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Characterization of Maleimide-Based Glycogen Synthase Kinase-3 (GSK-3) Inhibitors as Stimulators of Steroidogenesis

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Abstract

Inhibition of GSK-3 has been well documented to account for the behavioral actions of the mood stabilizer lithium in various animal models of mood disorders. Recent studies have showed that genetic or pharmacological inhibition of GSK-3 resulted in anxiolytic-like and pro-social behavior. In our ongoing efforts to develop GSK-3 inhibitors for the treatment of mood disorders, SAR studies on maleimide-based compounds were undertaken. We present herein for the first time that some of these GSK-3 inhibitors, in particular analogs **1** and **9**, were able to stimulate progesterone production in the MA-10 mouse tumor Leydig cell model of steroidogenesis without any significant toxicity. These two compounds were tested in the SmartCube® behavioral assay and showed anxiolytic-like signatures following daily dose administration (50 mg/kg, i.p.) for 13 days. Taken together, these results support the hypothesis that GSK-3 inhibition could influence neuroactive steroid production thereby mediating the modulation of anxiety-like behavior in vivo.

Keywords

kinase inhibitor; GSK-3; maleimides; steroidogenesis; lithium; anxiolytic

INTRODUCTION

The mood stabilizer lithium, commonly available in the carbonate form (Eskalith®), has remained as a primary treatment of choice for manic-depressive illness with demonstrated anti-suicidal and neuroprotective efficacies.^{1, 2} Although the exact molecular mechanisms underlying the actions of lithium are still not completely understood, it has been reported to directly inhibit a series of biologically relevant enzymes including, but not limited to,

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Author Contributions

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glycogen synthase kinase-3 (GSK-3), inositol monophosphatase, phosphoglucomutase, other inositol polyphosphatases, and monophosphoesterases.³ Other proposed mechanisms of lithium action in psychiatric disorders also emerged, such as the ionic hypothesis, modulation of neurotransmitter signaling, arachidonic acid metabolism, and modulation of the hypothalamus-pituitary-adrenal (HPA) axis activity.^{4, 5} Among these reported possible molecular mechanisms of lithium's action, the inhibition of GSK-3—either directly or through the destabilization of the Akt/ -arrestin/protein phosphatase 2A complex—has been well documented to account for the behavioral actions of lithium in animal models.⁶⁻¹⁰ GSK-3 is a multifunctional serine/threonine kinase responsible for the control of a number of central physiological events, such as cell membrane signal-to-gene transcription, metabolic homeostasis, cell development, neurogenesis, and cell survival/apoptosis.^{11, 12} Two homologs of GSK-3 exist: GSK-3 α (51 kDa) and GSK-3 β (47 kDa) which share more than 95% amino acid sequence homology in the catalytic site, with the latter being predominantly expressed in the nervous system. GSK-3 is constitutively active in basal non-stimulated cells, thereby catalyzing the phosphorylation of numerous signaling molecules, such as glycogen synthase, -catenin transcription factor, and tau proteins, leading to their degradation.^{13, 14} Phosphorylation of the Tyr279 and Tyr 216 on GSK-3 α and GSK-3 β , respectively, is required for their activation, while Ser21/Ser9 phosphorylation at GSK-3 α / β is necessary for their enzymatic inhibition. Significantly, GSK-3 has also been shown to be regulated not only by the mood stabilizers lithium and valproate but also by antidepressants and antipsychotics that modulate the key neurotransmitters signaling in the brain such as dopamine and serotonin.^{15, 16} Taken together, these findings support the role of GSK-3 inhibition as a key convergence point for at least some neuropsychiatric drugs routinely used for the treatment of mental disorders although the molecular mechanisms by which GSK-3 inhibition elicits mood stabilization remains elusive.^{17, 18}

Recently, selective deletion of forebrain GSK-3 β in mice was reported to exert anxiolytic-like and pro-social behavioral effects.¹⁹ In line with this study, lithium *per se* or in combination with other drugs has been demonstrated to suppress anxiety-like symptoms in different behavioral paradigms using mouse models of Fragile X syndrome, Huntington's disease, and cerebral ischemia.²⁰⁻²⁴ In the context of anxiety disorders, alteration of the levels of neuroactive steroids such as pregnenolone and progesterone has been implicated in the disease pathophysiology.²⁵ The anxiolytic effects of these neuroactive steroids are attributed to the binding to GABA_A receptors which manifests in the potentiation of GABA-induced Cl⁻ currents. A significant body of research in stress physiology has revealed the important roles of progesterone and its metabolite allopregnanolone in the modulation of HPA axis.²⁶ Of particular relevance to the pathophysiology of anxiety disorders, dysregulation of the HPA system has been observed in patients and normalization with lithium therapy suggested that interactions of lithium with the HPA axis may contribute to its therapeutic effects.⁵ Interestingly, inhibition of GSK-3 β (increased Ser9 phosphorylation) and the concomitant -catenin accumulation has been reported to be an important signaling pathway for the ability of luteal cells to induce progesterone secretion when exposed to luteinizing hormone.²⁷ Our group previously identified maleimide **1** (Figure 1) as a potent and selective, brain-penetrant, GSK-3 β inhibitor which attenuates hyperactivity in a mouse model of mania induced by amphetamine and chlordiazepoxide.²⁸ On the basis of the hypothesis that GSK-3 inhibition could induce the production of neurosteroids which in turn modulate the anxiety-like behavior *in vivo*, we sought to investigate the ability of these ATP-competitive maleimide-based GSK-3 β inhibitors to control steroid formation in a steroidogenic cell model.

CHEMISTRY

Based upon a body of SAR information accumulated over the years for the development of benzofuran-3-yl-(indol-3-yl)maleimides as GSK-3 inhibitors, 6-substitution on the indole ring has generally been shown to decrease GSK-3 inhibitory activity. On the other hand, substitution at the 5- or 7-position of the indole ring is generally well tolerated,²⁹ as exemplified by 5-halogen bearing analogs which include the previously reported maleimide **1**. A new generation of maleimides **2–31** was synthesized with the aim to improve upon potency and water solubility (Figure 1) following the synthetic routes depicted in Schemes 1–4. Commercially available indoles **32a** and **32b** were initially alkylated with *O*-trityl-3-bromopropan-1-ol or *O*-trityl-2-bromoethan-1-ol in *N,N*-dimethylformamide (DMF) as a solvent and sodium hydride as a base (Scheme 1). Treatment of the alkylated indoles **33** with ethyl chlorooxoacetate in diethyl ether afforded the glyoxalates **34**. Perkin-type condensation³⁰ between the glyoxalates and benzofuran-3-yl-acetamide²⁸ generated the desired maleimides **2–4**. Alkylation of **32a** and **32b** with *tert*-butyl (2-bromoethyl)carbamate followed by treatment of the intermediary indoles **35a** and **35b** with ethyl chlorooxoacetate gave the glyoxalates **36a** and **36b**. Maleimide formation with benzofuran-3-yl-acetamide and subsequent Boc deprotection using trifluoroacetic acid (TFA) in chloroform yielded the target compounds **5** and **6**. The *N*-methylaminopropyl (**7**, **8**) and 2-methoxyethyl analogs (**9–12**) were synthesized in a similar fashion. Commercially available 7-azaindoles **37a** and **37b** were subjected to the sequential *N*-alkylation, glyoxylation and condensation with benzofuran-3-yl-acetamide to give maleimides **13–16** (Scheme 2).^{31, 32} 6-Aza-5-methoxyindole was used as a starting material for target compounds **17** and **18**. 2-(Imidazo[1,2-*a*]pyridin-3-yl)acetamide³³ **38** was condensed with the appropriate glyoxalates to afford the final maleimides **19** and **20**. For compounds **21–23**, indole-7-carboxaldehyde **39** was first alkylated with the appropriate alkyl bromides and subjected to sequential Wittig reaction with methylenetriphenylphosphorane and then hydroboration. Alkylation of the intermediary alcohols **40a** and **40b** followed by treatment with ethyl chlorooxoacetate resulted in the formation of the glyoxalates **41a–c**, which upon condensation with benzofuran-3-yl-acetamide gave the desired maleimides **21–23**.

To access target compounds **24**, **25**, **27–31** (Scheme 3), indole-7-carboxaldehyde **39** or 5-fluoroindole-7-carboxaldehyde **42** were initially subjected to a reductive amination protocol using ethanolamine and hydrogen gas with palladium(0) catalyst following a previously reported literature protocol.³⁴ Sequential Boc protection of the secondary amine, tosylation of the hydroxyl group and sodium hydride-assisted cyclization³⁴ gave the intermediary 1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-*hi*]indoles **43a** and **43b**. Glyoxylation with ethyl chlorooxoacetate, condensation with benzofuran-3-yl-acetamide and subsequent Boc deprotection afforded the maleimides **24** and **25**. Amide coupling of the maleimides with the appropriate carboxylic acids using *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC·HCl) and HOBt as the coupling reagent or treatment with carbamoyl chlorides gave the target compounds **27–31**. For the bromo analog **26** (Scheme 4), 5-bromoindoline **44** was first alkylated with 2-chloroethylamine hydrochloride using potassium carbonate as a base in DMF in a pressure tube and treated with aqueous formaldehyde in acetic acid and sulfuric acid³⁵, followed by Boc protection. The resultant (*tert*-butyl 9-bromo-3,4,6,7-tetrahydro-[1,4]diazepino[6,7,1-*hi*]indole-2(1*H*)-carboxylate) **45** was subsequently treated with DDQ, followed by glyoxylation, condensation with benzofuran-3-yl-acetamide and Boc deprotection to yield the final maleimide **26**.

RESULTS AND DISCUSSION

GSK-3 Inhibition by Maleimides 2–31

Testing of all the newly synthesized compounds for their ability to inhibit GSK-3 was carried out by Reaction Biology, Inc. using their standard protocol (Tables 1 and 2). The hydroxyethyl analogue **2** was found to have a similar potency compared to the parent maleimide **1** (IC₅₀ value of 21 nM).³⁶ Substitution of the bromine by a fluorine atom (**2** vs **3**) at the 5-position of the indole ring resulted in an approximately 4-fold increase in the potency, whereas lengthening of the hydroxyethyl to a hydroxypropyl moiety (**3** vs **4**) gave a 3-fold decrease in potency. Subsequent chemical modifications were carried out to improve upon the water solubility of these maleimides, which is commonly achieved by the addition of an ionizable nitrogen group. The aminoethyl substitution at the indole nitrogen (**5** and **6**) diminished the GSK-3 inhibition by approximately 6- to 8-fold in comparison with the hydroxyethyl group (compounds **2** and **3**), with the 5-bromo analog **5** being 3-fold less potent than the 5-fluoro analog **6**. This trend was also observed with the *N*-methylaminopropyl analogs **7** and **8**. As a primary hydroxyl group generally poses a potential metabolic liability that arises from oxidation and bioconjugation processes, the hydroxyethyl moiety in analogs **2** and **3** was replaced with a 2-methoxyethyl group (compounds **9–12**). Within this series, the unsubstituted indole analog **9** was identified as the most potent GSK-3 inhibitor with an IC₅₀ value of 20 nM. The 5-fluoro and 5-bromo analogs **10** and **11** were found to be 2- and 4-fold less potent with IC₅₀ values of 40 and 82 nM respectively. Direct comparison of the methoxy analogs **10** and **11** vs the hydroxyl analogs **3** and **2**, respectively, showed that the hydroxyl analogs were approximately 4- to 8-fold more potent. The 5,6-difluoro analog **12** had a similar potency to the 5-fluoro analog with an IC₅₀ value of 36 nM.

The effects of: i) introduction of an azaindole ring in place of the indole ring (analogs **13–18**) and ii) replacement of the benzofuran ring with an imidazopyridine (analogs **19** and **20**) on the GSK-3 inhibition were examined.^{31, 32} To our delight, the azaindole analog **13** had an IC₅₀ value of 5 nM while the extension of the methyl group to 2-methoxyethyl (compound **14**) resulted in a 5-fold reduction in potency compared to **13**. Deletion of the 5-bromo group gave a further ~3-fold reduction in GSK-3 inhibitory activity (**15, 16** vs **13, 14**), while the 5-methoxy-6-azaindoles **17** and **18** had IC₅₀ values of 66 and 127 nM, respectively. Replacement of the benzofuran ring with an imidazopyridine (**19** and **20**) also resulted in a further increase in potency (IC₅₀ values of 5 and 0.9 nM respectively).

The next set of SAR modifications focused on the introduction of a 2-methoxyethyl group at 7-position of the indole ring. Previous SAR studies had shown that introduction of hydroxyalkyl groups at the 7-position resulted in single digit nanomolar GSK-3 inhibitors.²⁹ Towards this end, the 2-methoxyethyl attached at the 7-position of the indole ring of analog **21** led to a potent inhibitor with an IC₅₀ value of 11 nM (Table 2). Replacement of the methoxy group by a *tert*-butoxy was found to be deleterious for activity (**22** vs **21**). The bisalkylated analog **23** had similar potency to that of compound **22**. Inspired by the diazepinoindole scaffold originally reported by Eli Lilly & Co. in 2004,³⁴ cyclization of the two alkyl chains to a diazepinoindole scaffold exemplified by compounds **24–31**, resulted in highly potent GSK-3 inhibitors. The diazepinoindole analogs **24–26** all possessed IC₅₀ values between 4 and 12 nM and further extension of the diazepinoindole nitrogen atom to an amide moiety gave even more potent GSK-3 inhibitors **27–31**. Of a particular note, compound **31** was identified as the most potent GSK-3 inhibitor from this series with an IC₅₀ value of 13 pM, representing the most potent compound to date in our library of GSK-3 inhibitors. In addition, the aqueous solubility of compounds **5**, **20** and **24** was determined to be 180, 35 and 240 mg/L using an HPLC-based method.³⁸

Characterization of GSK-3 β Inhibitors as Stimulators of Steroidogenesis

In order to test the hypothesis that GSK-3 inhibition could induce the production of neuroactive steroids thereby mediating the modulation of anxiety-like behavior, 17 of the most potent, newly-synthesized GSK-3 inhibitors (Tables 1 and 2) were screened for their ability to induce progesterone expression in MA-10 mouse tumor Leydig cells. This cell line is a hormone-responsive steroidogenic cell model which has been extensively used to study the regulation of steroidogenesis whereby the biosynthesis of progesterone, the main steroid formed, is measured.³⁹ Initially, MA-10 cells were incubated in the presence of the GSK-3 inhibitors for 2 h at a concentration of 100 μ M and at the end of the incubation period, progesterone formation was measured by radioimmunoassay (RIA). As shown in Figure 2A, 7 of the 17 tested GSK-3 inhibitors, namely analogs **1**, **9**, **12**, **13**, **14**, **21**, and **26**, were bioactive in stimulating progesterone production. Dose-response experiments were next carried out for all the tested compounds ranging from 0.1 to 100 μ M, in which a statistically significant induction of progesterone synthesis was observed starting at a concentration of approximately 50 μ M (Figure 2B). As the high micromolar / low millimolar concentrations posed significant concern regarding cytotoxicity, and as steroid production can be facilitated by disruption of mitochondrial membrane integrity,⁴⁰ additional cytotoxicity profiling was performed. MA-10 Leydig cells were exposed to GSK-3 inhibitors for 2 h at 100 μ M concentration and cellular toxicity assessed by the MTT assay (Figure 2C). Some of the tested analogs showed low, but observable signs of cytotoxicity at the 100 μ M concentrations, for example compounds **16** and **23**, whereas maleimides **12**, **25-28**, **30**, and **31** were found to be significantly more cytotoxic at the tested concentration. Dose dependency curves for the cytotoxicity were also obtained for all the tested compounds, ranging from 0.1 to 100 μ M concentrations (Figure 2D). Examination of the overall progesterone production and cytotoxicity data presented in Figures 2A-D revealed that out of the seven compounds that stimulated progesterone production (Figure 2A), analogs **12** and **26** were also found to be toxic to the MA-10 cells. However, the remaining five GSK-3 inhibitors that stimulated steroidogenesis (compounds **1**, **9**, **13**, **14**, and **21**) showed either low or no apparent toxicity at a concentration of 100 μ M. To examine the time-dependency of these GSK-3 inhibitors in stimulating steroidogenesis, two of the most potent, non-toxic stimulators **1** and **9** were incubated with MA-10 cells at a concentration of 50 μ M for 0–120 min, media collected, and progesterone production was then assessed by RIA (Figure 3). There appears to be a biphasic time response to these two compounds, with the first maximum steroid production appearing within 15 min and remaining until 60 min, followed by a linear increase in steroid production between 60 and 120 min. These data altogether demonstrated for the first time that some of the maleimide-based GSK-3 inhibitors, namely **1**, **9**, **13**, **14**, and **21**, were able to stimulate progesterone production in MA-10 tumor Leydig cells in a dose-dependent manner with either low or no apparent signs of toxicity as assessed by the MTT assay.

In vivo SmartCube® Assay for GSK-3 Inhibitors **1** and **9**

Encouraged by the demonstrated ability of some of the newly synthesized GSK-3 inhibitors to stimulate steroidogenesis in vitro, two of the most potent, non-toxic stimulators **1** and **9** were administered to C57BL/6 mice in the proprietary SmartCube® system developed by Psychogenics, Inc.^{41, 42} This behavioral screening platform provides a sequence of challenges to mice and captures more than 1,400 features during the 45 min testing session. These features were analyzed with computer algorithms and data mining approaches to automate the study of mice behavior and compared to a database of behavioral signatures obtained using a set of diverse reference compounds, including antipsychotics, anxiolytics, and antidepressants.⁴¹ In this assay, mice were injected once a day with either compound **1** or **9** (50 mg/kg, i.p.) for 13 days, and the SmartCube® signatures were depicted in Figure 4. Neither compound demonstrated any classified behavioral activity after a single dose (15

min, day 1 test, Figure 4A). In contrast, a robust signature was obtained after chronic administration of both compounds over 13 days (Figure 4B). The signature classes for both compounds were found to be relatively similar, with the predominant portion of the signatures predicting anxiolytic-like activity, as well as some cognitive enhancer effects. In accordance with the ability of compounds **1** and **9** to stimulate steroidogenesis in MA-10 cells (Figure 2), the in vitro and in vivo data presented herein supported the hypothesis that GSK-3 inhibition could influence the formation of neuroactive steroids, which in turn mediate the anxiolytic-like behavior in living systems.

CONCLUSIONS

In this study, we presented our findings on the SAR exploration of the benzofuran-3-yl-(indol-3-yl)maleimides with the aim to improve their potency at GSK-3 inhibitory activity as well as their aqueous solubility. The replacement of indole ring in the original parent maleimide **1** with an azaindole or diazepinoindole moiety resulted in highly potent GSK-3 inhibitors with improved aqueous solubility. Compound **31** has been identified as the most potent GSK-3 inhibitor in our library thus far with an IC₅₀ value of 13 pM. In order to test the hypothesis that GSK-3 inhibition could indeed stimulate steroid production thereby mediating the modulation of anxiety-like behavior in vivo, 17 newly synthesized analogs were screened for their ability to induce steroidogenesis in a steroidogenic cell model, the MA-10 mouse tumor Leydig cell lines. Following dose-response experiments measuring the progesterone induction and cytotoxicity profiles, compounds **1**, **9**, **13**, **14**, and **21** were identified for the first time to be the most potent steroidogenesis stimulators in this assay without any significant toxicity at the tested concentration of 100 μM. The two most potent, non-toxic steroidogenesis stimulators **1** and **9** were subsequently tested in a proprietary SmartCube® assay. Daily administration of each compound at 50 mg/kg intraperitoneally resulted in anxiolytic-like signatures after 13 days. Taken together, these data supported the hypothesis that GSK-3 inhibition could be involved in the induction of steroidogenesis and thus neuroactive steroid production, thereby mediating the modulation of anxiety-like behavior in vivo. Continued efforts are directed towards the elucidation of the role of GSK-3 inhibition on the stimulation of steroidogenesis.

EXPERIMENTAL SECTION

Chemical Synthesis

General Methods—Starting materials, reagents, and solvents were purchased from commercial suppliers and used without further purification unless otherwise stated. Anhydrous THF and CH₂Cl₂ were obtained by distillation over sodium wire or CaH₂ respectively. All non-aqueous reactions were run under an argon atmosphere with exclusion of moisture from reagents. The progress of reactions was monitored by TLC on SiO₂. For flash chromatography, 230–400 mesh particle size SiO₂ was used. Melting points were determined on a Thomas-Hoover uni-melt apparatus and are uncorrected. Infrared absorption spectra were recorded on a Nicolet 6700 FT-IR spectrometer equipped with a Geon-KBr beamsplitter. ¹H NMR and ¹³C NMR spectra were recorded on a Bruker spectrometer at 400 and 100 MHz, respectively, and were referenced to the residual peaks of CDCl₃ at 7.26 ppm or DMSO-*d*₆ at 2.50 ppm (¹H NMR) and CDCl₃ at 77.2 ppm or DMSO-*d*₆ at 39.51 ppm (¹³C NMR). Chemical shifts were reported in parts per million (ppm) downfield of tetramethylsilane (TMS) and the following abbreviations used to denote coupling patterns: s = singlet; d = doublet; t = triplet; q = quartet; br = broad. ¹³C NMR chemical shifts are reported in ppm relative to the internal perdeuterated solvent resonance and are specified as quarternary (C), tertiary (CH), secondary (CH₂) or primary (CH₃) where appropriate, using additional DEPT experiments. Mass spectra were measured in the ESI mode at an ionization potential of 70 eV with an LC-MS MSD (Hewlett-Packard). HRMS

experiments were performed on a Micromass Q-TOF-2 tandem mass instrument. Purities of all final compounds (>96%) were established by analytical HPLC, which was carried out on an Agilent 1100 HPLC system with a Synergi 4 μ Hydro-RP 80A column, with detection at 254 and 280 nm on a Shimadzu SPD-10A VP detector; flow rate = 1.4 mL/min; gradient of 0–100% acetonitrile in water (both containing 0.05 vol % of TFA) in 25 min. Final products were purified by preparative HPLC under the following conditions: ACE 5 AQ column, 150 mm $\times$ 21.2 mm; flow rate = 17 mL/min; all solvents containing 0.05 vol % TFA; UV detection at 254 and 280 nm; gradient I: 40–100% MeOH in water over 35 min; Gradient II: 8–100% MeOH in water over 40 min.

General Procedure for the Indole or Azaindole N-Alkylation (Method A)—To a solution of substituted indole or azaindole (1 equiv) in dry DMF (3 mL/mmol) cooled with an ice bath was added NaH (55% suspension in mineral oil, 1.5 equiv). After 30 min stirring at 0 to 5 °C, an alkyl bromide (1.2 equiv) or methyl iodide (1.2 equiv.) was added in one portion. The reaction mixture was then heated to 60 °C and stirred overnight. Upon completion of the reaction as indicated by TLC, the reaction mixture was poured into ice-water and extracted with EtOAc (3 $\times$ 20 mL). The combined organic phases were washed with water (50 mL), brine (50 mL), dried over anhydrous Na₂SO₄, and concentrated in vacuo. The crude product was either purified by flash chromatography (hexane to EtOAc-hexane; 1:3) or directly used for further reaction without additional purification.

General Procedure for the Indole or Azaindole Glyoxylation (Method B)—To a solution of the substituted *N*-alkylindole (1 mmol) in Et₂O (5 mL/mmol) cooled with an ice bath was added ethyl chlorooxoacetate (5 equiv) dropwise. The reaction mixture was allowed to slowly warm to room temperature overnight until completion. Upon reaction completion as indicated by TLC, the reaction mixture was cooled with an ice bath and carefully quenched by slow addition of saturated aqueous NaHCO₃. The reaction mixture was extracted with CH₂Cl₂ (3 $\times$ 20 mL) and the combined organic phases were washed with saturated aqueous NaHCO₃ (2 $\times$ 40 mL), water (40 mL), brine (40 mL), dried over anhydrous Na₂SO₄ and concentrated in vacuo. The residue was purified by flash chromatography (EtOAc-hexane; 1:2) to give the substituted glyoxalates. For *N*-alkylazaindoles, a solution of the azaindole (1 equiv.) in CH₂Cl₂ was treated with AlCl₃ (5 equiv.) and stirred at ambient temperature for 1 h prior to the addition of ethyl chlorooxoacetate (5 equiv.). Quenching with MeOH and evaporation in vacuo gave the residue which was purified by flash chromatography (EtOAc-hexane; 2:3).

General Procedure for the Perkin-type Condensation to Give Maleimides (Method C)—To a suspension of indolyl-3-glyoxylate or azaindolyl-3-glyoxylate (1 equiv) and benzofuran-3-yl-acetamide (1.1 equiv) in dry THF or DMF (10 mL/mmol) at 0–5 °C was added a 1.0 M solution of *t*-BuOK in THF (2.5 equiv) in a dropwise manner. The reaction mixture was stirred at room temperature overnight. Upon completion of the reaction as ascertained by TLC, the reaction mixture was quenched with saturated aqueous NH₄Cl and extracted with EtOAc (3 $\times$ 20 mL). The combined organic phases were washed with water (50 mL), brine (50 mL), dried over Na₂SO₄ and evaporated in vacuo. The residue was purified by flash chromatography (1:2 to 1:1 EtOAc-hexane eluent) prior to further HPLC purification.

General Procedure for the Deprotection of N-Boc Amines (Method D)—To a solution of the *N*-Boc protected precursor (1 mmol) in CHCl₃ (5 mL) was added TFA (1 mL) at ambient temperature. The mixture was stirred for 2 h and concentrated in vacuo. The crude product was dissolved in DMF and filtered over a syringe filter

(polytetrafluoroethylene, 17 mm diameter, 0.45 μm pore size) prior to the preparative HPLC.

General Procedure for the Wittig Reaction (Method E)—To a suspension of the indole carboxaldehyde (1 equiv.) and methylenetriphenylphosphorane (3 equiv.) in dry THF (15 mL/mmol) was slowly added a 1.0 M solution of *t*-BuOK (3 equiv.) at 0 to 5 °C. The reaction mixture was slowly warmed to room temperature overnight with stirring and concentrated in vacuo. The residue was purified by flash chromatography (1:4 to 1:2 EtOAc-hexane eluent).

General Procedure for the Hydroboration (Method F)—To a solution of the vinyl indole (1 equiv.) in dry THF (6 mL/mmol) was added a solution of borane-tetrahydrofuran complex (1.0 M in THF, 1.1 equiv.) in a dropwise manner at ambient temperature. After 2.5 h stirring, 10% aqueous NaOH (1.3 equiv.) was added in one portion (effervescence was observed), followed by a hydrogen peroxide solution (35%, 1.3 equiv.). After 3.5 h stirring at ambient temperature, the reaction mixture was quenched by the addition of saturated aqueous NH_4Cl and diluted with water. Following extraction with EtOAc (3×50 mL), the combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography (1:4 to 1:1 EtOAc-hexane eluent).

General Procedure for the *tert*-Butyl Ether (Method G)—To a solution of 2-(1-methyl-1*H*-indol-7-yl)ethanol (1 mmol) in CH_2Cl_2 (1 mL/mmol) and cyclohexane (2 mL/mmol) was added *tert*-butyltrichloroacetimidate (1.1 mmol) at ambient temperature in one portion, followed by 30 μL of boron trifluoride diethyletherate. After stirring overnight, the reaction mixture was diluted with CH_2Cl_2 and directly purified by flash chromatography (1:7 to 1:3 EtOAc-hexane eluent) to give the desired product.

General Procedure for the Amide Coupling (Method H)—To a solution of the free amine (1 equiv.), carboxylic acid (1.2 equiv.), *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (1.3 equiv.) and HOBt (1.3 equiv.) in DMF (5 mL/mmol) was added *N*-methylmorpholine (3 equiv.) in one portion at ambient temperature. After overnight stirring, saturated aqueous NaHCO_3 was added, followed by extraction with CH_2Cl_2 (3×15 mL). The combined organic phases were washed with brine (40 mL), dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography (1:2 to 1:1 EtOAc-hexane or 3:97 MeOH: CH_2Cl_2 eluent) prior to further HPLC purification.

General Procedure for the Urea Formation (Method I)—To a solution of the free amine (1 equiv.) in MeOH (10 mL/mmol) was added carbamoyl chloride (1.5 equiv.) at ambient temperature, followed by *N,N*-diisopropylethylamine (3 equiv.) in one portion. After 3 h stirring, the reaction mixture was diluted with aqueous 1 M NaOH and extracted with EtOAc (3×15 mL). The combined organic phases were washed with brine (40 mL), dried over anhydrous Na_2SO_4 and concentrated in vacuo prior to purification by HPLC.

3-(Benzofuran-3-yl)-4-(5-bromo-1-(2-hydroxyethyl)-1*H*-indol-3-yl)-1*H*-pyrrole-2,5-dione (2)

The title compound was synthesized from commercially available 5-bromoindole (**32a**) and *O*-trityl-2-bromoethan-1-ol according to general procedures A–C, purified using gradient I preparative HPLC, and isolated as an orange solid in 15% yield over 3 steps. M.p. 152–157 °C. IR (ATR): ν_{max} 3140, 3057, 2944, 1702, 1700, 1544, 1356, 1114, 744 cm^{-1} . ^1H NMR (400 MHz, $\text{DMSO}-d_6$) 11.20 (s, 1H), 8.29 (s, 1H), 7.94 (s, 1H), 7.63 (d, $J = 8.4$ Hz, 1H), 7.50 (d, $J = 8.8$ Hz, 1H), 7.25 (t, $J = 7.2$ Hz, 1H), 7.18 (dd, $J = 8.8, 2.0$ Hz, 1H), 7.06 (d, $J = 2.0$ Hz, 1H), 6.94 (t, $J = 7.4$ Hz, 1H), 6.85 (d, $J = 7.6$ Hz, 1H), 4.93–4.89 (m, 1H), 4.27 (t, J

= 5.2 Hz, 2H), 3.69–3.65 (m, 2H). ^{13}C NMR (100 MHz, DMSO- d_6) 172.1 (C=O), 171.8 (C=O), 154.4 (C), 147.2 (CH), 135.3 (C), 135.1 (CH), 132.3 (C), 127.2 (C), 125.2 (C), 125.0 (CH), 124.4 (CH), 123.5 (CH), 123.0 (C), 122.9 (CH), 121.7 (CH), 112.9 (CH), 112.7 (C), 111.5 (CH), 111.4 (C), 103.7 (C), 60.1 (CH₂), 48.9 (CH₂). HRMS (ESI): m/z [M+H]⁺ calculated for C₂₂H₁₆BrN₂O₄: 451.0288, found: 451.0278. HPLC purity: 97.6%.

3-(Benzofuran-3-yl)-4-(5-fluoro-1-(2-hydroxyethyl)-1H-indol-3-yl)-1H-pyrrole-2,5-dione (3)

The title compound was synthesized from commercially available 5-fluorindole (**32b**) and *O*-trityl-2-bromoethan-1-ol according to general procedures A–C, purified using gradient I preparative HPLC, and isolated as an orange solid in 15% yield over 3 steps. M.p. 107–112 °C. IR (ATR): ν_{max} 3232, 1699, 1695, 1541, 1478, 1450, 1334, 741 cm⁻¹. ^1H NMR (400 MHz, DMSO- d_6) 11.19 (s, 1H), 8.28 (s, 1H), 7.98 (s, 1H), 7.62 (d, J = 8.0 Hz, 1H), 7.59–7.51 (m, 1H), 7.24 (t, J = 8.2 Hz, 1H), 6.97–6.85 (m, 3H), 6.58 (dd, J = 9.2, 2.0 Hz, 1H), 4.92 (br s, 1H), 4.29 (t, J = 5.2 Hz, 2H), 3.68 (t, J = 5.4 Hz, 2H). ^{13}C NMR (100 MHz, DMSO- d_6) 172.0 (C=O), 171.7 (C=O), 157.0 (d, $^1J_{\text{C-F}}$ = 232.6 Hz, C), 154.2 (C), 147.1 (CH), 135.4 (CH), 133.1 (C), 132.3 (C), 126.1 (d, $^3J_{\text{C-F}}$ = 11.0 Hz, C), 125.2 (C), 124.8 (CH), 122.8 (CH), 122.3 (C), 121.7 (CH), 112.0 (d, $^3J_{\text{C-F}}$ = 10.2 Hz, CH), 111.4 (CH), 111.3 (C), 110.0 (d, $^2J_{\text{C-F}}$ = 26.4 Hz, CH), 105.8 (d, $^2J_{\text{C-F}}$ = 24.9 Hz, CH), 104.0 (d, $^4J_{\text{C-F}}$ = 4.4 Hz, C), 60.0 (CH₂), 48.9 (CH₂). HRMS (ESI): m/z [M+H]⁺ calculated for C₂₂H₁₆FN₂O₄: 391.1089, found: 391.1104. HPLC purity: 99.1%.

3-(Benzofuran-3-yl)-4-(5-fluoro-1-(3-hydroxypropyl)-1H-indol-3-yl)-1H-pyrrole-2,5-dione (4)

The title compound was synthesized from commercially available 5-fluorindole (**32b**) and *O*-trityl-3-bromopropan-1-ol according to general procedures A–C, purified using gradient I preparative HPLC, and isolated as an orange solid in 25% yield over 3 steps. M.p. 101–106 °C. IR (ATR): ν_{max} 3140, 2944, 1704, 1699, 1545, 1479, 1451, 1336, 742 cm⁻¹. ^1H NMR (400 MHz, DMSO- d_6) 11.20 (s, 1H), 8.32 (s, 1H), 7.93 (s, 1H), 7.61 (d, J = 8.0 Hz, 1H), 7.55–7.50 (m, 1H), 7.22 (t, J = 8.4 Hz, 1H), 6.97–6.85 (m, 2H), 6.78 (d, J = 7.6 Hz, 1H), 6.64 (dd, J = 10.4, 2.4 Hz, 1H), 4.63 (br s, 1H), 4.30 (t, J = 6.8 Hz, 2H), 3.34 (t, J = 6.0 Hz, 2H), 1.85–1.79 (m, 2H). ^{13}C NMR (100 MHz, DMSO- d_6) 172.0 (C=O), 171.7 (C=O), 157.2 (d, $^1J_{\text{C-F}}$ = 233.5 Hz, C), 154.2 (C), 147.1 (CH), 134.8 (CH), 132.7 (C), 132.2 (C), 126.1 (d, $^3J_{\text{C-F}}$ = 11.0 Hz, C), 125.1 (C), 124.8 (CH), 122.8 (CH), 122.7 (C), 121.5 (CH), 111.7 (d, $^3J_{\text{C-F}}$ = 10.3 Hz, CH), 111.4 (CH), 111.2 (C), 110.2 (d, $^2J_{\text{C-F}}$ = 26.4 Hz, CH), 105.8 (d, $^2J_{\text{C-F}}$ = 26.4 Hz, CH), 104.1 (d, $^4J_{\text{C-F}}$ = 4.4 Hz, C), 57.4 (CH₂), 43.1 (CH₂), 32.7 (CH₂). HRMS (ESI): m/z [M+H]⁺ calculated for C₂₃H₁₈FN₂O₄: 405.1245, found: 405.1249. HPLC purity: 97.9%.

3-(1-(2-Aminoethyl)-5-bromo-1H-indol-3-yl)-4-(benzofuran-3-yl)-1H-pyrrole-2,5-dione trifluoroacetate (5)

The title compound was synthesized from commercially available 5-bromoindole (**32a**) and *tert*-butyl (2-bromoethyl)carbamate according to general procedures A–D, purified using gradient II preparative HPLC, and isolated as an orange solid in 12% yield over 3 steps. M.p. 153–157 °C. IR (ATR): ν_{max} 3054, 1704, 1699, 1545, 1451, 1343, 1185, 1119, 743, 722 cm⁻¹. ^1H NMR (400 MHz, DMSO- d_6) 11.26 (s, 1H), 8.28 (s, 1H), 7.99 (br s, 3H), 7.94 (s, 1H), 7.65 (d, J = 8.0 Hz, 1H), 7.56 (d, J = 8.8 Hz, 1H), 7.31–7.24 (m, 2H), 7.18 (s, 1H), 6.98 (d, J = 7.6 Hz, 1H), 6.91 (d, J = 8.0 Hz, 1H), 4.44 (t, J = 6.4 Hz, 2H), 3.17 (t, J = 5.6 Hz, 2H). ^{13}C NMR (100 MHz, DMSO- d_6) 171.9 (C=O), 171.6 (C=O), 158.3 (q, $^2J_{\text{C-F}}$ = 36.1 Hz, TFA), 154.4 (C), 147.4 (CH), 134.9 (C), 134.6 (CH), 131.8 (C), 127.3 (C), 125.0 (CH), 124.9 (CH), 123.9 (CH), 123.8 (C), 122.9 (CH), 121.8 (CH), 117.3 (d, $^1J_{\text{C-F}}$ = 298.2 Hz, TFA), 113.1 (C), 112.4 (CH), 111.5 (CH), 111.1 (C), 104.5 (C), 104.5 (C), 43.6 (CH₂),

38.5 (CH₂). HRMS (ESI): m/z [M+H]⁺ calculated for C₂₂H₁₇BrN₃O₃: 450.0448, found: 450.0458. HPLC purity: 98.2%.

3-(1-(2-Aminoethyl)-5-fluoro-1H-indol-3-yl)-4-(benzofuran-3-yl)-1H-pyrrole-2,5-dione trifluoroacetate (6)

The title compound was synthesized from commercially available 5-fluorindole (**32b**) and *tert*-butyl (2-bromoethyl)carbamate according to general procedures A–D, purified using gradient II preparative HPLC, and isolated as an orange solid in 16% yield over 3 steps. M.p. 144–148 °C. IR (ATR): $\nu_{\max}$ 3052, 1704, 1700, 1544, 1480, 1451, 1342, 1198, 1116, 742, 721 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆) 11.25 (s, 1H), 8.28 (s, 1H), 7.97 (s, 1H), 7.91 (br s, 3H), 7.64 (d, *J* = 8.0 Hz, 1H), 7.62–5.6 (m, 1H), 7.28 (t, *J* = 7.6 Hz, 1H), 7.05–6.91 (m, 3H), 6.69 (dd, *J* = 7.6, 2.5 Hz, 1H), 4.45 (t, *J* = 6.4 Hz, 2H), 3.19 (br s, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) 172.0 (C=O), 171.7 (C=O), 158.4 (q, ²*J*_{C-F} = 35.9 Hz, TFA), 157.4 (d, ¹*J*_{C-F} = 233.5 Hz, C), 154.3 (C), 147.4 (CH), 135.1 (CH), 132.8 (C), 131.9 (C), 126.2 (d, ³*J*_{C-F} = 10.6 Hz, C), 125.0 (CH), 125.0 (C), 123.3 (C), 122.9 (CH), 121.9 (CH), 117.1 (d, ¹*J*_{C-F} = 299.3 Hz, TFA), 111.6 (d, ³*J*_{C-F} = 9.5 Hz, CH), 111.5 (CH), 111.2 (C), 110.6 (d, ²*J*_{C-F} = 26.4 Hz, CH), 106.4 (d, ²*J*_{C-F} = 24.9 Hz, CH), 105.0 (d, ⁴*J*_{C-F} = 2.9 Hz, C), 43.7 (CH₂), 38.5 (CH₂). HRMS (ESI): m/z [M+H]⁺ calculated for C₂₂H₁₇FN₃O₃: 390.1249, found: 390.1256. HPLC purity: 98.0%.

3-(Benzofuran-3-yl)-4-(5-bromo-1-(3-(methylamino)propyl)-1H-indol-3-yl)-1H-pyrrole-2,5-dione trifluoroacetate (7)

The title compound was synthesized from commercially available 5-bromoindole (**32a**) and *tert*-butyl (3-bromopropyl)(methyl)carbamate according to general procedures A–D, purified using gradient II preparative HPLC, and isolated as an orange solid in 14% yield over 4 steps. M.p. 126–129 °C. IR (ATR): $\nu_{\max}$ 3041, 1713, 1671, 1547, 1452, 1337, 1202, 1127, 742, 721 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆) 11.25 (s, 1H), 8.33 (s, 1H), 8.29 (br s, 2H), 7.95 (s, 1H), 7.64 (d, *J* = 8.0 Hz, 1H), 7.55 (d, *J* = 8.8 Hz, 1H), 7.29–7.21 (m, 2H), 7.13 (d, *J* = 1.2 Hz, 1H), 6.93 (t, *J* = 7.4 Hz, 1H), 6.80 (d, *J* = 8.0 Hz, 1H), 4.33 (t, *J* = 6.4 Hz, 2H), 2.82–2.78 (m, 2H), 2.54 (s, 3H), 2.04–2.00 (m, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) 171.9 (C=O), 171.6 (C=O), 158.2 (q, ²*J*_{C-F} = 36.1 Hz, TFA), 154.3 (C), 147.2 (CH), 134.6 (C), 134.1 (CH), 131.9 (C), 127.3 (C), 125.0 (C), 124.9 (CH), 124.7 (CH), 123.6 (CH), 123.6 (C), 122.8 (CH), 121.5 (CH), 117.2 (d, ¹*J*_{C-F} = 298.2 Hz, TFA), 112.9 (C), 112.5 (CH), 111.5 (CH), 111.1 (C), 104.0 (C), 45.6 (CH₂), 43.2 (CH₂), 32.5 (CH₃), 26.2 (CH₂). HRMS (ESI): m/z [M+H]⁺ calculated for C₂₄H₂₁BrN₃O₃: 478.0761, found: 478.0768. HPLC purity: 99.1%.

3-(Benzofuran-3-yl)-4-(5-fluoro-1-(3-(methylamino)propyl)-1H-indol-3-yl)-1H-pyrrole-2,5-dione trifluoroacetate (8)

The title compound was synthesized from commercially available 5-fluorindole (**32b**) and *tert*-butyl (3-bromopropyl)(methyl)carbamate according to general procedures A–D, purified using gradient II preparative HPLC, and isolated as an orange solid in 16% yield over 4 steps. M.p. 120–125 °C. IR (ATR): $\nu_{\max}$ 3041, 1713, 1670, 1547, 1480, 1452, 1338, 1199, 1123, 745, 721 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆) 11.23 (s, 1H), 8.32 (s, 1H), 8.30 (br s, 2H), 7.99 (s, 1H), 7.65–7.56 (m, 2H), 7.25 (t, *J* = 8.2 Hz, 1H), 7.01–6.90 (m, 2H), 6.82 (d, *J* = 7.6 Hz, 1H), 6.65 (dd, *J* = 10.4, 2.6 Hz, 1H), 4.34 (t, *J* = 7.0 Hz, 2H), 2.85–2.79 (m, 2H), 2.54 (s, 3H), 2.07–2.01 (m, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) 172.0 (C=O), 171.7 (C=O), 158.2 (q, ²*J*_{C-F} = 31.5 Hz, TFA), 157.2 (d, ¹*J*_{C-F} = 234 Hz, C), 154.2 (C), 147.2 (CH), 134.6 (CH), 132.5 (C), 132.1 (C), 126.3 (d, ³*J*_{C-F} = 10.5 Hz, C), 125.1 (C), 124.9 (CH), 123.1 (C), 122.8 (CH), 121.6 (CH), 117.2 (d, ¹*J*_{C-F} = 298.2 Hz, TFA), 111.8 (d, ³*J*_{C-F} = 9.8 Hz, CH), 111.5 (CH), 111.1 (C), 110.4 (d, ²*J*_{C-F} = 25.8 Hz, CH), 106.1 (d, ²*J*_{C-F} = 24.9

Hz, CH), 104.5 (d, $^4J_{\text{C-F}} = 4.4$ Hz, C), 45.7 (CH₂), 43.3 (CH₂), 32.6 (CH₃), 26.2 (CH₂). HRMS (ESI): m/z [M+H]⁺ calculated for C₂₄H₂₁FN₃O₃: 418.1561, found: 418.1570. HPLC purity: 98.4%.

3-(Benzofuran-3-yl)-4-(1-(2-methoxyethyl)-1H-indol-3-yl)-1H-pyrrole-2,5-dione (9)

The title compound was synthesized from commercially available indole (**32c**) and 2-bromoethyl methyl ether according to general procedures A–C, purified using gradient I preparative HPLC, and isolated as a yellow solid in 35% yield over 3 steps. M.p. 147–150 °C. IR (ATR): ν_{max} 3144, 2928, 1710, 1548, 1451, 1326, 1099, 1072, 734 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆) 11.17 (s, 1H), 8.27 (s, 1H), 7.90 (s, 1H), 7.59 (d, $J = 8.0$ Hz, 1H), 7.52 (d, $J = 8.0$ Hz, 1H), 7.21 (t, $J = 7.6$ Hz, 1H), 7.06 (t, $J = 7.8$ Hz, 1H), 6.96–6.79 (m, 3H), 6.74 (t, $J = 7.6$ Hz, 1H), 4.41 (t, $J = 5.2$ Hz, 2H), 3.62 (t, $J = 5.2$ Hz, 2H), 3.19 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) 172.1 (C=O), 171.8 (C=O), 154.2 (C), 147.0 (CH), 136.2 (C), 133.6 (CH), 132.6 (C), 125.6 (C), 125.3 (C), 124.7 (CH), 122.7 (CH), 122.6 (C), 122.1 (CH), 121.7 (CH), 120.9 (CH), 120.1 (CH), 111.4 (C), 111.4 (CH), 110.7 (CH), 104.2 (C), 70.7 (CH₂), 58.2 (CH₃), 45.8 (CH₂). HRMS (ESI): m/z [M+H]⁺ calculated for C₂₃H₁₉N₂O₄: 387.1339, found: 387.1351. HPLC purity: 98.4%.

3-(Benzofuran-3-yl)-4-(5-fluoro-1-(2-methoxyethyl)-1H-indol-3-yl)-1H-pyrrole-2,5-dione (10)

The title compound was synthesized from commercially available 5-fluoroindole (**32b**) and 2-bromoethyl methyl ether according to general procedures A–C, purified using gradient I preparative HPLC, and isolated as an orange solid in 36% yield over 3 steps. M.p. 83–88 °C. IR (ATR): ν_{max} 3189, 2933, 1701, 1696, 1542, 1479, 1451, 1333, 1113, 742 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆) 11.19 (s, 1H), 8.31 (s, 1H), 7.92 (s, 1H), 7.62 (d, $J = 8.4$ Hz, 1H), 7.57–7.53 (m, 1H), 7.23 (t, $J = 8.0$ Hz, 1H), 6.96–6.87 (m, 2H), 6.80 (d, $J = 8.0$ Hz, 1H), 6.65 (dd, $J = 9.2, 2.6$ Hz, 1H), 4.40 (t, $J = 4.8$ Hz, 2H), 3.59 (t, $J = 5.2$ Hz, 2H), 3.18 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) 172.0 (C=O), 171.7 (C=O), 157.3 (d, $^1J_{\text{C-F}} = 233.5$ Hz, C), 154.2 (C), 147.1 (CH), 135.1 (CH), 133.0 (C), 132.2 (C), 126.1 (d, $^3J_{\text{C-F}} = 11.0$ Hz, C), 125.1 (C), 124.9 (CH), 122.8 (CH), 122.7 (C), 121.6 (CH), 112.0 (d, $^3J_{\text{C-F}} = 9.5$ Hz, CH), 111.4 (CH), 111.2 (C), 110.1 (d, $^2J_{\text{C-F}} = 25.9$ Hz, CH), 105.8 (d, $^2J_{\text{C-F}} = 24.7$ Hz, CH), 104.4 (d, $^4J_{\text{C-F}} = 4.4$ Hz, C), 70.8 (CH₂), 58.2 (CH₃), 46.1 (CH₂). HRMS (ESI): m/z [M+H]⁺ calculated for C₂₃H₁₈FN₂O₄: 405.1245, found: 405.1264. HPLC purity: 98.6%.

3-(Benzofuran-3-yl)-4-(5-bromo-1-(2-methoxyethyl)-1H-indol-3-yl)-1H-pyrrole-2,5-dione (11)

The title compound was synthesized from commercially available 5-bromoindole (**32a**) and 2-bromoethyl methyl ether according to general procedures A–C, purified using gradient I preparative HPLC, and isolated as an orange solid in 30% yield over 3 steps. M.p. 92–96 °C. IR (ATR): ν_{max} 3213, 2931, 1704, 1695, 1541, 1451, 1333, 1113, 742 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆) 11.21 (s, 1H), 8.30 (s, 1H), 7.87 (s, 1H), 7.61 (d, $J = 8.4$ Hz, 1H), 7.48 (d, $J = 8.4$ Hz, 1H), 7.23 (t, $J = 7.8$ Hz, 1H), 7.17 (dd, $J = 8.8, 1.6$ Hz, 1H), 7.13 (d, $J = 1.6$ Hz, 1H), 6.89 (t, $J = 7.8$ Hz, 1H), 6.76 (d, $J = 7.6$ Hz, 1H), 4.36 (t, $J = 5.0$ Hz, 2H), 3.56 (t, $J = 5.0$ Hz, 2H), 3.15 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) 172.1 (C=O), 171.7 (C=O), 154.4 (C), 147.2 (CH), 135.1 (C), 134.7 (CH), 132.2 (C), 127.3 (C), 125.1 (C), 125.0 (CH), 124.6 (CH), 123.5 (CH), 123.4 (C), 122.8 (CH), 121.6 (CH), 112.8 (C), 112.8 (CH), 111.5 (CH), 111.3 (C), 103.9 (C), 70.8 (CH₂), 58.2 (CH₃), 46.1 (CH₂). HRMS (ESI): m/z [M+H]⁺ calculated for C₂₃H₁₈BrN₂O₄: 465.0444, found: 465.0458. HPLC purity: 99.2%.

3-(Benzofuran-3-yl)-4-(5,6-difluoro-1-(2-methoxyethyl)-1H-indol-3-yl)-1H-pyrrole-2,5-dione (12)

The title compound was synthesized from commercially available 5,6-difluoroindole (**32d**) and 2-bromoethyl methyl ether according to general procedures A–C, purified using

gradient I preparative HPLC, and isolated as an orange solid in 37% yield over 3 steps. M.p. 98–103 °C. IR (ATR): $\max$ 3231, 3061, 2931, 1704, 1700, 1544, 1482, 1451, 1335, 1113, 854, 745 cm^{-1} . ^1H NMR (400 MHz, $\text{DMSO}-d_6$) 11.22 (s, 1H), 8.34 (s, 1H), 7.88 (s, 1H), 7.74–7.67 (m, 1H), 7.63 (d, $J = 8.6$ Hz, 1H), 7.24 (t, $J = 8.3$ Hz, 1H), 6.92–6.84 (m, 2H), 6.73 (d, $J = 8.1$ Hz, 1H), 4.37 (t, $J = 5.1$ Hz, 2H), 3.57 (t, $J = 4.8$ Hz, 2H), 3.17 (s, 3H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) 171.9 (C=O), 171.6 (C=O), 154.2 (C), 147.3 (CH), 147.1 (dd, $J = 237, 16.1$ Hz, C), 144.8 (dd, $J = 234, 14.6$ Hz, C), 134.9 (C), 134.8 (CH), 131.8 (C), 131.7 (C), 124.9 (CH), 123.6 (C), 122.8 (CH), 121.5 (CH), 121.2 (d, $^3J_{\text{C-F}} = 8.8$ Hz, C), 111.5 (CH), 111.0 (C), 107.6 (d, $^2J_{\text{C-F}} = 20.5$ Hz, CH), 104.5 (d, $^4J_{\text{C-F}} = 3.7$ Hz, C), 99.4 (d, $^2J_{\text{C-F}} = 22.0$ Hz, CH), 70.7 (CH_2), 58.1 (CH_3), 46.2 (CH_2). HRMS (ESI): m/z [M-H] $^-$ calculated for $\text{C}_{23}\text{H}_{15}\text{F}_2\text{N}_2\text{O}_4$: 421.1005, found: 421.1010. HPLC purity: 96.5%.

3-(Benzofuran-3-yl)-4-(5-bromo-1-methyl-1H-pyrrolo[2,3-b]pyridin-3-yl)-1H-pyrrole-2,5-dione (13)

The title compound was synthesized from commercially available 5-bromo-7-azaindole (**37a**) according to general procedures A–C, purified using gradient I preparative HPLC, and isolated as an orange solid in 25% yield over 3 steps. M.p. 244–248 °C. IR (ATR): $\max$ 3136, 3039, 2751, 1707, 1538, 1452, 1338, 1120, 744 cm^{-1} . ^1H NMR (400 MHz, $\text{DMSO}-d_6$) 11.28 (s, 1H), 8.36 (s, 1H), 8.27 (s, 1H), 8.13 (s, 1H), 7.65 (d, $J = 8.2$ Hz, 1H), 7.41 (s, 1H), 7.26 (t, $J = 7.7$ Hz, 1H), 6.96 (t, $J = 7.2$ Hz, 1H), 6.83 (d, $J = 7.8$ Hz, 1H), 3.85 (s, 3H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) 171.8 (C=O), 171.5 (C=O), 154.3 (C), 147.4 (CH), 145.9 (C), 143.3 (CH), 135.6 (CH), 131.4 (CH), 131.4 (C), 125.0 (CH), 124.9 (C), 123.8 (C), 122.9 (CH), 121.4 (CH), 119.4 (C), 111.5 (CH), 111.5 (C), 110.9 (C), 102.0 (C), 31.6 (CH_3). HRMS (ESI): m/z [M+H] $^+$ calculated for $\text{C}_{20}\text{H}_{13}\text{BrN}_3\text{O}_3$: 422.0135, found: 422.0124. HPLC purity: 98.3%.

3-(Benzofuran-3-yl)-4-(5-bromo-1-(2-methoxyethyl)-1H-pyrrolo[2,3-b]pyridin-3-yl)-1H-pyrrole-2,5-dione (14)

The title compound was synthesized from commercially available 5-bromo-7-azaindole (**37a**) and 2-bromoethyl methyl ether according to general procedures A–C, purified using gradient I preparative HPLC, and isolated as a yellow solid in 25% yield over 3 steps. M.p. 142–145 °C. IR (ATR): $\max$ 3133, 3040, 2752, 1714, 1547, 1455, 1335, 1108, 744 cm^{-1} . ^1H NMR (400 MHz, $\text{DMSO}-d_6$) 11.28 (s, 1H), 8.37 (s, 1H), 8.28 (d, $J = 1.8$ Hz, 1H), 8.02 (s, 1H), 7.65 (d, $J = 8.1$ Hz, 1H), 7.60 (d, $J = 8.0$ Hz, 1H), 7.26 (t, $J = 8.1$ Hz, 1H), 6.93 (t, $J = 7.4$ Hz, 1H), 6.75 (d, $J = 7.8$ Hz, 1H), 4.42 (t, $J = 5.2$ Hz, 2H), 3.60 (t, $J = 5.2$ Hz, 2H), 3.14 (s, 3H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) 171.8 (C=O), 171.5 (C=O), 154.4 (C), 147.5 (CH), 145.6 (C), 143.3 (CH), 134.8 (CH), 131.6 (CH), 131.4 (C), 125.0 (CH), 124.6 (C), 124.3 (C), 122.9 (CH), 121.5 (CH), 119.6 (C), 111.6 (C), 111.5 (CH), 110.7 (C), 102.3 (C), 70.2 (CH_2), 57.9 (CH_3), 44.0 (CH_2). HRMS (ESI): m/z [M+H] $^+$ calculated for $\text{C}_{22}\text{H}_{17}\text{BrN}_3\text{O}_4$: 466.0397, found: 466.0397. HPLC purity: 99.2%.

3-(Benzofuran-3-yl)-4-(1-methyl-1H-pyrrolo[2,3-b]pyridin-3-yl)-1H-pyrrole-2,5-dione (15)

The title compound was synthesized from commercially available 7-azaindole (**37b**) according to general procedures A–C, purified using gradient I preparative HPLC, and isolated as an orange solid in 14% yield over 3 steps. M.p. 219–223 °C. IR (ATR): $\max$ 3138, 3020, 2734, 1717, 1700, 1545, 1452, 1332, 1110, 978, 742 cm^{-1} . ^1H NMR (400 MHz, $\text{DMSO}-d_6$) 11.25 (s, 1H), 8.33 (s, 1H), 8.11 (d, $J = 4.5$ Hz, 1H), 8.13 (s, 1H), 7.62 (d, $J = 8.3$ Hz, 1H), 7.26–7.19 (m, 2H), 6.92 (t, $J = 7.2$ Hz, 1H), 6.87–6.78 (m, 2H), 3.88 (s, 3H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) 172.0 (C=O), 171.7 (C=O), 154.2 (C), 147.4 (C), 147.3 (CH), 143.3 (CH), 134.3 (CH), 131.9 (C), 129.1 (CH), 125.2 (C), 124.9 (CH), 123.0 (C), 122.8 (CH), 121.6 (CH), 117.9 (C), 116.3 (CH), 111.5 (CH), 111.1 (C), 102.4 (C), 31.4

(CH₃). HRMS (ESI): m/z [M+H]⁺ calculated for C₂₀H₁₄N₃O₃: 344.1030, found: 344.1041. HPLC purity: 99.9%.

3-(Benzofuran-3-yl)-4-(1-(2-methoxyethyl)-1H-pyrrolo[2,3-b]pyridin-3-yl)-1H-pyrrole-2,5-dione (16)

The title compound was synthesized from commercially available 7-azaindole (**37b**) and 2-bromoethyl methyl ether according to general procedures A–C, purified using gradient I preparative HPLC, and isolated as an orange solid in 15% yield over 3 steps. M.p. 180–183 °C. IR (ATR): $\nu_{\max}$ 3136, 2993, 2731, 1712, 1547, 1331, 1109, 982, 744 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆) 11.25 (s, 1H), 8.35 (s, 1H), 8.19 (d, *J* = 4.8 Hz, 1H), 8.04 (s, 1H), 7.62 (d, *J* = 8.0 Hz, 1H), 7.37 (d, *J* = 8.1 Hz, 1H), 7.23 (t, *J* = 7.3 Hz, 1H), 6.93–6.84 (m, 2H), 6.77 (d, *J* = 7.9 Hz, 1H), 4.47 (t, *J* = 5.3 Hz, 2H), 3.65 (t, *J* = 5.3 Hz, 2H), 3.18 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) 171.9 (C=O), 171.6 (C=O), 154.3 (C), 147.3 (CH), 147.2 (C), 143.3 (CH), 133.6 (CH), 131.9 (C), 129.3 (CH), 124.9 (CH), 124.9 (C), 123.6 (C), 122.8 (CH), 121.6 (CH), 118.0 (C), 116.5 (CH), 111.5 (CH), 110.9 (C), 102.7 (C), 70.3 (CH₂), 58.0 (CH₃), 43.8 (CH₂). HRMS (ESI): m/z [M+H]⁺ calculated for C₂₂H₁₈N₃O₄: 388.1292, found: 388.1298. HPLC purity: 98.6%.

3-(Benzofuran-3-yl)-4-(5-methoxy-1-methyl-1H-pyrrolo[2,3-c]pyridin-3-yl)-1H-pyrrole-2,5-dione (17)

The title compound was synthesized from commercially available 6-aza-5-methoxyindole according to general procedures A–C, purified using gradient I preparative HPLC, and isolated as an orange solid in 13% yield over 3 steps. M.p. 170–175 °C. IR (ATR): $\nu_{\max}$ 3039, 2732, 1717, 1678, 1652, 1547, 1186, 1119, 722 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆) 11.24 (s, 1H), 8.49 (s, 1H), 8.26 (s, 1H), 8.21 (s, 1H), 7.64 (d, *J* = 8.3 Hz, 1H), 7.28–7.24 (m, 1H), 7.00 (s, 2H), 6.03 (s, 1H), 3.94 (s, 3H), 3.49 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) 171.9 (C=O), 171.6 (C=O), 156.6 (C), 154.2 (C), 147.3 (CH), 140.9 (CH), 135.4 (C), 131.5 (C), 130.4 (C), 128.6 (CH), 125.5 (CH), 125.0 (C), 123.1 (C), 123.0 (CH), 121.5 (CH), 111.5 (CH), 111.2 (C), 103.0 (C), 97.3 (CH), 54.2 (CH₃), 33.7 (CH₃). HRMS (ESI): m/z [M+H]⁺ calculated for C₂₁H₁₆N₃O₄: 374.1135, found: 374.1138. HPLC purity: 99.7%.

3-(Benzofuran-3-yl)-4-(5-methoxy-1-(2-methoxyethyl)-1H-pyrrolo[2,3-c]pyridin-3-yl)-1H-pyrrole-2,5-dione (18)

The title compound was synthesized from commercially available 6-aza-5-methoxyindole and 2-bromoethyl methyl ether according to general procedures A–C, purified using gradient I preparative HPLC, and isolated as an orange solid in 15% yield over 3 steps. M.p. 183–188 °C. IR (ATR): $\nu_{\max}$ 3046, 2941, 2724, 1712, 1695, 1649, 1549, 1451, 1338, 1179, 1115, 743 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆) 11.24 (s, 1H), 8.54 (s, 1H), 8.29 (s, 1H), 8.12 (s, 1H), 7.64 (d, *J* = 8.2 Hz, 1H), 7.26 (t, *J* = 8.2 Hz, 1H), 6.96–6.87 (m, 2H), 6.19 (s, 1H), 4.47 (d, *J* = 5.2 Hz, 2H), 3.63 (d, *J* = 5.2 Hz, 2H), 3.53 (s, 3H), 3.20 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) 171.8 (C=O), 171.5 (C=O), 156.3 (C), 154.2 (C), 147.4 (CH), 140.5 (CH), 135.8 (C), 131.3 (C), 129.9 (C), 128.6 (CH), 125.2 (CH), 125.0 (C), 123.9 (C), 123.0 (CH), 121.4 (CH), 111.6 (CH), 111.0 (C), 103.6 (C), 97.4 (CH), 70.8 (CH₂), 58.2 (CH₃), 54.4 (CH₃), 46.6 (CH₂). HRMS (ESI): m/z [M+H]⁺ calculated for C₂₃H₂₀N₃O₅: 418.1398, found: 418.1406. HPLC purity: 99.9%.

3-(Imidazo[1,2-*a*]pyridin-3-yl)-4-(5-methyl-5H-[1,3]dioxolo[4,5-*f*]indol-7-yl)-1H-pyrrole-2,5-dione trifluoroacetate (19)

The title compound was synthesized from 5,6-methylenedioxyindole and 2-(imidazo[1,2-*a*]pyridin-3-yl)acetamide **38** according to general procedures A–C, purified using gradient II preparative HPLC, and isolated as a red solid in 15% yield over 3 steps. M.p. 134–139 °C.

IR (ATR): $\max$ 3129, 2725, 1711, 1671, 1471, 1334, 1182, 1100, 755 cm^{-1} . ^1H NMR (400 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$) 11.25 (s, 1H), 8.16 (s, 1H), 7.91 (s, 1H), 7.80–7.84 (m, 2H), 7.70 (t, $J = 7.8$ Hz, 1H), 7.04 (s, 1H), 6.92 (t, $J = 6.9$ Hz, 1H), 5.73 (s, 2H), 5.54 (s, 1H), 3.74 (s, 3H). ^{13}C NMR (100 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$) 170.7 (C=O), 170.5 (C=O), 145.6 (C), 144.1 (C), 135.0 (C), 134.3 (CH), 132.3 (C), 131.8 (CH), 127.2 (CH), 126.7 (C), 119.3 (C), 117.5 (C), 115.7 (CH), 112.9 (CH), 111.9 (C), 104.6 (C), 100.7 (CH), 100.7 (CH_2), 96.9 (CH), 91.2 (CH), 33.1 (CH_3). HRMS (ESI): m/z $[\text{M}+\text{H}]^+$ calculated for $\text{C}_{21}\text{H}_{15}\text{N}_4\text{O}_4$: 387.1088, found: 387.1087. HPLC purity: 99.0%.

3-(5-Bromo-1-methyl-1*H*-indol-3-yl)-4-(imidazo[1,2-*a*]pyridin-3-yl)-1*H*-pyrrole-2,5-dione trifluoroacetate (20)

The title compound was synthesized from 5-bromoindole and 2-(imidazo[1,2-*a*]pyridin-3-yl)acetamide **38** according to general procedures A–C, purified using gradient II preparative HPLC, and isolated as a yellow solid in 15% yield over 3 steps. M.p. 230–235 °C. IR (ATR): $\max$ 3163, 3081, 2940, 2708, 1717, 1656, 1552, 1330, 1201, 1178, 1126, 760 cm^{-1} . ^1H NMR (400 MHz, $\text{CD}_3\text{OD}/\text{DMSO}-d_6$) 11.24 (s, 1H), 8.33 (s, 1H), 8.17 (s, 1H), 7.92 (d, $J = 9.0$ Hz, 1H), 7.85 (d, $J = 6.9$ Hz, 1H), 7.70 (t, $J = 8.0$ Hz, 1H), 7.36 (d, $J = 8.7$ Hz, 1H), 7.13 (dd, $J = 8.7, 1.7$ Hz, 1H), 6.95 (t, $J = 6.9$ Hz, 1H), 6.21 (d, $J = 1.7$ Hz, 1H), 3.85 (s, 3H). ^{13}C NMR (100 MHz, $\text{CD}_3\text{OD}/\text{DMSO}-d_6$) 171.7 (C=O), 171.3 (C=O), 158.2 (q, $^2J_{\text{C-F}} = 36.1$ Hz, TFA), 141.7 (C), 137.9 (CH), 136.4 (CH), 135.5 (C), 133.0 (CH), 128.3 (CH), 128.1 (C), 127.6 (C), 125.9 (CH), 122.1 (CH), 117.8 (C), 117.2 (d, $^1J_{\text{C-F}} = 298.2$ Hz, TFA), 116.6 (C), 115.0 (C), 114.5 (CH), 114.0 (CH), 113.4 (CH), 104.2 (C), 33.7 (CH_3). HRMS (ESI): m/z $[\text{M}+\text{H}]^+$ calculated for $\text{C}_{20}\text{H}_{14}\text{BrN}_4\text{O}_2$: 421.02946, found: 421.0300. HPLC purity: 96.8%.

3-(Benzofuran-3-yl)-4-(7-(2-methoxyethyl)-1-methyl-1*H*-indol-3-yl)-1*H*-pyrrole-2,5-dione (21)

The title compound was synthesized from alcohol **40a** (generated from indole-7-carboxaldehyde **39** employing general methods E and F) and methyl iodide according to general procedures A–C, purified using gradient I preparative HPLC, and isolated as an orange solid in 26% yield over 3 steps. M.p. 91–96 °C. IR (ATR): $\max$ 3135, 2928, 1704, 1699, 1695, 1545, 1450, 1320, 1102, 743 cm^{-1} . ^1H NMR (400 MHz, $\text{DMSO}-d_6$) 11.17 (s, 1H), 8.22 (s, 1H), 7.85 (s, 1H), 7.58 (d, $J = 8.4$ Hz, 1H), 7.25–7.19 (m, 1H), 6.94–6.89 (m, 2H), 6.85 (d, $J = 7.6$ Hz, 1H), 6.70 (d, $J = 8.0$ Hz, 1H), 6.61 (t, $J = 7.6$ Hz, 1H), 4.11 (s, 3H), 3.59 (t, $J = 7.0$ Hz, 2H), 3.28 (t, $J = 7.2$ Hz, 2H), 3.24 (s, 3H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) 172.1 (C=O), 171.9 (C=O), 154.1 (C), 146.9 (CH), 136.0 (CH), 134.9 (C), 132.4 (C), 126.9 (C), 125.5 (C), 124.7 (CH), 124.5 (CH), 123.3 (C), 122.7 (CH), 122.4 (C), 121.7 (CH), 120.1 (CH), 119.0 (CH), 111.6 (C), 111.3 (CH), 103.5 (C), 73.3 (CH_2), 58.0 (CH_3), 37.2 (CH_3), 31.5 (CH_2). HRMS (ESI): m/z $[\text{M}+\text{H}]^+$ calculated for $\text{C}_{24}\text{H}_{21}\text{N}_2\text{O}_4$: 401.1496, found: 401.1510. HPLC purity: 98.1%.

3-(Benzofuran-3-yl)-4-(7-(2-(tert-butoxy)ethyl)-1-methyl-1*H*-indol-3-yl)-1*H*-pyrrole-2,5-dione (22)

The title compound was synthesized from alcohol **40a** (generated from indole-7-carboxaldehyde **39** employing general methods E and F) and *tert*-butyl 2,2,2-trichloroacetimidate (method G) according to general procedures A–C, purified using gradient I preparative HPLC, and isolated as an orange solid in 16% yield over 3 steps. M.p. 107–112 °C. IR (ATR): $\max$ 3211, 2971, 1704, 1699, 1545, 1451, 1322, 1106, 1077, 743 cm^{-1} . ^1H NMR (400 MHz, $\text{DMSO}-d_6$) 11.16 (s, 1H), 8.22 (s, 1H), 7.85 (s, 1H), 7.58 (d, $J = 8.4$ Hz, 1H), 7.24–7.18 (m, 1H), 6.93–6.88 (m, 2H), 6.85 (d, $J = 7.6$ Hz, 1H), 6.71 (dd, $J = 8.0, 1.0$ Hz, 1H), 6.61 (t, $J = 7.6$ Hz, 1H), 4.12 (s, 3H), 3.55 (t, $J = 7.0$ Hz, 2H), 3.18 (t, $J = 6.8$ Hz, 2H), 1.06 (s, 9H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) 172.2 (C=O), 171.9 (C=O),

154.1 (C), 146.9 (CH), 136.0 (CH), 135.0 (C), 132.4 (C), 126.9 (C), 125.5 (C), 124.7 (CH), 124.6 (CH), 123.7 (C), 122.7 (CH), 122.2 (C), 121.7 (CH), 120.1 (CH), 119.0 (CH), 111.5 (C), 111.3 (CH), 103.5 (C), 72.3 (CH₂), 62.9 (C), 37.1 (CH₃), 32.6 (CH₂), 27.2 (CH₃). HRMS (ESI): m/z [M+H]⁺ calculated for C₂₇H₂₇N₂O₄: 443.1965, found: 443.1983. HPLC purity: 96.9%.

3-(Benzofuran-3-yl)-4-(1,7-bis(2-methoxyethyl)-1H-indol-3-yl)-1H-pyrrole-2,5-dione (23)

The title compound was synthesized from alcohol **40b** (generated from indole-7-carboxaldehyde **39** employing general methods E and F) and 2-bromoethyl methyl ether according to general procedures A–C, purified using gradient I preparative HPLC, and isolated as an orange solid in 16% yield over 3 steps. M.p. 178–182 °C. IR (ATR): $\nu_{\max}$ 3146, 2892, 1697, 1559, 1337, 1109, 747 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆) 11.19 (s, 1H), 8.29 (s, 1H), 7.78 (s, 1H), 7.57 (d, *J* = 8.4 Hz, 1H), 7.20 (t, *J* = 7.6 Hz, 1H), 6.89–6.81 (m, 3H), 6.77 (d, *J* = 8.0 Hz, 1H), 6.67 (t, *J* = 7.6 Hz, 1H), 4.55 (t, *J* = 5.0 Hz, 2H), 3.63–3.54 (m, 4H), 3.25–3.15 (m, 8H). ¹³C NMR (100 MHz, DMSO-*d*₆) 172.0 (C=O), 171.8 (C=O), 154.1 (C), 147.1 (CH), 135.1 (CH), 134.2 (C), 132.2 (C), 127.3 (C), 125.2 (C), 124.8 (CH), 124.7 (CH), 123.4 (C), 122.8 (C), 122.6 (CH), 121.7 (CH), 120.1 (CH), 119.2 (CH), 111.4 (CH), 111.3 (C), 104.3 (C), 72.8 (CH₂), 71.7 (CH₂), 58.3 (CH₃), 58.1 (CH₃), 48.2 (CH₂), 32.0 (CH₂). HRMS (ESI): m/z [M+H]⁺ calculated for C₂₆H₂₅N₂O₅: 445.1758, found: 445.1777. HPLC purity: 99.4%.

3-(Benzofuran-3-yl)-4-(1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-*h*]indol-7-yl)-1H-pyrrole-2,5-dione trifluoroacetate (24)

The title compound was synthesized from diazepinoindole **43a** (generated from indole-7-carboxaldehyde **39**) according to general procedures B–D, purified using gradient II preparative HPLC, and isolated as an orange solid in 12% yield over 3 steps. M.p. 160–165 °C. IR (ATR): $\nu_{\max}$ 3038, 2731, 1706, 1695, 1669, 1545, 1451, 1340, 1180, 1116, 745 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆) 11.26 (s, 1H), 9.44 (br s, 2H), 8.26 (s, 1H), 7.97 (s, 1H), 7.61 (d, *J* = 8.4 Hz, 1H), 7.25 (t, *J* = 8.0 Hz, 1H), 7.05 (d, *J* = 7.2 Hz, 1H), 7.02–6.86 (m, 3H), 6.77 (t, *J* = 8.0 Hz, 1H), 4.68 (br s, 4H), 3.72 (br s, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) 172.0 (C=O), 171.7 (C=O), 158.2 (q, ²*J*_{C-F} = 36.1 Hz, TFA), 154.2 (C), 147.3 (CH), 135.3 (C), 134.4 (CH), 131.9 (C), 126.8 (C), 125.3 (C), 124.9 (CH), 123.9 (C), 122.9 (CH), 122.8 (CH), 121.8 (CH), 121.3 (CH), 120.5 (CH), 118.0 (C), 117.2 (d, ¹*J*_{C-F} = 298.2 Hz, TFA), 111.5 (CH), 111.3 (C), 104.9 (C), 46.9 (CH₂), 46.6 (CH₂), 45.2 (CH₂). HRMS (ESI): m/z [M+H]⁺ calculated for C₂₃H₁₈N₃O₃: 384.1343, found: 384.1362. HPLC purity: 98.2%.

3-(Benzofuran-3-yl)-4-(9-fluoro-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-*h*]indol-7-yl)-1H-pyrrole-2,5-dione trifluoroacetate (25)

The title compound was synthesized from diazepinoindole **43b** (generated from 5-fluoroindole-7-carboxaldehyde **42**)³⁴ according to general procedures B–D, purified using gradient II preparative HPLC, and isolated as an orange solid in 14% yield over 3 steps. M.p. 152–157 °C. IR (ATR): $\nu_{\max}$ 3136, 3050, 2738, 1710, 1670, 1547, 1452, 1340, 1181, 1120, 746 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆) 11.29 (s, 1H), 9.65 (br s, 2H), 8.30 (s, 1H), 7.99 (s, 1H), 7.63 (d, *J* = 8.4 Hz, 1H), 7.27 (t, *J* = 7.6 Hz, 1H), 7.04–6.94 (m, 2H), 6.90 (d, *J* = 7.6 Hz, 1H), 6.63 (d, *J* = 9.2 Hz, 1H), 4.66 (br s, 4H), 3.71 (br s, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) 171.9 (C=O), 171.6 (C=O), 158.2 (q, ²*J*_{C-F} = 36.1 Hz, TFA), 156.6 (d, ¹*J*_{C-F} = 233.4 Hz, C), 154.2 (C), 147.4 (CH), 135.9 (CH), 132.1 (C), 131.6 (C), 127.5 (d, ³*J*_{C-F} = 10.6 Hz, C), 125.1 (C), 125.0 (CH), 124.2 (C), 123.0 (CH), 121.6 (CH), 119.8 (d, ³*J*_{C-F} = 9.6 Hz, C), 117.3 (d, ¹*J*_{C-F} = 298.2 Hz, TFA), 111.6 (CH), 111.1 (C), 110.9 (d, ²*J*_{C-F} = 27 Hz, CH), 105.9 (d, ²*J*_{C-F} = 24.4 Hz, CH), 105.1 (d, ⁴*J*_{C-F} = 4.4 Hz, C), 46.7

(CH₂), 46.6 (CH₂), 45.6 (CH₂). HRMS (ESI): *m/z* [M+H]⁺ calculated for C₂₃H₁₇FN₃O₃: 402.1248, found: 402.1260. HPLC purity: 97.9%.

3-(Benzofuran-3-yl)-4-(9-bromo-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-*h*]indol-7-yl)-1*H*-pyrrole-2,5-dione trifluoroacetate (26)

The title compound was synthesized from 5-bromoindoline **45** (see Supplementary Information), purified using gradient II preparative HPLC, and isolated as an orange solid. M.p. 243–248 °C. IR (ATR): $\nu_{\max}$ 3138, 3050, 1709, 1700, 1695, 1623, 1545, 1336, 1179, 746 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆) 11.29 (s, 1H), 9.55 (br s, 2H), 8.31 (s, 1H), 7.96 (s, 1H), 7.65 (d, *J* = 7.8 Hz, 1H), 7.30–7.25 (m, 2H), 7.08 (s, 1H), 6.99 (t, *J* = 7.6 Hz, 1H), 6.88 (d, *J* = 8.8 Hz, 1H), 4.66 (br s, 4H), 3.70 (br s, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) 171.8 (C=O), 171.5 (C=O), 158.2 (q, ²*J*_{C-F} = 30.9 Hz, TFA), 154.4 (C), 147.4 (CH), 135.4 (CH), 134.1 (C), 131.5 (C), 128.4 (C), 125.0 (CH), 124.9 (C), 124.9 (CH), 124.6 (C), 123.6 (CH), 123.0 (CH), 121.6 (CH), 120.1 (C), 116.3 (d, ¹*J*_{C-F} = 294.3 Hz, TFA), 112.5 (C), 111.5 (CH), 111.1 (C), 104.6 (C), 46.5 (CH₂), 46.3 (CH₂), 45.3 (CH₂). HRMS (ESI): *m/z* [M+H]⁺ calculated for C₂₃H₁₇BrN₃O₃: 462.0448, found: 462.0448. HPLC purity: 98.9%.

3-(Benzofuran-3-yl)-4-(9-fluoro-2-(2-(pyridin-4-yl)acetyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-*h*]indol-7-yl)-1*H*-pyrrole-2,5-dione (27)

The title compound was synthesized from maleimide **24** and pyrazine-2-carboxylic acid according to general procedure H, purified using gradient II preparative HPLC, and isolated as an orange solid (30%). M.p. 167–172 °C. IR (ATR): $\nu_{\max}$ 3138, 3053, 1708, 1627, 1545, 1451, 1332, 1017, 744 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆, two rotamers observed) 11.22 (s, 1H), 11.21 (s, 1H), 8.87 (d, *J* = 1.5 Hz, 1H), 8.77 (d, *J* = 2.5 Hz, 1H), 8.75 (d, *J* = 2.5 Hz, 1H), 8.69 (dd, *J* = 2.5, 1.5 Hz, 1H), 8.66 (dd, *J* = 2.5, 1.5 Hz, 1H), 8.59 (d, *J* = 1.5 Hz, 1H), 8.25 (s, 2H), 7.96 (s, 1H), 7.85 (s, 1H), 7.61 (s, 1H), 7.59 (s, 1H), 7.27–7.21 (m, 2H), 7.03 (d, *J* = 6.8 Hz, 1H), 6.97–6.93 (m, 4H), 6.82–6.69 (m, 3H), 6.60 (t, *J* = 7.6 Hz, 1H), 6.52 (d, *J* = 6.8 Hz, 1H), 5.17 (s, 2H), 4.98 (s, 2H), 4.73–4.69 (m, 2H), 4.61–4.57 (m, 2H), 4.23–4.18 (m, 2H), 4.10–4.05 (m, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆, two rotamers observed) 172.0 (C=O), 171.8 (C=O), 166.1 (C=O), 165.9 (C=O), 154.2 (C), 149.3 (C), 149.2 (C), 147.2 (CH), 145.7 (CH), 145.6 (CH), 144.6 (CH), 143.8 (CH), 143.2 (CH), 143.0 (CH), 135.6 (C), 135.2 (C), 134.4 (CH), 134.3 (CH), 132.2 (C), 126.7 (C), 125.3 (C), 125.3 (C), 124.8 (CH), 123.3 (C), 123.3 (C), 123.2 (C), 122.8 (CH), 121.8 (CH), 121.7 (CH), 120.6 (CH), 120.3 (CH), 120.1 (CH), 120.0 (CH), 111.4 (CH), 104.7 (C), 104.5 (C), 50.3 (CH₂), 50.1 (CH₂), 48.7 (CH₂), 47.3 (CH₂), 46.9 (CH₂), 40.5 (CH₂). HRMS (ESI): *m/z* [M+H]⁺ calculated for C₂₈H₂₀N₅O₄: 490.1510, found: 490.1523. HPLC purity: 98.2%.

3-(Benzofuran-3-yl)-4-(9-fluoro-2-(2-(pyridin-4-yl)acetyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-*h*]indol-7-yl)-1*H*-pyrrole-2,5-dione trifluoroacetate (28)

The title compound was synthesized from maleimide **24** and 2-(pyridin-4-yl)acetic acid according to general procedure H, purified using gradient II preparative HPLC, and isolated as an orange solid (30%). M.p. 142–147 °C. IR (ATR): $\nu_{\max}$ 3063, 2725, 1711, 1645, 1548, 1451, 1338, 1180, 1127, 748 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆, two rotamers observed) 11.23 (s, 1H), 11.23 (s, 1H), 8.80 (s, 1H), 8.78 (s, 1H), 8.72 (s, 1H), 8.71 (s, 1H), 8.25 (s, 1H), 8.22 (s, 1H), 7.99 (s, 1H), 7.90 (s, 1H), 7.82 (s, 1H), 7.81 (s, 1H), 7.71 (s, 1H), 7.70 (s, 1H), 7.61 (s, 1H), 7.59 (s, 1H), 7.29–7.21 (m, 2H), 7.05–6.60 (m, 12H), 5.09 (s, 2H), 5.03 (s, 2H), 4.76 (br s, 2H), 4.60 (br s, 2H), 4.22–4.15 (m, 4H), 4.11 (s, 2H), 4.03 (s, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆, two rotamers observed) 172.1 (C=O), 172.1 (C=O), 171.8 (C=O), 171.8 (C=O), 168.0 (C=O), 167.9 (C=O), 158.5 (q, ²*J*_{C-F} = 35.1 Hz, TFA), 155.2 (C), 154.3 (C), 154.2 (C), 154.2 (C), 147.2 (CH), 147.1 (CH), 142.5 (CH), 142.1 (CH),

135.6 (C), 135.3 (C), 134.3 (CH), 132.3 (C), 132.2 (C), 128.2 (C), 127.7 (CH), 126.8 (CH), 126.5 (C), 125.5 (C), 125.3 (C), 124.8 (CH), 123.7 (C), 123.4 (C), 123.3 (C), 123.0 (C), 122.9 (CH), 122.7 (CH), 121.8 (CH), 121.4 (CH), 120.8 (CH), 120.2 (CH), 120.2 (CH), 119.9 (CH), 116.2 (d, $^1J_{C-F}$ = 294.3 Hz, TFA), 111.4 (CH), 111.4 (C), 104.6 (C), 104.5 (C), 49.2 (CH₂), 48.7 (CH₂), 48.1 (CH₂), 47.3 (CH₂), 47.0 (CH₂), 45.8 (CH₂), 39.4 (CH₂). HRMS (ESI): m/z [M+H]⁺ calculated for C₃₀H₂₃N₄O₄: 503.1714, found: 503.1727. HPLC purity: 99.4%.

3-(Benzofuran-3-yl)-4-(9-fluoro-2-(piperidine-1-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-*h*]indol-7-yl)-1*H*-pyrrole-2,5-dione (29)

The title compound was synthesized from maleimide **25** and piperidine-1-carbonyl chloride according to general procedure I, purified using gradient II preparative HPLC, and isolated as an orange solid (40%). M.p. 129–134 °C. IR (ATR): $\max$ 3136, 3053, 2934, 2855, 2731, 1714, 1614, 1548, 1451, 1336, 1116, 746 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆) 11.21 (s, 1H), 8.28 (s, 1H), 7.90 (s, 1H), 7.61 (d, J = 7.8 Hz, 1H), 7.25 (t, J = 8.0 Hz, 1H), 6.93 (t, J = 7.2 Hz, 1H), 6.89–6.82 (m, 2H), 6.45 (d, J = 8.4 Hz, 1H), 4.65 (s, 2H), 4.50–4.44 (m, 2H), 3.87–3.81 (m, 2H), 3.05–2.97 (m, 4H), 1.51–1.43 (m, 6H). ¹³C NMR (100 MHz, DMSO-*d*₆) 172.0 (C=O), 171.7 (C=O), 163.3 (C=O), 156.8 (d, $^1J_{C-F}$ = 245.9 Hz, C), 154.3 (C), 147.3 (CH), 135.7 (CH), 132.3 (C), 132.0 (C), 127.2 (d, $^3J_{C-F}$ = 10.7 Hz, C), 126.9 (d, $^3J_{C-F}$ = 8.6 Hz, C), 125.2 (C), 125.0 (CH), 123.3 (C), 122.8 (CH), 121.7 (CH), 111.5 (CH), 111.3 (C), 109.3 (d, $^2J_{C-F}$ = 30.0 Hz, CH), 104.8 (d, $^4J_{C-F}$ = 4.4 Hz, C), 104.2 (d, $^2J_{C-F}$ = 22.0 Hz, CH), 50.9 (CH₂), 49.1 (CH₂), 49.0 (CH₂), 47.7 (CH₂), 25.2 (CH₂), 24.2 (CH₂). HRMS (ESI): m/z [M+H]⁺ calculated for C₂₉H₂₆N₄O₄: 513.1933, found: 513.1955. HPLC purity: 97.3%.

3-(2-([1,4'-Bipiperidine]-1'-carbonyl)-9-fluoro-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-*h*]indol-7-yl)-4-(benzofuran-3-yl)-1*H*-pyrrole-2,5-dione trifluoroacetate (30)

The title compound was synthesized from maleimide **25** and 4-piperidino-1-piperidine-carbonyl chloride according to general procedure I, purified using gradient II preparative HPLC, and isolated as a yellow solid (40%). M.p. 190–195 °C. IR (ATR): $\max$ 3138, 3046, 2946, 2725, 1712, 1695, 1616, 1451, 1338, 1180, 1112, 748 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆) 11.23 (s, 1H), 8.97 (br s, 1H), 8.29 (s, 1H), 7.93 (s, 1H), 7.63 (d, J = 8.1 Hz, 1H), 7.26 (t, J = 6.3 Hz, 1H), 6.98–6.85 (m, 3H), 6.47 (d, J = 9.9 Hz, 1H), 4.71 (s, 2H), 4.53–4.49 (m, 2H), 3.90–3.86 (m, 2H), 3.60–3.46 (m, 5H), 2.96–2.89 (m, 2H), 2.77–2.67 (m, 2H), 1.95–1.33 (m, 10H). ¹³C NMR (100 MHz, DMSO-*d*₆) 172.0 (C=O), 171.7 (C=O), 162.6 (C=O), 158.2 (q, $^2J_{C-F}$ = 31.6 Hz, TFA), 156.7 (d, $^1J_{C-F}$ = 233.4 Hz, C), 154.2 (C), 147.2 (CH), 135.8 (CH), 132.2 (C), 131.9 (C), 127.3 (d, $^3J_{C-F}$ = 10.8 Hz, C), 126.6 (d, $^3J_{C-F}$ = 8.6 Hz, C), 125.2 (C), 124.9 (CH), 123.4 (C), 122.8 (CH), 121.7 (CH), 117.0 (d, $^1J_{C-F}$ = 294.8 Hz, TFA), 111.4 (CH), 111.2 (C), 109.3 (d, $^2J_{C-F}$ = 25.6 Hz, CH), 104.8 (d, $^4J_{C-F}$ = 4.3 Hz, C), 104.4 (d, $^2J_{C-F}$ = 23.4 Hz, CH), 62.6 (CH₂), 50.7 (CH₂), 49.0 (CH₂), 48.9 (CH₂), 48.9 (CH₂), 45.4 (CH₂), 25.5 (CH₂), 22.8 (CH₂), 21.4 (CH₂). HRMS (ESI): m/z [M-H]⁻ calculated for C₃₄H₃₃N₅O₄: 594.2522, found: 594.2531. HPLC purity: 99.0%.

3-(Benzofuran-3-yl)-4-(9-fluoro-2-(morpholine-4-carbonyl)-1,2,3,4-tetrahydro-[1,4]diazepino[6,7,1-*h*]indol-7-yl)-1*H*-pyrrole-2,5-dione (31)

The title compound was synthesized from maleimide **25** and morpholine-4-carbonyl chloride according to general procedure I, purified using gradient I preparative HPLC, and isolated as an orange solid (50%). M.p. 157–160 °C. IR (ATR): $\max$ 3139, 3058, 2962, 2856, 1711, 1700, 1615, 1547, 1458, 1334, 1111, 746 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆) 11.22 (s, 1H), 8.28 (s, 1H), 7.91 (s, 1H), 7.62 (d, J = 8.1 Hz, 1H), 7.26 (t, J = 8.3 Hz, 1H), 6.94 (t, J = 7.3 Hz, 1H), 6.89–6.85 (m, 2H), 6.46 (d, J = 9.4 Hz, 1H), 4.71 (s, 2H), 4.53–4.49

(m, 2H), 3.90–3.86 (m, 2H), 3.60–3.54 (m, 4H), 3.09–3.03 (m, 4H). ^{13}C NMR (100 MHz, DMSO- d_6) 171.9 (C=O), 171.7 (C=O), 163.0 (C=O), 156.8 (d, $^1J_{\text{C-F}} = 234.9$ Hz, C), 154.2 (C), 147.2 (CH), 135.7 (CH), 132.2 (C), 131.9 (C), 127.2 (d, $^3J_{\text{C-F}} = 10.7$ Hz, C), 126.6 (d, $^3J_{\text{C-F}} = 8.6$ Hz, C), 125.2 (C), 124.9 (CH), 123.3 (C), 122.7 (CH), 121.7 (CH), 111.4 (CH), 111.2 (C), 109.5 (d, $^2J_{\text{C-F}} = 26.4$ Hz, CH), 104.7 (d, $^4J_{\text{C-F}} = 4.4$ Hz, C), 104.3 (d, $^2J_{\text{C-F}} = 24.9$ Hz, CH), 65.7 (CH₂), 50.3 (CH₂), 49.0 (CH₂), 48.8 (CH₂), 47.2 (CH₂). HRMS (ESI): m/z [M-H][−] calculated for C₂₈H₂₂FN₄O₅: 513.1580, found: 513.1605. HPLC purity: 97.3%.

Steroidogenesis assay

Cell culture—MA-10 mouse Leydig tumor cells were a gift from Dr. Mario Ascoli (University of Iowa). Cells were cultured in 75 cm²-cell culture flasks with Dulbecco's modified Eagle's medium (DMEM)/nutrient mixture F-12 Ham supplemented with 5% fetal bovine serum and 2.5% heat-inactivated horse serum, as previously described.⁴³

Steroid biosynthesis—For steroid synthesis experiments in the presence of medium with the compounds under investigation, MA-10 cells were plated into 96-well plates at 2.5×10^4 cells per well. After allowing cells to adhere for 18 h, the cells were washed with PBS and exposed to the respective treatment (0–100 μM tested compound in medium). All media conditions were serum-free. Compounds were prepared as DMSO stock solutions and used at a final DMSO concentration of <0.02% in media. Controls contained the same amount of DMSO. At the end of the incubation, culture medium was collected and tested for progesterone production using RIA with anti-progesterone antisera (MP Biomedicals, Solon, OH), following the manufacturer's recommended conditions. Progesterone production was normalized for the amount of protein in each well. RIA data were analyzed using Prism 5.03 from GraphPad.

Protein measurement—Proteins were quantified using the dye-binding assay of Bradford⁴⁴ with bovine serum albumin (BSA) as the standard.

SmartCube® assay—Drugs were injected 15 min before the test, during which multiple challenges were presented over the course of the test session. At least 12 mice were used in each treatment group. Digital videos of the subjects were processed with computer vision algorithms to extract over 1,400 dependent measures including frequency and duration of behavioral states such as grooming and rearing and many other features obtained during the test session. These data were compared against a standard database of therapeutic class signatures obtained with the same experimental protocol. The database comprises 14 classes of drugs with some of the major classes, such as the antidepressant class, comprising several subclasses with representatives of most of the drugs on the market. Using machine learning techniques, the reference database was combed to find reference drug class signatures. The best performing techniques were chosen from our evaluation tests and used to build classifiers that capture the therapeutic class signatures. The behavioral signatures of the test compound were then assessed quantitatively with these classifiers to predict total and specific potential therapeutic utility.^{41, 42}

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Kovacsics CE, Gottesman II, Gould TD. Lithium's antisuicidal efficacy: elucidation of neurobiological targets using endophenotype strategies. *Annu Rev Pharmacol Toxicol.* 2009; 49:175–198. [PubMed: 18834309]
2. Wada A. Lithium and neuropsychiatric therapeutics: neuroplasticity via glycogen synthase kinase-3 β , beta-catenin, and neurotrophin cascades. *J Pharmacol Sci.* 2009; 110:14–28. [PubMed: 19423950]
3. Phiel CJ, Klein PS. Molecular targets of lithium action. *Annu Rev Pharmacol Toxicol.* 2001; 41:789–813. [PubMed: 11264477]
4. Marmol F. Lithium: bipolar disorder and neurodegenerative diseases Possible cellular mechanisms of the therapeutic effects of lithium. *Prog Neuropsychopharmacol Biol Psychiatry.* 2008; 32:1761–1771. [PubMed: 18789369]
5. Spencer CM, Jahng JW, Ryu V, Houtp TA. Lithium-induced gene expression of inducible cyclic adenosine monophosphate early repressor in the rat adrenal gland. *J Neurosci Res.* 2005; 82:273–282. [PubMed: 16175568]
6. Beaulieu JM, Marion S, Rodriguiz RM, Medvedev IO, Sotnikova TD, Ghisi V, Wetsel WC, Lefkowitz RJ, Gainetdinov RR, Caron MG. A beta-arrestin 2 signaling complex mediates lithium action on behavior. *Cell.* 2008; 132:125–136. [PubMed: 18191226]
7. Jope RS. Glycogen synthase kinase-3 in the etiology and treatment of mood disorders. *Front Mol Neurosci.* 2011; 4(16):1–11. [PubMed: 21441980]
8. Valvezan AJ, Klein PS. GSK-3 and Wnt Signaling in Neurogenesis and Bipolar Disorder. *Front Mol Neurosci.* 2012; 5(1):1–13. [PubMed: 22319467]
9. O'Brien WT, Huang J, Buccafusca R, Garskof J, Valvezan AJ, Berry GT, Klein PS. Glycogen synthase kinase-3 is essential for beta-arrestin-2 complex formation and lithium-sensitive behaviors in mice. *J Clin Invest.* 2011; 121:3756–3762. [PubMed: 21821916]
10. Gould TD, Manji HK. The Wnt signaling pathway in bipolar disorder. *Neuroscientist.* 2002; 8:497–511. [PubMed: 12374432]
11. Doble BW, Woodgett JR. GSK-3: tricks of the trade for a multi-tasking kinase. *J Cell Sci.* 2003; 116:1175–1186. [PubMed: 12615961]
12. Jope RS, Yuskaitis CJ, Beurel E. Glycogen synthase kinase-3 (GSK3): inflammation, diseases, and therapeutics. *Neurochem Res.* 2007; 32:577–595. [PubMed: 16944320]
13. Boulahjar R, Ouach A, Matteo C, Bourg S, Ravache M, le Guevel R, Marionneau S, Oullier T, Lozach O, Meijer L, Guguen-Guillouzo C, Lazar S, Akssira M, Troin Y, Guillaumet G, Routier S. Novel Tetrahydropyrido[1,2-a]isoindolone Derivatives (Valmerins): Potent Cyclin-Dependent Kinase/Glycogen Synthase Kinase 3 Inhibitors with Antiproliferative Activities and Antitumor Effects in Human Tumor Xenografts. *J Med Chem.* 2012; 55:9589–9606. [PubMed: 23083119]
14. Fugel W, Oberholzer AE, Gschloessl B, Dzikowski R, Pressburger N, Preu L, Pearl LH, Baratte B, Ratin M, Okun I, Doerig C, Kruggel S, Lemcke T, Meijer L, Kunick C. 3,6-Diamino-4-(2-halophenyl)-2-benzoylthieno[2,3-b]pyridine-5-carbonitriles Are Selective Inhibitors of Plasmodium falciparum Glycogen Synthase Kinase-3. *J Med Chem.* 2013; 56:264–275. [PubMed: 23214499]
15. Sutton LP, Rushlow WJ. The effects of neuropsychiatric drugs on glycogen synthase kinase-3 signaling. *Neuroscience.* 2011; 199:116–124. [PubMed: 22001305]
16. Beaulieu JM, Gainetdinov RR, Caron MG. Akt/GSK3 signaling in the action of psychotropic drugs. *Annu Rev Pharmacol Toxicol.* 2009; 49:327–347. [PubMed: 18928402]
17. Beaulieu JM. A role for Akt and glycogen synthase kinase-3 as integrators of dopamine and serotonin neurotransmission in mental health. *J Psychiatry Neurosci.* 2012; 37:7–16. [PubMed: 21711983]

18. Beurel E, Song L, Jope RS. Inhibition of glycogen synthase kinase-3 is necessary for the rapid antidepressant effect of ketamine in mice. *Mol Psychiatry*. 2011; 16:1068–1070. [PubMed: 21502951]
19. Latapy C, Rioux V, Guitton MJ, Beaulieu JM. Selective deletion of forebrain glycogen synthase kinase 3 β reveals a central role in serotonin-sensitive anxiety and social behaviour. *Philos Trans R Soc Lond B Biol Sci*. 2012; 367:2460–2474. [PubMed: 22826345]
20. Mines MA, Yuskaitis CJ, King MK, Beurel E, Jope RS. GSK3 influences social preference and anxiety-related behaviors during social interaction in a mouse model of fragile X syndrome and autism. *PLoS One*. 2010; 5(e9706):1–12.
21. Mines MA, Jope RS. Glycogen synthase kinase-3: a promising therapeutic target for fragile x syndrome. *Front Mol Neurosci*. 2011; 4(35):1–8. [PubMed: 21441980]
22. Liu ZH, Chuang DM, Smith CB. Lithium ameliorates phenotypic deficits in a mouse model of fragile X syndrome. *Int J Neuropsychopharmacol*. 2011; 14:618–630. [PubMed: 20497624]
23. Chiu CT, Liu G, Leeds P, Chuang DM. Combined treatment with the mood stabilizers lithium and valproate produces multiple beneficial effects in transgenic mouse models of Huntington's disease. *Neuropsychopharmacology*. 2011; 36:2406–2421. [PubMed: 21796107]
24. Yan XB, Wang SS, Hou HL, Ji R, Zhou JN. Lithium improves the behavioral disorder in rats subjected to transient global cerebral ischemia. *Behav Brain Res*. 2007; 177:282–289. [PubMed: 17210190]
25. Schule C, Eser D, Baghai TC, Nothdurfter C, Kessler JS, Rupprecht R. Neuroactive steroids in affective disorders: target for novel antidepressant or anxiolytic drugs? *Neuroscience*. 2011; 191:55–77. [PubMed: 21439354]
26. Wirth MM. Beyond the HPA Axis: Progesterone-Derived Neuroactive Steroids in Human Stress and Emotion. *Front Endocrinol (Lausanne)*. 2011; 2(19):1–14. [PubMed: 22649356]
27. Roy L, McDonald CA, Jiang C, Maroni D, Zeleznik AJ, Wyatt TA, Hou X, Davis JS. Convergence of 3',5'-cyclic adenosine 5'-monophosphate/protein kinase A and glycogen synthase kinase-3 β /beta-catenin signaling in corpus luteum progesterone synthesis. *Endocrinology*. 2009; 150:5036–5045. [PubMed: 19819952]
28. Kozikowski AP, Gaisina IN, Yuan H, Petukhov PA, Blond SY, Fedolak A, Caldarone B, McGonigle P. Structure-based design leads to the identification of lithium mimetics that block mania-like effects in rodents. possible new GSK-3 β therapies for bipolar disorders. *J Am Chem Soc*. 2007; 129:8328–8332. [PubMed: 17552518]
29. Gaisina IN, Gallier F, Ougolkov AV, Kim KH, Kurome T, Guo S, Holzle D, Luchini DN, Blond SY, Billadeau DD, Kozikowski AP. From a natural product lead to the identification of potent and selective benzofuran-3-yl-(indol-3-yl)maleimides as glycogen synthase kinase 3 β inhibitors that suppress proliferation and survival of pancreatic cancer cells. *J Med Chem*. 2009; 52:1853–1863. [PubMed: 19338355]
30. Faul MM, Wimmeroski LL, Krumrich CA. A new one step synthesis of maleimides by condensation of glyoxylate esters with acetamides. *Tetrahedron Lett*. 1999; 40:1109–1112.
31. Routier S, Ayerbe N, Merour JY, Coudert G, Bailly C, Pierre A, Pfeiffer B, Caignard DH, Renard P. Synthesis and biological evaluation of 7-azaindolocarbazoles. *Tetrahedron*. 2002; 58:6621–6630.
32. O'Neill DJ, Shen L, Prouty C, Conway BR, Westover L, Xu JZ, Zhang HC, Maryanoff BE, Murray WV, Demarest KT, Kuo GH. Design, synthesis, and biological evaluation of novel 7-azaindolyl-heteroaryl-maleimides as potent and selective glycogen synthase kinase-3 β (GSK-3 β) inhibitors. *Bioorg Med Chem*. 2004; 12:3167–3185. [PubMed: 15158785]
33. Chedid, M. Hematopoietic neoplasm chemotherapy. US20100261712A1. 2010.
34. Engler TA, Henry JR, Malhotra S, Cunningham B, Furness K, Brozinick J, Burkholder TP, Clay MP, Clayton J, Diefenbacher C, Hawkins E, Iversen PW, Li Y, Lindstrom TD, Marquart AL, McLean J, Mendel D, Misener E, Briere D, O'Toole JC, Porter WJ, Queener S, Reel JK, Owens RA, Brier RA, Eessalu TE, Wagner JR, Campbell RM, Vaughn R. Substituted 3-imidazo[1,2-a]pyridin-3-yl-4-(1,2,3,4-tetrahydro-[1,4]diazepino-[6,7,1-hi]indol-7-yl)pyrrole-2,5-diones as highly selective and potent inhibitors of glycogen synthase kinase-3. *J Med Chem*. 2004; 47:3934–3937. [PubMed: 15267232]

35. Magnus NA, Ley CP, Pollock PM, Wepsiec JP. Pictet-Spengler based synthesis of a bisarylmaleimide glycogen synthase kinase-3 inhibitor. *Org Lett*. 2010; 12:3700–3703. [PubMed: 20704418]
36. Chen PC, Gaisina IN, El-Khodori BF, Ramboz S, Makhortova NR, Rubin LL, Kozikowski AP. Identification of a Maleimide-Based Glycogen Synthase Kinase-3 (GSK-3) Inhibitor, BIP-135, that Prolongs the Median Survival Time of 7 SMA KO Mouse Model of Spinal Muscular Atrophy. *ACS Chem Neurosci*. 2012; 3:5–11. [PubMed: 22348181]
37. <http://www.reactionbiology.com/>.
38. Braeuning A, Buchmann A. The Glycogen Synthase Kinase Inhibitor 3-(2,4-Dichlorophenyl)-4-(1-methyl-1H-indol-3-yl)-1H-pyrrole-2,5-dione (SB216763) Is a Partial Agonist of the Aryl Hydrocarbon Receptor. *Drug Metab Dispos*. 2009; 37:1576–1580. [PubMed: 19448134]
39. Diemer T, Allen JA, Hales KH, Hales DB. Reactive oxygen disrupts mitochondria in MA-10 tumor Leydig cells and inhibits steroidogenic acute regulatory (StAR) protein and steroidogenesis. *Endocrinology*. 2003; 144:2882–2891. [PubMed: 12810543]
40. Privalle CT, McNamara BC, Dhariwal MS, Jefcoate CR. ACTH control of cholesterol side-chain cleavage at adrenal mitochondrial cytochrome P-450_{scc}. Regulation of intramitochondrial cholesterol transfer. *Mol Cell Endocrinol*. 1987; 53:87–101. [PubMed: 2822509]
41. Roberds SL, Filippov I, Alexandrov V, Hanania T, Brunner D. Rapid, computer vision-enabled murine screening system identifies neuropharmacological potential of two new mechanisms. *Front Neurosci*. 2011; 5(103):1–4. [PubMed: 21390287]
42. Zhang HK, Eaton JB, Yu LF, Nys M, Mazzolari A, van Elk R, Smit AB, Alexandrov V, Hanania T, Sabath E, Fedolak A, Brunner D, Lukas RJ, Vistoli G, Ulens C, Kozikowski AP. Insights into the structural determinants required for high-affinity binding of chiral cyclopropane-containing ligands to alpha4beta2-nicotinic acetylcholine receptors: an integrated approach to behaviorally active nicotinic ligands. *J Med Chem*. 2012; 55:8028–8037. [PubMed: 22928944]
43. Hauet T, Yao ZX, Bose HS, Wall CT, Han Z, Li W, Hales DB, Miller WL, Culty M, Papadopoulos V. Peripheral-type benzodiazepine receptor-mediated action of steroidogenic acute regulatory protein on cholesterol entry into leydig cell mitochondria. *Mol Endocrinol*. 2005; 19:540–554. [PubMed: 15498831]
44. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*. 1976; 72:248–254. [PubMed: 942051]

ABBREVIATIONS

GSK-3	glycogen synthase kinase 3
HPA	hypothalamus pituitary adrenal
SAR	structure-activity relationship
TLC	thin layer chromatography
HPLC	high performance liquid chromatography
rt	room temperature
TFA	trifluoroacetic acid
RIA	radioimmunoassay
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
ATR	attenuated total reflectance

Figure 1.

Synthetic modifications to improve the potency and water solubility of 3-(benzofuran-3-yl)-4-(5-bromo-1-methyl-1H-indol-3-yl)-1H-pyrrole-2,5-dione **1**.

Figure 2.

Steroidogenesis stimulation of maleimide-based GSK-3 inhibitors in MA-10 tumor Leydig cells. MA-10 tumor Leydig cells were incubated in the presence or absence of GSK-3 inhibitor, and at the end of incubation, steroid (progesterone) production and cellular toxicity were evaluated by RIA and MTT assay, respectively. (A). Steroid production by MA-10 cells exposed to 100 μ M GSK-3 inhibitors. MA-10 Leydig cells were exposed to GSK-3 inhibitor for 2 h and steroid production assessed by RIA. ***, $p < 0.001$ as assessed by one-way ANOVA with Newman-Keuls post-test. (B). Dose-dependent steroid production by MA-10 cells exposed to 0–100 μ M GSK-3 inhibitors. MA-10 Leydig cells were exposed to GSK-3 inhibitor for 2 h and steroid production assessed by RIA. Only those that showed significant stimulation of steroid production are presented; the remaining tested compounds (16, 17, 20, 23, 25, 27–31) showed <30 ng progesterone/mg protein at all the tested concentrations after 2 h incubation. (C). Cellular toxicity of MA-10 cells exposed to 100 μ M GSK-3 inhibitors. MA-10 Leydig cells were exposed to GSK-3 inhibitor for 2 h and cellular toxicity assessed by MTT assay. *, $p < 0.05$ (orange); ***, $p < 0.001$ (red) as assessed by one-way ANOVA with Newman-Keuls post-test. (D). Dose-dependent cellular toxicity of MA-10 cells exposed to 0–100 μ M GSK-3 inhibitors. MA-10 Leydig cells were exposed to GSK-3 inhibitor for 2 h and cellular toxicity assessed by MTT assay (red = toxic, orange =

slightly toxic, gray = non-toxic). Results are presented as the mean $\pm$ S.E.M. (n=3) for A and C, and presented as nonlinear regression curve fit of data points for B and D.

Figure 3. Steroidogenic time-response of GSK-3 inhibitors **1** and **9**. MA-10 cells were cultured with 50 μ M of **1** (panel A) or **9** (panel B) for 0–120 min and progesterone production was assessed by RIA. Results are presented as the mean $\pm$ S.E.M (n=2).

Figure 4. Smartcube® signatures of GSK-3 inhibitors **1** and **9** after 1 (acute) or 13 days (chronic) of treatment, 15 min following one dose of each compound at 50 mg/kg i.p.

Scheme 1. Synthesis of Target Compounds 2–12.^a

^a Reagents and conditions: (a) (i) NaH (1.5 equiv.), DMF, rt, 0.5 h; (ii) *O*-trityl-3-bromopropan-1-ol or *O*-trityl-2-bromoethan-1-ol (1.2 equiv.), 60 °C, 16 h, 80–99%; (b) ethyl chlorooxoacetate (5 equiv.), Et₂O, 0 °C to rt, 16 h, 42–74%; (c) benzofuran-3-yl-acetamide (1.1 equiv.), *t*-BuOK (2.5 equiv.), THF or DMF, 0 °C to rt, 16 h, 22–51%; (d) (i) NaH (1.5 equiv.), DMF, rt, 0.5 h; (ii) *tert*-butyl (2-bromoethyl)carbamate (1.2 equiv.), 60 °C, 16 h, 70–90%; (e) trifluoroacetic acid, CHCl₃, rt, 2 h; (f) (i) NaH (1.5 equiv.), DMF, rt, 0.5 h; (ii) *tert*-butyl *N*-(3-bromopropyl)-*N*-methylcarbamate (1.2 equiv.), 60 °C, 16 h, 76–99%.

Scheme 2. Synthesis of Target Compounds 13–23.^a

^a Reagents and conditions: (a) (i) NaH (1.5 equiv.), DMF, rt, 0.5 h; (ii) $R^2\text{Br}$ (1.2 equiv.), 60 °C, 16 h, 85–99%; (b) ethyl chlorooxoacetate (5 equiv.), Et₂O, 0 °C to rt, 16 h or (i) AlCl₃ (5 equiv.), CH₂Cl₂, rt, 1 h; (ii) ethyl chlorooxoacetate (5 equiv.), Et₂O, 0 °C to rt, 16 h, 31–83%; (c) benzofuran-3-yl-acetamide (1.1 equiv.), *t*-BuOK (2.5 equiv.), THF or DMF, 0 °C to rt, 16 h, 42–60%; (d) ethyl 2-(5-bromo-1-methyl-1*H*-indol-3-yl)-2-oxoacetate or ethyl 2-(5-methyl-5*H*-[1,3]dioxolo[4,5-*f*]indol-7-yl)-2-oxoacetate, *t*-BuOK (2.5 equiv.), THF or DMF, 0 °C to rt, 16 h, 30–40%; (e) Ph₃PCH₃Br (3 equiv.), *t*-BuOK (3 equiv.), THF, 0 °C to rt, 16 h, 80–90%; (f) (i) BH₃·THF (1.1 equiv.), THF, rt, 2.5 h; (ii) 10% aq. NaOH (1.3 equiv.), 35% H₂O₂ (1.3 equiv.), rt, 3.5 h, 55–70%; (g) (i) NaH (1.5 equiv.), DMF, rt, 0.5 h; (ii) MeI (1.2 equiv.), 60 °C, 16 h, 90–99%; (h) *tert*-butyl 2,2,2-trichloroacetimidate (1.1 equiv.), cyclohexane, CH₂Cl₂, cat. BF₃·OEt₂, rt, 16 h, 53%.

Scheme 3. Synthesis of Target Compounds 24, 25 and 27–31.^a

^a Reagents and conditions: (a) (i) ethanolamine (2 equiv.), 10% Pd/C (cat.), MeOH, rt, 1 h; (ii) H_2 , 1 atm, rt, 3 h, 84–95%; (b) $(Boc)_2O$ (1.2 equiv.), THF, aq. K_2CO_3 , 0 °C to rt, 5 h, 85–95%; (c) MsCl (1.2 equiv.), *N,N*-diisopropylethylamine, THF, 0 °C to rt, 16 h, 72–96%; (d) NaH (1.5 equiv.), DMF, 0 °C, 1 h, 84–90%; (e) ethyl chlorooxoacetate (5 equiv.), Et_2O , 0 °C to rt, 16 h, 42–49%; (f) benzofuran-3-yl-acetamide (1.1 equiv.), *t*-BuOK (2.5 equiv.), THF or DMF, 0 °C to rt, 16 h, 30–40%; (g) trifluoroacetic acid, $CHCl_3$, rt, 2 h; (h) R^4CO_2H (1.2 equiv.), EDC·HCl (1.3 equiv.), HOBt (1.3 equiv.), DMF, *N*-methylmorpholine (3 equiv.), rt, 16 h, 30–50%; (i) R^4COCl (1.5 equiv.), *N,N*-diisopropylethylamine (3 equiv.), MeOH, rt, 3 h, 30–50%.

Scheme 4. Synthesis of Target Compound 26.^a

^a Reagents and conditions: (a) 2-chloroethylamine hydrochloride (1.1 equiv.), K₂CO₃, DMF, sealed tube, 110 °C, 16 h, 25%; (b) 36% aq. HCHO (1.2 equiv.), AcOH, H₂SO₄, 70 °C, 16 h; (c) (Boc)₂O (1 equiv.), THF, aq. K₂CO₃, 0 °C to rt, 5 h, 65% over 2 steps; (d) DDQ (1.2 equiv.), Et₂O, PhMe, rt, 3 h, 50%; (e) ethyl chlorooxacetate (5 equiv.), Et₂O, 0 °C to rt, 16 h, 39%; (f) benzofuran-3-yl-acetamide (1.1 equiv.), *t*-BuOK (2.5 equiv.), THF or DMF, 0 °C to rt, 16 h, 30%; (g) trifluoroacetic acid, CHCl₃, rt, 2 h.

Table 1

Inhibition of GSK-3 by Maleimides 2–20.

Cmpd.	R ¹	X	R ²	IC ₅₀ (nM) ^a
1	5-Br	CH	–CH ₃	21.1±1.8
2	5-Br	CH	–(CH ₂) ₂ OH	21.4±3.1
3	5-F	CH	–(CH ₂) ₂ OH	4.8±0.2
4	5-F	CH	–(CH ₂) ₃ OH	15.0±2.7
5	5-Br	CH	–(CH ₂) ₂ NH ₂	116±9
6	5-F	CH	–(CH ₂) ₂ NH ₂	41.1±0.7
7	5-Br	CH	–(CH ₂) ₃ NHMe	404±23
8	5-F	CH	–(CH ₂) ₃ NHMe	202±5
9	H	CH	–(CH ₂) ₂ OMe	19.8±5.8
10	5-F	CH	–(CH ₂) ₂ OMe	39.7±0.6
11	5-Br	CH	–(CH ₂) ₂ OMe	82.3±5.7
12	5,6-difluoro	CH	–(CH ₂) ₂ OMe	35.7±1.9
13	5-Br	N	Me	5.3±0.8
14	5-Br	N	–(CH ₂) ₂ OMe	25.2±1.2
15	H	N	Me	17.6±1.0
16	H	N	–(CH ₂) ₂ OMe	58.2±3.5
17	-	-	Me	65.9±3.6
18	-	-	–(CH ₂) ₂ OMe	127±5
19	5,6-methylenedioxy	-	Me	5.3±1.0
20	5-Br	-	Me	0.87±0.11

^aThe synthesized maleimides were evaluated for their ability to inhibit phosphorylation of primed substrate (YRRAVPPSPSLSRHSSPHQ(pS)EDEEE; 20 μM) by human GSK-3 in the presence of 10 μM ATP concentration. These compounds were tested at Reaction Biology Inc.³⁷ and IC₅₀ values are presented as average of duplicates ± SEM.

Table 2

Inhibition of GSK-3 by Maleimides 21–31.

Cmpd.	R ¹	R ²	R ³	R ⁴	IC ₅₀ (nM) ^a
21	H	Me	Me	-	11.2±3.1
22	H	Me	<i>t</i> -Bu	-	148±15
23	H	-(CH ₂) ₂ OMe	Me	-	114±16
24	H	-	-	-	4.2±0.7
25	F	-	-	-	3.7±0.5
26	Br	-	-	-	11.5±2.2
27	H	-	-	2-pyrazinyl	0.20±0.06
28	H	-	-	4-pyridylmethyl	0.59±0.11
29	F	-	-	1-piperidinyl	1.9±0.4
30	F	-	-	4-piperidinopiperidin-1-yl	0.53±0.01
31	F	-	-	4-morpholinyl	0.013±0.005

^aThe synthesized maleimides were evaluated for their ability to inhibit phosphorylation of primed substrate (YRRAVPPSPSLRHSSPHQ(pS)EDEEE; 20 μM) by human GSK-3 in the presence of 10 μM ATP concentration. These compounds were tested at Reaction Biology Inc.³⁷ and IC₅₀ values are presented as either average of duplicates ± SEM.