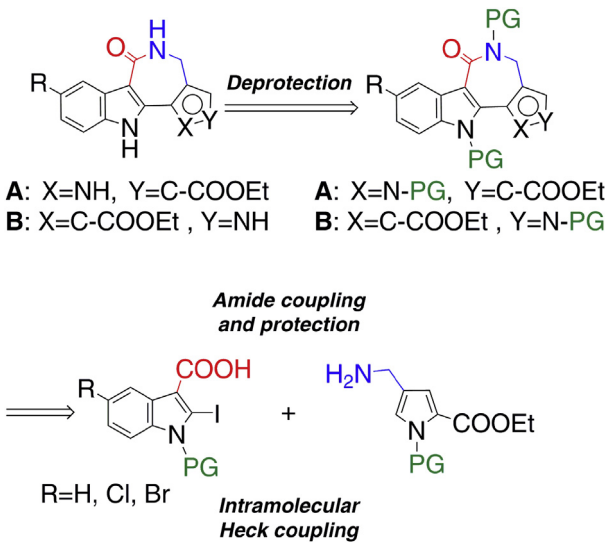
Consequently, the incorporation of a seven-membered lactam moiety into a multicyclic scaffold might contribute to the identification of new kinase inhibitor chemotypes with potential applications to other molecular targets.

Inspired by these observations and due to our interest in the identification of new kinase interacting molecules,[17] we have focused on the generation of new azepinone containing scaffolds[18] as a starting point for the discovery of potential kinase inhibitors. Particularly, we envisaged the regioisomeric 2-carboxyethyl-1*H*-pyrrole-annulated indoloazepinone scaffolds **A** and **B** (Fig. 1) as interesting core structures for the induction of high affinity interactions with the ATP-binding site of kinases. To explore the scope and limitations of our synthetic methodology and gain initial insights into structure activity relationships, the introduction of two halogen substituents at the C(8) position of the new tetracyclic molecules was planned. The target scaffolds can be considered structurally related to paullone family. To date, a notable number of paullone analogues have been synthesized and much attention has been paid to introducing structural variations on the paullone parent skeleton either modifying the benzazepinone segment or replacing the indole core with other heteroaromatic rings.[19] Nevertheless, such regioisomeric pyrrole-annulated tetracyclic heterocycles have not been reported previously in the literature.

Herein, we described a straightforward methodology for the effective synthesis of small tetracyclic heterocycles bearing the regioisomeric scaffolds **A** and **B** via assembly of appropriate indole and pyrrole precursors followed by an intramolecular palladium-catalyzed Heck coupling reaction. Preliminary evaluation of a number of newly synthesized derivatives indicated their kinase inhibitory activities and revealed promising selectivity profiles against different kinase targets.

2. Results and discussion

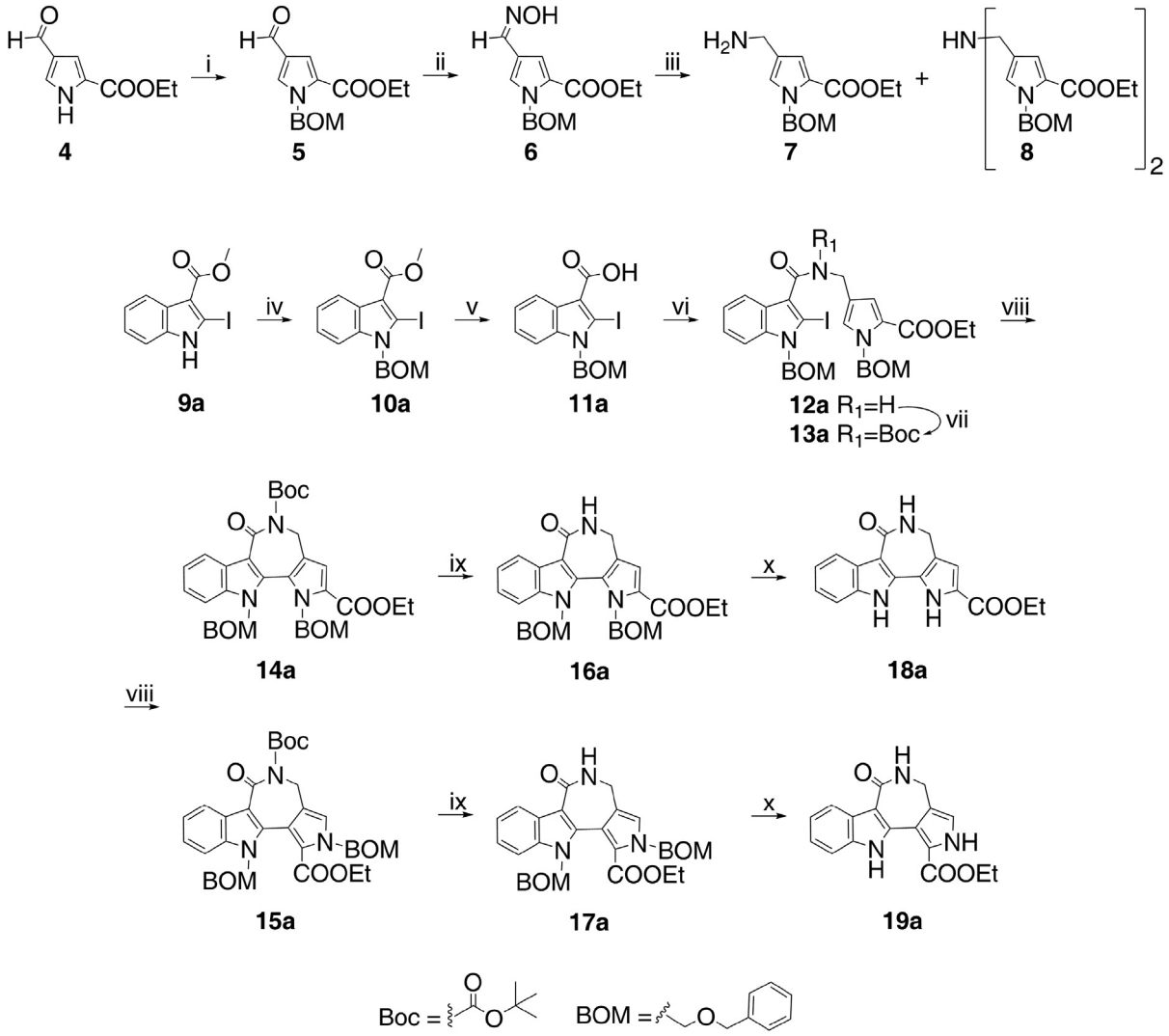
Initially, a simple retrosynthetic plan was considered towards the regioisomeric 2-carboxyethyl-1*H*-pyrrole-annulated tetracyclic scaffolds **A** and **B** (Scheme 1). This involved the amide bond formation between appropriately substituted *N*-protected 2-iodo-1*H*-indole-3-carboxylic acids and an *N*-protected 2-ethoxycarbonyl-1*H*-pyrrole-4-methanamine followed by *N*-protection of the generated amide linkage. *N*-protection of both the heterocyclic precursors was considered important not only for the directed substitution of the cores, but also for the following palladium-catalyzed synthetic step. Subsequently, palladium-mediated intramolecular Heck coupling reaction between the 2-iodo-substituted indole position and either the unsubstituted C(5) or the C(3) of the pyrrole ring was expected to generate the fully protected tetracyclic scaffold **A** or **B**, correspondingly. Finally, sequential removal of the protective groups would afford the target compounds of scaffold **A** or **B**.



Scheme 1. Retrosynthetic plan towards the construction of the scaffolds **A** and **B**.

The synthesis of the pyrrole precursor **7** is depicted in Scheme 2. Initially, the *N*-protection of the known 4-formyl pyrrole derivative **4**[20] was performed. An electron-donating protective group was expected to promote the upcoming intramolecular palladium-mediated coupling reaction enriching the electron density of the pyrrole ring. Among the known electron-donating protective groups, the benzyloxymethyl (BOM) group was chosen. Thus, **4** was *N*-protected using benzyloxymethyl chloride (BOM-Cl) in the presence of potassium *tert*-butoxide (*t*-BuOK) providing the *N*-BOM protected intermediate **5** in very good yield (85%). Oximation of the 4-formyl group using hydroxylamine hydrochloride and sodium carbonate furnished a mixture of *E*,*Z*-pyrrole-4-carbaldehyde oximes **6** in excellent yield (91%). The two geometric isomers were isolated and characterized, but full assignment of the structures was not completed. The mixture of the *E*,*Z*-oximes was reduced upon treatment with sodium borohydride-nickel chloride hexahydrate giving the *N*-protected 1*H*-pyrrole-4-methanamine precursor **7** in moderate yield (42%), while the dimerized by-product **8** was also isolated in a small amount.

With the pyrrole precursor **7** in hand, our attention was turned to the synthesis of the *N*-protected 2-iodo-1*H*-indole-3-carboxylic acid precursor **11a** (Scheme 2). The introduction of the *N*-BOM group also on the indole fragment was considered indispensable for facilitating the final cleavage of the protective groups and the generation of the fully deprotected tetracyclic compounds. Thus, treatment of the previously described 2-iodo-substituted indole **9a**[21] with BOM-Cl in the presence of sodium hydride furnished the *N*-BOM protected indole **10a** quantitatively which after alkaline



Scheme 2. Reagents and conditions: (i) *t*-BuOK, $BnOCH_2Cl$, 18-Crown-6, THF, 0 °C, 30 min, 85%; (ii) $H_2NOH{\cdot}HCl$, Na_2CO_3, $EtOH/H_2O$, 90 °C, 1 h, 91%; (iii) $NiCl_2{\cdot}6H_2O$, $NaBH_4$, MeOH, 0 °C to rt, 1.5 h, **7**=42%, **8**=10%; (iv) NaH, $BnOCH_2Cl$, THF, rt, overnight, 100%; (v) $NaOH/H_2O$, MeOH, 65 °C, overnight, 85%; (vi) **7**, 4-DMAP, EDCI·HCl, DCM, 0 °C to rt, overnight, 81%; (vii) 4-DMAP, Boc_2O, DCM, 0 °C to rt, overnight, 97%; (viii) $Pd(OAc)_2$, PPh_3, Ag_2CO_3, DMF, 80 °C, 30 min, **14a**=44%, **15a**=14%; (ix) TFA, DCM, 0 °C to rt, 7 h for **16a** and 5 h for **17a**, **16a**=94%, **17a**=92%; (x) 10% Pd/C, $HCOONH_4$, EtOH abs, 68 °C, 1 h for **18a** and 8 h for **19a**, **18a**=91%, **19a**=85%.

hydrolysis provided the corresponding 2-iodo-1*H*-indole-3-carboxylic acid precursor **11a** in 85% yield.

Having already synthesized the precursors **7** and **11a**, we focused on the construction of the corresponding tetracyclic scaffolds **A** and **B**. Amide coupling between **11a** and **7** using *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDCI) and 4-(dimethylamino)pyridine (4-DMAP) in DCM provided the amide conjugate **12a** in a very good yield (81%) (Scheme 2). *N*-amide protection was effected by treatment with Boc_2O in the presence of 4-DMAP. Then, the *N*-Boc protected amide **13a** was subjected to palladium-mediated intramolecular Heck coupling reaction. The cyclization was carried out using palladium(II) acetate/PPh_3 in the presence of Ag_2CO_3 as base, a catalytic system which has previously used efficiently in intramolecular Heck reactions for the synthesis of tricyclic or tetracyclic nitrogen heterocycles.[22] Under these conditions, cyclization at the C(5) of the pyrrole ring resulted in the formation of tetracyclic compound **14a** in 44% yield, while cyclization at the C(3) led to the regioisomeric product **15a** in 14% yield. Furthermore, cleavage of the *N*-Boc protective group by treatment with trifluoroacetic acid (TFA) in DCM led to the corresponding compounds **16a** and **17a**.

The assignment for the structural elucidation of the regioisomeric products **16a** and **17a** was performed with a combination of 1D (^1H, ^{13}C, APT) and 2D (H,H COSY, edited HSQC, HMBC) NMR experiments. With these methods a complete assignment of all chemical shifts to the corresponding hydrogen and carbon atoms was possible. Especially the HMBC spectrum, showing cross correlation signals between proton and carbon atoms connected via two and three bonds, is very helpful to distinguish both isomers. In both **16a** and **17a** the isolated proton of the pyrrole ring gives a singlet in the ^1H spectrum at 7.08 and 7.06 ppm, respectively, each showing in HMBC couplings via three bonds to the carbon atoms of the CH_2 group in the corresponding seven-membered lactam ring (38.02 and 37.43 ppm) and to the quaternary carbons in the pyrrole ring (130.40 and 125.69 ppm) (Fig. 2). In contrary, compound **17a** shows a three bond coupling of the pyrrole ring proton (7.06 ppm) to the N–CH_2–O methylene carbon (77.80 ppm), which is missed in compound **16a**. In that case this proton (7.08 ppm) correlates to the carbonyl group at 160.71 ppm.

Subsequently, the complete cleavage of the *N*-BOM protective groups from the tetracyclic derivatives **16a** and **17a** was attempted (Scheme 2). To this purpose, compound **16a** was subjected to catalytic hydrogenolysis conditions over 10% Pd/C in the presence of ammonium formate. The fully deprotected analogue **18a** was obtained in excellent yield (91%). Applying the same hydrogenolysis

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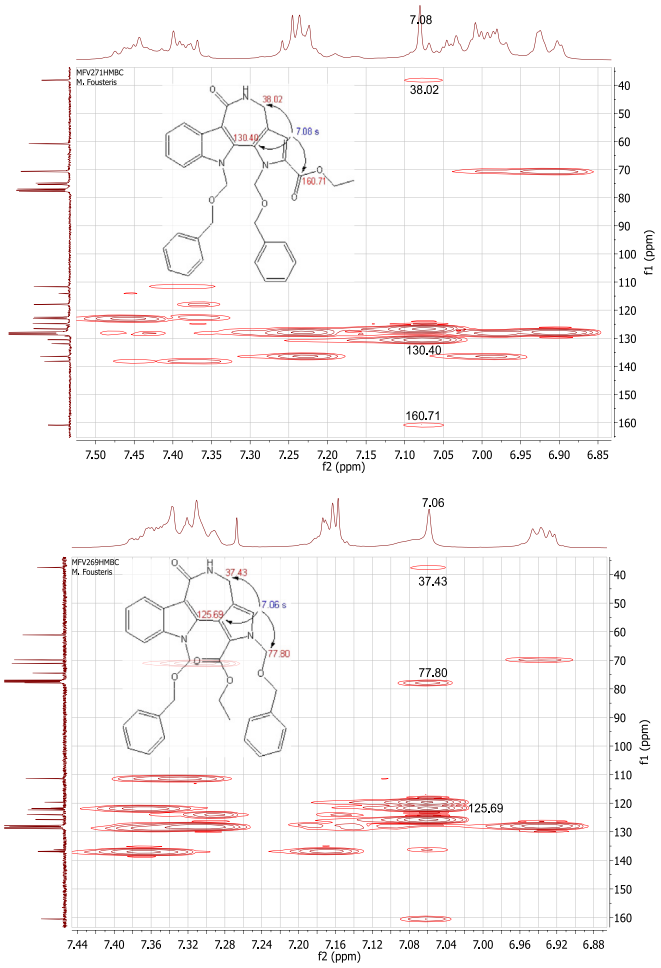

Fig. 2. HMBC spectra of compounds **16a** and **17a**.

conditions to **17a**, the regioisomeric analogue **19a** was also isolated in very good yield (85%).

Prompted by these results, we decided to extend our study to the synthesis of the regioisomeric C(8)-halogenated tetracyclic analogues **16**(**b**–**c**) and **17**(**b**–**c**), respectively. To achieve our goal, we first investigated the access to the 5-halogenated 2-iodo-1*H*-indole-3-carboxylic acids **11**(**b**–**c**).

Thus, commercially available 5-chloro-indole **20b** was transformed easily to 1*H*-indole-3-carboxylic acid methyl ester **22b**[23] via trichloroacetylation followed by basic methanolysis (Scheme 3). Protection of the indole nitrogen atom with the electron-withdrawing benzenesulfonyl group was expected to promote the C(2) iodo-substitution. Benzenesulfonylation using standard conditions provided the *N*-benzenesulfonylated indole **23b** in 85% yield which was introduced to C(2) iodination conditions. Initial attempts to obtain the 2-iodo-substituted indole **24b** using LDA/I_2 or mesityllithium/1,2-diiodoethane (ICH_2CH_2I),[24] led either to the recovery of unreacted **23b** or to its partial decomposition. These results prompted us to replace the *N*-benzenesulfonyl group with the *N*-*tert*-butyloxycarbonyl (*N*-Boc) group. Thus, *N*-Boc protected indole **25b** was obtained from **22b** upon treatment with Boc anhydride and 4-DMAP in quantitative yield.

Various experimental conditions were investigated for the effective 2-iodo-substitution of substrate **25b** (Table 1). Initially, lithiation of **25b** was examined using equimolar amount of LDA at −78 °C. The attempt to trap the lithiated species using excess of I_2 resulted in the recovery of unreacted **25b** (Table 1, entry 1). Using excess of ICH_2CH_2I and performing the iodination reaction over a broad range of temperatures, the 2-iodo-indole **26b** obtained only in very low yields (Table 1, entries 2–4) together with small amounts of the *N*-deprotected 2-iodo derivative **9b**. In an effort to improve the formation of **26b** and diminish the extended decomposition of **25b** which was occurred at elevated temperatures, excess (2 equiv) of LDA was used and the temperature of the iodination reaction was maintained steady at −60 °C. Under these conditions, a slightly better yield was recorded for **26b** (14%), while a great amount of **25b** (62%) was recovered intact (Table 1, entry 5). Further increase of the LDA amount (3 equiv) did not improve the formation of **26b** (Table 1, entry 6). Interestingly, increasing the reaction scale and prolonging the iodination reaction time led to a remarkable improvement in the yield of **26b** (51%), whereas recovered **25b** (23%) and deprotected indole **9b** (19%) were also isolated (Table 1, entry 7). Extending the reaction time to almost 33 h caused no significant alteration in the yield of **26b** (46%) (Table 1, entry 8). Surprisingly, carrying out the reaction on a much bigger scale, **26b** was obtained in optimum yield (81%) (Table 1, entry 9), possibly due to the more precise monitoring of the reaction progress.

Removal of the *N*-Boc protective group upon treatment with TFA furnished the deprotected indole **9b** in 87% yield (Scheme 3). Finally, *N*-BOM protection followed by alkaline hydrolysis of the methyl ester provided the desired 5-chloro-indole precursor **11b** in 75% yield.

Following the above established sequence of reactions, we succeeded to obtain the *N*-BOM protected 5-bromo-2-iodo-1*H*-indole-3-carboxylic acid **11c** starting from commercially available 5-bromo-1*H*-indole **20c** (Scheme 3). Notably, the 2-iodo-indole **26c** was obtained in optimum yield (79%) by adjusting the temperature of the iodination reaction at −50 °C.

Applying the conditions described in Scheme 2, the conjugation between the indole precursors **11**(**b**–**c**) and the pyrrole fragment **7** furnished amides **12**(**b**–**c**) which were easily transformed to the corresponding *N*-Boc protected derivatives **13**(**b**–**c**) in excellent yields (Scheme 3). The protected amides **13b** and **13c** were submitted to intramolecular Heck coupling reaction providing the regioisomeric cyclized C(8)-halogenated products, **14b**–**15b** and **14c**–**15c**, respectively. Furthermore, treatment of compounds **14**(**b**–**c**) and **15**(**b**–**c**) with TFA in DCM resulted in the removal of the *N*-Boc protective group affording compounds **16**(**b**–**c**) and **17**(**b**–**c**), respectively.

Finally, the removal of *N*-BOM groups from the tetracyclic compounds **16**(**b**–**c**) and **17**(**b**–**c**), was investigated. The BOM-deprotection of bromo-derivative **16c** was proven non-trivial. Catalytic hydrogenolysis over 10% Pd/C in the presence of ammonium formate led not only to the cleavage of the BOM groups, but also to debromination of the skeleton affording the unsubstituted derivative **18a** (Scheme 4). Classical hydrogenolysis using H_2 over various catalysts (10% Pd/C, $Pd(OH)_2$, 5% Pt/C sulfided) neither prevent the debromination nor led to the removal of the BOM groups providing the known intermediate **16a**. Alternatively, the acidic cleavage of BOM-protective groups of **16c** was examined. Thus, various acidic conditions such as *p*-TsOH/DCM, $AlCl_3$/DCM or BBr_3/DCM were used but they resulted either in the recovery or decomposition of **16c**. Prolonged treatment of **16c** with TFA in a mixture of $CHCl_3$/toluene furnished the *N*,*N*′-di(hydroxymethyl) compound **27c** in 60% yield. Extensive efforts to eliminate the hydroxymethyl groups upon basic treatment (aq K_2CO_3/acetone, 1 N aq NaOH/THF, CH_3COONa/EtOH, NH_3 7 M in CH_3OH/CH_3OH, 40% Triton B in CH_3OH/THF or CH_3CN, $^iPr_2NEt$/, ethylenediamine/CH_3OH, Et_3N/CH_3OH) failed to afford the fully deprotected compound **18c**.

Notably, treatment both of **16b** and **16c** with 6 N aq HCl in ethanol provided a mixture of partially deprotected products. ^1H NMR analysis of the isolated compounds detected a halogenated mono-*N*-BOM protected and a halogenated mono-*N*-hydroxymethyl compound. Nevertheless, all attempts to complete the

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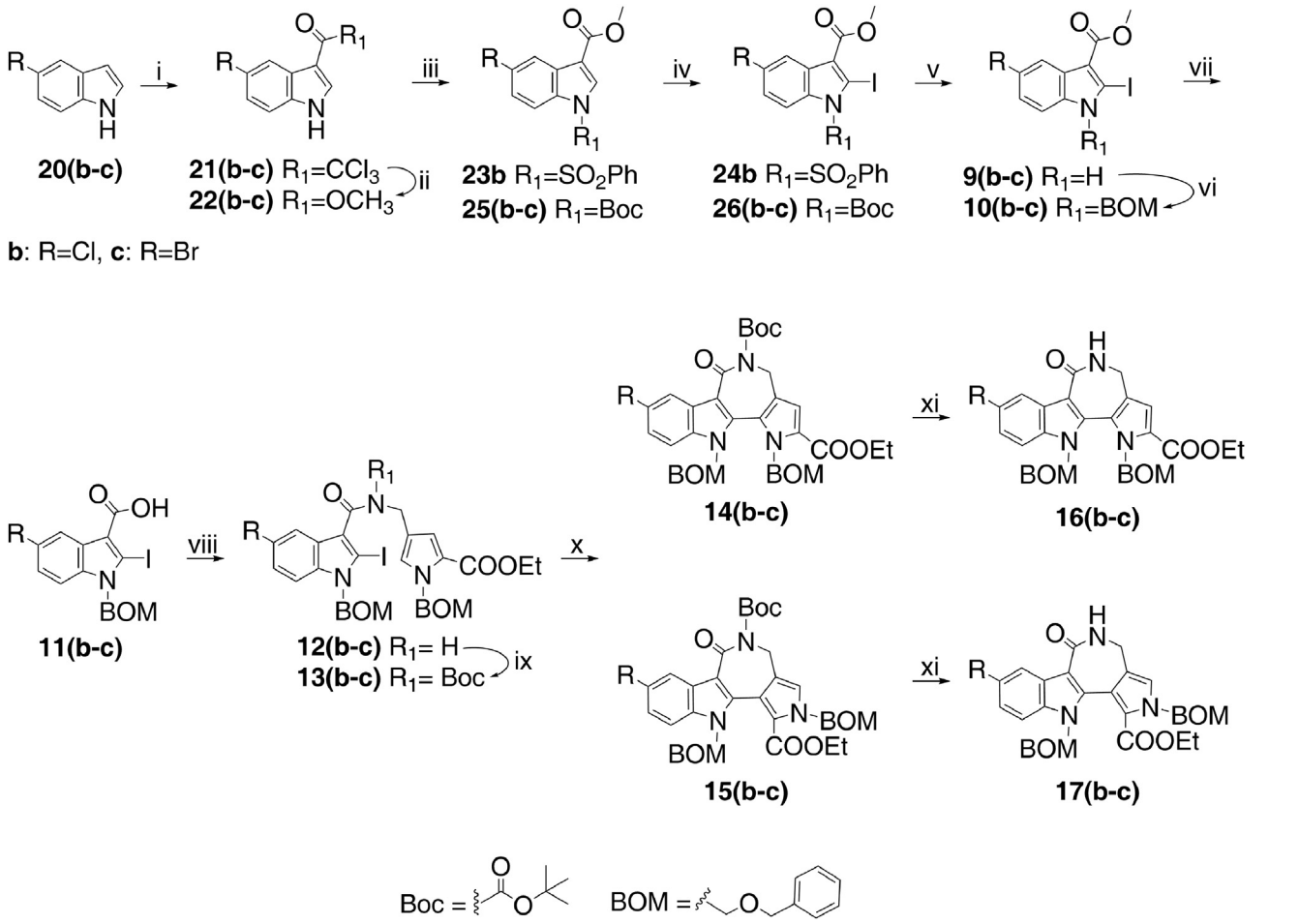

Scheme 3. Reagents and conditions: (i) Cl_3CCOCl, C_5H_5N, THF, 0 °C to rt, overnight, **21b**=95%, **21c**[25]=100%; (ii) KOH/H_2O, MeOH, 85 °C, 3 h, **22b**=83%, **22c**[26]=86%; (iii) R_1=SO_2Ph: NaH, $PhSO_2Cl$, THF, rt, overnight, **23b**=85%; R_1=Boc: 4-DMAP, Boc_2O, THF, 0 °C to rt, 1 h, **25b**=99%, **25c**=96%; (iv) ICH_2CH_2I, LDA, THF, −78 °C to −60 °C for **26b** and −78 °C to −50 °C for **26c**, overnight, **26b**=81%, **26c**=79%; (v) TFA, DCM, rt, overnight, **9b**=87%, **9c**=79%; (vi) NaH, $BnOCH_2Cl$, THF, rt, overnight, **10b**=84%, **10c**=80%; (vii) $NaOH/H_2O$, MeOH, 78 °C, overnight, **11b**=75%, **11c**=90%; (viii) **7**, 4-DMAP, EDCI·HCl, DCM, 0 °C to rt, overnight, **12b**=93%, **12c**=95%; (ix) 4-DMAP, Boc_2O, DCM, 0 °C to rt, overnight, **13b**=92%, **13c**=93%; (x) $Pd(OAc)_2$, PPh_3, Ag_2CO_3, DMF, 80 °C, 30 min, **14b**=36%, **15b**=26%, **14c**=39%, **15c**=13%; (xi) TFA, DCM, 0 °C to rt, 4 h for **16b** and **17b**, 21.5 h for **16c** and 16 h for **17c**, **16b**=100%, **17b**=91%, **16c**=75%, **17c**=97%.

Table 1
2-Iodo-substitution of the *N*-Boc protected indole **25b**

Entry	**25b** (mmol)	LDA[a] (equiv)	ICH_2CH_2I[b] (equiv)	Iodination conditions T (°C)/time (h)	**26b** (%)	**25b** (%) recovered	**9b** (%)
1	65×10^{-3}	1.1	I_2[c]/3	−78→25/2.5	—	74	—
2	65×10^{-3}	1.1	3	−78→25/25.5	7	14	—
3	65×10^{-3}	1.1	3	−78→−30/10	7	28	6
4	65×10^{-3}	1.1	3	−78→−50/13	9	88	8
5	65×10^{-3}	2	3	−78→−60/13.5	14	62	7
6	65×10^{-3}	3	3	−78→−60/10.5	10	24	10
7	323×10^{-3}	2	3	−78→−60/22.5	51	23	19
8	323×10^{-3}	2	3	−78→−60/33.5	46	18	19
9	2.5	2	3	−78→−60/28.5	81	3	14

[a] LDA solution 2 M in THF/heptane/ethylbenzene; Lithiation conditions: −78 °C/0.5 h.
[b] 1,2-diiodoethane solution 0.74 M in THF.
[c] I_2 solution 0.74 M in THF.

deprotection step by prolonged exposure of the mixture of the partially deprotected products to 6 N aq HCl led to decomposition.

On the other hand, exposure of the regioisomeric halogenated derivatives **17b** and **17c** to 6 N aq HCl in ethanol led to the isolation of the desired deprotected compounds **19b** and **19c** in 62% and 91%, respectively (Scheme 5). Unexpectedly, in both reactions a halogenated mono-*N*-EOM protected derivative (**28b** or **28c**) was formed, possibly via acid catalyzed transetherification of the corresponding *N*-hydroxymethyl intermediates. The latter were easily converted to the desired deprotected products upon further treatment with 6 N aq HCl.

A preliminary study was conducted concerning the in vitro inhibitory activities of the new tetracyclic compounds **18a** and **19**(**a**–**c**) against an extended panel of protein kinases (MRC-PPU Express Screen, International Centre for Kinase profiling, University

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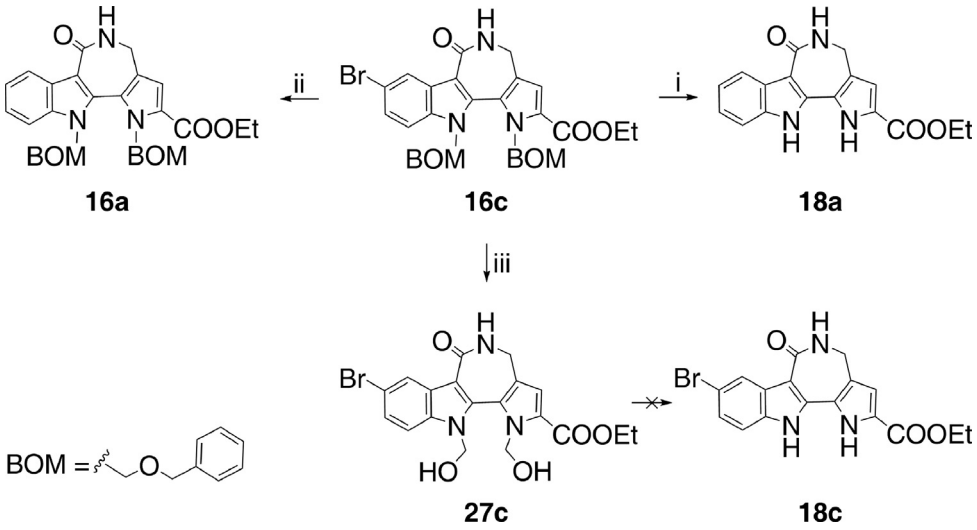
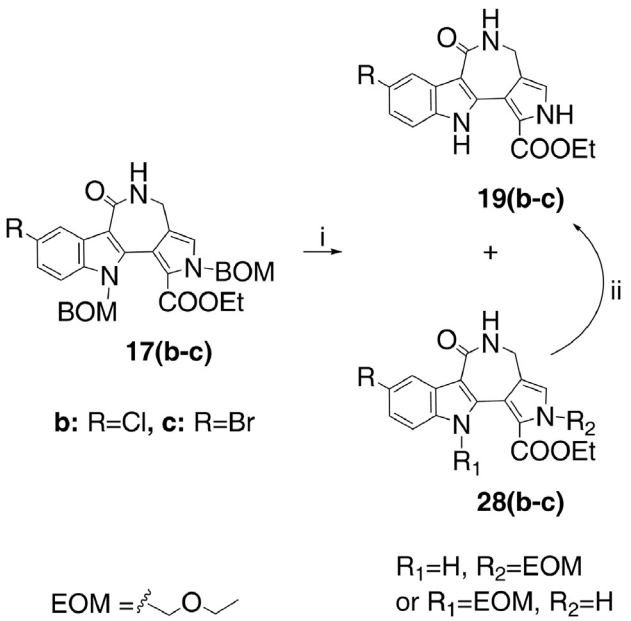


Scheme 4. Reagents and conditions: (i) 10% Pd/C, $HCOONH_4$, abs. EtOH, 68 °C, 1 h; (ii) Classical hydrogenolysis conditions (see text for details); (iii) TFA, $CHCl_3$/toluene (1/2), 98 °C, 25 h, **27c**=60%.



Scheme 5. Reagents and conditions: (i) 6 N aq HCl, EtOH abs, sealed tube, 130 °C, 2 h, **19b**=62%, **19c**=91%, **28b**=33%, **28c**=7%; (ii) 6 N aq HCl, EtOH abs, sealed tube, 130 °C, 3.5 h, **19b**=56%, **19c**=54%.

of Dundee).[27] The compounds were screened at a single concentration of 10 μM (in duplicate) and the kinase remaining activity (%) was determined. As it is presented in Table 2, compound **18a** showed more than 80% inhibition of AMPK activity, while it also displayed good PIM1 (62%) kinase. Interestingly, the regioisomeric derivative **19a** proved the most selective among the tested compounds displaying a 78% decrease of TAK1 activity, while it did not exhibit any significant inhibitory effect against the rest of the kinase targets. Considering the detected inhibition of TAK1 activity by **18a** was up to 58%, it is concluded that the framework of the tetracyclic scaffold may affect the compound's selectivity. Furthermore, the C(8)-halogenated derivatives **19b** and **19c** presented a quite similar activity profile against PLK1, MST2, TRKA, VEG-FR and SmMLCK kinase (64–76%). Notably, TAK1 activity was reduced in only 28% and 39% by **19b** and **19c** correspondingly, indicating that except for the framework also the substitution pattern of the tetracyclic skeleton may contribute to the inhibitory profile of the new compounds. Although a limited number of substituents were introduced at C(8), it is pointed out that this particular position is correlated probably with the selective activity against different enzymes. Thus, a detailed structure-activity relationship study around the basic scaffold **B** will define the structural requirements for optimized TAK1 inhibition. To this direction, compound **19a** may serve as a starting point of a medicinal chemistry campaign aiming at the discovery of new TAK1 inhibitors.

Finally, the antiproliferative activity of derivatives **18a** and **19**(**a**–**c**) against an MCF-7 breast cancer cell line, was investigated. The compounds were tested in a concentration range from 1 μM to 500 μM (each concentration was checked three times) utilizing MCF-7 cells and 20% DMSO as positive and negative controls, respectively. Unfortunately, none of the compounds showed any significant antiproliferative activity (See Fig. S1, Electronic Supplementary data).

3. Conclusions

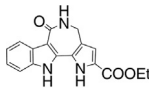
In summary, we have developed the synthesis of small tetracyclic heterocycles, derivatives of the two new regioisomeric 2-carboxyethyl-1*H*-pyrrole-annulated indoloazepinone scaffolds **A** and **B**, via sequential amide conjugation of appropriate indole and pyrrole precursors and intramolecular Heck coupling reaction. Preliminary kinase selectivity profiling data suggest that the azepinone containing scaffolds **A** and **B** can serve as new chemotypes for the identification of new protein kinase inhibitors. Importantly, derivative **19a** figures as a promising selective compound for the discovery of new TAK1 inhibitors.

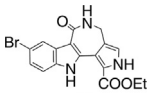
4. Experimental section

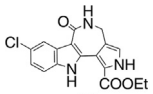
4.1. General

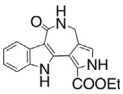
All reagents were obtained commercially from Alfa Aesar, Sigma-Aldrich or Merck and used without further purification. Reactions involving moisture-sensitive reactants were conducted in flame-dried glassware under an atmosphere of argon. Reagents and anhydrous solvents were transferred via syringe. Analytical TLC was performed on Merck Silica gel 60 F_{254} on precoated silica gel plates. Visualization was accomplished under UV light (254 and 365 nm), by exposure to iodine vapours and by use of Seebach

Table 2
Kinase selectivity profiling of compounds **18a** and **19**(**a**–**c**) screened at a concentration of 10 μM (in duplicate) against a panel of 50 kinases. The results are displayed as the mean percentage of kinase remaining activity (%) with a standard deviation (SD). Remaining kinase activities >75% have been omitted for clarity (See Table S1, Electronic Supplementary data)


18a


19c


19b


19a

Kinase	%	SD	Kinase	%	SD	Kinase	%	SD	Kinase	%	SD
AMPK(hum)	19	4	PLK1	24	0	MST2	24	2	TAK1	22	4
Aurora B	31	9	MST2	25	2	TrkA	25	4	TrkA	66	8
PLK1	36	4	TrkA	28	3	PLK1	25	5	VEG-FR	71	5
PIM1	38	10	VEG-FR	28	1	SmMLCK	31	0	PLK1	74	6
TAK1	42	21	SmMLCK	30	4	VEG-FR	36	6			
MLK3	45	3	IRAK4	39	9	IRAK4	38	11			
TBK1	47	0	TBK1	47	2	TBK1	51	7			
MARK3	49	11	TAK1	61	33	MLK3	67	3			
CAMK1	51	7	MLK3	62	0	RIPK2	68	5			
CHK2	55	2	Aurora B	63	0	TAK1	72	28			
CAMKKb	62	1	RSK1	66	1	CHK2	73	11			
VEG-FR	65	2	RIPK2	67	8	CAMKKb	73	0			
PDK1	65	5	CHK2	68	5	AMPK (hum)	75	5			
DYRK1A	66	3	SGK1	71	9	RSK1	75	1			
RSK1	66	3	AMPK(hum)	72	2						
MST2	67	0	HIPK2	73	1						
SmMLCK	71	1									
MSK1	72	4									
SGK1	73	15									
IGF-1R	74	2									

staining solution. Flash column chromatography was performed on silica gel (SDS 60A, 40–63 μm). Melting points were determined on an Electrothermal IA9200 digital melting point apparatus in capillary tubes and are uncorrected. The IR spectra were recorded on a FTIR Jasco spectrophotometer. NMR spectra (^{1}H and ^{13}C) were recorded on a Brucker DPX 400 MHz (400 MHz for ^{1}H, 100 MHz for ^{13}C) or a Bruker Avance III High-Definition four-channel 700 MHz (700 MHz for ^{1}H, 176 MHz for ^{13}C) spectrometer. Also, NMR spectra (^{1}H, ^{13}C, APT, H,H COSY, edited HSQC, and HMBC) were recorded on a VARIAN MERCURY 400 plus (400 MHz for ^{1}H, 100 MHz for ^{13}C) and a VARIAN MERCURY 300 plus (300 MHz for ^{1}H, 75 MHz for ^{13}C) spectrometer. The chemical shifts (δ) are reported in parts per million (ppm) and the residual solvent signal is used as an internal standard. The following abbreviations are used for the proton spectra multiplicities: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad signal (br) and combinations thereof. Coupling constants (J) are given in Hertz (Hz). Electron-spray ionization (ESI) mass spectra were recorded at 30V, on a Micromass-Platform LC spectrometer using methanol or acetonitrile as solvent. High resolution mass spectra were obtained on a Bruker Daltonics ESI-FT-ICR-MS 'APEX II' [7T] mass spectrometer.

Analytical samples of the final products were provided after semi-preparative Reverse Phase HPLC using a Millipore Waters 501 solvent delivery system with a Waters Spherisorb S5 ODS2 column (5 μm, 250×10 mm) and a Waters Lambda-Max Model 481 LC Spectrometer (λ 254 nm). The mobile phase consisted of acetonitrile containing 0.08% TFA (solvent A) and water containing 0.08% TFA (solvent B). Elution conditions are provided for each compound (flow rate 3.0 mL/min). Purity of the final products was determined by a combination of ^{1}H, ^{13}C NMR and HLPC techniques and was found to be >95%.

4.2. General procedure for the synthesis of the tetracyclic derivatives 14(a–c) and 15(a–c)

To a solution of an appropriate precursor **13**(**a**–**c**) (1 equiv) in dry DMF, $Pd(OAc)_2$, (0.09 equiv), PPh_3 (0.2 equiv) and Ag_2CO_3 (4 equiv) were added under argon atmosphere. The reaction mixture was stirred at 80 °C for the indicated time. After cooling, it was diluted with H_2O and extracted with EtOAc (3×). The combined organic layers were washed with brine (1×), dried over anhydrous Na_2SO_4, filtered and the solvents were removed under reduced pressure. The crude residue was purified by silica gel flash column chromatography using either mixtures of *n*-hexane/EtOAc or toluene/EtOAc to afford the corresponding regioisomeric tetracyclic derivatives **14**(**a**–**c**) and **15**(**a**–**c**).

*4.2.1. 5-(tert-Butyl) 2-ethyl 1,11-bis(benzyloxymethyl)-6-oxo-1,4,6,11-tetrahydro-5H-pyrrolo[2′,3′:5,6]azepino[4,3-b]indole-2,5-dicarboxylate (**14a**) and 5-(tert-Butyl) 1-ethyl 2,11-bis(benzyloxymethyl)-6-oxo-2,4,6,11-tetrahydro-5H-pyrrolo[3′,4′:5,6]azepino[4,3-b]indole-1,5-dicarboxylate (**15a**).* Compounds **14a** and **15a** were synthesized according to the general procedure from **13a** (120 mg, 0.154 mmol) in dry DMF (3.3 mL) using $Pd(OAc)_2$ (3.11 mg, 0.014 mmol), PPh_3 (8.1 mg, 0.031 mmol) and Ag_2CO_3 (170 mg, 0.616 mmol). The reaction time was 30 min. The crude residue was purified by silica gel flash column chromatography (*n*-hexane/EtOAc 90:10 to 80:20). **14a** (44 mg, 44%), yellow solid; mp 122.7–123.6 °C; R_f (*n*-hexane/EtOAc 7:3) 0.48; IR (KBr): ν_{max} 1711, 1678, 1219, 1138, 1091 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.45–8.42 (m, 1H), 7.48 (dq, J=4.7 Hz, J=2.5 Hz, 1H), 7.44–7.40 (m, 2H), 7.30–7.26 (m, 4H), 7.12–7.06 (m, 3H), 7.02 (dd, J=6.4 Hz, J=3.1 Hz, 2H), 6.98 (dd, J=7.5 Hz, J=1.9 Hz, 2H), 6.04 (d, J=9.8 Hz, 1H), 5.61 (d, J=10.5 Hz, 1H), 5.48 (dd, J=10.2 Hz, J=6.9 Hz, 2H), 5.24 (d, J=15.2 Hz, 1H), 4.46–4.38 (m, 2H), 4.29 (d, J=11.9 Hz, 1H), 4.21 (q, J=7.1 Hz, 2H), 4.02–3.96 (m, 2H), 1.56 (s, 9H), 1.45 (t, J=7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 164.6, 160.7, 152.0, 137.9, 136.2, 136.1, 132.1, 130.5, 128.6, 128.6 (2×C), 128.3 (2×C), 128.3, 127.9, 127.8 (2×C), 127.8 (2×C), 126.9, 126.4, 124.9, 123.4, 122.8, 118.3, 114.2, 111.5, 83.1, 75.4, 74.8, 70.6, 70.6, 60.8, 41.3, 28.3 (3×C), 14.5. MS (ESI$^+$) (m/z): 650.33 $[M+H]^+$; HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{38}H_{39}N_3O_7$: 672.26802, found: 672.26749, $[2M+Na]^+$ calcd for $C_{38}H_{39}N_3O_7$: 1321.54682, found: 1321.54536. **15a** (14 mg, 14%), white solid; mp 55–56.6 °C; R_f (*n*-hexane/EtOAc 7:3) 0.36; IR (KBr): ν_{max} 1705, 1670, 1211, 1138, 1105, 617 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.37 (dd,

J=6.7 Hz, J=1.9 Hz, 1H), 7.6 (dd, J=6.7 Hz, J=1.5 Hz, 1H), 7.39–7.28 (m, 7H), 7.19 (s, 1H), 7.16–7.14 (m, 3H), 6.9–6.87 (m, 2H), 5.81 (d, J=10.2 Hz, 1H), 5.77 (d, J=11.1 Hz, 1H), 5.71 (d, J=10.2 Hz, 1H), 5.45 (d, J=11.1 Hz, 1H), 5.21 (d, J=14.9 Hz, 1H), 4.50 (s, 2H), 4.19 (q, J=7.1 Hz, 2H), 4.02 (d, J=11.9 Hz, 1H), 3.96 (d, J=14.8 Hz, 1H), 3.86 (d, J=11.9 Hz, 1H), 1.53 (s, 9H), 1.13 (t, J=7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 165.4, 160.2, 152.3, 136.8, 136.7, 136.7, 136.5, 128.8 (2×C), 128.40 (2×C), 128.4, 128.3, 128.0, 128.0 (2×C), 127.6 (2×C), 125.0, 124.2, 124.2, 122.8, 122.4, 121.2, 119.8, 111.9, 111.5, 83.0, 77.7, 74.6, 70.7, 69.9, 61.2, 40.5, 28.3 (3×C), 14.1; MS (ESI$^+$) (m/z): 672.58 [M+Na]$^+$; HRMS-ESI (m/z): [M+H]$^+$ calcd for $C_{38}H_{39}N_3O_7$: 650.28608, found: 650.28543.

*4.2.2. 5-(tert-Butyl) 2-ethyl 1,11-bis(benzyloxymethyl)-8-chloro-6-oxo-1,4,6,11-tetrahydro-5H-pyrrolo[2′,3′:5,6]azepino[4,3-b]indole-2,5-dicarboxylate (**14b**) and 5-(tert-Butyl) 1-ethyl 2,11-bis(benzyloxymethyl)-8-chloro-6-oxo-2,4,6,11-tetrahydro-5H-pyrrolo[3′,4′:5,6]azepino[4,3-b]indole-1,5-dicarboxylate (**15b**).* Compounds **14b** and **15b** were synthesized according to the general procedure from **13b** (168 mg, 0.21 mmol) in dry DMF (4.5 mL) using $Pd(OAc)_2$ (4.2 mg, 0.019 mmol), PPh_3 (11 mg, 0.042 mmol) and Ag_2CO_3 (231.6 mg, 0.84 mmol). The reaction time was 30 min. The crude residue was purified by silica gel flash column chromatography (toluene/EtOAc 98:2). **14b** (52 mg, 36%), yellow crystalline solid; mp 63–64.7 °C; R_f (toluene/EtOAc 95:5) 0.58; IR (KBr): ν_{max} 1714, 1672, 1221, 1138, 1093 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz): δ 8.20 (d, J=1.9 Hz, 1H), 7.51 (d, J=8.8 Hz, 1H), 7.33 (dd, J=8.8 Hz, J=2.1 Hz, 1H), 7.17–7.14 (m, 4H), 6.96 (dd, J=5.0 Hz, J=1.8 Hz, 3H), 6.91–6.89 (m, 2H), 6.81–6.79 (m, 2H), 6.03 (d, J=10.2 Hz, 1H), 5.68 (d, J=10.8 Hz, 1H), 5.37 (d, J=10.8 Hz, 1H), 5.28 (d, J=10.2 Hz, 1H), 5.10 (d, J=15.4 Hz, 1H), 4.33 (qd, J=7.1 Hz, J=3.3 Hz, 2H), 4.22 (d, J=12.2 Hz, 1H), 4.08–3.94 (m, 3H), 3.71 (d, J=11.9 Hz, 1H), 1.46 (s, 9H), 1.36 (t, J=7.1 Hz, 3H); ^{13}C NMR (CD_3OD, 100 MHz): δ 166.6, 161.8, 152.9, 137.6, 137.3, 135.3, 131.3, 130.8, 130.1, 129.4 (2×C), 129.2 (2×C), 129.0, 129.0, 128.9, 128.7 (2×C), 128.6 (2×C), 128.3, 128.3, 126.0, 122.4, 119.2, 114.6, 113.6, 84.4, 76.6, 75.8, 71.5, 71.0, 61.9, 42.5, 28.3 (3×C), 14.7; MS (ESI$^+$) (m/z): 706.19 [M+Na]$^+$; HRMS-ESI (m/z): [M+H]$^+$ calcd for $C_{38}H_{38}ClN_3O_7$: 684.24710, found: 684.24671. **15b** (37 mg, 26%), white solid; mp 130.6–131.8 °C; R_f (toluene/EtOAc 95:5) 0.37; IR (KBr): ν_{max} 1703, 1672, 1209, 1140, 1101, 590 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz): δ 8.20 (d, J=1.8 Hz, 1H), 7.68 (d, J=8.8 Hz, 1H), 7.45 (s, 1H), 7.34 (dd, J=8.7 Hz, J=2.1 Hz, 1H), 7.3–7.19 (m, 5H), 7.12 (dd, J=5.0 Hz, J=1.0 Hz, 3H), 6.85–6.83 (m, 2H), 5.90 (d, J=10.2 Hz, 1H), 5.82 (d, J=11.2 Hz, 1H), 5.70 (d, J=10.2 Hz, 1H), 5.38 (d, J=11.2 Hz, 1H), 5.17 (d, J=15.2 Hz, 1H), 4.50 (d, J=3.2 Hz, 2H), 4.20–4.15 (m, 2H), 4.10 (d, J=12.4 Hz, 1H), 3.99 (d, J=15.1 Hz, 1H), 3.90 (d, J=12.4 Hz, 1H), 1.51 (s, 9H), 1.10 (t, J=7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 165.0, 160.1, 152.3, 137.7, 136.7, 136.3, 135.1, 129.4, 128.8, 128.7 (2×C), 128.4 (2×C), 128.3, 128.1, 127.9 (2×C), 127.6 (2×C), 125.1, 124.5, 124.2, 121.9, 120.8, 119.9, 112.6, 111.4, 83.1, 77.8, 74.8, 70.8, 70.0, 61.3, 40.4, 28.3 (3×C), 14.1; MS (ESI$^+$) (m/z): 706.07 [M+Na]$^+$; HRMS-ESI (m/z): [M+H]$^+$ calcd for $C_{38}H_{38}ClN_3O_7$: 684.24710, found: 684.24690.

*4.2.3. 5-(tert-Butyl) 2-ethyl 1,11-bis(benzyloxymethyl)-8-bromo-6-oxo-1,4,6,11-tetrahydro-5H-pyrrolo[2′,3′:5,6]azepino[4,3-b]indole-2,5-dicarboxylate (**14c**)and 5-(tert-Butyl) 1-ethyl 2,11-bis(benzyloxymethyl)-8-bromo-6-oxo-2,4,6,11-tetrahydro-5H-pyrrolo[3′,4′:5,6]azepino[4,3-b]indole-1,5-dicarboxylate (**15c**).* Compounds **14c** and **15c** were synthesized according to the general procedure from **13c** (300 mg, 0.35 mmol) in dry DMF (7.45 mL) using $Pd(OAc)_2$ (7.1 mg, 0.032 mmol), PPh_3 (18.4 mg, 0.07 mmol) and Ag_2CO_3 (386 mg, 1.4 mmol). The reaction time was 23 h. The crude residue was purified by silica gel flash column chromatography (toluene/EtOAc 98:2). **14c** (100 mg, 39%), white solid; mp 54.8–56 °C; R_f (*n*-hexane/EtOAc 7:3) 0.52; IR (KBr): ν_{max} 1712, 1672, 1221, 1136, 1091, 603 cm^{-1}; ^{1}H NMR (CD_3COCD_3, 400 MHz): δ 8.47 (d, J=2.0 Hz, 1H), 7.63 (d, J=8.8 Hz, 1H), 7.51 (dd, J=8.7 Hz, J=2.0 Hz, 1H), 7.34–7.22 (m, 5H), 7.15–7.08 (m, 3H), 7.04–7.02 (m, 1H), 6.97–6.95 (m, 1H), 6.20 (d, J=10.1 Hz, 1H), 5.93 (d, J=10.9 Hz, 1H), 5.63 (d, J=10.9 Hz, 1H), 5.54 (d, J=10.1 Hz, 1H), 5.20 (d, J=15.4 Hz, 1H), 4.34 (dd, J=9.1 Hz, J=7.1 Hz, 2H), 4.24 (q, J=7.1 Hz, 2H), 4.14–4.06 (m, 2H), 3.95 (d, J=11.6 Hz, 1H), 1.45 (s, 9H), 1.37 (t, J=7.1 Hz, 3H); ^{13}C NMR (CD_3COCD_3, 100 MHz): δ 164.6, 160.9, 152.8, 137.6, 137.6, 137.5, 134.7, 131.2, 130.9, 129.1 (2×C), 128.9 (2×C), 128.7, 128.5 (2×C), 128.4, 128.4 (2×C), 128.2, 128.2, 127.9, 125.4, 118.8, 116.7, 114.9, 113.3, 82.7, 76.7, 75.8, 70.9, 70.7, 61.2, 41.5, 28.2 (3×C), 14.7; MS (ESI$^+$) (m/z): 750.15 [M+Na]$^+$; HRMS-ESI (m/z): [M+H]$^+$ calcd for $C_{38}H_{38}BrN_3O_7$: 728.19659, found: 728.19673. **15c** (32 mg, 13%), white solid; mp 136.4–138 °C; R_f (*n*-hexane/EtOAc 7:3) 0.43; IR (KBr): ν_{max} 1703, 1672, 1205, 1141, 1101, 607 cm^{-1}; ^{1}H NMR (CD_3COCD_3, 400 MHz): δ 8.46 (dd, J=2.0 Hz, J=0.5 Hz, 1H), 7.71 (dd, J=8.7 Hz, J=0.5 Hz, 1H), 7.57 (s, 1H), 7.48 (dd, J=8.7 Hz, J=2.0 Hz, 1H), 7.34–7.27 (m, 5H), 7.18–7.16 (m, 3H), 6.93–6.91 (m, 2H), 5.96–5.93 (m, 2H), 5.83 (d, J=10.1 Hz, 1H), 5.50 (d, J=11.3 Hz, 1H), 5.23 (d, J=15.2 Hz, 1H), 4.56 (s, 2H), 4.22 (q, J=7.1 Hz, 2H), 4.15–4.08 (m, 2H), 3.94 (d, J=12.1 Hz, 1H), 1.48 (s, 9H), 1.12 (t, J=7.1 Hz, 3H); ^{13}C NMR (CD_3COCD_3, 100 MHz): δ 165.3, 160.6, 152.9, 139.2, 138.5, 137.7, 136.5, 131.0, 129.2 (2×C), 129.0 (2×C), 128.6, 128.5, 128.4 (2×C), 128.3 (2×C), 127.1, 126.5, 125.2, 125.1, 121.1, 120.9, 116.1, 114.7, 111.4, 82.6, 78.8, 75.3, 71.2, 70.3, 61.7, 40.8, 28.2 (3×C), 14.3; MS (ESI$^+$) (m/z): 750.03 [M+Na]$^+$; HRMS-ESI (m/z): [M+H]$^+$ calcd for $C_{38}H_{38}BrN_3O_7$: 728.19659, found: 728.19647.

4.3. General procedure for the synthesis of the tetracyclic derivatives 18a and 19a

A mixture of **16a** or **17a** (1 equiv), 10% Pd/C (0.3 equiv) and $HCOONH_4$ (10.6 equiv) in EtOH abs was heated to reflux for the indicated time. The resulting reaction mixture was filtered through a Celite pad and the filtrate was concentrated under reduced pressure. The crude residue was partitioned between a saturated solution of $NaHCO_3$ and EtOAc. The two phases were separated and the aqueous phase was extracted with EtOAc (2×). The combined organic layers were washed with brine (1×), dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The resulting solid residue was purified by recrystallization with EtOAc and drops of *n*-hexane to afford the corresponding fully deprotected tetracyclic products **18a** or **19a**.

*4.3.1. Ethyl 6-oxo-4,5,6,11-tetrahydro-1H-pyrrolo[2′,3′:5,6]azepino[4,3-b]indole-2-carboxylate (**18a**).* Compound **18a** was synthesized according to the general procedure from **16a** (6 mg, 0.011 mmol) upon treatment with 10% Pd/C (3.5 mg) and $HCOONH_4$ (7.35 mg, 0.117 mmol) in EtOH abs (0.34 mL). The reaction time was 1 h **18a** (3.1 mg, 91%), yellow solid; mp 227.1 °C (dec); R_f (*n*-hexane/EtOAc 2:8) 0.22; IR (KBr): ν_{max} 3281, 1687, 1610, 1205 cm^{-1}; ^{1}H NMR (DMSO-d_6, 700 MHz): δ 12.05 (s, 1H), 11.5 (s, 1H), 8.11 (d, J=7.9 Hz, 1H), 7.51 (t, J=4.8 Hz, 1H), 7.49 (d, J=8.1 Hz, 1H), 7.17 (dt, J=54.4 Hz, J=7.9 Hz, 2H), 6.87 (d, J=2.3 Hz, 1H), 4.31 (q, J=7.1 Hz, 2H), 4.02 (d, J=4.8 Hz, 2H), 1.32 (t, J=7.1 Hz, 3H); ^{13}C NMR (DMSO-d_6, 176 MHz): δ 167.3, 160.3, 135.8, 130.8, 128.3, 127.3, 124.4, 122.9, 122.9, 122.0, 120.8, 113.4, 111.4, 108.4, 60.1, 37.0, 14.4; MS (ESI$^+$) (m/z): 353.70 [M+2Na-2H]$^+$; HRMS-ESI (m/z): [M+H]$^+$ calcd for $C_{17}H_{15}N_3O_3$: 310.11862, found: 310.11855; HPLC: Isocratic elution AcN (+0.08% TFA)/H_2O (+0.08% TFA)=70/30; t_R=5.488 min; purity=96.1%.

*4.3.2. Ethyl 6-oxo-4,5,6,11-tetrahydro-2H-pyrrolo[3′,4′:5,6]azepino[4,3-b]indole-1-carboxylate (**19a**).* Compound **19a** was synthesized according to the general procedure from **17a** (12 mg, 0.022 mmol) upon treatment with 10% Pd/C (6.96 mg) and $HCOONH_4$ (14.5 mg, 0.23 mmol) in EtOH abs (0.67 mL). The reaction time was 8 h **19a** (5.75 mg, 85%), white solid; mp 126.4 °C; R_f (*n*-hexane/EtOAc 2:8) 0.14; IR (KBr): ν_{max} 3406, 1682, 1614, 1205 cm^{-1}; ^{1}H NMR (DMSO-d_6, 700 MHz): δ 12.11 (br s, 1H), 11.43 (br s, 1H), 8.05 (d, J=8.0 Hz, 1H), 7.65 (t, J=5.0 Hz, 1H), 7.53 (d, J=8.1 Hz, 1H), 7.18 (t, J=7.5 Hz, 1H), 7.14 (d, J=3.0 Hz, 1H), 7.1 (t, J=7.5 Hz, 1H), 4.35 (q, J=7.1 Hz, 2H), 3.96 (d,

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J=4.9 Hz, 2H), 1.33 (t, J=7.1 Hz, 3H); ^{13}C NMR (DMSO-d_6, 176 MHz): δ 167.8, 160.8, 135.3, 133.1, 128.0, 124.9, 122.5, 121.5, 120.4, 120.3, 120.0, 117.6, 111.4, 108.8, 60.6, 36.3, 14.2; MS (ESI$^+$) (m/z): 310.48 [M+H]$^+$; HRMS-ESI (m/z): [M+H]$^+$ calcd for $C_{17}H_{15}N_3O_3$: 310.11862, found: 310.11881, [M+Na]$^+$ calcd for $C_{17}H_{15}N_3O_3$: 332.10056, found: 332.10054, [2M+Na]$^+$ calcd for $C_{17}H_{15}N_3O_3$: 641.21190, found: 641.21144; HPLC: Isocratic elution AcN (+0.08% TFA)/H_2O (+0.08% TFA)=70/30; t_R=7.156 min; purity=97.2%.

4.4. Procedures for the synthesis of the tetracyclic derivatives 19(b–c) and 28(b–c)

*4.4.1. Ethyl 8-chloro-6-oxo-4,5,6,11-tetrahydro-2H-pyrrolo[3′,4′:5,6] azepino[4,3-b]indole-1-carboxylate (**19b**) and compound **28b**.* To a solution of **17b** (7.66 mg, 0.013 mmol) in EtOH abs (2.6 mL), 6 N aq HCl (1.91 mL) was added and the mixture was heated at 130 °C for 2 h in a sealed tube. After the completion of the reaction, the mixture was neutralized with 25% aq NH_3 and the solvents were evaporated under reduced pressure. The residue was extracted with EtOAc (3×). The combined organic phases were washed with brine (1×), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified by silica gel flash column chromatography using DCM/MeOH 99:1 to afford compounds **19b** and **28b**. **19b** (2.76 mg, 62%), white solid; mp 257.4 °C (dec); R_f (n-hexane/EtOAc 3:7) 0.23; IR (KBr): ν_{max} 3331, 1685, 1639, 1217 cm^{-1}; ^{1}H NMR (DMSO-d_6, 700 MHz): δ 12.18 (br s, 1H), 11.59 (br s, 1H), 8.03 (d, J=2.1 Hz, 1H), 7.74 (t, J=5.0 Hz, 1H), 7.57 (d, J=8.6 Hz, 1H), 7.19 (dd, J=8.6 Hz, J=2.2 Hz, 1H), 7.15 (d, J=3.0 Hz, 1H), 4.35 (q, J=7.1 Hz, 2H), 3.97 (d, J=4.9 Hz, 2H), 1.32 (t, J=7.1 Hz, 3H); ^{13}C NMR (DMSO-d_6, 176 MHz): δ 167.5, 160.7, 134.6, 133.8, 129.1, 125.0, 124.9, 122.4, 120.4, 120.2, 119.8, 117.9, 113.1, 108.4, 60.7, 36.3, 14.1; MS (ESI$^+$) (m/z): 366.37 [M+Na]$^+$; HRMS-ESI (m/z): [M+H]$^+$ calcd for $C_{17}H_{14}ClN_3O_3$: 344.07965, found: 344.07946, [M+Na]$^+$ calcd for $C_{17}H_{14}ClN_3O_3$: 366.06159, found: 366.06140, [2M+Na]$^+$ calcd for $C_{17}H_{14}ClN_3O_3$: 709.13396, found: 709.13352; HPLC: Isocratic elution AcN (+0.08% TFA)/H_2O (+0.08% TFA)=70/30; t_R=8.296 min; purity=97.6%. **28b** (1.74 mg, 33%), yellow solid; mp 220.8 °C (dec); R_f (n-hexane/EtOAc 3:7) 0.34; IR (KBr): ν_{max} 2924, 1682, 1614, 1222, 1095 cm^{-1}; ^{1}H NMR (CD_3COCD_3, 400 MHz): δ 11.45 (br s, 1H), 8.25 (d, J=2.5 Hz, 1H), 7.6 (d, J=8.6 Hz, 1H), 7.34 (s, 1H), 7.21 (dd, J=8.6 Hz, J=2.2 Hz, 1H), 6.95 (br s, 1H), 5.74 (s, 2H), 4.43 (q, J=7.1 Hz, 2H), 4.15 (d, J=4.3 Hz, 2H), 3.49 (q, J=7.0 Hz, 2H), 1.38 (t, J=7.1 Hz, 3H), 1.12 (t, J=7.0 Hz, 3H); ^{13}C NMR (CD_3COCD_3, 176 MHz): δ 168.5, 162.6, 135.3, 134.9, 130.6, 126.8, 126.0, 124.7, 124.2, 123.8, 122.2, 119.8, 113.6, 110.4, 79.8, 64.8, 62.3, 37.4, 15.2, 14.3; MS (ESI$^+$) (m/z): 424.50 [M+Na]$^+$; HRMS-ESI (m/z): [M+Na]$^+$ calcd for $C_{20}H_{20}ClN_3O_4$: 424.10345, found: 424.10307, [2M+Na]$^+$ calcd for $C_{20}H_{20}ClN_3O_4$: 825.21769, found: 825.21684.

*4.4.1.1. Deprotection of compound **28b**.* To a solution of **28b** (1.79 mg, 4.45 μmol) in EtOH abs (0.89 mL), 6 N aq HCl (0.65 mL) was added and the mixture was heated at 130 °C for 3.5 h in a sealed tube. After the completion of the reaction, the mixture was neutralized with 25% aq NH_3 and the solvents were evaporated under reduced pressure. The residue was extracted with EtOAc (3×). The combined organic phases were washed with brine (1×), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified by silica gel flash column chromatography using DCM/MeOH 99:1 to afford compound **19b** (0.86 mg, 56%).

*4.4.2. Ethyl 8-bromo-6-oxo-4,5,6,11-tetrahydro-2H-pyrrolo[3′,4′:5,6] azepino[4,3-b]indole-1-carboxylate (**19c**) and compound **28c**.* To a solution of **17c** (15 mg, 0.024 mmol) in EtOH abs (4.8 mL), 6 N aq HCl (3.59 mL) was added and the mixture was heated at 130 °C for 2 h in a sealed tube. After the completion of the reaction, the mixture was neutralized with 25% NH_3 and the solvents were removed with evaporation under reduced pressure. The residue was extracted with EtOAc (3×). The combined organic phases were washed with brine (1×), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified by silica gel flash column chromatography using DCM/MeOH 99:1 to afford compounds **19c** and **28c**. **19c** (8.5 mg, 91%), white solid; mp 257.4 °C (dec); R_f (n-hexane/EtOAc 3:7) 0.23; IR (KBr): ν_{max} 3288, 1678, 1622, 1203 cm^{-1}; ^{1}H NMR (DMSO-d_6, 700 MHz): δ 12.18 (br s, 1H), 11.59 (br s, 1H), 8.19 (d, J=2.0 Hz, 1H), 7.75 (t, J=5.0 Hz, 1H), 7.53 (d, J=8.6 Hz, 1H), 7.3 (dd, J=8.6 Hz, J=2.0 Hz, 1H), 7.16 (d, J=3.0 Hz, 1H), 4.34 (q, J=7.1 Hz, 2H), 3.97 (d, J=4.9 Hz, 2H), 1.32 (t, J=7.1 Hz, 3H); ^{13}C NMR (DMSO-d_6, 176 MHz): δ 167.4, 160.6, 134.4, 134.1, 129.7, 124.9 (2×C), 123.4, 120.1, 119.7, 117.9, 113.6, 113.0, 108.3, 60.7, 36.3, 14.1; MS (ESI$^+$) (m/z): 410.35 [M+Na]$^+$; HRMS-ESI (m/z): [M+Na]$^+$ calcd for $C_{17}H_{14}BrN_3O_3$: 388.02913, found: 388.02876; HPLC: Isocratic elution AcN (+0.08% TFA)/H_2O (+0.08% TFA)=70/30; t_R=8.688 min; purity=98.2%. **28c** (0.72 mg, 7%), yellow solid; mp 220.1 °C (dec); R_f (n-hexane/EtOAc 3:7) 0.34; IR (KBr): ν_{max} 2924, 1699, 1616, 1224, 1103 cm^{-1}; ^{1}H NMR (CD_3COCD_3, 400 MHz): δ 11.46 (br s, 1H), 8.42–8.41 (m, 1H), 7.56 (d, J=8.6 Hz, 1H), 7.35–7.34 (m, 1H), 7.33 (d, J=2.0 Hz, 1H), 6.95 (br s, 1H), 5.74 (s, 2H), 4.43 (q, J=7.1 Hz, 2H), 4.15 (d, J=4.3 Hz, 2H), 3.49 (q, J=7.0 Hz, 2H), 1.38 (t, J=7.1 Hz, 3H), 1.12 (t, J=7.0 Hz, 3H); ^{13}C NMR (CD_3COCD_3, 176 MHz): δ 168.5, 162.5, 135.2, 131.2, 126.4, 126.0, 125.3, 124.7, 124.2, 119.8, 114.5, 114.0, 113.9, 110.3, 79.8, 64.8, 62.3, 37.4, 15.2, 14.3; MS (ESI$^+$) (m/z): 468.41 [M+Na]$^+$; HRMS-ESI (m/z): [M+H]$^+$ calcd for $C_{20}H_{20}BrN_3O_4$: 446.07100, found: 446.07060, [2M+3H]$^+$ calcd for $C_{20}H_{20}BrN_3O_4$: 893.14927, found: 893.13245;

*4.4.2.1. Deprotection of compound **28c**.* To a solution of **28c** (2.07 mg, 4.6 μmol) in EtOH abs (0.92 mL), 6 N aq HCl (0.66 mL) was added and the mixture was heated at 130 °C for 3.5 h in a sealed tube. After the completion of the reaction, the mixture was neutralized with 25% aq NH_3 and the solvents were evaporated under reduced pressure. The residue was extracted with EtOAc (3×). The combined organic phases were washed with brine (1×), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified by silica gel flash column chromatography using DCM/MeOH 99:1 to afford compound **19c** (0.96 mg, 54%).

4.5. Protein kinase profiling

Compounds **18a** and **19**(**a**–**c**) were profiled against a panel of 50 kinases (Exrpess Screen) at the International Centre for Kinase Profiling, Division of Signal Transduction Therapy, University of Dundee (http://www.kinase-screen.mrc.ac.uk/).[27]

4.6. Antiproliferative activity

Cell proliferation was measured using a cell proliferation ELISA, BrdU assay kit (Roche, Cat. No. 11669915001). The assay was performed according to the instructions. Therefore, 12000 MCF-7 cells were seeded in each well of a 96 well microplate (Greiner bio-one, flat bottomed, white, μclear) with a final volume of 100 μl. The medium was removed after 24 h and the cells were incubated for additional 24 h with 100 μl of different concentrations of the compounds (500, 250, 100, 50, 10, 5 and 1 μM), while after 4 h the cells were incubated with 5-bromo-2′-deoxyuridine (BrdU) labelling solution. Though, the solvent concentration was always lower than 0.5% (v/v). After removal of the medium the cells were fixed and incubated with anti-BrdU-POD solution. In the end, the cells were washed, incubated with the substrate solution and the

bottom of the microplate was covered with a white foil for measuring chemiluminescence using an Orion Microplate Luminometer.

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Supplementary data

Supplementary data (Synthetic procedures and spectroscopic data for compounds **4**, **5**, **6**, **7**, **8**, **21**(**b**–**c**), **22**(**b**–**c**), **23b**, **25**(**b**–**c**), **26**(**b**–**c**), **9**(**a**–**c**), **10**(**a**–**c**), **11**(**a**–**c**), **12**(**a**–**c**), **13**(**a**–**c**), **16**(**a**–**c**), **17**(**a**–**c**). Full kinase selectivity profiling data for compounds **18a** and **19**(**a**–**c**). Antiproliferative activity data for compounds **18a** and **19**(**a**–**c**). Copies of 1D- and 2D-NMR spectra of compounds **16a** and **17a** associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2016.03.048.

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