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# Analogues of the marine alkaloids oroidin, clathrocin, and hymenidin induce apoptosis in human HepG2 and THP-1 cancer cells†

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The marine alkaloids clathrocin, oroidin, and hymenidin, which were isolated from *Agelas* sponges, possess diverse biological activities. Herein, we describe the design of a library of their analogues and the evaluation of their apoptosis-inducing activities against the human hepatocellular carcinoma HepG2 and acute monocytic leukaemia THP-1 cell lines. The screening of the complete library of 96 compounds using the HepG2 cell line allowed us to determine key structural elements and physicochemical properties that are responsible for the apoptosis-inducing activity. The indole-based compounds **24c**, **28c**, **29c**, and **34c** were found to be the most potent inducers of apoptosis in HepG2 and THP-1 cell lines with EC<sub>50</sub> values in the low micromolar range. Cell cycle analysis assays confirmed that compounds **24c**, **28c**, **29c**, and **34c** induce the apoptosis of THP-1 cells at 25 μM, which highlights these oroidin analogues as interesting candidates for further evaluation of their anticancer activity.

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## Introduction

Marine organisms, particularly sponges, have been recognised as a rich source of natural products that possess anticancer activity.<sup>1–3</sup> Clathrocin, hymenidin, and oroidin (Fig. 1) are pyrrole-2-aminoimidazole<sup>4</sup> alkaloids that were initially isolated from the sponges of the genus *Agelas*. Oroidin acts as a chemical defence agent against the predatory reef fish and can be considered a biogenetic precursor of a variety of secondary metabolites that exhibit great structural complexity and diversity and present a range of biological activities.<sup>3,5</sup> The oroidin class of marine alkaloids has been reported to display modulatory activities on voltage-gated sodium<sup>6</sup> and calcium<sup>7</sup> channels, and oroidin and its analogues have also been extensively studied as inhibitors of bacterial biofilm formation.<sup>8</sup> However, the apoptosis-inducing activity of the marine alkaloids

clathrocin, hymenidin, and oroidin, and their synthetic analogues has not yet been evaluated.

The oroidin class of alkaloids is structurally relatively simple (Fig. 1) and possesses drug-like properties according to Lipinski's rule of five;<sup>9</sup> thus, this class is well suited for use as a starting point for the design of novel analogues and mimetics that can be screened for their biological activities. Recently, inspired by the reported ability of clathrocin to block human voltage-gated sodium channels,<sup>6</sup> we designed and synthesised a library of clathrocin and oroidin analogues and evaluated some

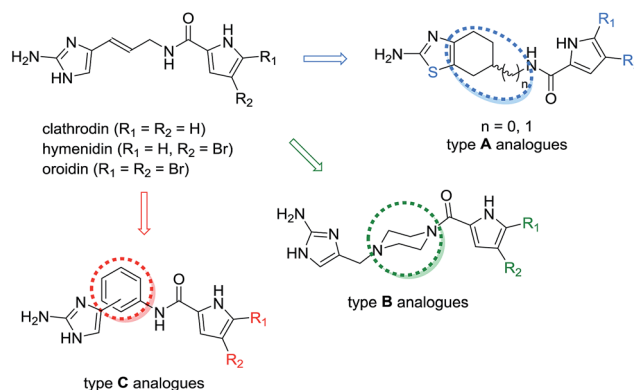


Fig. 1 Structures of the pyrrole-2-aminoimidazole alkaloids clathrocin, hymenidin, and oroidin, and their structural modifications (type A–C analogues).

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† Electronic supplementary information (ESI) available: Experimental procedures, compound characterization data, <sup>1</sup>H and <sup>13</sup>C spectra of the most active compounds, analysis of molecular descriptors, biological activity data, and description of biological assays. See DOI: 10.1039/c4md00286e

of the resulting compounds to determine their modulatory activity on human voltage-gated sodium channels<sup>10,11</sup> and their antibacterial activity.<sup>12</sup>

The clathrocin, hymenidin, and oroidin molecules possess a potentially unstable double bond in the linker between the 2-aminoimidazole and pyrrole moieties.<sup>13</sup> Therefore, we designed and synthesised a library of their analogues by modifying the central part of the molecule (Fig. 1, type **A** to **C** analogues) to obtain more stable and conformationally restricted compounds as well as the western (Fig. 1, type **A–C** analogues) and eastern parts (type **A–C** analogues) of the molecule to enable structure–activity relationship studies. In the present work, we studied the apoptosis-inducing activity of clathrocin, oroidin, and hymenidin, and their type **A–C** analogues against HepG2 and THP-1 human cancer cell lines to evaluate their potential as anticancer agents against hepatocellular carcinoma and acute monocytic leukaemia.

Hepatocellular carcinoma is the most common type of liver cancer, and its high incidence has been attributed to persistent infection with hepatitis B or C virus, contact with hepatocarcinogens (*e.g.*, aflatoxins), and cirrhosis. The development of drug resistance in hepatocellular carcinoma tumour cells after drug therapy indicates the important need for the discovery of novel anticancer agents for the successful treatment of liver cancer.<sup>14</sup>

Acute monocytic leukaemia, a type of acute myeloid leukaemia, is a hematopoietic cancer characterised by a disorder of hematopoietic progenitor cells, which lose their ability for normal differentiation and response to normal regulators of proliferation. Its incidence increases with age. Considering the aging population and the fact that acute myeloid leukaemia has the lowest survival rate of all leukaemias, new anticancer agents against acute myeloid leukaemia are urgently needed to fight this type of cancer in the future.<sup>15</sup>

## Results and discussion

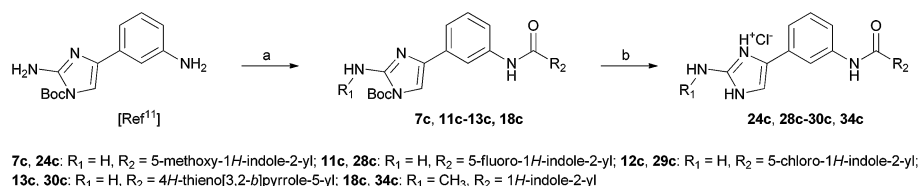
To evaluate the potential of the marine alkaloids clathrocin, oroidin, and hymenidin, and their type **A–C** analogues (a library of 96 compounds) to induce apoptosis in an *in vitro* human liver cancer model, we conducted a primary screening of all these compounds on the human hepatocellular carcinoma cell line HepG2 (ATCC® HB-8065™) using the annexin V/propidium iodide (PI) apoptosis assay with microcapillary flow cytometry (Guava EasyCyte™, Millipore/Merck, CA, USA) as the readout.<sup>16</sup> Annexin V was used to assess the loss of membrane asymmetry, which is characterised by externalisation of phosphatidylserine,

an early indicator of apoptosis.<sup>17</sup> Propidium iodide, which is widely used as a DNA intercalating dye for the evaluation of cell viability or DNA content in cell cycle analyses, was used to determine the membrane integrity.<sup>18</sup> Using this double-staining apoptosis assay, we were able to distinguish between living cells (annexin V-negative, PI-negative), early apoptotic cells (annexin V-positive, PI-negative) and late apoptotic/secondary necrotic cells (annexin V-positive, PI-positive).<sup>19</sup> All the compounds were tested at 50  $\mu$ M in four independent experiments (Tables S1–S4 in the ESI†). The HPLC purity of the tested compounds was above 95% monitored at 254 nm. Syntheses of the majority of compounds are reported elsewhere<sup>10–12,20,21</sup> and their analytical data (<sup>1</sup>H- and <sup>13</sup>C-NMR, mass spectra) can be found in the ESI.† Synthesis of the most active compounds **24c**, **28c–30c** and **34c** is summarised in Scheme 1.

The marine alkaloids clathrocin, oroidin and hymenidin, which were synthesised in our laboratory,<sup>20</sup> and their analogues **1–4** were found to possess only weak apoptosis-inducing activity in the HepG2 cell line with 25–38% apoptotic cells at 50  $\mu$ M (Table S1†).

In the series of type **A** analogues (Fig. 1), conformational restriction was achieved by replacing the (*E*)-5-(3-aminoprop-1-enyl)-1*H*-imidazol-2-amine moiety by the 4,5,6,7-tetrahydrobenzo[*d*]thiazol-2-amine core in which the imidazole was isosterically replaced by the thiazole ring.<sup>10</sup> However, type **A** analogues **1a–14a** containing also various modifications in the central and eastern parts of the parent molecules (Table S2†) did not display improved apoptosis-inducing activity compared with oroidin (20–38% apoptotic HepG2 cells at 50  $\mu$ M).

The type **B** analogues **1b–10b** were obtained by modification of the central part through replacement of the 3-aminoprop-1-enyl linker between the 2-aminoimidazole and pyrrole moieties by the less flexible *N*-methylenepiperazine group (Fig. 1).<sup>21</sup> Modification of the central part together with variations in the eastern and western parts of the parent molecules gave only weakly active compounds **1b–10b** (Table S3,† 20–34% apoptotic HepG2 cells at 50  $\mu$ M). In the type **C** analogues, the 1,3- or 1,4-disubstituted phenyl ring was incorporated in place of the prop-1-enyl linker to obtain a conformationally restricted central part of the molecule without changing the length of the molecule (Fig. 1).<sup>11</sup> The analysis of the apoptosis-inducing activity of the type **C** analogues revealed a similar trend to those observed with the type **A** and **B** analogues. If the substituent in the eastern part of the molecule was a five-membered pyrrole (**1c–3c**, **14c**, **19c**, **20c**, **31c**, **37c**, **38c**, and **43c**), pyrrolidine (**4c**, **21c**, **40c**, **41c**, and **44c**), furan (**15c**, **17c**, and **32c**), or imidazole (**39c**) ring, the



**Scheme 1** Synthesis of the most active compounds **24c**, **28c–30c** and **34c**. Reagents and conditions: (a) corresponding carboxylic acid, TBTU, NMM, CH<sub>2</sub>Cl<sub>2</sub>, 35 °C, 24 h; (b) HCl(g), THF–EtOH, rt, 5 h.

compounds were only weakly active (Table S4, Fig. S1,† 13–44% apoptotic HepG2 cells at 50  $\mu$ M). The only exceptions were the pyrrole-based compounds **35c** and **36c**, which contained a benzyl moiety on the imidazole ring nitrogen and induced apoptosis in 54% and 86% of HepG2 cells at a concentration of 50  $\mu$ M (Table S4†), respectively. In contrast, a significant gain in the apoptosis-inducing activity was observed in the compounds containing an indole or substituted indole moiety in place of the pyrrole ring of the parent marine alkaloids.

The indole-based compounds **5c–12c**, **16c**, **18c**, and **42c** and 4*H*-thieno[3,2-*b*]pyrrole **13c** containing a *tert*-butoxycarbonyl (Boc) group on the imidazole *N*<sup>1</sup> showed improved activity with 43–91% apoptotic HepG2 cells at 50  $\mu$ M. A substitution at position 5 of the indole ring with a methoxy (**7c**), benzyloxy (**8c**), fluoro (**11c**), or chloro (**12c**) group resulted in improved activity compared to the non-substituted indoles **5c** and **6c**, whereas a hydroxy (**9c**) or trifluoromethoxy (**10c**) substitution decreased the potency. A methyl substituent at the imidazole 2-amino group increased the percentage of apoptotic cells compared with those found with the indole-based compounds (**5c** vs. **18c**), whereas a reduction of the imidazole to obtain a 2-amino-imidazoline ring reduced the activity (**18c** vs. **16c**). Compound **42c** with a 1,4-disubstituted phenyl ring in the central part and an indole moiety in the eastern part of the molecule was among the most active apoptosis-inducing compounds and was more active than its 1,3-phenylene counterpart **5c**.

In general, the Boc-protected indole-based compounds **22c–29c**, **33c**, and **34c** and the 4*H*-thieno[3,2-*b*]pyrrole-based compound **30c** retained their apoptosis-inducing activity against the HepG2 cell line. Similar to their Boc-protected analogues, the compounds with the 6-fluoro- (**28c**), 6-chloro-indole (**29c**), and 4*H*-thieno[3,2-*b*]pyrrole (**30c**) moieties were the most active with more than 90% apoptotic HepG2 cells at 50  $\mu$ M.

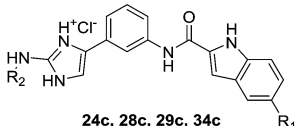
We also evaluated the apoptosis-inducing activity of compounds **45c–65c** (Table S4†), which were identified in the 3D similarity searching, based on the indole **22c**, in the ZINC database of drug-like compounds.<sup>22</sup> Interestingly, most of the indoles (**45c**, **53c**, **55c**, **57c**, **62c**, and **63c** but not **58c** and **64c**) induced apoptosis in more than 50% of HepG2 cells at 50  $\mu$ M, regardless of the ring type in the western part of the molecule, which indicates that the indole moiety is a crucial feature for significant apoptosis-inducing activity in type C oroidin analogues. Among compounds **45c–65c**, only compound **52c** showed improved apoptosis-inducing activity (92% apoptotic HepG2 cells at 50  $\mu$ M) compared with the template compound **22c**.

The molecular descriptor analysis of our library of oroidin analogues showed that most of the compounds possess drug-like properties according to Lipinski's rule of five<sup>9</sup> (Fig. S2†). The presented charts show that most of the active compounds (>50% apoptotic HepG2 cells at 50  $\mu$ M) are more lipophilic (log *D* values between 3 and 5) and have higher molecular weights (MW between 300 and 500) compared with their inactive counterparts (log *D* values between –1 and 5, MW between 200 and 500). In contrast, the number of hydrogen bond donors and acceptors is similarly distributed between the actives and inactives.

Of the 96 compounds screened, compounds **24c**, **28c–30c**, and **34c**, which were among the most active type C oroidin analogues, were selected for further characterisation. First, the EC<sub>50</sub> values for their apoptosis-inducing activity against the HepG2 cell line were determined using the annexin V/PI apoptosis assay (Table 1). 6-Fluoroindole **28c** was found to be the most active compound with an EC<sub>50</sub> value of 13  $\mu$ M, followed by 6-chloroindole **29c** (EC<sub>50</sub> = 16  $\mu$ M), 6-methoxy-indole **24c** (EC<sub>50</sub> = 18  $\mu$ M), indole **34c** (EC<sub>50</sub> = 20  $\mu$ M), and 4*H*-thieno[3,2-*b*]pyrrole **30c** (EC<sub>50</sub> = 42  $\mu$ M).

Considering the noteworthy HepG2 apoptosis-inducing activity of compounds **24c**, **28c–30c**, and **34c**, these were further evaluated using the human monocytic leukaemia THP-1 cell line (ATCC® TIB-202™) (Table 1). The screening of these compounds at 50  $\mu$ M against the THP-1 cell line showed that compounds **24c**, **28c**, **29c**, and **34c** induce apoptosis (87–97% apoptotic THP-1 cells), whereas compound **30c** was found to be inactive (12% apoptotic THP-1 cells). The dose-response curves showed that compounds **24c**, **28c**, **29c**, and **34c** exhibited similar activities against the THP-1 cell line with EC<sub>50</sub> values ranging from 20  $\mu$ M to 24  $\mu$ M (Table 1). Because activation of the apoptotic pathways is a key mechanism through which anticancer drugs kill tumour cells,<sup>23</sup> it was important to confirm that compounds **24c**, **28c**, **29c**, and **34c** *de facto* exert their cytotoxic effect against the THP-1 cell line *via* the induction of apoptosis and not by necrosis. Hence, THP-1 cells were subjected to a cell cycle analysis after exposure to **24c**, **28c**, **29c**, and **34c** to determine the incidence of fragmented DNA (sub-G1 population) by PI staining of the nuclei.<sup>24</sup> DMSO (0.25%), which was used as a negative control, did not affect the cell cycle in THP-1 cells (Fig. 2). The results of the cell cycle analysis after the incubation of THP-1 cells with compounds **24c**, **28c**, **29c**, or **34c** at 25  $\mu$ M for 24 h and 48 h (Fig. 2 and 3 and Table S5†) show the presence of a sub-G1 cell population, which confirmed the presence of programmed cell death, *i.e.* apoptosis. Compounds **28c** and **29c** showed similar potency: 41% and 40% of the cells were found in the sub-G1 peak after 48 h of treatment,

Table 1 Cytomic apoptosis-inducing activities of the type C analogues **24c**, **28c–30c**, and **34c**

Compound			HepG2 <sup>a</sup> EC <sub>50</sub>	THP-1 <sup>a</sup> EC <sub>50</sub>
	R <sub>1</sub>	R <sub>2</sub>		
<b>24c</b>	OCH <sub>3</sub>	H	18 ± 1 $\mu$ M	20 ± 2 $\mu$ M
<b>28c</b>	F	H	13 ± 7 $\mu$ M	23 ± 4 $\mu$ M
<b>29c</b>	Cl	H	16 ± 6 $\mu$ M	24 ± 5 $\mu$ M
<b>30c</b>	—	—	42 ± 16 $\mu$ M	>50 $\mu$ M
<b>34c</b>	H	CH <sub>3</sub>	20 ± 5 $\mu$ M	24 ± 1 $\mu$ M

<sup>a</sup> The values are the mean ± SD of three independent experiments performed in triplicate.

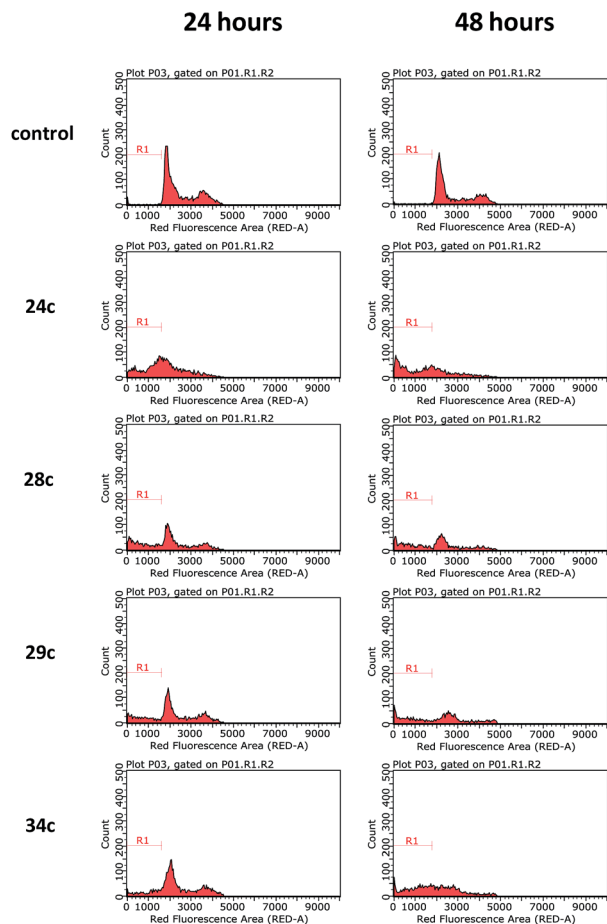


Fig. 2 Cell cycle analysis of THP-1 cells after incubation with compounds **24c**, **28c**, **29c**, and **34c** at 25  $\mu$ M for 24 h and 48 h. DMSO (0.25%) in culture medium was used as a negative control. Representative histograms of three independent experiments are displayed.

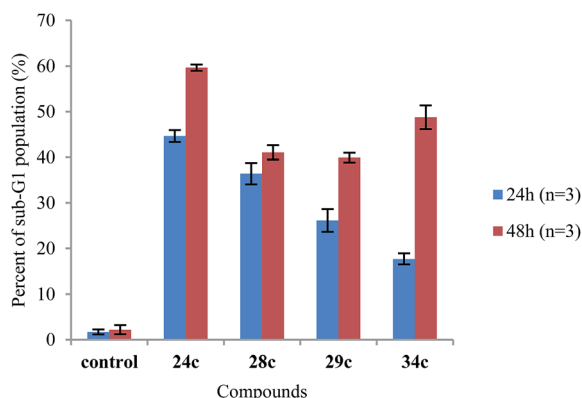


Fig. 3 Comparison of the sub-G1 cell cycle population after the treatment of THP-1 cells with compounds **24c**, **28c**, **29c**, and **34c** at 25  $\mu$ M for 24 h and 48 h ( $n = 3$  independent experiments).

respectively. Compounds **34c** and **24c** displayed more potent apoptosis-inducing activity with 49% and 60% of the cells in the sub-G1 population, although the  $EC_{50}$  values of all four compounds were very similar (20–24  $\mu$ M).

## Conclusions

In conclusion, the marine alkaloids clathrocin, oroidin, and hymenidin and a library of their synthetic analogues were tested to determine their apoptosis-inducing activities against the human hepatocellular carcinoma HepG2 cell line. Although the three tested marine alkaloids were found to be only weakly active, the library compounds, particularly their indole-based type C analogues, displayed promising activities. Compounds **24c**, **28c–30c**, and **34c**, which were the most active compounds in the library, exhibited  $EC_{50}$  values between 13  $\mu$ M and 42  $\mu$ M against the HepG2 cell line and between 20  $\mu$ M and 24  $\mu$ M against the acute monocytic leukaemia THP-1 cell line. Through cell cycle analysis, it was confirmed that compounds **24c**, **28c**, **29c**, and **34c** induce apoptosis at 25  $\mu$ M in THP-1 cells. These results render compounds **24c**, **28c**, **29c**, and **34c** interesting hits for further optimisation towards more potent oroidin, hymenidin and clathrocin analogues that induce apoptosis of human hepatic and blood cancer cells. Additional investigation of their mechanism of induction of apoptosis at the molecular level will be necessary to assess their potential for development towards new anticancer agents.

## Experimental section

### Chemistry

#### General procedure A

**Synthesis of compounds 24c, 28c–30c and 34c.** A solution of Boc-protected starting compound (1 mmol) in a mixture of THF and EtOH = 1 : 2 (5 mL) was saturated with gaseous HCl and stirred at room temperature for 5 h. The solvent was removed under reduced pressure, and the solid was filtered off and washed with diethyl ether and dichloromethane, to afford the title compound.

**2-Amino-4-(3-(5-methoxy-1H-indole-2-carboxamido)phenyl)-1H-imidazol-3-ium chloride (24c).** Prepared from **7c** according to General procedure A. Yield: 96%; off-white solid; mp 237–241  $^{\circ}$ C; IR (KBr)  $\nu = 3301$  (N–H), 3138 (C–H), 2955 (C–H), 2761 (C–H), 1673 (C=O), 1653, 1625, 1585, 1541, 1452, 1418, 1336, 1281, 1238, 1208, 1177, 1153, 1132, 1116, 1022, 883, 839, 788, 755  $cm^{-1}$ .  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  3.79 (s, 3H, OCH<sub>3</sub>), 6.89 (dd, 1H,  $^3J = 9.2$  Hz,  $^4J = 2.4$  Hz, Ar–H), 7.15 (d, 1H,  $^4J = 2.4$  Hz, Ar–H), 7.33 (s, 1H, Ar–H), 7.37–7.49 (m, 6H, 4  $\times$  Ar–H, NH<sub>2</sub>), 7.69–7.72 (m, 1H, Ar–H), 8.08 (s, 1H, Ar–H), 10.42 (s, 1H, NH), 11.70 (s, 1H, NH), 12.16 (s, 1H, NH), 12.85 (s, 1H, NH);  $^{13}C$  NMR (DMSO- $d_6$ )  $\delta$  55.25 (OCH<sub>3</sub>), 102.02, 104.14, 109.43, 113.23, 115.19, 116.46, 119.74, 120.33, 126.39, 127.26, 128.12, 129.30, 131.54, 132.16, 139.45, 147.82, 153.84, 159.72; MS (ESI)  $m/z$  (%) = 348.2 ([M – Cl]<sup>+</sup>, 100). HRMS for C<sub>19</sub>H<sub>18</sub>N<sub>5</sub>O<sub>2</sub>: calculated, 348.1461; found, 348.1459. HPLC: Phenomenex Luna 5  $\mu$ m C18 column (4.6 mm  $\times$  150 mm); mobile phase: 60–90% of MeOH in TFA (0.1%) in 20 min; flow rate: 1.0 mL min<sup>–1</sup>; injection volume: 10  $\mu$ L; retention time: 3.029 min (98.2% at 254 nm, 98.7% at 280 nm).

**2-Amino-4-(3-(5-fluoro-1H-indole-2-carboxamido)phenyl)-1H-imidazol-3-ium chloride (28c).** Prepared from **11c** according to General procedure A. Yield: 77%; off-white solid; mp 202–205  $^{\circ}$ C; IR (KBr)  $\nu = 3443$  (N–H), 3275 (N–H), 3145 (C–H), 2764 (C–H),



1662 (C=O), 1628, 1607, 1544, 1486, 1449, 1411, 1327, 1287, 1244, 1231, 1204, 1145, 1103, 954, 840, 780, 752, 727 cm<sup>-1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 7.11 (dt, 1H, <sup>3</sup>*J* = 9.2 Hz, <sup>4</sup>*J* = 2.0 Hz, Ar-H), 7.33 (s, 1H, Ar-H), 7.40–7.50 (m, 7H, 5 × Ar-H, NH<sub>2</sub>), 7.69–7.71 (m, 1H, Ar-H), 8.08 (t, 1H, <sup>4</sup>*J* = 1.6 Hz, Ar-H), 10.49 (s, 1H, NH), 11.95 (s, 1H, NH), 12.14 (s, 1H, NH), 12.83 (s, 1H, NH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) δ 104.34 (d, 1C, <sup>4</sup>*J*<sub>C-F</sub> = 5 Hz), 105.89 (d, 1C, <sup>2</sup>*J*<sub>C-F</sub> = 23 Hz), 109.51, 112.65 (d, 1C, <sup>2</sup>*J*<sub>C-F</sub> = 26 Hz), 113.63 (d, 1C, <sup>3</sup>*J*<sub>C-F</sub> = 9 Hz), 116.52, 119.93, 120.39, 126.37, 127.00 (d, 1C, <sup>3</sup>*J*<sub>C-F</sub> = 9 Hz), 128.17, 129.35, 132.96, 133.57, 139.28, 147.81, 157.20 (d, 1C, <sup>1</sup>*J*<sub>C-F</sub> = 231 Hz), 159.47; <sup>19</sup>F NMR (DMSO-*d*<sub>6</sub>) δ -123.59 (s, 1F); MS (ESI) *m/z* (%) = 336.1 ([M - Cl]<sup>+</sup>, 100). HRMS for C<sub>18</sub>H<sub>15</sub>N<sub>5</sub>O: calculated, 336.1261; found, 336.1264. HPLC: Phenomenex Luna 5 μm C18 column (4.6 mm × 150 mm); mobile phase: 60–90% of MeOH in TFA (0.1%) in 20 min; flow rate: 1.0 mL min<sup>-1</sup>; injection volume: 10 μL; retention time: 3.585 min (99.4% at 254 nm, 99.1% at 280 nm).

**2-Amino-4-(3-(5-chloro-1H-indole-2-carboxamido)phenyl)-1H-imidazol-3-ium chloride (29c).** Prepared from **12c** according to General procedure A. Yield: 71%; white solid; mp 201–204 °C; IR (KBr) ν = 3410 (N-H), 3260 (N-H), 3145 (C-H), 3032 (C-H), 2761 (C-H), 1693 (C=O), 1667, 1610, 1542, 1485, 1442, 1412, 1326, 1301, 1275, 1245, 1224, 1190, 1124, 1056, 914, 854, 798, 782, 754, 725 cm<sup>-1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 7.25 (dd, 1H, <sup>3</sup>*J* = 8.8 Hz, <sup>4</sup>*J* = 2.0 Hz, Ar-H), 7.33 (s, 1H, Ar-H), 7.40–7.51 (m, 6H, 4 × Ar-H, NH<sub>2</sub>), 7.69–7.71 (m, 1H, Ar-H), 7.79 (d, 1H, <sup>4</sup>*J* = 2.0 Hz, Ar-H), 8.07 (s, 1H, Ar-H), 10.53 (s, 1H, NH), 12.05 (s, 1H, NH), 12.14 (s, 1H, NH), 12.84 (s, 1H, NH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) δ 103.87, 109.52, 114.01, 116.53, 119.98, 120.40, 120.82, 123.98, 124.42, 126.37, 127.98, 128.18, 129.36, 132.75, 135.21, 139.23, 147.80, 159.41; MS (ESI) *m/z* (%) = 352.1 ([M - Cl]<sup>+</sup>, 100). HRMS for C<sub>18</sub>H<sub>15</sub>N<sub>5</sub>OCl: calculated, 352.0965; found, 352.0959. HPLC: Phenomenex Luna 5 μm C18 column (4.6 mm × 150 mm); mobile phase: 60–90% of MeOH in TFA (0.1%) in 20 min; flow rate: 1.0 mL min<sup>-1</sup>; injection volume: 10 μL; retention time: 5.338 min (98.4% at 254 nm, 98.8% at 280 nm).

**4-(3-(4H-Thieno[3,2-b]pyrrole-5-carboxamido)phenyl)-2-amino-1H-imidazol-3-ium chloride (30c).** Prepared from **13c** according to General procedure A. Yield: 78%; off-white solid; mp 198–202 °C; IR (KBr) ν = 3241 (N-H), 3135 (C-H), 3047 (C-H), 2763 (C-H), 1677 (C=O), 1625, 1541, 1488, 1460, 1385, 1348, 1308, 1231, 1191, 1115, 1084, 963, 877, 827, 748, 711 cm<sup>-1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 7.03 (dd, 1H, <sup>3</sup>*J* = 5.2 Hz, <sup>4</sup>*J* = 0.8 Hz, Ar-H), 7.31 (s, 1H, Ar-H), 7.36–7.49 (m, 6H, 4 × Ar-H, NH<sub>2</sub>), 7.66–7.69 (m, 1H, Ar-H), 8.06 (t, 1H, <sup>4</sup>*J* = 1.6 Hz, Ar-H), 10.24 (s, 1H, NH), 11.99 (s, 1H, NH), 12.14 (s, 1H, NH), 12.82 (s, 1H, NH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) δ 103.91, 109.43, 111.90, 116.27, 119.48, 120.15, 122.94, 126.45, 128.08, 128.28, 129.26, 130.49, 139.64, 141.32, 147.77, 159.41; MS (ESI) *m/z* (%) = 324.1 ([M - Cl]<sup>+</sup>, 100). HRMS for C<sub>16</sub>H<sub>14</sub>N<sub>5</sub>OS: calculated, 324.0919; found, 324.0911. HPLC: Phenomenex Luna 5 μm C18 column (4.6 mm × 150 mm); mobile phase: 60–90% of MeOH in TFA (0.1%) in 20 min; flow rate: 1.0 mL min<sup>-1</sup>; injection volume: 10 μL; retention time: 2.790 min (96.5% at 254 nm, 97.0% at 280 nm).

**4-(3-(1H-Indole-2-carboxamido)phenyl)-2-(methylamino)-1H-imidazol-3-ium chloride (34c).** Prepared from **18c** according to General procedure A. Yield: 95%; white solid; mp 190–194 °C; IR

(KBr) ν = 3269, 3182, 3049, 2934, 2865, 2746, 1685, 1635, 1602, 1541, 1494, 1414, 1368, 1334, 1312, 1248, 1190, 1145, 1115, 1060, 1018, 989, 919, 971, 819, 796, 775, 750 cm<sup>-1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 2.95 (d, 3H, <sup>3</sup>*J* = 4.8 Hz, CH<sub>3</sub>), 7.09 (dt, 1H, <sup>3</sup>*J* = 6.8 Hz, <sup>4</sup>*J* = 1.2 Hz, indole-H), 7.24 (dt, 1H, <sup>3</sup>*J* = 6.8 Hz, <sup>4</sup>*J* = 1.2 Hz, indole-H), 7.43–7.50 (m, 5H, 5 × Ar-H), 7.68–7.72 (m, 2H, 2 × Ar-H), 7.84 (q, 1H, <sup>3</sup>*J* = 4.8 Hz, NH), 8.11 (t, 1H, <sup>4</sup>*J* = 2.0 Hz, Ar-H-2'), 10.44 (s, 1H, NH), 11.82 (s, 1H, NH), 12.40 (br s, 1H, NH), 12.62 (br s, 1H, NH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) δ 30.07 (CH<sub>3</sub>), 105.00, 110.20, 112.88, 117.28, 120.42, 120.67, 120.87, 122.25, 124.35, 127.43, 127.45, 128.69, 129.72, 131.80, 137.33, 139.87, 149.08, 160.25; MS (ESI) *m/z* (%) = 332 ([M - Cl]<sup>+</sup>, 100). HRMS for C<sub>19</sub>H<sub>18</sub>N<sub>5</sub>O: calculated, 332.1511; found, 332.1499. HPLC: Phenomenex Luna 5 μm C18 column (4.6 mm × 150 mm); mobile phase: 10–90% of MeOH in TFA (0.1%) in 20 min; flow rate 1.0 mL min<sup>-1</sup>; injection volume: 20 μL; retention time: 17.146 min (99.2% at 254 nm, 99.2% at 280 nm).

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