

Cite this: *Org. Biomol. Chem.*, 2011, **9**, 2925

www.rsc.org/obc

PAPER

# Soluble polymer supported divergent synthesis of tetracyclic benzene-fused pyrazino/diazepino indoles: an advanced synthetic approach to bioactive scaffolds†

Po-Tsung Lin, Deepak B. Salunke, Li-Hsun Chen and Chung-Ming Sun\*

Received 6th December 2010, Accepted 27th January 2011

DOI: 10.1039/c0ob01126f

The synthesis of indoline substituted nitrobenzene on a PEG support and its further elaboration to structurally diverse benzene-fused pyrazino/diazepino indoles is disclosed. A reagent based diversification approach coupled with Pictet–Spengler type condensation reactions furnished these fused polycyclic scaffolds. Microwave irradiation was used as a means of rate acceleration for soluble polymer-supported reactions. The efficiency of these fused heterocyclic molecules to inhibit the vascular endothelial growth factor receptor 3 (VEGFR-3) was examined *in vitro* using kinase receptor activation enzyme-linked immunosorbant assay (KIRA-ELISA). Based on the preliminary results obtained, a small set of potential drug candidates were identified as novel leads in this therapeutic area to be further explored as anti-metastatic agents.

## Introduction

The majority of biologically relevant chemical space is explored by heterocyclic structures. Among the top 20 best-selling drugs currently in use, 70% are organic small molecules and 10 out of them comprise one or more heterocyclic rings. The presence of a heterocyclic core is directly linked to some bioactivity, and such motifs are frequently found in 'privileged structures'.<sup>1</sup> On the other hand, a recent survey revealed that the CAS registry database consists of more than 24 million organic chemical substances but half of these molecules can be described by just 143 shapes.<sup>2</sup> This scenario demands the clear need for new heterocyclic scaffold design from the virtual chemical space to explore newer dimensions in the drug discovery process. During our search for novel fused heterocyclic ring systems, the benzene fused heterocycles are found to be the second generation of structural motifs,<sup>3</sup> whereas plenty of indole fused ring systems with a range of biological activities are reported.<sup>4</sup> These chimeric heterocyclic scaffolds are found to be the outcome of scientific curiosity, natural product analoguing as well as SAR development.<sup>5</sup> Such chimeric motifs are designed with the vision that the biological activity of several new hybrids exceeds that of the parent compounds.<sup>6</sup>

This practice has unimaginable ends, since each scaffold with its pharmacophoric residues can occupy a unique chemical space.

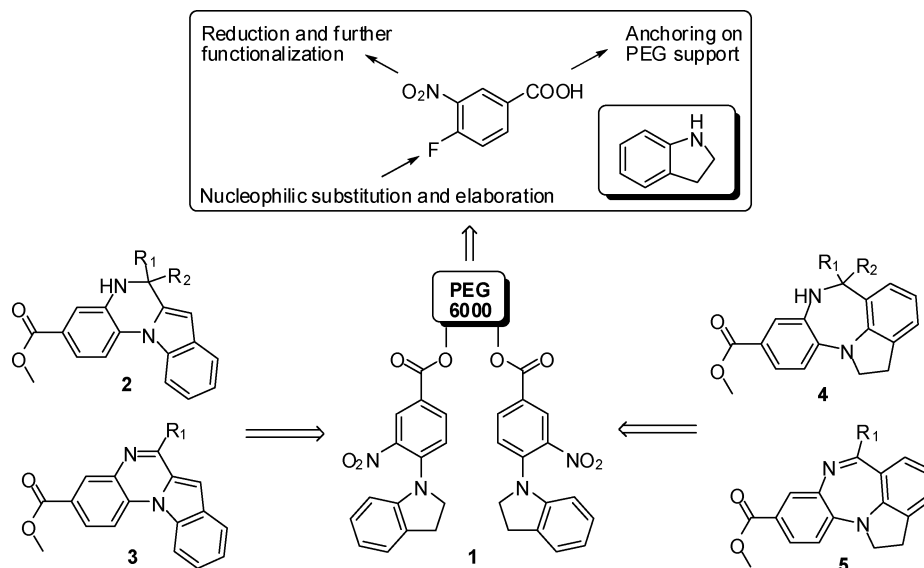
In recent years, various protocols for the construction of such fused heterocyclic compounds *via* polymer supported strategies have been developed.<sup>7</sup> Drug hunters have greatly adopted combinatorial synthesis for lead generation, target validation and lead optimization in drug discovery.<sup>8</sup> To speed up this discovery process and maintain the balance between new chemical entity synthesis and screening, several powerful techniques such as parallel synthesis<sup>9</sup> and multicomponent reactions<sup>10</sup> have been developed. Still there is a need for new tools to provide a fast and more efficient way to synthesize novel chemical compounds. With this in view, the present article describes a rapid and diversity-oriented synthesis of benzo derivatives of pyrazino/diazepino indoles on a soluble polymer support.

The Pictet–Spengler type condensation reaction was utilized as a key reaction to furnish these polycyclic skeletons in a library fashion. Most of the Pictet–Spengler reactions have involved an aliphatic amine instead of aromatic amines.<sup>11</sup> Very few articles concerning the Pictet–Spengler cyclization reaction involving an aromatic amine in combination with indole, imidazole or electron rich phenyl ring systems are reported.<sup>12</sup> Based on this, a multi-disciplinary synergistic methodology<sup>13</sup> is proposed for the present synthesis which takes advantage of a reagent based diversification approach<sup>14</sup> and liquid-phase combinatorial synthesis,<sup>15</sup> as well as microwave mediated reaction acceleration.<sup>16</sup>

A retrosynthetic strategy towards the four different complex heterocycles **2–5** *via* chemically robust poly(ethylene glycol) (PEG, Mol. Wt.  $\approx$  6000) anchored intermediate **1** from 4-fluoro-3-nitrobenzoic acid (FNBA) is depicted in Scheme 1.

Laboratory of Combinatorial Drug Discovery, Department of Applied Chemistry National Chiao Tung University, Hsinchu 300-10, Taiwan.  
E-mail: cmsun@mail.nctu.edu.tw

† Electronic supplementary information (ESI) available: Stepwise proton NMR monitoring on a PEG support, X-ray crystallographic data for **2a** and **4k**, copies of HPLC, <sup>1</sup>H and <sup>13</sup>C NMR, MS, HRMS and IR spectra. CCDC reference numbers 782682 and 782683. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c0ob01126f



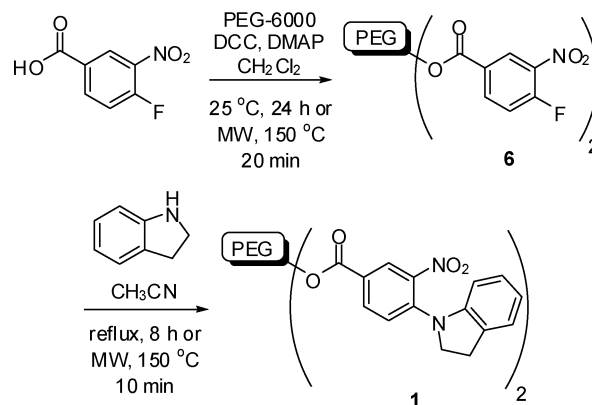
**Scheme 1** Retrosynthetic analysis for the benzo derivatives of pyrazino/diazepino indoles 2–5.

FNBA was chosen as a starting material, since the three different functionalities on the phenyl ring can be expertly utilized for the construction of the desired molecules on support. These scaffolds incorporate the structural features of many biologically important heterocycles such as diazepine, benzo-diazepine, diazepino-indole, pyrazine, benzo-pyrazine and pyrazino-indole. One of the important and common structural components in these scaffolds is *N*-fused indole, which has a great biological and pharmaceutical significance.<sup>4,17</sup> The closely related indolopyrazine/diazepine nucleus was identified as a potent antiproliferative agent and also demonstrated its antidepressant, anti-obesity, and analgesic as well as anti-inflammatory effects.<sup>18</sup> Incorporation of tetrahedral carbon atoms in the present ring systems (2 and 4) was intended to disrupt the typical one dimensional planarity of aromatic skeletons. This may create multidimensional concave and convex surfaces in the molecule and can provide these skeletons as privileged structures to design novel high affinity ligands for future drug discovery.<sup>19</sup> Increased hit rates at comparably small library size are expected from such non-flat skeletal compound collections.

## Results and discussion

A synthetic route to the targeted benzene-fused pyrazino/diazepino indoles is described in Schemes 2 and 3. Esterification of the commercially available 4-fluoro-3-nitro benzoic acid with polymer support using DCC and catalytic DMAP under microwave irradiation<sup>20</sup> (150 °C) for 20 min furnished PEG linked nitro-fluoro benzoate **6** (Scheme 2).

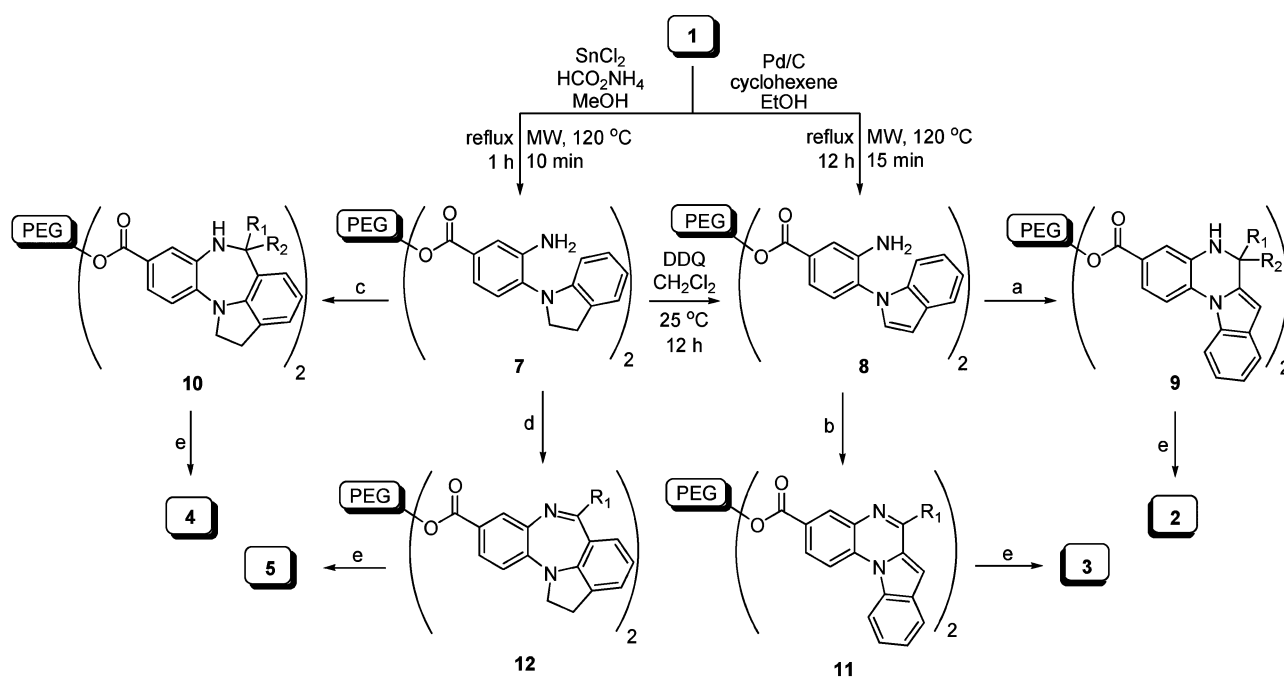
Using this recipe and following the conventional protocol reaction required 24 h and double loading of the reagents for complete conversion. To monitor the progression of reaction, a small portion of the reaction mixture was pulled out, the compound was precipitated and washed with cold ether and dried to record the proton NMR spectrum. The appearance of three signals for aromatic protons along with the  $\alpha$ -methylene protons and methylene bulk related to polymer support confirmed



**Scheme 2** Synthesis of indoline substituted nitrobenzene on a PEG support.

the successful immobilization of the starting material FNBA (Fig. 1B). Upon completion of the reaction, the polymer bound compound mixtures were purified by the same precipitation and washing protocol.

In the next step, the aromatic substitution on immobilized *o*-fluoro nitrobenzene **6** by indoline was achieved in refluxing acetonitrile. The reaction took 8 h for the complete disappearance of the starting materials as monitored by regular proton NMR (Fig. 1C). Microwave promoted acceleration in this *ipso*-fluoro displacement reaction was observed. This indoline substitution was completed in 10 min of microwave irradiation at 150 °C. From this point onwards, the synthetic design was branched out using a reagent based diversification approach (Scheme 3). We presumed that 3-amino-4-(1-indolyl)benzoate **7**, might undergo an electrophilic cyclization with aldehydes or ketones at the phenyl ring,<sup>21</sup> while 3-amino-4-(1-indolyl)benzoate **8**, could cyclize onto its electron rich pyrrole ring.<sup>22</sup> Selection of the appropriate reducing conditions to accomplish compounds **7** and **8** from the anchored nitro intermediate **1** was the key to realize our envisioned and diversified strategy.



Reagents and conditions: (a) ketones, TFA,  $\text{MgSO}_4$ ,  $\text{CHCl}_3$ , MW, 150 °C, 10 min; (b) aldehydes, TFA,  $\text{MgSO}_4$ ,  $\text{CHCl}_3$ , reflux, 10 h or MW, 120 °C, 10 min; (c) ketones, TFA,  $\text{MgSO}_4$ ,  $\text{CHCl}_3$ , MW, 150 °C, 10 min or aldehydes, TFA,  $\text{MgSO}_4$ ,  $\text{CHCl}_3$ , reflux, 10 h or MW, 120 °C, 10 min; (d) *p*-nitrobenzaldehyde, TFA,  $\text{MgSO}_4$ ,  $\text{CHCl}_3$ , reflux, 10 h or MW, 120 °C, 10 min; (e) KCN, MeOH, 25 °C, 12 h.

Scheme 3 Synthesis of desired heterocycles using reagent based diversification.

Several reducing conditions were attempted for the reduction of the nitro functionality in **1**. We have observed an interesting resistance of this nitro group towards various reducing agents such as zinc, samarium iodide and iron(II) chloride. Finally, the desired amino-indolinyl intermediate **7** was obtained using tin(II) chloride in the presence of ammonium formate in refluxing methanol. The indoline ring in this polymer conjugate **7** was further oxidized to the indole substituted aniline conjugate **8** through the application of DDQ in methylene chloride at 25 °C. This two step transformation was realized in one-pot by the exposure of **1** to catalytic transfer hydrogenation conditions using palladium and cyclohexene (refluxing ethanol for 12 h or under microwave irradiation at 120 °C, 15 min) to furnish indole substituted aniline conjugate **8**. Simultaneous nitro reduction as well as palladium catalyzed dehydrogenation<sup>23</sup> of indoline to indole was accomplished in one pot during this transformation. It should be mentioned that under these conditions no additional oxidants are required for the further oxidation of indoline to indole. Thus, compound **8** was obtained *via* two routes: in a single step by reductive dehydrogenation from nitro intermediate **1** or from the oxidation of amino conjugate **7**. During all these reductive transformations, no yield loss was detected which suggested the presence of intact polymer support. Stepwise proton NMR monitoring toward the formation of indolinyl/indolyl-aminobenzoates **7** and **8**, respectively from key intermediate **1** is shown in Fig. 2. The presence of the methylene protons of the

indoline ring in conjugate **7** and the appearance of additional aromatic protons in **8** were clearly observed in the proton NMR spectra.

The penultimate key step in this process was to accomplish the possible Pictet–Spengler type heterocyclization on a soluble polymer support. Indole substituted conjugate **8** on treatment with various ketones in the presence of TFA and  $\text{MgSO}_4$  in a microwave cavity (150 °C) for 10 min furnished the desired benzene-fused dihydropyrazino-indole scaffolds **9**. Whereas, with a similar recipe, using aldehydes (MW, 120 °C, 10 min) in place of ketones, a benzene-fused pyrazino-indole scaffold **11** was obtained on the polymer support. The intermediate dihydropyrazine ring was found to be easily air oxidized during this transformation to deliver more stable fully aromatic flat skeleton. In contrast, under these acid catalyzed dehydrating conditions (using both aldehydes as well as ketones), indoline linked polymer conjugate **7** yielded benzene-fused tetrahydrodiazepino-indole scaffolds **10**. Only in the case of *p*-nitrobenzaldehyde (Table 1, entry 5), cyclization followed by subsequent oxidation of the benzodiazepine ring was observed, resulting in the formation of benzene-fused dihydrodiazepino-indole skeleton **12**.

To understand the significance of dielectric heating over conventional heating experiments, the Pictet–Spengler type cyclizations described herein (using both aldehydes as well as ketones) were additionally attempted by reflux heating. It is remarkable to observe that the cyclizations by conventional reflux heating were

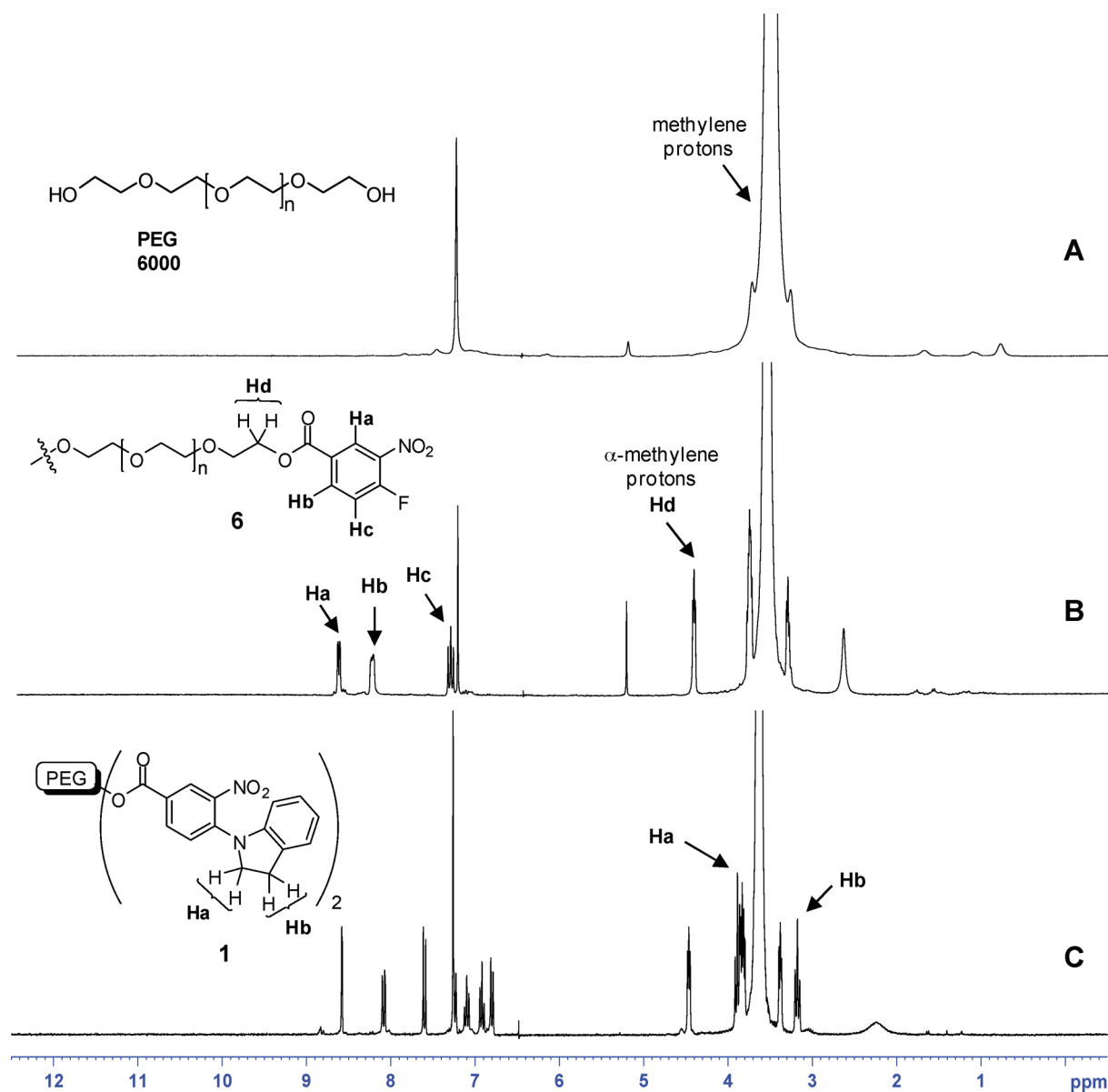


Fig. 1 Stepwise proton NMR monitoring for the synthesis of key intermediate 1.

successful only with the aldehydes; our several efforts to obtain the cyclized products by reflux heating using ketones failed. In the case of ketones, microwave irradiation at a higher temperature (150 °C) than that of aldehydes (120 °C) was required for the complete conversion. This can be attributed to the additional three dimensional obstacles during the ketone cyclization to furnish the preferred hetero systems with tetrahedral carbon atoms in the skeleton. Overall, the aromatic aldehydes were found to be more reactive than the substituted aliphatic aldehydes followed by cyclic, symmetrical or asymmetrical ketones.

During these Pictet–Spengler type condensation reactions, the iminium ion generated *in situ* undergoes C–C bond formation with the C-2 of the indole ring in compound 8 or the C-7 of the indoline ring in compound 7, respectively to furnish benzene fused pyrazino or diazepino indoles as novel heterosystems. The ring nitrogen in indole or indoline played a crucial role in directing the electron cloud at the C-2 or C-7 positions of the ring. The presence of a

sufficiently reactive aromatic nucleus as well as conformational restrictions due to anilinic amines (compared to aliphatic amines in classical Pictet–Spengler isoquinoline synthesis), synergistically promoted the observed cyclization.

Finally cleavage of the soluble polymer support was achieved using potassium cyanide (1%) in methanol at room temperature to obtain polymer-free benzene-fused pyrazino/diazepino indoles 2–5 in good to excellent yields (68–93%, Table 1). The complete cleavage during this reaction was verified by recording proton NMR spectrum of the recovered polymer support. Upfield shift of the α-methylene protons from 4.4 to 3.5 δ ppm confirmed this absolute transformation. Reaction progress and stepwise transformations on a polymer support were clearly observed in proton NMR spectra.<sup>24</sup> To understand the efficiency of this polymer supported combinatorial synthetic process the crude products were subsequently analyzed by HPLC (purity ranges from 70–99%, Table 1). It is noteworthy to mention that with

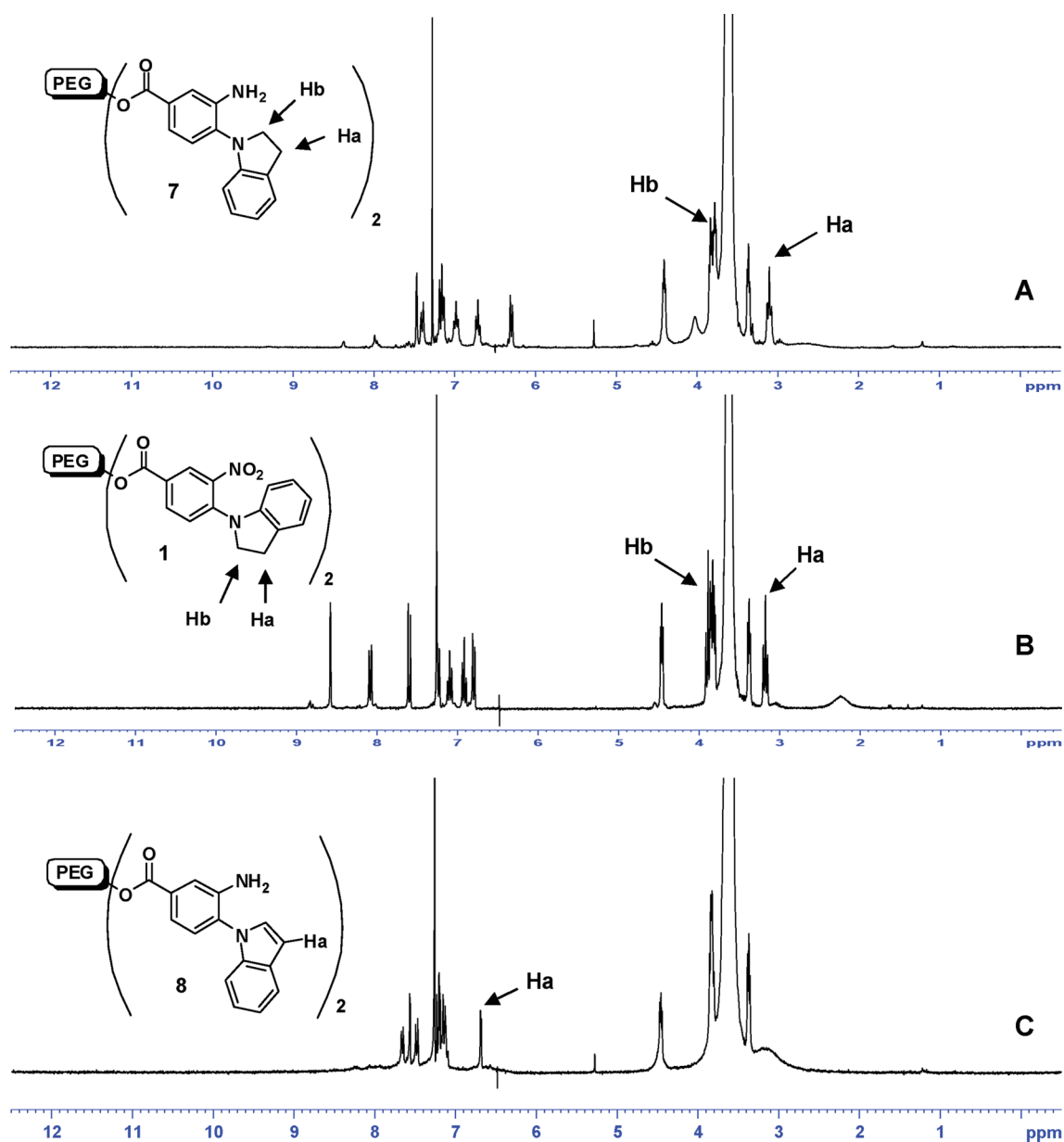


Fig. 2 Proton NMR monitoring toward the formation of **7** and **8** from key intermediate **1**.

the aid of microwave mediated reaction acceleration this macrogel supported synergistic synthesis furnished the desired heterocycles in a very short time. Lower yield (59%) as well as poor HPLC purity (38%) of the product **4j** with *o*-nitro benzaldehyde was attributed to the additional steric hindrance during the cyclization. All the final products were confirmed by spectroscopic analysis. The complete data is included in the ESI.†

To gain more insight about the non-flat tetracyclic benzene-fused pyrazino/diazepino indole skeletons **2** and **4**, single crystals of the representative compounds **2a** and **4k** were grown from a hot saturated filtered solution of these compounds in  $\text{CH}_2\text{Cl}_2$ - $\text{CH}_3\text{OH}$  mixtures. Suitable crystals were obtained by slow evaporation of the solvent at room temperature. Compound **2a** was crystallized as yellow lump where as crystals of compound **4k** were obtained as pale yellow lump. The best amongst them were selected and the X-ray diffraction data was collected.†<sup>25</sup> Non-planarity in

the basic skeleton was clearly noticed in the ORTEP<sup>26</sup> diagrams (Fig. 3).

A twist between the two planes comprising the indole/indoline ring and the phenyl ring connected by N2 and C10 in **2a** or N2 and C9 in **4k** was observed. These two twisted planes are connected by a linker with tetrahedral carbon ( $\text{sp}^3$  hybridized C1) and a nitrogen atom (N1) resulting in a six-membered pyrazino or seven-membered diazepino ring junction with partial envelope and twist conformations. The residue at C1 (nitrophenyl ring in **4k**) distinctly occupies the space perpendicular to the basic skeleton. The X-ray structures clearly revealed that by varying the aldehydes or ketones during the Pictet–Spengler cyclization and further N1 alkylation will result in molecules with three-dimensional (3D) orientations having concave and convex surfaces. Suitable solubility and salt forming properties (important for oral absorption and bioavailability) can be expected from these

**Table 1** Benzene-fused pyrazino/diazepino indoles (**2–5**)








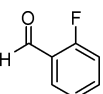
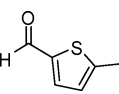
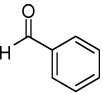


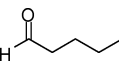


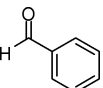
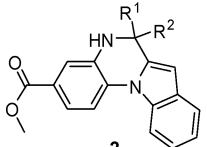
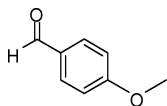
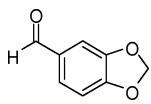
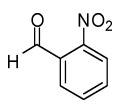
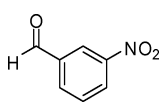
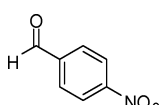
Entry	R <sup>1</sup> COR <sup>2</sup>	LRMS <sup>a</sup>	Yield <sup>b</sup> (%)	Purity <sup>c</sup>	VEGFR-3 Inhibition <sup>d</sup>
<b>2a</b>		307	88	99	73
<b>2b</b>		361	93	87	96
<b>2c</b>		369	80	97	30
<b>2d</b>		321	73	79	20
<b>2e</b>		335	90	91	32
<b>2f</b>		333	75	79	47
<b>3a</b>		305	71	70	62
<b>3b</b>		371	81	88	85
<b>3c</b>		373	83	90	<i>NT</i>
<b>3d</b>		353	85	84	96
<b>4a</b>		309	92	98	<i>NT</i>
<b>4b</b>		323	91	94	82
<b>4c</b>		337	68	70	40
<b>4d</b>		349	80	84	86
<b>4e</b>		309	82	80	66
<b>4f</b>		357	87	96	36

Table 1 (Contd.)

Entry	R <sup>1</sup> COR <sup>2</sup>	LRMS <sup>a</sup>	Yield <sup>b</sup> (%)	Purity <sup>c</sup>	VEGFR-3 Inhibition <sup>d</sup>
4g		363	89	96	73
4h		387	80	77	73
4i		401	85	87	51
4j		424 <sup>e</sup>	59	38	64
4k		424 <sup>e</sup>	75	56	81
5		400	77	71	67

<sup>a</sup> [M+H]<sup>+</sup>. <sup>b</sup> isolated yields determined on weight of purified samples. <sup>c</sup> HPLC recorded after the cleavage followed by precipitation and washing. Compounds with <90% purity were further column purified to achieve the required purity for biological screening. <sup>d</sup> *in vitro* VEGFR-3 kinase inhibition at 1 μM conc. analyzed using KIRA-ELISA; sorafenib (1 μM) was used as an internal standard (86% inhibition). <sup>e</sup> [M+Na]<sup>+</sup>. NT: Not Tested.

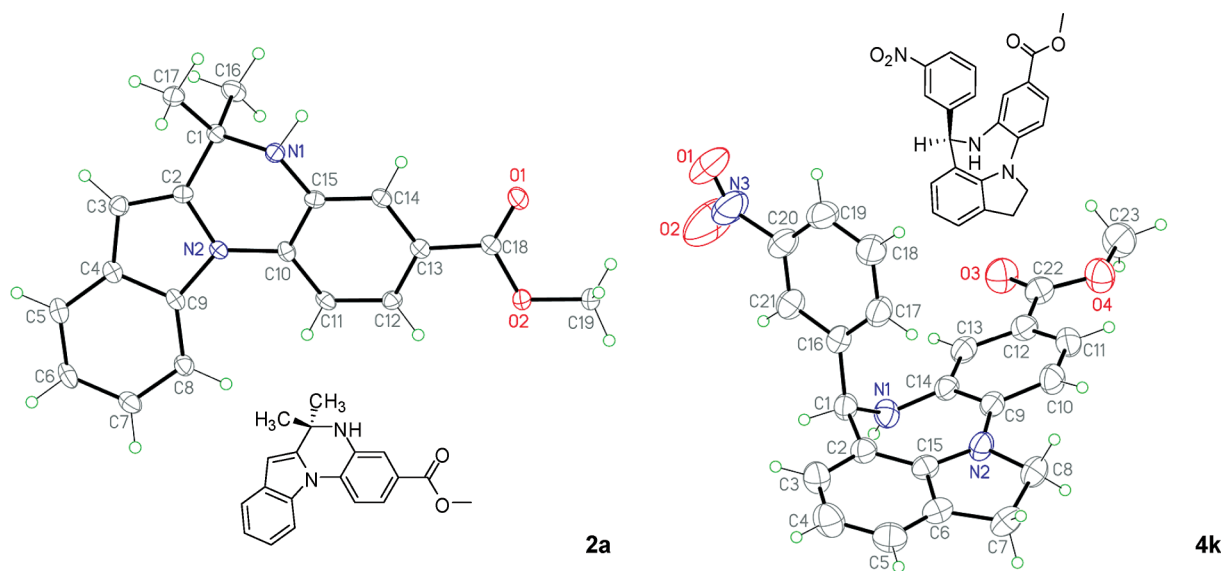


Fig. 3 ORTEP view of compounds 2a and 4k.

heterocycles having two nitrogen atoms as well as a carboxylate functionality. The carboxylate functionality can be further utilized as per the SAR requirements.

Structurally similar tetracyclic heterocycles are known to bind cyclin-dependent kinases.<sup>27</sup> Recently, the imidazo-pyrazines have been reported as dual inhibitors of Aurora kinases A and B with picomolar inhibitory activity<sup>28</sup> whereas pyrimido-diazepines have been designed as adenine mimics to inhibit receptor tyrosine kinases such as KDR, VEGFR-2 and c-Kit.<sup>29</sup> With various such advantages, the synthesized library of these tetracyclic heterocycles was exposed to vascular endothelial growth factor receptor 3 (VEGFR-3) so as to identify new chemotypes as structural leads to control lymphangiogenesis process during tumor migration. The role of lymphangiogenesis in promoting metastasis *via* the lymphatic system has been the subject of extensive research.<sup>30</sup> VEGFR-3 is a major mediator of lymphangiogenesis and is activated by its specific ligand VEGF-C.<sup>31</sup> Clinical studies also revealed that increased expression of VEGF-C was associated with lymph node metastasis in a variety of cancers in humans.<sup>32</sup> In the present study, the efficiency of the synthesized library was examined with an *in vitro* inhibition of VEGFR-3 kinase cell based assay (KIRA-ELISA) at 1  $\mu\text{M}$  concentration. Among the entire 20 compounds tested, 6 test compounds showed more than 80% inhibition of VEGF receptor 3, whereas 8 showed inhibitions in the range of 50–80% (Table 1). A small set of potential lead candidates were identified from this preliminary screening. Further SAR study towards the development of new drug candidates is on the way in our laboratory and the results will be published in due course.

## Conclusion

Three challenges in the synthesis of biologically relevant organic small molecules were addressed. A class of heterocyclic motif with known biological activity was selected and the structural diversity around the skeleton was expanded. The benzene-fused pyrazino/diazepino indoles were designed with ample opportunities for functionalization in SAR efforts. A very short and efficient synthetic path was created for these scaffolds using liquid phase combinatorial technique coupled with the application of microwave irradiation. The required diversity during the synthesis was achieved by incorporating an indoline in the scaffold, whereby Pictet–Spengler type reaction occurred in two possible ways resulting in pyrazino or diazepino fused rings. The efficacy of these fused heterocyclic drug-like molecules was identified with *in vitro* inhibition of VEGFR-3 kinase cell based assay. During this preliminary screening, some of the tested compounds exhibited moderate to good inhibition against VEGFR-3 which is related to the invasion and migration of cancer cells. A small set of potential lead candidates based on these scaffolds was identified to be further explored as anti-metastatic therapeutic agents.

## Experimental section

### General remarks

All reactions were performed under an inert atmosphere with unpurified reagents and dry solvents. Analytical thin-layer chro-

matography (TLC) was performed using 0.25 mm silica gel coated Kiselgel 60 F254 plates. Compound purification was carried out using flash chromatography grade silica gel 60 (230–400 mesh). IR spectra were recorded on an FT-IR spectrophotometer. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on a 300 and 75 MHz spectrometer, respectively. Chemical shifts are reported in parts per milliom (ppm) on the scale from an internal standard. Mass spectra were recorded on a time-of-flight mass spectrometer, samples being introduced by infusion method using the electrospray ionization technique. All starting compounds were purchased from commercial sources and used without purification.

### Microwave irradiation and conventional experimental methods

A monomode CEM Discover<sup>TM</sup> microwave reactor with standard configuration operating at a maximum power of 300 W and equipped with an infrared pyrometer for the control of temperature and compressed air system for cooling was used. All experiments were carried out in sealed microwave process vials. To understand the significance of dielectric heating, the experiments were also carried out using the conventional method in round bottom flasks, using the same reaction mixtures at room temperature or by classical heating in an oil bath as mentioned in Scheme 2 and Scheme 3. To monitor the progression of reaction on a polymer support, a small portion of the reaction mixture was pulled out, the compound was precipitated and washed with cold ether, subsequently dried and its proton NMR spectrum was recorded. The reaction progress and the stepwise transformations on a polymer support after each stage of the synthetic sequence from 4-fluoro-3-nitrobenzoic acid to the desired products **2–5** were cleanly observed in proton NMR spectra.

### Synthesis of indoline substituted nitrobenzene on a PEG support (**1**)

PEG 6000 (1.2 g, 0.2 mmol), 4-fluoro-3-nitro-benzoic acid (0.222 g, 1.2 mmol), *N,N'*-dicyclohexylcarbodiimide (DCC) (0.247 g, 1.2 mmol) and 4-dimethylaminopyridine (DMAP) (0.015 g, 0.12 mmol) in dry  $\text{CH}_2\text{Cl}_2$  (15 mL) were placed in a pressure-resistant test tube provided with a magnetic stirring bar. The tube was sealed with a septum, placed in the CEM MW apparatus, and subjected to MW irradiation at 150 °C for 20 min. Upon completion of the reaction,  $\text{CH}_2\text{Cl}_2$  (10 mL) was added, and the reaction mixture was cooled to 0 °C. The solid dicyclohexylurea (DCU) formed was filtered off and  $\text{CH}_2\text{Cl}_2$  layer was evaporated. To this crude material cold diethyl ether (10 mL) was added to precipitate the PEG-bound fluoro-nitro aryl intermediate **6**. The precipitate was then collected on a sintered glass funnel and thoroughly washed with diethyl ether (10 mL  $\times$  2) to remove the excess reagents and dried. To this crude material **6** (1.25 g, 0.2 mmol) in dry  $\text{CH}_3\text{CN}$  (15 mL) was added indoline (0.167 g, 1.4 mmol) in a sealed vial and it was irradiated in a microwave reactor at 150 °C for 10 min. The crude product **1** was purified by precipitating and washing with excess cold ether (20 mL  $\times$  3) and dried *in vacuo*. This was used as it is for the further transformations. The stepwise proton NMR monitoring for these two transformations is shown in Fig. 1.



### Synthesis of PEG-bound 3-amino-4-(1-indolyl)benzoate (**7**) and PEG-bound 3-amino-4-(1-indolyl)benzoate (**8**) from intermediate (**1**)

Tin(II) chloride (0.1 g, 10%) and ammonium formate (20.0 equiv., 0.202 g, 3.2 mmol) were added to a solution of polymer bound fluoro-nitro aryl intermediate **6** (1.0 g, 0.16 mmol) in MeOH (20 mL), and the reaction mixture was subjected to microwave irradiation at 120 °C for 10 min. Upon completion of reaction, the mixtures were filtered through Celite to remove insoluble tin(II) chloride, and the filtrate was collected and concentrated under reduced pressure. Dichloromethane (15 mL) was added to precipitate ammonium formate, and the mixture was again passed through a thin layer of Celite to remove ammonium formate. The organic layer was concentrated and the crude product (**7**) obtained was used as it is for the further transformations. In a separate experiment, to the solution of polymer bound fluoro-nitro aryl intermediate **6** (1.0 g, 0.16 mmol) in EtOH (20 mL) was added 10% Pd/C (0.1 g, 10 Wt% of **6**) followed by cyclohexene (2 mL) was added and the reaction mixture was irradiated in a microwave reactor at 120 °C for 15 min. The solid palladium on charcoal was filtered off and the solvent was evaporated. PEG-bound 3-amino-4-(1-indolyl)benzoate **8** was isolated by the same precipitation and washing technique using diethyl ether. Compound **8** was also obtained from intermediate **7**: a solution of 3-amino-4-(1-indolyl)benzoate **7** (1.0 g, 0.15 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was stirred at room temperature in the presence of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) for overnight. The reaction mixture was filtered and the precipitate was washed with CH<sub>2</sub>Cl<sub>2</sub> (50 mL). The combined organic layers were concentrated and used as it is for the further transformations.

### General procedure for the Pictet–Spengler cyclization and cleavage from support

To a solution of PEG-bound 3-amino-4-(1-indolyl)benzoate **7** or PEG-bound 3-amino-4-(1-indolyl)benzoate **8** (1.0 equiv.) in CHCl<sub>3</sub> (10 mL), aldehyde or ketone (3.0 equiv.), anhydrous magnesium sulfate (20%) and 2 drops of trifluoroacetic acid (TFA) were added. Upon the addition of TFA, the faint yellow solution turned to dark red color. The resulting reaction mixture in a sealed vial was irradiated in a microwave reactor for 10 min at 120 °C. After completion of the reaction, the compound mixtures were passed through a thin layer of Celite to remove MgSO<sub>4</sub>. The solvent was removed under reduced pressure and diluted with slow addition of excess of cold ether (50 mL). The precipitated benzene fused pyrazino/diazepino indole conjugates **9–12** were filtered through a fritted funnel and washed with excess cold ether (20 mL × 3) and dried *in vacuo*. Potassium cyanide (0.01 equiv.) was added to a solution of conjugates **9–12** in methanol (10 mL). The mixtures were stirred at ambient temperature for 12 h. The solvent was removed under reduced pressure, and the mixtures were precipitated and washed with ether (50 mL × 3). The filtrates were collected, and products **2–5** were obtained in good yields and purity (Table 1).

### Methyl 6,6-dimethyl-5,6-dihydroindolo[1,2-*a*]quinoxaline-3-carboxylate (**2a**)

IR (neat)  $\nu_{\max}$  3338, 2958, 1711, 1597 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  8.01 (d, *J* = 8.4 Hz, 1H), 7.95 (d, *J* = 8.4 Hz, 1H), 7.71

(dd, *J* = 8.4, 1.8 Hz, 1H), 7.65 (d, *J* = 7.9 Hz, 1H), 7.57 (d, *J* = 1.8 Hz, 1H), 7.34–7.20 (m, 2H), 6.44 (s, 1H), 3.95 (s, 3H), 1.60 (s, 6H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  166.8, 143.1, 135.9, 134.0, 130.4, 130.1, 125.3, 122.7, 121.6, 121.4, 121.0, 117.1, 115.9, 111.9, 97.2, 52.0, 51.8, 28.6 (2C); MS (ESI) *m/z* 307 (MH<sup>+</sup>); HRMS (ESI) calcd for C<sub>19</sub>H<sub>19</sub>N<sub>2</sub>O<sub>2</sub>; *m/z* 307.1446; Found 307.1445.

### Methyl 5'*H*-spiro[cycloheptane-1,6'-indolo[1,2-*a*]quinoxaline]-3'-carboxylate (**2b**)

IR (neat)  $\nu_{\max}$  3352, 2925, 2852, 1709, 1599 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  8.00 (d, *J* = 8.3 Hz, 1H), 7.93 (d, *J* = 8.4 Hz, 1H), 7.67 (dd, *J* = 8.4, 1.8 Hz, 1H), 7.65 (d, *J* = 7.3 Hz, 1H), 7.58 (d, *J* = 1.8 Hz, 1H), 7.31–7.20 (m, 2H), 6.49 (s, 1H), 3.95 (s, 3H), 2.19–2.08 (m, 2H), 1.99–1.92 (m, 2H), 1.84–1.62 (m, 8H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  166.8, 144.4, 135.6, 133.9, 130.8, 130.1, 125.3, 122.6, 121.5, 121.3, 121.0, 117.2, 115.8, 111.8, 97.7, 57.0, 52.0, 39.4 (2C), 30.0 (2C), 22.5 (2C); MS (ESI) *m/z* 361 (MH<sup>+</sup>); HRMS (ESI) calcd for C<sub>23</sub>H<sub>25</sub>N<sub>2</sub>O<sub>2</sub>; *m/z* 361.1916; Found 361.1919.

### Methyl 6-methyl-6-phenyl-5,6-dihydroindolo[1,2-*a*]quinoxaline-3-carboxylate (**2c**)

IR (neat)  $\nu_{\max}$  3332, 2991, 2970, 1699, 1599 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  8.01 (d, *J* = 8.3 Hz, 1H), 7.87 (d, *J* = 8.3 Hz, 1H), 7.69 (d, *J* = 7.5 Hz, 1H), 7.64 (dd, *J* = 8.3, 1.8 Hz, 1H), 7.61 (d, *J* = 1.8 Hz, 1H), 7.36–7.17 (m, 7H), 6.51 (s, 1H), 3.92 (s, 3H), 1.99 (s, 3H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  167.3, 150.0, 141.9, 136.5, 134.6, 131.3, 130.3, 128.8 (2C), 127.7, 126.3 (2C), 125.7, 123.4, 122.1, 122.0, 121.7, 117.8, 116.6, 112.3, 100.5, 58.0, 52.5, 26.1; MS (ESI) *m/z* 369 (MH<sup>+</sup>); HRMS (ESI) calcd for C<sub>24</sub>H<sub>21</sub>N<sub>2</sub>O<sub>2</sub>; *m/z* 369.1603; Found 369.1605.

### Methyl 6-ethyl-6-methyl-5,6-dihydroindolo[1,2-*a*]quinoxaline-3-carboxylate (**2d**)

IR (neat)  $\nu_{\max}$  3348, 2952, 2929, 1701, 1596 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  8.01 (d, *J* = 8.3 Hz, 1H), 7.93 (d, *J* = 8.3 Hz, 1H), 7.70–1.64 (m, 2H), 7.55 (d, *J* = 1.8 Hz, 1H), 7.34–7.20 (m, 2H), 6.41 (s, 1H), 3.94 (s, 3H), 1.89–1.70 (m, 2H), 1.59 (s, 3H), 0.95 (t, *J* = 7.4 Hz, 3H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  166.8, 142.0, 135.8, 134.0, 130.4, 130.1, 125.3, 122.6, 121.4, 121.0, 117.0, 115.8, 111.9, 98.5, 54.7, 52.0, 33.1, 30.9, 25.6, 8.5; MS (ESI) *m/z* 321 (MH<sup>+</sup>); HRMS (ESI) calcd for C<sub>20</sub>H<sub>21</sub>N<sub>2</sub>O<sub>2</sub>; *m/z* 321.1603; Found 321.1601.

### Methyl 6,6-diethyl-5,6-dihydroindolo[1,2-*a*]quinoxaline-3-carboxylate (**2e**)

IR (neat)  $\nu_{\max}$  3344, 2972, 2918, 2848, 1703, 1597 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  8.04 (d, *J* = 8.1 Hz, 1H), 7.94 (d, *J* = 8.2 Hz, 1H), 7.67 (d, *J* = 8.1 Hz, 2H), 7.57 (s, 1H), 7.34–7.22 (m, 2H), 6.39 (s, 1H), 3.95 (s, 3H), 1.86 (q, *J* = 7.4 Hz, 4H), 0.96 (t, *J* = 7.4 Hz, 6H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  167.3, 140.5, 136.6, 134.5, 130.6, 130.4, 125.7, 122.9, 121.8, 121.4, 121.4, 117.1, 116.0, 112.6, 100.0, 58.5, 52.5, 31.8 (2C), 8.7 (2C); MS (ESI) *m/z* 335 (MH<sup>+</sup>); HRMS (ESI) calcd for C<sub>21</sub>H<sub>23</sub>N<sub>2</sub>O<sub>2</sub>; *m/z* 335.1759; Found 335.1761.

**Methyl 5'-H-spiro[cyclopentane-1,6'-indolo[1,2-a]quinoxaline]-3'-carboxylate (2f)**

IR (neat)  $\nu_{\max}$  3346, 2947, 2866, 1697, 1597  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.00 (d,  $J = 7.5$  Hz, 1H), 7.94 (d,  $J = 8.4$  Hz, 1H), 7.71 (dd,  $J = 8.4, 1.9$  Hz, 1H), 7.65 (dd,  $J = 7.5, 0.5$  Hz, 1H), 7.57 (d,  $J = 1.8$  Hz, 1H), 7.33–7.17 (m, 2H), 6.42 (s, 1H), 3.95 (s, 3H), 2.12–2.11 (m, 2H), 1.98–1.85 (m, 6H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  166.8, 142.9, 136.3, 134.2, 131.0, 130.0, 125.2, 122.5, 121.7, 121.4, 121.0, 117.3, 116.0, 111.8, 97.2, 62.7, 52.0, 38.9 (2C), 21.0 (2C); MS (ESI)  $m/z$  333 ( $\text{MH}^+$ ); HRMS (ESI) calcd for  $\text{C}_{21}\text{H}_{21}\text{N}_2\text{O}_2$ ;  $m/z$  333.1603; Found 333.1605.

**Methyl 6-ethylindolo[1,2-a]quinoxaline-3-carboxylate (3a)**

IR (neat)  $\nu_{\max}$  2976, 2912, 2864, 1716, 1614  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.63 (d,  $J = 2.0$  Hz, 1H), 8.42 (t,  $J = 8.7$  Hz, 2H), 8.21 (dd,  $J = 8.7, 2.0$  Hz, 1H), 7.96 (d,  $J = 8.1$ , 1H), 7.57 (dt,  $J = 7.0, 1.2$  Hz, 1H), 7.47 (dt,  $J = 7.0, 1.2$  Hz, 1H), 7.20 (s, 1H), 4.00 (s, 3H), 3.14 (q,  $J = 7.5$  Hz, 2H), 1.52 (t,  $J = 7.5$  Hz, 3H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  166.8, 160.9, 135.8, 133.8, 133.4, 131.7, 129.7, 129.6, 129.2, 125.9, 125.1, 123.6, 123.2, 115.0, 114.7, 101.1, 52.6, 29.2, 12.2; MS (ESI)  $m/z$  305 ( $\text{MH}^+$ ); HRMS (ESI) calcd for  $\text{C}_{19}\text{H}_{17}\text{N}_2\text{O}_2$ ;  $m/z$  305.1290; Found 305.1293.

**Methyl 6-(2-fluorophenyl)indolo[1,2-a]quinoxaline-3-carboxylate (3b)**

IR (neat)  $\nu_{\max}$  2920, 2852, 1722, 1612  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.77 (d,  $J = 1.9$  Hz, 1H), 8.57 (d,  $J = 8.7$ , 1H), 8.50 (d,  $J = 8.7$  Hz, 1H), 8.33 (dd,  $J = 8.7, 1.9$  Hz, 1H), 7.94 (d,  $J = 7.9$  Hz, 1H), 7.78 (dd,  $J = 7.5, 1.7$  Hz, 1H), 7.65–7.55 (m, 2H), 7.49 (t,  $J = 7.5$  Hz, 1H), 7.38 (t,  $J = 7.5$  Hz, 1H), 7.33 (m, 1H), 7.06 (d,  $J = 1.7$  Hz, 1H), 4.00 (s, 3H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  166.7, 160.6 ( $^1J_{\text{CF}} = 247.5$  Hz), 153.3, 136.0, 134.0, 133.6, 132.6, 132.1 ( $^2J_{\text{CF}} = 8.5$  Hz), 131.5, 131.4, 130.3, 129.8, 129.7, 126.2, 126.0 ( $^2J_{\text{CF}} = 22.5$  Hz), 125.5, 125.0 ( $^4J_{\text{CF}} = 3.2$  Hz), 123.8, 123.5, 116.9 ( $^2J_{\text{CF}} = 22.5$  Hz), 115.0 ( $^3J_{\text{CF}} = 8.5$  Hz), 104.0, 52.7; MS (ESI)  $m/z$  371 ( $\text{MH}^+$ ); HRMS (ESI) calcd for  $\text{C}_{23}\text{H}_{16}\text{FN}_2\text{O}_2$ ;  $m/z$  371.1196; Found 371.1194.

**Methyl 6-(5-methylthiophen-2-yl)indolo[1,2-a]quinoxaline-3-carboxylate (3c)**

IR (neat)  $\nu_{\max}$  2945, 2922, 1716, 1616  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.61 (d,  $J = 1.9$  Hz, 1H), 8.41 (t,  $J = 7.9$  Hz, 2H), 8.17 (dd,  $J = 8.7, 1.9$  Hz, 1H), 7.94 (d,  $J = 7.9$  Hz, 1H), 7.84 (d,  $J = 3.6$  Hz, 1H), 7.55 (t,  $J = 8.7$  Hz, 2H), 7.45 (t,  $J = 7.8$  Hz, 1H), 6.91 (d,  $J = 3.6$  Hz, 1H), 3.98 (s, 3H), 2.61 (s, 3H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  166.8, 149.9, 145.2, 139.7, 135.8, 133.5, 133.3, 131.9, 130.0, 129.6, 129.2, 128.0, 126.7, 126.0, 125.3, 123.7, 123.3, 115.1, 114.7, 103.0, 52.7, 16.1; MS (ESI)  $m/z$  373 ( $\text{MH}^+$ ); HRMS (ESI) calcd for  $\text{C}_{22}\text{H}_{17}\text{N}_2\text{O}_2\text{S}$ ;  $m/z$  373.1011; Found 373.1009.

**Methyl 6-phenylindolo[1,2-a]quinoxaline-3-carboxylate (3d)**

IR (neat)  $\nu_{\max}$  3057, 2924, 2852, 1722, 1616  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.80 (s, 1H), 8.54 (d,  $J = 8.8$  Hz, 1H), 8.50 (d,  $J = 8.7$ , 1H), 8.29 (dd,  $J = 8.7, 2.0$  Hz, 1H), 8.07–8.04 (m, 2H), 7.96

(d,  $J = 8.0$  Hz, 1H), 7.68–7.60 (m, 4H), 7.50 (t,  $J = 7.5$  Hz, 1H), 7.34 (s, 1H), 4.00 (s, 3H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  166.8, 157.4, 137.9, 135.9, 133.8, 133.7, 132.3, 130.9, 129.9, 129.9, 129.2, 129.1, 126.2, 125.6, 123.8, 123.5, 115.0, 114.9, 104.6, 52.7; MS (ESI)  $m/z$  353 ( $\text{MH}^+$ ); HRMS (ESI) calcd for  $\text{C}_{23}\text{H}_{17}\text{N}_2\text{O}_2$ ;  $m/z$  353.1290; Found 353.1288.

**Methyl 6,6-dimethyl-1,2,6,7-tetrahydroindolo[1,7-ab][1,5]benzodiazepine-9-carboxylate (4a)**

IR (neat)  $\nu_{\max}$  3344, 2924, 2852, 1709, 1591  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.71 (dd,  $J = 8.6, 1.9$  Hz, 1H), 7.55 (s, 1H), 7.07 (d,  $J = 7.6$  Hz, 2H), 6.83 (d,  $J = 8.6$  Hz, 1H), 6.77 (t,  $J = 7.6$  Hz, 1H), 4.07 (t,  $J = 8.6$  Hz, 2H), 3.90 (s, 3H), 3.21 (t,  $J = 8.6$  Hz, 2H), 1.50 (s, 6H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  167.6, 143.3, 141.8, 136.1, 132.2, 131.6, 125.6, 124.4, 123.8, 123.4, 120.9, 119.7, 114.4, 57.0, 52.3, 52.2 (2C), 31.8, 27.8; MS (ESI)  $m/z$  309 ( $\text{MH}^+$ ); HRMS (ESI) calcd for  $\text{C}_{19}\text{H}_{21}\text{N}_2\text{O}_2$ ;  $m/z$  309.1603; Found 309.1606.

**Methyl 6-ethyl-6-methyl-1,2,6,7-tetrahydroindolo[1,7-ab][1,5]benzodiazepine-9-carboxylate (4b)**

IR (neat)  $\nu_{\max}$  3346, 2986, 2854, 1705, 1591  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.66 (dd,  $J = 8.3, 1.8$  Hz, 1H), 7.44 (d,  $J = 1.8$  Hz, 1H), 7.04 (d,  $J = 7.6$  Hz, 1H), 6.98 (d,  $J = 7.7$  Hz, 1H), 6.76 (t,  $J = 8.3$  Hz, 2H), 4.09–3.95 (m, 2H), 3.87 (s, 3H), 3.18 (t,  $J = 7.4$  Hz, 2H), 1.93–1.78 (m, 2H), 1.50 (s, 3H), 0.86 (t,  $J = 7.4$  Hz, 3H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  167.6, 143.6, 141.7, 135.7, 132.3, 130.2, 125.4, 125.3, 123.7, 123.5, 120.9, 119.5, 114.4, 59.8, 52.3, 52.2, 36.1, 29.1, 27.8, 8.7; MS (ESI)  $m/z$  323 ( $\text{MH}^+$ ); HRMS (ESI) calcd for  $\text{C}_{20}\text{H}_{23}\text{N}_2\text{O}_2$ ;  $m/z$  323.1759; Found 323.1757.

**Methyl 6-butyl-1,2,6,7-tetrahydroindolo[1,7-ab][1,5]benzodiazepine-9-carboxylate (4c)**

IR (neat)  $\nu_{\max}$  3354, 2953, 2931, 2870, 1712, 1593  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.64 (m, 1H), 7.45 (s, 1H), 7.02 (d,  $J = 7.2$  Hz, 1H), 6.84 (d,  $J = 7.2$ , 1H), 6.77–6.71 (m, 2H), 4.18–4.11 (m, 2H), 3.97–3.93 (m, 1H), 3.88 (s, 3H), 3.18–3.14 (m, 2H), 1.49–1.41 (m, 2H), 1.26 (sextet,  $J = 6.8$  Hz, 2H), 0.82 (t,  $J = 6.9$  Hz, 3H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  167.5, 143.4, 141.4, 134.6, 132.1, 127.1, 125.3, 123.8, 123.4, 120.9, 120.7, 119.6, 115.6, 61.1, 52.3, 52.1, 37.4, 28.7, 27.7, 22.8, 14.3; MS (ESI)  $m/z$  337 ( $\text{MH}^+$ ); HRMS (ESI) calcd for  $\text{C}_{21}\text{H}_{25}\text{N}_2\text{O}_2$ ;  $m/z$  337.1916; Found 337.1917.

**Methyl 1',2'-dihydro-7'-H-spiro[cyclohexane-1,6'-indolo[1,7-ab][1,5]benzodiazepine]-9'-carboxylate (4d)**

IR (neat)  $\nu_{\max}$  3379, 2927, 2854, 107, 1589  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.66 (dd,  $J = 8.5, 1.9$  Hz, 1H), 7.49 (d,  $J = 1.9$  Hz, 1H), 7.13 (d,  $J = 7.5$  Hz, 1H), 7.04 (d,  $J = 7.5$  Hz, 1H), 6.77 (d,  $J = 8.5$  Hz, 2H), 4.04 (t,  $J = 8.8$  Hz, 2H), 3.90 (s, 3H), 3.19 (t,  $J = 8.8$  Hz, 2H), 1.86–1.80 (m, 6H), 1.67–1.57 (m, 4H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  167.6, 143.9, 142.1, 133.0, 132.3, 130.2, 125.1, 123.8, 123.3, 123.2, 120.5, 119.8, 114.5, 58.2, 52.4, 52.2, 36.3, 27.7 (2C), 25.5, 21.6 (2C); MS (ESI)  $m/z$  349 ( $\text{MH}^+$ ); HRMS (ESI) calcd for  $\text{C}_{22}\text{H}_{25}\text{N}_2\text{O}_2$ ;  $m/z$  349.1916; Found 349.1914.

**Methyl 6-ethyl-1,2,6,7-tetrahydroindolo[1,7-ab][1,5]benzodiazepine-9-carboxylate (4e)**

IR (neat)  $\nu_{\max}$  3348, 2954, 2922, 1704, 1591  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.66 (d,  $J = 7.5$  Hz, 1H), 7.52 (s, 1H), 7.05 (d,  $J = 7.5$  Hz, 1H), 6.86 (d,  $J = 7.4$  Hz, 1H), 6.79 (s, 1H), 6.76 (s, 1H), 6.73 (t,  $J = 7.4$  Hz, 1H), 4.19–3.94 (m, 3H), 3.89 (s, 3H), 3.30–3.10 (m, 2H), 1.66–1.49 (m, 2H), 0.98 (t,  $J = 7.3$  Hz, 3H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  167.5, 143.5, 141.5, 135.0, 132.1, 127.2, 127.1, 125.1, 123.8, 123.3, 120.7, 119.6, 114.9, 62.8, 52.3, 52.2, 30.8, 27.7, 11.4; MS (ESI)  $m/z$  309 ( $\text{MH}^+$ ); HRMS (ESI) calcd for  $\text{C}_{19}\text{H}_{21}\text{N}_2\text{O}_2$ ;  $m/z$  309.1603; Found 309.1605.

**Methyl 6-phenyl-1,2,6,7-tetrahydroindolo[1,7-ab][1,5]benzodiazepine-9-carboxylate (4f)**

IR (neat)  $\nu_{\max}$  3344, 2949, 2853, 1707, 1593  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.65 (dd,  $J = 8.5, 1.8$  Hz, 1H), 7.36–7.26 (m, 4H), 7.20 (dd,  $J = 8.0, 1.8$  Hz, 2H), 7.08 (d,  $J = 7.2$  Hz, 1H), 6.83 (d,  $J = 8.5$  Hz, 1H), 6.66 (t,  $J = 7.5$  Hz, 1H), 6.52 (d,  $J = 7.5$  Hz, 1H), 5.30 (s, 1H), 4.14 (td,  $J = 8.4, 2.5$  Hz, 2H), 3.84 (s, 3H), 3.28 (td,  $J = 8.4, 2.5$  Hz, 2H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  167.3, 144.3, 142.4, 141.4, 136.8, 131.8, 129.2 (2C), 128.6 (2C), 128.2, 128.0, 125.8, 125.6, 124.2, 123.3, 121.1, 119.6, 115.2, 65.6, 52.4, 52.1, 27.8; MS (ESI)  $m/z$  357 ( $\text{MH}^+$ ); HRMS (ESI) calcd for  $\text{C}_{23}\text{H}_{21}\text{N}_2\text{O}_2$ ;  $m/z$  357.1603; Found 357.1600.

**Methyl 6-(thiophen-2-yl)-1,2,6,7-tetrahydroindolo[1,7-ab][1,5]benzodiazepine-9-carboxylate (4g)**

IR (neat)  $\nu_{\max}$  3348, 2916, 2848, 1683, 1589  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.62 (dd,  $J = 8.6, 2.0$  Hz, 1H), 7.32 (d,  $J = 2.0$  Hz, 1H), 7.14 (dd,  $J = 5.1, 1.0$  Hz, 1H), 7.08 (dd,  $J = 7.1, 1.0$  Hz, 1H), 6.84–6.79 (m, 2H), 6.75 (d,  $J = 8.7$  Hz, 1H), 6.70 (d,  $J = 7.5$  Hz, 1H), 6.69 (s, 1H), 5.67 (s, 1H), 4.80 (t,  $J = 8.0$  Hz, 2H), 3.83 (s, 3H), 3.22 (t,  $J = 8.0$  Hz, 2H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  167.3, 146.5, 143.7, 141.5, 135.5, 132.2, 127.9, 127.0, 126.3, 125.9, 125.9, 125.2, 124.5, 124.0, 121.1, 119.8, 115.1, 60.7, 52.4, 52.1, 27.8; MS (ESI)  $m/z$  363 ( $\text{MH}^+$ ); HRMS (ESI) calcd for  $\text{C}_{21}\text{H}_{19}\text{N}_2\text{O}_2\text{S}$ ;  $m/z$  363.1167; Found 363.1168.

**Methyl 6-(4-methoxyphenyl)-1,2,6,7-tetrahydroindolo[1,7-ab][1,5]benzodiazepine-9-carboxylate (4h)**

IR (neat)  $\nu_{\max}$  3344, 2949, 2835, 1709, 1593  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.62 (dd,  $J = 8.6, 2.0$  Hz, 1H), 7.28 (d,  $J = 2.0$  Hz, 1H), 7.10 (d,  $J = 8.6$  Hz, 2H), 7.05 (d,  $J = 7.5, 1\text{H}$ ), 6.83 (d,  $J = 8.6$  Hz, 2H), 6.80 (d,  $J = 8.6$  Hz, 1H), 6.63 (t,  $J = 7.5$  Hz, 1H), 6.51 (d,  $J = 7.5$  Hz, 1H), 5.24 (s, 1H), 4.11 (td,  $J = 7.7, 3.3$  Hz, 2H), 3.82 (s, 3H), 3.78 (s, 3H), 3.22 (td,  $J = 7.7, 3.3$  Hz, 2H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  167.4, 159.4, 144.3, 141.4, 137.4, 134.9, 131.8, 129.7, 127.9, 126.2, 125.6, 124.0, 123.2, 121.1, 119.5, 115.1, 114.5, 65.0, 55.6, 52.4, 52.1, 27.8; MS (ESI)  $m/z$  387 ( $\text{MH}^+$ ); HRMS (ESI) calcd for  $\text{C}_{24}\text{H}_{23}\text{N}_2\text{O}_3$ ;  $m/z$  387.1709; Found 387.1707.

**Methyl 6-(1,3-benzodioxol-5-yl)-1,2,6,7-tetrahydroindolo[1,7-ab][1,5]benzodiazepine-9-carboxylate (4i)**

IR (neat)  $\nu_{\max}$  3344, 2946, 2900, 1707, 1592  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.64 (d,  $J = 8.5$  Hz, 1H), 7.29 (d,  $J = 7.6$  Hz,

1H), 7.07 (d,  $J = 7.0$  Hz, 1H), 6.82 (d,  $J = 8.5$  Hz, 1H), 6.74 (d,  $J = 7.6$  Hz, 1H), 6.68 (d,  $J = 6.1$  Hz, 2H), 6.64 (s, 1H), 6.57 (d,  $J = 7.6$  Hz, 1H), 5.94 (s, 2H), 5.25 (s, 1H), 4.10 (t,  $J = 8.8$  Hz, 2H), 3.85 (s, 3H), 3.23 (t,  $J = 8.8$  Hz, 2H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  166.9, 147.9, 146.9, 143.7, 140.9, 136.7, 136.3, 131.4, 127.5, 125.3, 125.2, 123.6, 122.7, 121.6, 120.6, 119.1, 114.6, 108.3, 108.1, 101.0, 64.8, 51.9, 51.7, 26.4; MS (ESI)  $m/z$  401 ( $\text{MH}^+$ ); HRMS (ESI) calcd for  $\text{C}_{24}\text{H}_{21}\text{N}_2\text{O}_4$ ;  $m/z$  401.1501; Found 401.1503.

**Methyl 6-(2-nitrophenyl)-1,2,6,7-tetrahydroindolo[1,7-ab][1,5]benzodiazepine-9-carboxylate (4j)**

IR (neat)  $\nu_{\max}$  3357, 2949, 2856, 1701, 1594  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.97 (dd,  $J = 7.9, 1.8$  Hz, 1H), 7.57 (dd,  $J = 8.6, 1.8$  Hz, 1H), 7.30–7.17 (m, 2H), 7.12 (dd,  $J = 6.2, 1.9$  Hz, 1H), 7.04 (d,  $J = 1.9$  Hz, 1H), 6.80 (d,  $J = 8.6$  Hz, 1H), 6.76–6.69 (m, 3H), 5.99 (s, 1H), 4.29–3.99 (m, 2H), 3.78 (s, 3H), 3.38–3.23 (m, 2H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  167.1, 149.2, 145.1, 141.8, 138.0, 135.0, 133.4, 132.3, 130.6, 128.7, 128.5, 125.9, 125.5, 124.7, 123.7, 123.3, 121.7, 120.1, 114.9, 61.0, 52.3, 52.1, 27.8; MS (ESI)  $m/z$  424 ( $\text{M}+\text{Na}^+$ ); HRMS (ESI) calcd for  $\text{C}_{23}\text{H}_{19}\text{N}_3\text{O}_4\text{Na}$ ;  $m/z$  424.1273; Found 424.1270.

**Methyl 6-(3-nitrophenyl)-1,2,6,7-tetrahydroindolo[1,7-ab][1,5]benzodiazepine-9-carboxylate (4k)**

IR (neat)  $\nu_{\max}$  3340, 2949, 2854, 1707, 1593  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.07 (d,  $J = 5.9$  Hz, 1H), 8.00 (s, 1H), 7.62 (d,  $J = 8.4$  Hz, 1H), 7.45 (s, 2H), 7.22 (s, 1H), 7.14 (d,  $J = 6.9$  Hz, 1H), 6.83 (d,  $J = 8.4$  Hz, 1H), 6.72 (t,  $J = 7.4$  Hz, 1H), 6.63 (d,  $J = 7.4$  Hz, 1H), 5.52 (s, 1H), 4.17 (dt,  $J = 18.5, 8.4$  Hz, 1H), 4.11 (dt,  $J = 18.5, 8.4$  Hz, 1H), 3.83 (s, 3H), 3.28 (dd,  $J = 8.4, 8.4$  Hz, 2H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  167.2, 148.7, 145.0, 141.6, 135.5, 134.4, 132.4, 132.4, 130.6, 130.0, 127.9, 126.0, 124.7, 123.7, 123.3, 123.1, 121.4, 120.0, 115.3, 64.8, 52.4, 52.2, 27.8; MS (ESI)  $m/z$  424 ( $\text{M}+\text{Na}^+$ ); HRMS (ESI) calcd for  $\text{C}_{23}\text{H}_{19}\text{N}_3\text{O}_4\text{Na}$ ;  $m/z$  424.1273; Found 424.1272.

**Methyl 6-(4-nitrophenyl)-1,2-dihydroindolo[1,7-ab][1,5]benzodiazepine-9-carboxylate (5)**

IR (neat)  $\nu_{\max}$  2949, 2844, 1712, 1518  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.23 (d,  $J = 8.8$  Hz, 2H), 7.72 (d,  $J = 8.8$  Hz, 2H), 7.71 (d,  $J = 2.1$  Hz, 1H), 7.64 (dd,  $J = 8.4, 2.1$  Hz, 1H), 7.10 (dd,  $J = 7.7, 1.1$  Hz, 1H), 6.68 (t,  $J = 7.7$  Hz, 1H), 6.58 (d,  $J = 8.4$  Hz, 1H), 6.51 (dd,  $J = 7.7, 1.1$  Hz, 1H), 3.87 (s, 3H), 3.73 (t,  $J = 8.4$  Hz, 2H), 3.01 (t,  $J = 8.4$  Hz, 2H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  167.6, 166.7, 156.9, 148.9, 148.8, 147.3, 139.0, 133.5, 133.0, 130.9, 130.8 (2C), 130.6, 129.0, 125.0, 123.6 (2C), 123.2, 122.4, 115.7, 52.3, 49.9, 27.7; MS (ESI)  $m/z$  400 ( $\text{MH}^+$ ); HRMS (ESI) calcd for  $\text{C}_{23}\text{H}_{18}\text{N}_3\text{O}_4$ ;  $m/z$  400.1297; Found 400.1301.

**VEGFR-3 inhibition assay**

This assay was performed in two microtiter plates. The first plate was used to culture an adherent cell line expressing the VEGF receptor 3 and to stimulate the receptor with a test compound. The second plate was used to capture the solubilized membrane receptor, which was then probed for phosphotyrosine content with phosphotyrosine specific antibody. H928 cells ( $2 \times 10^5$ ) in 100  $\mu\text{L}$

medium were added to each well in a flat-bottom 24-well culture plate and cultured overnight at 37 °C in 5% CO<sub>2</sub>. After the supernatants were removed, the cells were serum-starved for 24 h. A medium containing a test compound was added into each well and the cell culture was incubated for 30 min before it was stimulated by recombinant VEGF-C for 15 min. After the supernatants were removed, 100 µL of lysis buffer was added into each well to lyse the cells and solubilize the VEGFR-3. The lysis buffer included 150 mM NaCl containing 50 mM Hepes (Genentech media prep), 0.5% Triton-X 100 (Genentech media prep), 0.01% thimerosol, 30 kIU/mL aprotinin (ICN Biochemicals, Aurora, Ohio), 1 mM 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF; ICN Biochemicals) and 2 mM sodium orthovanadate. The plate was then put on a plate shaker (Bellco Instruments Vineland, NJ) and the substance in each well was allowed to undergo mixing for 60 min at room temperature. While the cells were being solubilized, an ELISA microtiter plate (Nunc Maxisorp, Inter Med, Denmark) coated overnight at 4 °C with the affinity-purified polyclonal anti-VEGFR-3 (2.5 µg mL<sup>-1</sup> in phosphate buffered saline (PBS), 100 µL/well) was decanted, tamped on a paper towel, and blocked with 150 µL/well block buffer (PBS containing 0.5% BSA and 0.01% thimerosol) for 60 min at room temperature with gentle agitation. The anti-VEGFR-3 coated plate was subsequently washed twice with wash buffer (PBS containing 0.05% Tween 20 and 0.01% thimerosol). The lysate containing solubilized VEGFR-3 from the cell-culture microtiter well was transferred (85 µL/well) to the anti-VEGFR-3 coated ELISA plate and incubated for 2 h at room temperature with gentle agitation. The unbound receptors were removed by washing with wash buffer. 100 µL of biotinylated 4G10 (antiphosphotyrosine) diluted to 0.2 µg mL<sup>-1</sup> in dilution buffer (PBS containing 0.5% BSA, 0.05% Tween 20, 5 mM EDTA, and 0.01% thimerosol) was added into each well. After incubation for 2 h at room temperature, the plate was washed and 100 µL HRP-conjugated streptavidin (Zymed Laboratories, S. San Francisco, CA) diluted 1 : 2000 in dilution buffer was further added. The free avidin conjugate was washed away and 100 µL freshly prepared substrate solution (tetramethyl benzidine, TMB) was added to each well. The reaction was allowed to proceed for 10 min and the color development was stopped by the addition of 1.0 M H<sub>3</sub>PO<sub>4</sub> (100 µL/well). The absorbances at 450 nm with a reference wavelength of 650 nm (A<sub>450/650</sub>), were recorded using an ELISA reader. The inhibition efficacy of each test compound was expressed as an inhibition percentage calculated as 1 - [(C - A)/(B - A)]. Wherein, 'A' is the basal amount of phosphotyrosine detected in a blank control, 'B' is the amount of phosphotyrosine detected with VEGF-C only, and 'C' is the amount of phosphotyrosine detected with a test compound and VEGF-C.

## Acknowledgements

National Science Council of Taiwan for the financial assistance.

## References

- 1 A. A. Patchett and R. P. Nargund, *Annu. Rep. Med. Chem.*, 2000, **35**, 289–298.
- 2 A. H. Lipkus, Q. Yuan, K. A. Lucas, S. A. Funk, W. F. Bartelt III, R. J. Schenck and A. J. Trippe, *J. Org. Chem.*, 2008, **73**, 4443–4451.
- 3 C. Gil and S. Brase, *J. Comb. Chem.*, 2009, **11**, 175–197.
- 4 F. R. de Sa Alves, E. J. Barreiro and C. A. M. Fraga, *Mini-Rev. Med. Chem.*, 2009, **9**, 782–793.
- 5 (a) D. A. Horton, G. T. Bourne and M. L. Smythe, *Chem. Rev.*, 2003, **103**, 893–930; (b) N. R. Candeias, L. C. Branco, P. M. Gois, C. A. M. Afonso and A. F. Trindade, *Chem. Rev.*, 2009, **109**, 2703–2802; (c) K. Kumar and H. Waldmann, *Angew. Chem., Int. Ed.*, 2009, **48**, 3224–3242.
- 6 L. F. Tietze, H. P. Bell and S. Chandrasekhar, *Angew. Chem., Int. Ed.*, 2003, **42**, 3996–4028.
- 7 T. E. Nielsen and M. Meldal, *Curr. Opin. Drug Discovery Dev.*, 2009, **12**, 798–810.
- 8 J. P. Kennedy, L. Williams, T. M. Bridges, R. N. Daniels, D. Weaver and C. W. Lindsley, *J. Comb. Chem.*, 2008, **10**, 345–354.
- 9 R. N. Lawrence, *Drug Discovery Today*, 2000, **5**, 172–175.
- 10 *Multicomponent reactions*, (ed.: J. Zhu H. Bienayme) Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, 2005, 468 pp.
- 11 E. D. Cox and J. M. Cook, *Chem. Rev.*, 1995, **95**, 1797–1842.
- 12 (a) E. J. Glamkowski and J. M. Fortunato, *J. Heterocycl. Chem.*, 1979, **16**, 865–869; (b) J. Yang, X. Che, Q. Dang, Z. Wei, S. Gao and X. Bai, *Org. Lett.*, 2005, **7**, 1541–1543; (c) B. Kundu, D. Sawant, P. Partani and A. P. Kesarwani, *J. Org. Chem.*, 2005, **70**, 4889–4892; (d) M. A. J. Duncton, L. M. Smith II, S. Burdzovic-Wizeman, A. Burns, H. Liu, Y. Mao, W. C. Wong and A. S. Kiselyov, *J. Org. Chem.*, 2005, **70**, 9629–9631; (e) P. K. Agarwal, D. Sawant, S. Sharma and B. Kundu, *Eur. J. Org. Chem.*, 2009, 292–303.
- 13 (a) B. Maiti, K. Chanda and C. M. Sun, *Org. Lett.*, 2009, **11**, 4826–4829; (b) Y. S. Hsiao, G. S. Yellol, L. H. Chen and C. M. Sun, *J. Comb. Chem.*, 2010, **12**, 723–732; (c) J. J. Lai, D. B. Salunke and C. M. Sun, *Org. Lett.*, 2010, **12**, 2174–2177; (d) G. S. Yellol, T. W. Chung and C. M. Sun, *Chem. Commun.*, 2010, **46**, 9170–9172.
- 14 (a) K. M. Brummond and D. Chen, *Org. Lett.*, 2008, **10**, 705–708; (b) A. R. Kelly, J. Wei, S. Kesavan, J. C. Marie, N. Windmon, D. W. Young and L. A. Marcaurelle, *Org. Lett.*, 2009, **11**, 2257–2260.
- 15 C. M. Sun, *Methods Mol. Biol.*, 2002, **201**, 141–166.
- 16 C. O. Kappe, *Angew. Chem., Int. Ed.*, 2004, **43**, 6250–6284.
- 17 K. Fuchibe, T. Kaneko, K. Mori and T. Akiyama, *Angew. Chem., Int. Ed.*, 2009, **48**, 8070–8073.
- 18 (a) S. Laliberte, P. K. Dornan and A. Chen, *Tetrahedron Lett.*, 2010, **51**, 363–366; (b) R. H. Dodd, C. Ouannes, M. C. Potier, L. P. de Carvalho, J. Rossier and P. Potier, *J. Med. Chem.*, 1987, **30**, 1248–1254; (c) E. J. Glamkowski, J. M. Fortunato, H. H. Ong, R. C. Allen, J. C. Wilker and H. M. Geyer III, *J. Med. Chem.*, 1984, **27**, 81–83.
- 19 (a) F. Lovering, J. Bikker and C. Humblet, *J. Med. Chem.*, 2009, **52**, 6752–6756; (b) D. B. Salunke, C. M. Sun, *Chiral Synthesis on Polymer Support: A Combinatorial Approach. In Polymeric Chiral Catalyst Design and Chiral Polymer Synthesis*, (Ed.: S. Itsuno) Wiley-Blackwell: USA, 2010, Chapter 6.
- 20 All the microwave-assisted polymer-supported reactions were performed in CEM Discover Microwave in closed vessel system.
- 21 L. H. Zhang, W. Meier, E. Wats, T. D. Costello, P. Ma, C. L. Ensinger, J. M. Rodgers, I. C. Jacobson and P. Rajagopalan, *Tetrahedron Lett.*, 1995, **36**, 8387–8390.
- 22 S. Raines, S. Y. Chai and F. P. Palopoli, *J. Heterocycl. Chem.*, 1976, **13**, 711–716.
- 23 T. Hara, K. Mori, T. Mizugaki, K. Ebitani and K. Kaneda, *Tetrahedron Lett.*, 2003, **44**, 6207–6210.
- 24 The stepwise <sup>1</sup>H NMR monitoring for these transformations carried out on a PEG support is included in the supporting information.
- 25 The single crystals were selected using Olympus stereo zoom microscope and the X-ray diffraction data was collected on a Bruker X8 APEX CCD diffractometer with omega and phi scan mode, kMoKa = 0.71073 Å at T = 100(2) K. All the data were corrected for Lorentzian, polarization, and absorption effects using Bruker's SAINT and SAD-ABS programs. The crystal structures were solved by direct methods using SHELXS-97 and the refinement was performed by full matrix least squares on F<sup>2</sup> using SHELXTL software package (Sheldrick, G. M. *Acta Crystallogr.* 2008, **A64**, 112–122). Hydrogen atoms were included in the refinement as per the riding model. Crystallographic data for the structural analysis of **2a** and **4k** have been deposited with the Cambridge Crystallographic Data Centre, CCDC No. 782683 and 782682, respectively.† Copies of this information can be obtained free of charge from The Director, CCDC, 12 Union road, Cambridge CB2 1EZ, UK (fax: C44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk) or via www.ccdc.cam.ac.uk/conts/retrieving.html.

- 26 M. N. Burnett, C. K. Johnson, *ORTEP III, report ORNL-6895*, Oak Ridge National Laboratory, TN, USA, 1996.
- 27 C. Che, J. Xiang, G. X. Wang, R. Fathi, J. M. Quan and Z. Yang, *J. Comb. Chem.*, 2007, **9**, 982–989.
- 28 T. Yu, J. R. Tagat, A. D. Kerekes, R. J. Doll, Y. Zhang, Y. Xiao, S. Esposito, D. B. Belanger, P. J. Curran, A. K. Mandal, M. A. Siddiqui, N. Y. Shih, A. D. Basso, M. Liu, K. Gray, S. Tevar, J. Jones, S. Lee, L. Liang, S. Ponery, E. B. Smith, A. Hruza, J. Voigt, L. Ramanathan, W. Prorise and M. Hu, *ACS Med. Chem. Lett.*, 2010, **1**, 214–218.
- 29 V. Gracias, Z. Ji, I. Akritopoulou-Zanze, C. Abad-Zapatero, J. R. Huth, D. Song, P. J. Hajduk, E. F. Johnson, K. B. Glaser, P. A. Marcotte, L. Pease, N. B. Soni, K. D. Stewart, S. K. Davidsen, M. R. Michaelides and S. W. Djuric, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 2691–2695.
- 30 (a) M. G. Achen and S. A. Stackler, *Int. J. Cancer*, 2006, **119**, 1755–1760; (b) Y. Cao and Q. Liu, *Adv. Cancer Res.*, 2007, **97**, 203–224; (c) J. P. Sleeman and W. Thiele, *Int. J. Cancer*, 2009, **125**, 2747–2756; (d) S. Hirakawa, *Cancer Sci.*, 2009, **100**, 983–989.
- 31 (a) J. L. Su, P. C. Yang, J. Y. Shih, C. Y. Yang, L. H. Wei, C. Y. Hsieh, C. H. Chou, Y. M. Jeng, M. Y. Wang, K. J. Chang, M. C. Hung and M. L. Kuo, *Cancer Cell*, 2006, **9**, 209–223; (b) S. Maekawa, A. Iwasaki, T. Shirakusa, S. Enatsu, T. Kawakami, M. Kuroki and M. Kuroki, *Anticancer Res.*, 2007, **27**, 3735–3741.
- 32 (a) F. Chen, K. Takenaka, E. Ogawa, K. Yanagihara, Y. Otake, H. Wada and F. Tanaka, *Clin. Cancer Res.*, 2004, **10**, 8548–8553; (b) X. E. Liu, X. D. Sun and J. M. Wu, *World J. Gastroenterol.*, 2004, **10**, 352–355; (c) X. M. Yu, C. Y. Lo, W. F. Chan, K. Y. Lam, P. Leung and J. M. Luk, *Clin. Cancer Res.*, 2005, **11**, 8063–8069; (d) V. Cohen-Kaplan, I. Naroditsky, A. Zetser, N. Ilan, I. Vlodaysky and I. Doweck, *Int. J. Cancer*, 2008, **123**, 2566–2573.