Published in final edited form as:

Eur J Med Chem. 2017 September 08; 137: 476–487. doi:10.1016/j.ejmech.2017.06.011.

Evaluation of the Antidepressant Therapeutic Potential of Isocyanine and Pseudoisocyanine Analogues of the Organic Cation Decynium-22

Anwen M. Krause-Heuer^{1,‡}, Rheaclare Fraser-Spears^{2,‡}, Jeremy C. Dobrowolski⁵, Mark E. Ashford¹, Naomi A. Wyatt¹, Maxine P. Roberts¹, Georgianna G. Gould², Wai-Ching Cheah¹, Clarissa K. L. Ng¹, Mohan Bhadbhade⁵, Bo Zhang⁶, Ivan Greguric¹, Nial J. Wheate⁶, Naresh Kumar⁵, Wouter Koek^{3,4}, Paul D. Callaghan¹, Lynette C. Daws^{2,4,*}, and Benjamin H. Fraser^{1,*}

¹The Australian Nuclear Science and Technology Organisation, Locked Bag 2001 Kirrawee DC NSW 2232, Australia

²Department of Cellular and Integrative Physiology, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900, USA

³Department of Psychiatry, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900, USA

⁴Department of Pharmacology, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900, USA

⁵School of Chemistry, University of New South Wales, Sydney, NSW 2052, Australia

⁶Faculty of Pharmacy, University of Sydney, Sydney, NSW 2006, Australia

Abstract

Herein we describe the synthesis and evaluation of antidepressant properties of seven analogues (1–7) of the low affinity/high capacity transporter blocker decynium-22 (D-22). All analogues (1–7) were synthesized via base promoted coupling reactions between *N*-alkylated-2-methylquinolinium iodides or *N*-alkylated-4-methylquinolinium iodides and electrophilic *N*-alkylated-2-iodoquinolinium iodides. All final compounds were purified by recrystallization or preparative HPLC and initial evaluation studies included; 1) screening for *in vitro* α 1-adrenoceptor activity (a property that can lead to unwanted side-effects), 2) measuring antidepressant-like activity in a mouse tail suspension test (TST), and 3) measuring effects upon mouse locomotion. The results showed some analogues have lower affinities at α 1-adrenoceptors compared to D-22 and showed antidepressant-like activity without the need for co-administration of SSRIs. Additionally, many analogues did not affect mouse locomotion to the same extent as D-22. Plans for additional evaluations of these promising analogues, including measurement of

*Corresponding Author: benjamin.fraser@ansto.gov.au. Phone: +61-02-97173887, Fax: +61-02-97179262; daws@uthscsa.edu. Phone: +1-210-5674174, Fax: +1-210-5674410.

‡These authors contributed equally.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

antidepressant-like activity with co-administration of selective serotonin re-uptake inhibitors (SSRIs), are outlined.

Graphical abstract

Keywords

depression; antidepressants; decynium-22; adrenoceptor; SSRIs; antidepressant-like activity

1. Introduction

Depression and its related mood disorders cause much suffering and lost productivity worldwide.[1] This problem is compounded by the fact that the most commonly prescribed antidepressants – the selective serotonin re-uptake inhibitors (SSRIs) – are not effective for a significant proportion of patients.[2] SSRIs act by blocking the high-affinity serotonin transporter (SERT) and the subsequent increase in extracellular serotonin[3] is thought to be critical for promoting downstream therapeutic effects.[4] A potential explanation for the limited therapeutic efficacy of SSRIs is the presence of low-affinity/high capacity transporters for serotonin in the brain [i.e. organic cation transporters (OCTs) and plasma monoamine transporter (PMAT)], which may limit the ability of SSRIs to increase the amount of extracellular serotonin.[5] Previously the Daws group have demonstrated that decynium-22 (D-22) (Figure 1) – a potent inhibitor of OCTs and/or PMAT – enhances the ability of the SSRI fluvoxamine to inhibit clearance of serotonin from extracellular fluid and to produce antidepressant-like activity in wild type mice.[6] Moreover, they showed that when SERT is genetically compromised, D-22 inhibited serotonin clearance and produced antidepressant-like effects when given alone.[7] This is of special interest due to the existence of common polymorphisms in the human SERT gene, which are linked to psychiatric disorders and their treatment.[8, 9] Since OCTs and PMAT can also take up other biogenic amines, including norepinephrine and dopamine, it is possible that their blockade may also potentiate the therapeutic efficacy of antidepressant drugs that act by blocking the norepinephrine and dopamine transporters (NET and DAT, respectively).

Given the significant potential of D-22 as an adjunct treatment for depression we have synthesized a small library of D-22 analogues (isocyanines **1–2** and pseudoisocyanines **3–7**) and evaluated their potential for further development as therapeutics for depression and other psychiatric disorders (Figure 1). Although D-22 produces antidepressant-like effects when SERT is genetically or pharmacologically compromised, at higher doses it markedly reduces locomotor activity, an undesirable side-effect. In addition, D-22 has appreciable activity at α_1 -adrenoceptors which could lead to unwanted side-effects such as orthostatic

hypertension, syncope and nasal congestion.[10] Therefore, our aims were to synthesize and evaluate several D-22 analogues (**1–7**) for, firstly, their potential for less off-target effects via decreased activity at α 1-adrenoceptors (compared with D-22) and, secondly, for their ability to retain or have increased antidepressant-like effects without impairing locomotor activity. Isocyanines **1** and **2** and pseudoisocyanines **3** and **7** have been synthesized previously but with only limited chemical characterization data.[11, 12] Pseudoisocyanines **4**, **5** and **6** are novel analogues and their synthesis and full chemical characterization is reported herein.

2. Results and discussion

2.1 Synthetic chemistry

Synthesis of *N*-alkylated intermediates for base promoted pseudoisocyanine and isocyanine coupling reactions were completed using adaptations of previously reported procedures (Scheme 1).[12–16] 4-Methylquinoline (**8**) and 2-chloroquinoline (**9**) were both alkylated using an excess of methyl iodide and/or 2-iodopropane to give 1,4-dimethylquinolinium iodide (**10**), 1-isopropylquinolinium iodide (**11**) and 2-iodo-1-methylquinolinium iodide (**12**) in good yields. For the subsequent base promoted coupling reactions, the appropriate *N*-alkylated precursor (**10–11**) and 2-iodo-1-methylquinolinium iodide (**12**) were suspended in dry ethanol and treated with ethanolic potassium hydroxide following an adaptation of procedure of Russ *et al.* (Scheme 1).[12]

Upon addition of ethanolic potassium hydroxide, an immediate color change to purple was observed which was expected due to formation of the isocyanine product – a known class of dye compound.[17] The purple solids were collected by filtration and analysis by HPLC, however, showed both samples contained the desired isocyanines **1** and **2** but in purity levels less than 95%. Attempts to purify **1** and **2** by crystallization were unreliable and gave products with purity levels ranging from 79–95% by microanalysis. This was attributed to varying amounts of ethanol and salt (NaI or NaOH) by-product being co-crystallised with the isocyanine product. The procedure of Russ and co-workers does not report HPLC or microanalytical data for compounds **1** and **2** but only ^1H and ^{13}C NMR data which would not detect by-product salt content.[12] Given the concerns regarding the purity of isocyanines **1** and **2**, conditions for purification by preparative HPLC were developed. The solids were first dissolved in excess trifluoroacetic acid (TFA) (~5 mL) and heated to 80 °C in order to exchange the iodide counter-ion for trifluoroacetate. It was necessary to convert the iodides into trifluoroacetate salts before preparative HPLC as the trifluoroacetate salts purified more readily under reverse phase HPLC conditions. This also ensured the isocyanine products contained a single counter-ion for chemical and biological characterization. For HPLC purification, the crude solids were dissolved in the minimum amount of ACN/DMSO (90/10) and were eluted using a water/ACN mobile phase with a 10% TFA modifier. This gave the isocyanines **1** and **2** in moderate yields (54% and 48%) and purity greater than 98% (HPLC). Elemental analysis confirmed the presence of the trifluoroacetate counter ion with no detectable amount (<0.3%) of iodide counter ion. Characterization data including ^1H and ^{13}C NMR, LRMS, HRMS, IR and elemental analysis were all consistent with the structures shown for isocyanines **1** and **2** (Scheme 1). ^1H NMR data for both isocyanines **1** and **2** were near identical to the previously

published data for their corresponding iodide salts.[12] The ^1H signal assigned to the methine hydrogen ($\text{C}=\text{CH}-\text{C}$) appears at 6.48 ppm for both the iodide and trifluoroacetate salt compounds[12] (See supplementary information for details).

For synthesis of pseudoisocyanine analogues (**3–7**), the *N*-alkylated coupling precursors (**19–24**) were prepared by reaction of 6-substituted-2-methylquinolines (**13–17**) or 2-chloroquinoline (**18**) with an excess of iodoethane at 90 °C for 2–7 days in a sealed tube (Scheme 2).

Although all previous methylation reactions were complete in 24 h the ethylation reactions took considerably longer (2–7 days) owing to the increased steric hindrance of the 6-substituted-2-methylquinoline starting materials and the ethyl iodide electrophile.[18, 19] It was generally observed that alkylation reactions performed at temperatures above 90 °C gave diminished yields and significant amounts of unreacted starting material. This observation is consistent with the known phenomenon for *N*-alkylation reactions, where equilibrium lies back in favour of starting materials at higher temperatures.[19] Alkylation reactions performed with excess alkyl iodide (3 equiv.) in a sealed tube at 85–90 °C minimized this effect and consistently gave the highest yields of *N*-alkylated products (see supplementary information for details). All characterization data for the electrophilic coupling partner 1-ethyl-2-iodoquinolinium iodide (**24**) were consistent with that previously reported,[12] however additionally, single crystals of **24** were obtained from ethanol and were suitable for x-ray diffraction crystallography. The resulting crystal structure confirmed the presence of the 2-iodo leaving group and an iodide counter ion. This is the first report of an x-ray crystal structure for 1-ethyl-2-iodoquinolinium iodide (**24**) which is used extensively in coupling reactions for the synthesis of cationic organic compounds and dyes (Figure 2) (see supplementary data for details).

Base promoted coupling reactions for synthesis of pseudoisocyanines **3–7** were also performed following an adaptation of the procedure of Russ *et al.* (Scheme 2).[12] A suspension of the appropriate 6-substituted-2-methylquinolinium iodide (**19–23**) and 2-iodo-1-ethylquinolinium iodide (**24**) in dry ethanol were treated with potassium hydroxide in ethanol and heated to 35 °C for 2 days. An immediate color change to purple/red was observed and after concentration by rotary evaporation and filtration of the solid product, analysis by HPLC indicated a purity of less than 95%. The final compounds were then either treated with TFA (~5 mL) and purified by preparative HPLC (pseudoisocyanine **4**) or recrystallized from ethanol (pseudoisocyanines **3** and **5–7**). Microanalysis confirmed the presence of a trifluoroacetate counter ion for pseudoisocyanine **4** with no detectable (<0.3%) amount of iodide present. For pseudoisocyanines **3** and **5–7** elemental analysis were consistent with that expected for the products with an iodide counter ion. After recrystallization of pseudoisocyanine **6**, single crystals suitable for x-ray diffraction crystallography were obtained from ethanol (Figure 3).

The x-ray crystal structure of pseudoisocyanine **6** confirmed the presence of an iodide counter-ion, two molecules of co-crystallized water (supported by elemental analysis) and (*E*)-geometry of the central methine ($\text{C}-\text{CH}=\text{C}$) group; the carbon-carbon bond lengths for the methine unit ($\text{C6A}-\text{C5BA}$ (1.387 Å) and $\text{C5BA}-\text{C6B}$ (1.394 Å)) are intermediate

between single and double bonds which is consistent with π -electron delocalization over the two heterocyclic ring systems (see supplementary information for details).

2.2 Biological evaluation studies

2.2.1 *In vitro* α 1-adrenoceptor activity—Analogues **1–7** were first screened for *in vitro* α 1-adrenoceptor activity (decreased activity being a predictor of less undesirable side effects *in vivo*), and then *in vivo* for antidepressant-like activity in the TST and for effect upon mouse locomotion. Affinity of analogues **1–7** for α 1-adrenoceptors was measured by displacement of [3 H]prazosin (Perkin Elmer, 81.7–84.2 Ci/mmol), a selective α -1 receptor antagonist. D-22 has appreciable affinity for α 1-adrenoceptors[10] and since this is an off-target site of action, with potential unwanted side-effects, including orthostatic hypertension, syncope and nasal congestion, it was important to compare the affinities of D-22, and lead analogues (those not lethal at a high dose), for α 1-adrenoceptors. Although analogues **1**, **3**, and **4** showed similar α -1 adrenoceptor activity to that of decynium-22 at a single concentration (IC₅₀ not determined), promising analogues with lower affinity were identified as analogues **2**, **5**, **6** and **7**. [3 H]Prazosin binding and competition binding assays were based on prior studies[10, 20, 21] (See supplementary information for details). Figure 4 shows the concentration response analysis for displacement of [3 H]prazosin by each competing ligand. Prazosin displacement by D-22 and **2**, **5**, **6** and **7** was compared with the displacement by the high affinity adrenoceptor blocker, tamsulosin. All competing ligands were less potent than tamsulosin, with D-22 being at least 10-fold less potent and **2**, **5**, **6** and **7** at least 100-fold less potent. Corticosterone, a known inhibitor of OCT3,[22] was found here to be a weak inhibitor at adrenoceptors. Summarized IC₅₀ values obtained from displacement experiments are provided in Table 1. Comparison of non-linear regression curves for each ligand with either tamsulosin or D-22 was performed to determine the statistical significance of the observed differences. The near 2 nM IC₅₀ for the high-affinity adrenoceptor ligand tamsulosin was 30-fold lower than the IC₅₀ for D-22 (IC₅₀ = 60 nM, p =0.0003), and was 150-500-fold lower than the D-22 analogues (p <0.0001): analogue **2** (IC₅₀ $\approx$ 1 μ M), analogue **5** (IC₅₀ = 0.3 μ M), analogue **6** (IC₅₀ = 0.5 μ M), and analogue **7** (IC₅₀ = 0.7 μ M). In terms of structure/activity relationship, moving the methine link to the 4-position (analogue **2**) or the presence of 6-substituents on pseudoisocyanines (analogues **5–7**) markedly reduces affinity for α 1-adrenoceptors expressed in the hippocampus. This warrants their potential application as therapeutic analogues essentially devoid of α 1-adrenergic receptor-mediated behavioral effects.

2.2.2 Antidepressant-like activity assay (tail suspension test)—Given the significant potential for further development of analogues **2**, **5**, **6** and **7** (having decreased *in vitro* α 1-adrenoceptor activity) and that analogues **1**, **3** and **4** retained similar α 1-adrenoceptor activity compared to D-22, all analogues **1–7** were then evaluated for antidepressant-like activity in a mouse tail suspension test (TST) assay. The TST has been used to investigate antidepressant-like effects of drugs in mice, and shows predictive validity for drugs with antidepressant-like properties[23] (see supplementary information for details). Figure 5 shows the effect of D-22 and analogues **1–7** on immobility time in the TST. With the exception of **5** and **6**, none of the analogues, including D-22, displayed antidepressant-like activity (i.e. a reduction in immobility time). This is not surprising since

in our previously published studies, we found D-22 to be without effect on immobility time unless paired with an SSRI[6] or given to mice with a constitutive reduction in SERT expression,[7] where OCT3 expression is increased as a compensatory result. In contrast, a 3.2 mg/kg dose of analogues **5** and **6** caused mice to spend significantly less time immobile than saline treated controls (Figure 5). Given that this dose, if it affects locomotion, likely suppresses locomotor activity (see dose-response relationships in Figure 5F–G), this increase in time spent mobile in the TST is indicative of these analogues having independent antidepressant-like activity. In terms of SAR, the electron withdrawing substituents for **5** (fluoro) and **6** (chloro) impart independent antidepressant-like activity, while the larger and slightly weaker electron withdrawing substituents for **3** (methoxy), **4** (bromo) and **7** (iodo) and moving the methine linker to the 4-position (**1** and **2**) do not impart independent antidepressant-like activity.

2.2.3 Locomotor activity assay—Given analogues **5** and **6** show significant potential due to independent antidepressant like activity (not requiring co-administration of an SSRI) and that analogues **1–4** and **7** will be evaluated in future work with co-administration of an SSRI, all analogues **1–7** were then evaluated for their effect upon locomotor activity.

Locomotor activity was measured by placing the mouse in a chamber containing infrared emitters and receivers (see supplementary information for details). Mice received an intraperitoneal (ip) injection of D-22 (0.01 – 10.0 mg/kg), or equivalent dose of new analogue (**1–7**) immediately before being placed in the chamber. Locomotion was measured as infrared beam breaks per 5 min period during a four-hour session. Data are shown for cumulative beam breaks occurring 60 to 120 minutes following injection (Figure 6) (see supplementary information for details).

Figure 6A shows the dose-response relationship for D-22 to influence locomotor activity. This relationship is replotted in Figure 6B–H to facilitate comparison with analogues **1–7**. At 1.0 mg/kg, D-22 completely suppressed locomotor activity, whereas none of the analogues caused complete suppression of activity at this dose. These data suggest that all analogues (**1–7**) may have a more favorable profile for use *in vivo* than D-22. For analogues **1–6** (Figure 6B–G) maximal or near maximal suppression of locomotor activity was observed at a dose of 10 mg/kg. For analogue **1**, **3** and **4** (Figure 6B, D & E), one out of six mice in each test group died at this 10 mg/kg dose. Of great interest, 10 mg/kg of analogue **7** reduced locomotor activity by only half that of saline-injected control mice (Figure 6H), indicating analogue **7** is an excellent candidate for further development for use *in vivo*. One-way ANOVA followed by Dunnett's test was used 1) to examine the effects of D-22 on locomotion, 2) to compare the effects of 0.1 and 1 mg/kg of each analogue (**1–7**) with the effects of 0.1 and 1 mg/kg of D-22, and 3) to compare the maximal effect of each analogue with the maximal effect of D-22. D-22 decreased locomotion at 1 mg/kg ($p<0.0001$) but not at 0.1 mg/kg ($p=0.44$). At 0.1 mg/kg, none of the analogues had effects on locomotion that differed significantly from those of D-22 ($F[7,53]=1.82$, $p=0.10$). At 1 mg/kg, **6** and **7** affected locomotion significantly less than D-22 ($p=0.046$ and 0.013 , respectively). At 10 mg/kg, only analogue **7** had significantly less effect on locomotion than the maximally effective dose (1 mg/kg) of D-22 ($p=0.0096$). In terms of overall SAR, moving the methine

linker to the 4-position (analogues **1–2**) affords less decrease in locomotor activity, particularly at higher doses of 1 mg/kg and 10 mg/kg compared to D-22. For the pseudoisocyanines, the electron donating methoxy substituent (**3**) does not significantly affect locomotor activity compared to D-22, but electron withdrawing substituents, especially bromo (**6**) and iodo (**7**) give significantly less decreases in locomotor activity.

3. Conclusions

We have evaluated two isocyanine (**1–2**) and five pseudoisocyanine (**3–7**) analogues based upon the non-selective, parent analogue D-22, for their potential as novel antidepressants. The majority of analogues, and in particular analogues **6** and **7**, were less potent than D-22 in suppressing spontaneous locomotor activity in mice, indicating a much improved safety margin for *in vivo* applications of these analogues in comparison to D-22. Tail suspension tests revealed that **5** and **6** reduced immobility time suggesting that these analogues have potential independent action as antidepressants at the highest tested dose. Our data also showed **2**, **5**, **6** and **7** to have significantly reduced activity at adrenergic receptors. Antagonism at α 1-adrenergic receptors by prazosin has been reported to decrease the behavioral effects of antidepressants.[24] Despite its activity at adrenoceptors shown here and previously,[10] D-22 did enhance the antidepressant-like effects of the SSRI fluvoxamine,[6] suggesting that its activity at α 1-adrenoceptors is insufficient to obscure its ability to enhance the antidepressant-like effects of fluvoxamine. As our current results show **2**, **5**, **6** and **7** to have less affinity at α 1-adrenoceptors, the behavioral effects of these analogues in combination with selective serotonin reuptake inhibitors should be evaluated. Given their lower affinity for α 1-adrenoceptors, one prediction is that their ability to enhance the antidepressant-like effect of SSRIs might be considerably greater than that of D-22. Indeed, their reduced affinity at α 1-adrenoceptors might contribute to the ability of **5** and **6** to produce antidepressant-like effects by themselves. At this stage the mode of action of these analogues is most likely due to inhibition of OCTs and/or PMAT but this has yet to be proven. In order to determine this we are currently developing cell based assays and studies are planned that will evaluate the relative potency and selectivity of the D-22 derived analogues at specific low-affinity/high-capacity transporters, OCT2, OCT3 and PMAT that are widely expressed in the brain. Although D-22 does not have any appreciable affinity for the high-affinity/low capacity biogenic amine neurotransmitter transporters (SERT, NET and DAT) it will also be important to assess affinity values for D-22 derived analogues at these transporters in order to understand their true potential as novel antidepressants.

4. Experimental section

4.1 Commercial compounds

6-fluoro-2-methylquinoline, 6-chloro-2-methylquinoline, 6-bromo-2-methylquinoline, 6-methoxy-2-methylquinoline, 6-iodo-2-methylquinoline, 4-methylquinoline, 2-chloroquinoline, methyl iodide, ethyl iodide, 2-iodopropane, potassium hydroxide, trifluoroacetic acid, copper(I) iodide, sodium iodide, *N,N'*-dimethyl-cyclohexane-1,2-diamine, dichloromethane, ethanol and dioxane were of reagent grade quality and purchased from Sigma-Aldrich. Acetonitrile, water and methanol (HPLC grade) were purchased from

Merck and were dried using the Braun MB SPS-800 solvent purification system. Deuterated solvents were purchased from Cambridge Isotope Laboratories (Novachem, Collingwood, Australia).

4.2 General chemistry methods

Nuclear magnetic resonance (^{13}C and ^1H NMR) spectra were recorded using a Bruker Avance DPX-400, Bruker Avance III 300 or a Bruker Avance III 600 Cryo spectrometer. Low-resolution mass spectrometry (LRMS) were performed on a Micromass ZQ quadrupole mass spectrometer, and high-resolution mass spectrometry (HRMS) was performed at the University of Wollongong, Australia using a Bruker Daltonics BioApex-II 7T FTICR spectrometer equipped with an off-axis analytical electron spray ionization source. Melting points were measured using an Optimelt melting point apparatus and are uncorrected. Infrared (IR) spectra were collected on an Agilent Cary 630 FTIR spectrometer fitted with a diamond attenuated total reflectance (ATR) sample interface. Flash chromatography was performed on a Reveleris[®] Flash Chromatography System fitted with an ELSD (isopropanol support) and dual-wavelength UV (254 nm and 280 nm) detectors; solvent systems, column sizes and flow-rates are described where appropriate. Elemental or microanalysis was performed by the Campbell Microanalytical Laboratory, Chemistry Department, University of Otago, Dunedin, New Zealand. HPLC purity of all final analogues was greater than 95% and was performed using a Waters Empower 2 system with a Waters 600 pump, Waters in-line degasser AF, Waters temperature control module II, Waters 717 autosampler and Waters 2996 PDA. An Alltech (C18 (150 × 4.6 mm, 5 μm pore size) analytical column was used, with absorbance measured using a photo-diode array (PDA) detector. Samples were prepared as 1 mg/mL, with a 10 μL injection. Percentage purity was calculated from the peak area under using Empower software (Waters). Solvent system: A = ACN, B = H_2O , C = H_2O (1% TFA). Gradient profile 5%A 85%B (with constant 10%C) to 90%A (with constant 10%C) over 30 mins. UV detection wavelength = 254 nm. Preparative HPLC was performed using a Waters Empower 2 system with a Waters 600 pump, Waters 600 controller, Waters in-line degasser AF, Waters 717 autosampler and Waters 486 tunable absorbance detector. Column and eluent specifications are provided below for each compound.

4.3 Synthetic chemistry procedures

4.3.1 1,4-Dimethylquinolinium iodide (10)—An adaptation of the procedure of Russ *et al* was used.[12] 4-methylquinoline (**8**) (1 equiv, 5.27 g, 36.8 mmol) and methyl iodide (1.5 equiv, 7.85 g, 55.2 mmol) were dissolved in DCM (20 mL) and added to a 50 mL RBF. The solution was stirred at room temperature (rt) for 16 h and filtration of the resulting yellow solid and washing with DCM (3 × 5 mL) afforded the *title compound* (**10**) as a yellow crystalline solid (8.50 g, 29.8 mmol, 81%). Mp: decomposed 176–177 °C. ^1H NMR (300 MHz, $\text{d}_6\text{-DMSO}$): δ 9.35 (1H, d, J = 6.0 Hz), 8.54 (1H, dd, J = 8.5 Hz, 1.0 Hz), 8.48 (1H, d, J = 8.9 Hz), 8.26–8.28 (1H, m), 8.04–8.08 (2H, m), 4.58 (3H, s), 3.00 (3H, s). ^{13}C NMR (100 MHz, $\text{d}_6\text{-DMSO}$): δ 158.12, 148.97, 137.68, 134.92, 129.64, 128.45, 126.79, 122.45, 119.52, 45.01, 19.60. IR (neat); ν = 3057 (s), 3027 (s), 3010 (s), 1525 (s), 783 (s) cm^{-1} . ESI-MS (LRMS, ES^+ , 30 V) m/z [M] $^+$ for $\text{C}_{11}\text{H}_{12}\text{N}$ predicted 158.10; found 158.06. ESI-MS (HRMS, TOF MS AP^+) m/z [M] $^+$ for $\text{C}_{11}\text{H}_{12}\text{N}$ predicted 158.0964; found 158.0961.

HPLC purity = 99.5%, retention time = 7.30 mins, solvent system: A = ACN, B = H₂O, C = H₂O (1% TFA). Gradient profile 5% A 85% B (with constant 10% C) to 90% A (with constant 10% C) over 30 mins.

4.3.2 1-Isopropyl-4-methylquinolinium iodide (11)—An adaptation of the procedure of Russ *et al* was used.[12] 4-methylquinoline (**8**) (1 equiv, 5.01 g, 35.0 mmol) and isopropyl iodide (2 equiv, 11.9 g, 70.0 mmol) were added to a sealed tube reaction vessel. The solution was stirred and heated to 90 °C for 48 h. Filtration of the resulting yellow solid and trituration with diethyl ether (3 × 20 mL) afforded the *title compound* (**11**) as a yellow crystalline solid (8.22 g, 26.2 mmol, 75%). Mp 122–123 °C. ¹H NMR (400 MHz, DMSO): δ 9.48 (1H, d, *J* = 6.2 Hz), 8.72 (1H, d, *J* = 9.0 Hz), 8.55 (1H, dd, *J* = 1.2, 8.5 Hz), 8.22–8.30 (1H, m), 8.03–8.12 (2H, m), 5.87 (1H, septuplet, *J* = 6.6 Hz), 3.02 (3H, s), 1.71 (6H, d, *J* = 6.6 Hz). ¹³C NMR (100 MHz, d₆-DMSO): δ 158.02, 144.82, 136.90, 135.08, 129.43, 128.96, 127.27, 122.87, 118.83, 56.62, 22.14, 19.69. IR (neat); ν = 3020 (s), 2983 (s), 2929 (s), 1615 (s), 770 (s), 707 (s) cm⁻¹. ESI-MS (LRMS, ES⁺, 30 V) *m/z* [M]⁺ for C₁₃H₁₆N predicted 186.13; found 186.24. ESI-MS (HRMS, TOF MS AP+) *m/z* [M]⁺ for C₁₃H₁₆N predicted 186.1277; found 186.1279. HPLC purity = 98.9%, retention time = 7.31 mins, solvent system: A = ACN, B = H₂O, C = H₂O (1% TFA). Gradient profile 5% A 85% B (with constant 10% C) to 90% A (with constant 10% C) over 30 mins.

4.3.3 2-Iodo-1-methylquinolinium iodide (12)—2-chloroquinoline (**9**) (1 equiv, 5.00 g, 30.6 mmol) and methyl iodide (2 equiv, 8.68 g, 61.1 mmol) were dissolved up in DCM (30 mL) and the solution was heated to reflux for 48 h. Filtration of the resulting yellow solid and washing with DCM (3 × 10 mL) afforded the *title compound* (**12**) as a yellow crystalline solid (11.22 g, 92%). Mp: Decomposed 220–221 °C. ¹H NMR (300 MHz, d₆-DMSO): δ 7.90 (1H, d, *J* = 9.0 Hz), 7.71 (1H, dd, *J* = 7.7, 1.6 Hz), 7.62 (1H, ddd, *J* = 8.6, 7.1, 1.6 Hz, 1H), 7.47–7.57 (1H, m), 7.26 (1H, ddd, *J* = 7.7, 7.1, 1.1 Hz), 6.61 (1H, d, *J* = 9.5 Hz), 3.62 (3H, s). ¹³C NMR (100 MHz, d₆-DMSO): δ 161.06, 139.71, 139.19, 130.78, 128.71, 121.92, 121.06, 120.05, 114.59, 29.00. IR (neat); ν = 3038 (s), 3003 (s), 1617 (s), 830 (s), 758 (s) cm⁻¹. ESI-MS (LRMS) (ES⁺, 40 V) *m/z* [M]⁺ for C₁₀H₉IN predicted 270.0; found 269.94. ESI-MS (HRMS, TOF MS AP+) *m/z* [M]⁺ for C₁₀H₉IN predicted 269.9774; found 269.9774. HPLC purity = 98.6%, retention time = 7.31 mins, solvent system: A = ACN, B = H₂O, C = H₂O (1% TFA). Gradient profile 5% A 85% B (with constant 10% C) to 90% A (with constant 10% C) over 30 mins.

4.3.4 6-Methoxy-1-ethyl-2-methylquinolinium iodide (19)—6-methoxy-2-methylquinoline (**13**) (1 equiv, 2.00 g, 11.6 mmol) and ethyl iodide (5 equiv, 57.8 mmol, 9.01 g) were heated to 90 °C in a sealed tube for 3 days. Filtration of the resulting yellow/brown solid and washing with DCM (3 × 10 mL) afforded the *title compound* (**19**) as a brown/yellow solid (3.57 g, 94%). Mp: decomposition 200–201 °C. ¹H NMR (300 MHz, d₆-DMSO): δ 8.94 (1H, dd, *J* = 8.7, 0.7 Hz), 8.53 (1H, d, *J* = 10.6 Hz), 8.06 (1H, d, *J* = 8.6 Hz), 7.78–7.89 (2H, m), 4.96 (2H, q, *J* = 7.2 Hz), 3.99 (3H, s), 3.05 (3H, s), 1.51 (3H, t, *J* = 7.2 Hz). ¹³C NMR (75 MHz, DMSO): δ 158.49, 157.19, 144.01, 133.60, 130.20, 126.73, 125.85, 120.56, 108.57, 56.24, 47.25, 21.84, 13.57. IR (neat); ν = 3196 (s), 2895 (s), 854 (s), 674 (s) cm⁻¹. ESI-MS (LRMS, ES⁺, 15 V) *m/z* [M]⁺ for C₁₃H₁₆NO predicted 202.12;

found 202.10. ESI-MS (HRMS, TOF MS AP+) m/z [M]⁺ for C₁₃H₁₆NO predicted 202.1232; found 202.1227. HPLC purity = 99.2%, retention time = 9.87 mins, solvent system: A = ACN, B = H₂O, C = H₂O (1% TFA). Gradient profile 5% A 85% B (with constant 10% C) to 90% A (with constant 10% C) over 30 mins.

4.3.5 6-Bromo-1-ethyl-2-methylquinolinium iodide (20)—6-bromo-2-methylquinoline (**14**) (1 equiv, 4.00 g, 18.0 mmol) and ethyl iodide (5 equiv, 90.1 mmol, 14.05 g) were added to a sealed tube reaction vessel. The solution was stirred and heated to 90 °C for 7 days. Filtration of the resulting yellow/green solid and washing with DCM (3 × 10 mL) afforded the *title compound* (**20**) as a yellow/green solid (5.31 g, 78%). Mp: 208–209 °C. ¹H NMR (300 MHz, d₆-DMSO): δ 9.02 (1H, d, J = 8.6 Hz), 8.74 (1H, d, J = 2.3 Hz), 8.57 (1H, d, J = 9.5 Hz), 8.36 (1H, dd, J = 9.5, 2.3 Hz), 8.17 (1H, d, J = 8.6 Hz), 4.99 (2H, q, J = 7.2 Hz), 3.10 (3H, s), 1.52 (3H, t, J = 7.2 Hz). ¹³C NMR (75 MHz, d₆-DMSO): δ 161.71, 144.97, 138.08, 137.57, 132.79, 130.08, 127.26, 122.55, 121.72, 47.99, 22.90, 13.89. IR (neat); ν = 3012 (s), 2977 (s), 1510 (s), 894 (s), 813 (s) cm⁻¹. ESI-MS (LRMS, ES⁺, 30 V) m/z [M]⁺ for C₁₂H₁₃BrN predicted 250.0; found 249.8. ESI-MS (HRMS, TOF MS AP+) m/z [M]⁺ for C₁₂H₁₃BrN predicted 250.0226; found 250.0220. HPLC purity = 98.6%, retention time = 10.02 mins, solvent system: A = ACN, B = H₂O, C = H₂O (1% TFA). Gradient profile 5% A 85% B (with constant 10% C) to 90% A (with constant 10% C) over 30 mins.

4.3.6 6-Fluoro-1-ethyl-2-methylquinolinium iodide (21)—6-fluoro-2-methylquinoline (**15**) (1 equiv, 0.52 g, 3.24 mmol) and ethyl iodide (5 equiv, 16.2 mmol, 2.53 g) were added to a sealed tube reaction vessel. The solution was stirred and heated to 90 °C for 5 days. Filtration of the resulting yellow solid and washing with DCM (3 × 10 mL) afforded the *title compound* (**21**) as a yellow solid (0.75 g, 73%). Mp: decomposed 150–170 °C. ¹H NMR (300 MHz, d₆-DMSO): 9.05 (1H, dd, J = 8.8, 0.7 Hz), 8.73 (1H, dd, J = 9.8, 4.4 Hz), 8.29 (1H, dd, J = 8.4, 3.0 Hz), 8.23–8.10 (2H, m), 5.01 (2H, q, J = 7.3 Hz), 3.12 (3H, s), 1.53 (3H, t, J = 7.3 Hz). ¹³C NMR (100 MHz, d₆-DMSO): δ 160.43, 160.86, 160.84, 159.11, 145.29, 145.23, 135.72, 130.33, 130.19, 127.18, 125.17, 124.82, 122.95, 122.83, 114.67, 114.37, 48.25, 22.89, 13.99. IR (neat); ν = 3449 (s), 3071 (s), 2971 (s), 817 (s) cm⁻¹. ESI-MS (LRMS, ES⁺, 25 V) m/z [M]⁺ for C₁₂H₁₃NF predicted 190.02; found 190.10. ESI-MS (HRMS, TOF MS AP+) m/z [M]⁺ for C₁₂H₁₃NF predicted 190.1032; found 190.1098. HPLC purity = 98.7%, retention time = 9.49 mins, solvent system: A = ACN, B = H₂O, C = H₂O (1% TFA), gradient profile 5% A 85% B (with constant 10% C) to 90% A (with constant 10% C) over 30 mins.

4.3.7 6-Chloro-1-ethyl-2-methylquinolinium iodide (22)—6-chloro-2-methylquinoline (**16**) (1 equiv, 1.00 g, 5.63 mmol) and ethyl iodide (5 equiv, 28.2 mmol, 4.39 g) were added to a sealed tube reaction vessel. The solution was stirred and heated to 90 °C for 4 days. Filtration of the resulting yellow solid and washing with DCM (3 × 10 mL) afforded the *title compound* (**22**) as a yellow solid (1.84 g, 98%). Mp: decomposed 207–208 °C. ¹H NMR (300 MHz, d₆-DMSO): δ 9.02 (1H, d, J = 8.4 Hz), 8.65 (1H, d, J = 9.6 Hz), 8.59 (1H, d, J = 2.4 Hz), 8.23 (1H, dd, J = 2.8, 9.6 Hz), 8.17 (1H, d, J = 8.4 Hz), 4.99 (2H, q, J = 7.2 Hz), 3.11 (3H, s, 3H), 1.52 (t, J = 7.2 Hz, 3H). ¹³C NMR (75 MHz, d₆-

DMSO): δ 161.19, 144.56, 136.81, 135.01, 133.50, 129.25, 129.03, 126.84, 121.33, 47.60, 22.42, 13.42. IR (neat); ν = 3062 (s), 2983 (s), 904 (s), 684 (s) cm^{-1} . LRMS (ES^+ , 30 V) m/z $[\text{M}]^+$ for $\text{C}_{12}\text{H}_{13}\text{ClN}$ predicted 206.1; found 206.1. HRMS (TOF MS AP^+) m/z $[\text{M}]^+$ for $\text{C}_{12}\text{H}_{13}\text{ClN}$ predicted 206.0731; found 206.0741. HPLC purity = 99.0%, retention time = 9.48 mins.

4.3.8 6-Iodo-1-ethyl-2-methylquinolinium iodide (23)—6-iodo-2-methylquinoline (**17**) (1 equiv, 0.55 g, 2.04 mmol) and ethyl iodide (5 equiv, 10.2 mmol, 1.60 g) were added to a sealed tube reaction vessel. The solution was stirred and heated to 90 °C for 5 days. Filtration of the resulting yellow solid and washing with DCM (3×10 mL) afforded the *title compound* (**23**) as a yellow solid (0.56 g, 64%). Mp: decomposed 251–253 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 8.97 (1H, d, J = 9.4 Hz), 8.88 (1H, d, J = 2.0 Hz), 8.46 (1H, dd, J = 9.3, 2.0 Hz), 8.37 (1H, d, J = 9.4 Hz), 8.13 (1H, d, J = 8.6 Hz), 4.96 (2H, q, J = 7.3 Hz), 3.07 (3H, s), 1.50 (3H, t, J = 7.2 Hz). ^{13}C NMR (75 MHz, d_6 -DMSO): δ 161.47, 144.75, 143.39, 139.05, 137.92, 130.26, 126.94, 121.09, 96.41, 47.79, 22.88, 13.87. IR (neat); ν = 3013 (s), 2972 (s), 1510 (s), 820 (s) cm^{-1} . ESI-MS (LRMS, ES^+ , 25 V) m/z $[\text{M}]^+$ for $\text{C}_{12}\text{H}_{13}\text{NI}$ predicted 298.01; found 297.96. ESI-MS (HRMS, TOF MS AP^+) m/z $[\text{M}]^+$ for $\text{C}_{12}\text{H}_{13}\text{NI}$ predicted 298.0093; found 298.0080. HPLC purity = 98.5%, retention time = 11.04 mins, solvent system: A = ACN, B = H_2O , C = H_2O (1% TFA). Gradient profile 5%A 85%B (with constant 10%C) to 90%A (with constant 10%C) over 30 mins.

4.3.9 2-Iodo-1-ethylquinolinium iodide (24)—2-chloroquinoline (**9**) (1 equiv, 5.00 g, 30.6 mmol) and ethyl iodide (5 equiv, 152.8 mmol, 23.8 g) were heated to 90 °C in a sealed tube for 5 days. Filtration of the resulting yellow solid and washing with DCM (3×10 mL) afforded the *title compound* (**24**) as a yellow solid (7.29 g, 58%). Mp: Decomposed 212–213 °C. ^1H NMR (300 MHz, d_6 -DMSO): δ 7.90 (1H, dt, J = 9.5, 0.5 Hz), 7.72 (1H, dd, J = 7.7, 1.5 Hz), 1.69–7.52 (2H, m), 7.26 (1H, ddd, J = 7.7, 6.6, 1.5 Hz), 6.60 (1H, d, J = 9.5 Hz), 4.27 (2H, q, J = 7.1 Hz), 1.21 (3H, t, J = 7.1 Hz). ^{13}C NMR (75 MHz, d_6 -DMSO): δ 161.65, 139.29, 138.59, 130.85, 129.03, 121.77, 121.08, 120.34, 114.32, 36.44, 12.67. IR (neat); ν = 3041 (s), 2988 (s), 1565 (s), 823 (s), 770 (s) cm^{-1} . ESI-MS (LRMS, ES^+ , 30 V) m/z $[\text{M}]^+$ for $\text{C}_{11}\text{H}_{11}\text{IN}$ predicted 284.0; found 284.3. ESI-MS (HRMS, TOF MS AP^+) m/z $[\text{M}]^+$ for $\text{C}_{11}\text{H}_{11}\text{IN}$ predicted 283.9931; found 283.9930. HPLC purity = 99.4%, retention time = 9.92 mins, solvent system: A = ACN, B = H_2O , C = H_2O (1% TFA). Gradient profile 5%A 85%B (with constant 10%C) to 90%A (with constant 10%C) over 30 mins.

4.3.10 1,1'-Dimethyl-2,4'-cyanine trifluoroacetate (1)—An adaptation of the procedure of Russ *et al.* was used.[12] 1,4-dimethylquinolinium iodide (**10**) (1 equiv, 0.20 g, 0.70 mmol) and 2-iodo-1-methylquinolinium iodide (**12**) (1 equiv, 0.28 g, 0.70 mmol) were dispersed in dry EtOH (10 mL) at rt. To this suspension was added a solution of KOH (1.5 equiv, 0.06 g, 1.40 mmol) in dry EtOH (5 mL). The solution turned immediately dark red/purple and was stirred for 18 h at rt. Filtration of the solution gave a purple solid which was dried under vacuum and then dissolved up in TFA (5 mL). The solution was then heated to 80 °C for 18 h and then cooled to rt. The excess TFA was removed by distillation under reduced pressure to give purple solid which was then purified by prep. HPLC (column: Atlantis T3 (10 μm 30 $\times$ 150mm); isocratic (10% MeOH, 80% H_2O , 10% H_2O (1% TFA));

flow rate: 25 mL/min; inj. volume: 300 μ L). The appropriate fractions were evaporated under reduced pressure to remove ACN and then the remaining water lyophilized to give the *title compound* (**1**) as a purple solid (156 mg, 54%). Mp: 114–115 °C. ^1H NMR (400 MHz, d_6 -DMSO): δ 8.55 (1H, d, J = 8.4 Hz), 8.22 (1H, d, J = 7.2 Hz), 8.07 (1H, d, J = 9.5 Hz), 7.90–8.02 (4H, m), 7.88 (1H, d, J = 7.7 Hz), 7.77–7.83 (1H, m), 7.65–7.72 (1H, m), 7.47–7.53 (1H, m), 7.40 (1H, d, J = 7.2 Hz), 6.48 (1H, s), 4.07 (3H, s), 4.00 (3H, s). ^{13}C NMR (100 MHz, d_6 -DMSO): δ 153.99, 149.37, 143.51, 140.00, 138.44, 137.15, 132.89, 132.43, 128.84, 126.27, 125.83, 124.89, 124.45, 124.30, 121.29, 117.70, 116.70, 108.54, 94.42, 41.77, 37.85. IR (neat); ν = 3036 (s), 3010 (s), 2976 (s), 895 (s), 811 (s) cm^{-1} . ESI-MS (LRMS, ES^+ , 40 V) m/z [M] $^+$ for $\text{C}_{21}\text{H}_{19}\text{N}_2$ predicted 299.15; found 299.04. ESI-MS (HRMS, TOF MS AP^+) m/z [M] $^+$ for $\text{C}_{21}\text{H}_{19}\text{N}_2$ predicted 299.1548; found 299.1556. HPLC purity = 99.7%, retention time = 6.81 mins, solvent system: A = ACN, B = H_2O , C = H_2O (1% TFA). Gradient profile 5% A 85% B (with constant 10% C) to 90% A (with constant 10% C) over 30 mins. Elemental analysis ($\text{C}_{23}\text{H}_{19}\text{F}_3\text{N}_2\text{O}_2 \cdot 1.2\text{TFA}$) predicted %C: 55.55, H: 3.71, N: 5.1, I: 0.0. Found %C: 55.46 and 55.59, H: 3.82 and 3.82, N: 5.39 and 5.40, I: <0.3 and <0.3.

4.3.11 1-Methyl-1'-isopropyl-2,4'-cyanine trifluoroacetate (2)—An adaptation of the procedure of Russ *et al.* was used.[12] 1-isopropyl-4-methyl-quinolinium iodide (**11**) (1 equiv, 0.15 g, 0.48 mmol) and 2-iodo-1-methylquinolinium iodide (**12**) (1 equiv, 0.20 g, 0.48 mmol) were dispersed in dry EtOH (8 mL) at rt. To this suspension was added a solution of KOH (1.5 equiv, 0.96 mmol, 40 mg) in dry EtOH (5 mL). The solution turned immediately dark red/purple and was stirred for 18 h at rt. Filtration of the solution gave a purple solid which was dried under vacuum and then dissolved up in TFA (4 mL). The solution was then heated to 80 °C for 18 h and then cooled to rt. The excess TFA was removed by distillation under reduced pressure to give a purple solid which was dissolved up in ACN (1.0 mL) and purified by prep. HPLC (column: Atlantis T3 (10 μ m 30 $\times$ 150mm); isocratic (20% MeOH, 70% H_2O , 10% H_2O (1% TFA)); flow rate: 25 mL/min; inj. volume: 300 μ L). The appropriate fractions were evaporated under reduced pressure to remove ACN and then the remaining water lyophilized to give the *title compound* (**2**) as a purple solid (126 mg, 48%). Mp: 240–241 °C. ^1H NMR (400 MHz, d_6 -DMSO): δ 8.56 (1H, d, J = 7.7 Hz), 8.36 (1H, d, J = 7.4 Hz), 8.16 (1H, d, J = 8.8 Hz), 8.08 (1H, d, J = 9.4 Hz), 7.86–8.01 (4H, m), 7.77–7.82 (1H, m), 7.61–7.68 (1H, m), 7.47–7.52 (1H, m), 7.43 (1H, d, J = 7.4 Hz), 6.48 (1H, s), 5.31 (1H, septuplet J = 6.5 Hz), 4.01 (3H, s), 1.55 (6H, d, J = 6.5 Hz). ^{13}C NMR (100 MHz, d_6 -DMSO): δ 154.27, 149.16, 140.12, 138.31, 137.80, 137.20, 133.02, 132.47, 128.89, 126.34, 126.07, 124.95, 124.58, 121.38, 117.02, 116.74, 109.26, 94.56, 51.93, 37.81, 27.72. IR (neat); ν = 3036, 3010, 2976, 895, 811 cm^{-1} . ESI-MS (LRMS, ES^+ , 40 V) m/z [M] $^+$ for $\text{C}_{23}\text{H}_{23}\text{N}_2$ predicted 327.19; found 327.10. ESI-MS (HRMS, TOF MS AP^+) m/z [M] $^+$ for $\text{C}_{23}\text{H}_{23}\text{N}_2$ predicted 327.1861; found 327.1875. HPLC purity 99.3%, retention time = 8.15 min, solvent system: A = ACN, B = H_2O , C = H_2O (1% TFA). Gradient profile 5% A 85% B (with constant 10% C) to 90% A (with constant 10% C) over 30 mins. Elemental analysis ($\text{C}_{25}\text{H}_{23}\text{F}_3\text{N}_2\text{O}_2 \cdot 1.8\text{TFA}$) predicted %C: 53.20, H: 3.87, N: 4.34, I: 0.0. Found %C: 53.18 and 53.4, H: 3.87 and 3.98, N: 4.34 and 4.44, I: <0.3 and <0.3.

4.3.12 (E)-1-Ethyl-2-((1-ethylquinolin-2(1H)-ylidene)methyl)-6-methoxy-4a,8a-dihydro-quinolinium iodide (3)—6-methoxy-1-ethyl-2-methylquinolinium iodide (**19**) (1 equiv, 0.50 g, 1.51 mmol) and 1-ethyl-2-iodoquinolinium iodide (**24**) (1 equiv, 0.49 g, 1.51 mmol) were dispersed in dry EtOH (40 mL) at rt. To this suspension was added a solution of KOH (1.5 equiv, 0.18 g, 2.27 mmol) in dry EtOH (30 mL). The solution turned immediately purple and was heated to 35 °C for 48 h. After this time the solution was filtered to give a purple solid which was then re-crystallized from EtOH to give the *title compound* (**3**) as dark purple crystals (0.24 g, 32%). Mp: 266–267 °C. ¹H NMR (400 MHz, d₆-DMSO): δ 8.18 (1H, d, *J* = 9.4 Hz), 8.00 (2H, t, *J* = 10.7 Hz), 7.86–7.91 (3H, m), 7.77 (1H, t, *J* = 7.2 Hz), 7.71 (1H, d, *J* = 9.4 Hz), 7.52 (2H, dd, *J* = 2.8, 17.8 Hz), 7.46 (1H, t, *J* = 7.2 Hz), 5.58 (1H, s), 4.63–4.59 (2H, m), 4.48–4.52 (2H, m), 3.90 (3H, s), 1.53–1.47 (6H, m). ¹³C NMR (100 MHz, d₆-DMSO): δ 156.53, 152.21, 151.83, 138.82, 137.98, 136.84, 132.53, 129.13, 124.47, 122.56, 122.52, 121.35, 118.42, 115.95, 109.84, 88.37, 55.85, 44.88, 43.77, 12.14, 11.60. IR (neat); ν = 2986 (s), 2692 (s), 854 (s) cm⁻¹. ESI-MS (LRMS, ES⁺, 20 V) *m/z* [M]⁺ for C₂₄H₂₅N₂O predicted 357.20; found 357.09. ESI-MS (HRMS, TOF MS AP⁺) *m/z* [M]⁺ for C₂₄H₂₅N₂O predicted 357.1961; found 357.1960. HPLC purity = 99.9%, retention time = 12.91 mins, solvent system: A = ACN, B = H₂O, C = H₂O (1% TFA). Gradient profile 20% A 70% B (with constant 10% C) to 90% A (with constant 10% C) over 30 mins. Elemental analysis (C₂₄H₂₇N₂O·0.1(H₂O)·0.1EtOH) predicted %C: 58.98, H: 5.69, N: 5.68, I: 25.75. Found %C: 59.22, H: 5.30, N: 5.71, I: 25.86.

4.3.13 (E)-6-Bromo-1-ethyl-2-((1-ethylquinolin-2(1H)-ylidene)methyl)quinolinium 2,2,2-trifluoroacetate (4)—6-bromo-1-ethyl-2-methylquinolinium iodide (**20**) (1 equiv, 0.20 g, 0.53 mmol) and 1-ethyl-2-iodoquinolinium iodide (**24**) (1 equiv, 0.22 g, 0.53 mmol) were dispersed in dry EtOH (10 mL) at rt. To this suspension was added a solution of KOH (1.5 equiv, 45 mg, 0.80 mmol) in dry EtOH (10 mL). The solution turned immediately dark red/purple and was stirred for 18 h at rt. After this time, filtration of the solution gave a purple/red solid which was dried under vacuum and dissolved up in TFA (5 mL). The solution was heated to 80 °C for 18 h and then cooled to rt. The excess TFA was removed by distillation under reduced pressure to give a purple/red solid which was purified by prep. HPLC (column: Atlantis T3, 10 μm 30 × 150 mm); isocratic (45% MeOH, 45% H₂O, 10% H₂O (1% TFA)); flow rate: 25 mL/min; inj. volume: 300 μL). The appropriate fractions were evaporated under reduced pressure to remove ACN and the remaining water lyophilized to give the *title compound* (**4**) (79 mg, 22%). Mp: 265–266 °C. ¹H NMR (400 MHz, d₆-DMSO): δ 8.24 (1H, d, *J* = 9.2 Hz), 8.18 (1H, d, *J* = 2.0 Hz), 8.07 (1H, d, *J* = 8.6 Hz), 7.99–8.01 (2H, m), 7.84–7.92 (4H, m), 7.82 (1H, d, *J* = 9.4 Hz), 7.55–7.63 (1H, m), 5.66 (1H, s), 4.48–4.56 (2H, m), 4.58–4.69 (2H, m), 1.53 (3H, t, *J* = 7.2 Hz), 1.48 (3H, t, *J* = 7.2 Hz). ¹³C NMR (100 MHz, d₆-DMSO): δ 153.65, 152.35, 138.70, 138.63, 137.92, 136.01, 134.83, 133.10, 130.93, 129.44, 126.21, 125.55, 125.13, 122.86, 122.02, 118.48, 116.98, 116.74, 89.64, 44.75, 44.17, 12.00, 11.63. IR (neat); ν = 3423 (s), 3034 (s), 1499 (s), 1149 (s), 649 (s) cm⁻¹. ESI-MS (LRMS, ES⁺, 15 V) *m/z* [M]⁺ for C₂₃H₂₂NBr₂ predicted 405.10; found 405.02. ESI-MS (HRMS, TOF MS AP⁺) *m/z* [M]⁺ for C₂₃H₂₂NBr₂ predicted 405.0961; found 405.0967. HPLC purity = 97.4%, retention time = 20.05 mins, solvent system: A = ACN, B = H₂O, C = H₂O (1% TFA). Gradient profile 20% A 70% B (with constant 10% C) to 90% A (with constant 10% C)

over 30 mins. Elemental analysis ($C_{25}H_{24}BrF_3N_2O_2 \cdot 1.25TFA$) predicted %C: 49.75, H: 3.83, N: 4.22. Found %C: 49.64 and 49.68, H: 3.59 and 3.57, N: 4.29 and 4.28, I: <0.1 and <0.1.

4.3.14 (E)-1-Ethyl-2-((1-ethylquinolin-2(1H)-ylidene)methyl)-6-fluoro-4a,8a-dihydro quinolinium iodide (5)—6-fluoro-1-ethyl-2-methylquinolinium iodide (**21**) (1 equiv, 0.50 g, 1.57 mmol) and 1-ethyl-2-iodoquinolinium iodide (**24**) (1 equiv, 0.65 g, 1.57 mmol) were dispersed in dry EtOH (40 mL) at rt. To this suspension was added a solution of KOH (1.5 equiv, 0.13 g, 2.35 mmol) in dry EtOH (30 mL). The solution turned immediately red and was heated to 35 °C and stirred for 48 h after which the solution was filtered to give a purple solid which was re-crystallized from EtOH to give the *title compound* (**5**) as purple crystals (0.18 g, 24%). Mp: 275–276 °C. 1H NMR (400 MHz, d_6 -DMSO): δ 8.18 (1H, d, J = 9.2 Hz), 8.01 (2H, dd, J = 18.1, 9.4 Hz), 7.84–7.93 (3H, m), 7.70–7.81 (2H, m), 7.54 (1H, d, J = 2.9 Hz), 7.43–7.52 (2H, m), 5.58 (1H, s), 4.58–4.65 (2H, m), 4.47–4.55 (2H, m), 1.52 (3H, t, J = 7.2 Hz), 1.49 (3H, t, J = 7.2 Hz). ^{13}C NMR (151 MHz, d_6 -DMSO): δ 159.34, 157.72, 153.20, 152.61, 138.44 (d, J = 49 Hz), 136.81, 135.63, 132.94, 129.36, 126.03 (d, J = 10.6 Hz), 125.24, 124.87, 123.10, 121.78, 120.60 (d, J = 24.2 Hz), 118.98 (d, J = 9.06 Hz), 116.52, 113.77 (d, J = 22.7 Hz), 89.16, 44.53 (d, J = 19.6 Hz), 11.83 (d, J = 13.6 Hz). IR (neat); ν = 2983 (s), 2935 (s), 1523 (s), 845 (s), 766 (s) cm^{-1} . ESI-MS (LRMS, ES^+ , 20 V) m/z [M] $^+$ for $C_{23}H_{22}FN_2$ predicted 345.18; found 345.13. ESI-MS (HRMS, TOF MS AP^+) m/z [M] $^+$ for $C_{23}H_{22}FN_2$ predicted 345.1767; found 345.1760. HPLC purity = 97.8%, retention time = 19.40 mins, solvent system: A = ACN, B = H_2O , C = H_2O (1% TFA). Gradient profile 20%A 70%B (with constant 10%C) to 90%A (with constant 10%C) over 30 mins. Elemental analysis ($C_{23}H_{24}FIN_2 \cdot 0.5H_2O$) predicted %C: 57.15, H: 5.21, N: 5.8, I: 26.25. Found %C: 57.39, H: 4.82, N: 5.82, I: 26.36.

4.3.15 (E)-6-Chloro-1-ethyl-2-((1-ethylquinolin-2(1H)-ylidene)methyl)quinolinium iodide (6)—6-chloro-1-ethyl-2-methylquinolinium iodide (**22**) (1 equiv, 0.50 g, 1.50 mmol) and 1-ethyl-2-iodoquinolinium iodide (**24**) (1 equiv, 0.48 g, 1.50 mmol) were dispersed in dry EtOH (40 mL) at rt. To this suspension was added a solution of KOH (1.5 equiv, 2.25 mmol, 0.13 g) in dry EtOH (30 mL). The solution turned immediately purple and was heated to 35 °C and stirred for 48 h. After this time, the solution was filtered to give a purple solid which was re-crystallized from EtOH to give the *title compound* (**6**) as a purple crystals (0.27 g, 28%). MP: 273–274 °C. 1H NMR (400 MHz, d_6 -DMSO): δ 8.24 (1H, d, J = 8 Hz), 8.09–7.98 (2H, m), 7.98–8.03 (2H, m), 7.96 (1H, d, J = 8 Hz), 7.79–7.91 (4H, m), 7.55–7.63 (1H, m), 5.67 (1H, s), 4.49–4.56 (2H, m), 4.63–4.69 (2H, m), 1.53 (3H, t, J = 7.2 Hz), 1.49 (3H, t, J = 7.2 Hz). ^{13}C NMR (100 MHz, d_6 -DMSO): δ 153.63, 152.40, 138.68, 138.64, 137.59, 136.12, 133.10, 132.14, 129.44, 128.89, 127.89, 125.82, 125.54, 125.11, 122.95, 122.00, 118.35, 116.73, 89.58, 44.73, 44.25, 12.00, 11.65. IR (neat); ν = 3364 (s), 2694 (s), 1449 (s), 760 (s) cm^{-1} . ESI-MS (LRMS, ES^+ , 15 V) m/z [M] $^+$ for $C_{23}H_{22}N_2Cl$ predicted 361.15; found 361.12. ESI-MS (HRMS, TOF MS AP^+) m/z [$M+H$] $^+$ $C_{23}H_{23}N_2Cl$ predicted 489.0594; found 489.0573. HPLC purity = 98.1%, retention time = 19.41 mins, Solvent system: A = ACN, B = H_2O , C = H_2O (1% TFA). Gradient profile 20%A 70%B (with constant 10%C) to 90%A (with constant 10%C) over 30 mins.

Elemental analysis ($C_{23}H_{24}ClIN_2 \cdot 2H_2O$) predicted %C: 52.43, H: 5.36, N: 5.32, I: 24.09. Found %C: 52.64, H: 4.99, N: 5.34, I: 24.18.

4.3.16 (E)-1-Ethyl-2-((1-ethylquinolin-2(1H)-ylidene)methyl)-6-iodo-4a,8a-dihydro-quinolinium iodide (7)—6-iodo-1-ethyl-2-methylquinolinium iodide (**23**) (1 equiv, 0.50 g, 1.17 mmol) and 1-ethyl-2-iodoquinolinium iodide (**24**) (1 equiv, 0.48 g, 1.17 mmol) were dispersed in dry EtOH (40 mL) at rt. To this suspension was added a solution of KOH (1.5 equiv, 0.10 g, 1.76 mmol) in dry EtOH (30 mL). The solution turned immediately purple and was heated to 35 °C for 48 h. After this time, the solution was filtered to give a purple solid which was re-crystallized from EtOH to give the *title compound* (**7**) as purple crystals (0.13 g, 19%). Mp: decomposed 255–256 °C. 1H NMR (400 MHz, d_3 -MeCN): δ 8.18 (1H, d, J = 2.0 Hz), 8.09 (1H, d, J = 9.3 Hz), 8.04 (1H, d, J = 2.1, 9.1 Hz), 7.90–7.94 (2H, m), 7.87 (1H, dt, J = 1.6, 7.0 Hz), 7.79 (2H, d, J = 9.4 Hz), 7.72 (1H, d, J = 9.4 Hz), 7.56–7.60 (2H, m), 5.61 (1H, s), 4.57 (1H, q, J = 7.3 Hz), 4.43 (1H, q, J = 7.3 Hz), 1.61 (1H, t, J = 7.3 Hz), 1.56 (1H, t, J = 7.3 Hz). ^{13}C NMR (100 MHz, d_3 -MeCN): δ 153.56, 152.35, 140.43, 138.65, 138.59, 138.34, 137.00, 135.98, 133.07, 129.42, 126.54, 125.50, 125.09, 122.54, 121.99, 118.34, 116.70, 89.62, 89.51, 44.69, 44.07, 11.98, 11.64. IR (neat); ν = 3363 (s), 3026 (s), 1516 (s), 1131 (s), 689 (s) cm^{-1} . ESI-MS (LRMS, ES^+ , 25 V) m/z [M] $^+$ for $C_{23}H_{22}N_2I$ predicted 453.08; found 452.89. ESI-MS (HRMS, TOF MS AP^+) m/z [M] $^+$ for $C_{23}H_{22}N_2I$ predicted 453.0828; found 453.0809. Purity = 99.5%, retention time = 19.41 mins, solvent system: A = ACN, B = H_2O , C = H_2O (1% TFA). Gradient profile 20% A 70% B (with constant 10% C) to 90% A (with constant 10% C) over 30 mins. Elemental analysis ($C_{23}H_{24}I_2N_2 \cdot H_2O$) predicted %C: 46.02, H: 4.37, N: 4.67, I: 42.28 Found %C: 46.23, H: 4.19, N: 4.61, I: 42.02.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We gratefully acknowledge Priscilla Barba-Escobedo and Corey M. Smolik for technical assistance with behavioral and radio-ligand assays, respectively.

Funding Sources

This study was supported by National Institutes of Health (NIH) awards MH093320 (LCD and WK), T32DA031115 (RF-S), K12GM111726 (RF-S), a sub-award from GM097632 (CMS) and United States Department of Defense award AR110109 (GGG). BZ is grateful to the Australian Institute of Nuclear Scientists and Engineers (AINSE) for a 2016 post-graduate award.

Appendix A. Supplementary data

Complete chemical and biological characterization data (and detailed methods) including 1H NMR spectra, ^{13}C NMR spectra, HPLC chromatograms, mouse locomotor activity data, mouse TST antidepressant-like activity and *in vitro* α -1 adrenoceptor affinity are included in the supplementary data.

References

1. Kessler RC. The costs of depression. *Psychiatr Clin North Am.* 2012; 35:1–14. [PubMed: 22370487]
2. Kirsch I, Deacon BJ, Huedo-Medina TB, Scoboria A, Moore TJ, Johnson BT. Initial severity and antidepressant benefits: a meta-analysis of data submitted to the Food and Drug Administration. *PLoS Med.* 2008; 5:e45. [PubMed: 18303940]
3. Blakely RD, Ramamoorthy S, Schroeter S, Qian Y, Apparsundaram S, Galli A, DeFelice LJ. Regulated phosphorylation and trafficking of antidepressant-sensitive serotonin transporter proteins. *Biol Psychiatry.* 1998; 44:169–178. [PubMed: 9693389]
4. Charney DS, Menkes DB, Heninger GR. Receptor sensitivity and the mechanism of action of antidepressant treatment. *Arch Gen Psychiatry.* 1981; 38:1160–1180. [PubMed: 6271089]
5. Daws LC, Koek W, Mitchell NC. Revisiting Serotonin Reuptake Inhibitors and the Therapeutic Potential of “Uptake-2” in Psychiatric Disorders. *ACS Chem Neurosci.* 2013; 4:16–21. [PubMed: 23336039]
6. Horton RE, Apple DM, Owens WA, Baganz NL, Cano S, Mitchell NC, Vitela M, Gould GG, Koek W, Daws LC. Decynium-22 enhances SSRI-induced antidepressant-like effects in mice: uncovering novel targets to treat depression. *J Neurosci.* 2013; 33:10534–10543. [PubMed: 23785165]
7. Baganz NL, Horton RE, Calderon AS, Owens WA, Munn JL, Watts LT, Koldzic-Zivanovic N, Jeske NA, Koek W, Toney GM, Daws LC. Organic cation transporter 3: keeping the brake on extracellular serotonin in serotonin-transporter-deficient mice. *Proc Natl Acad Sci U S A.* 2008; 105:18976–18981. [PubMed: 19033200]
8. Daws LC. Unfaithful neurotransmitter transporters: Focus on serotonin uptake and implications for antidepressant efficacy. *Pharmacol Ther.* 2009; 121:89–99. [PubMed: 19022290]
9. Caspi A, Hariri AR, Holmes A, Uher R, Moffitt TE. Genetic sensitivity to the environment: the case of the serotonin transporter gene and its implications for studying complex diseases and traits. *Am J Psychiatry.* 2010; 167:509–527. [PubMed: 20231323]
10. Russ H, Friedgen B, Königs B, Schumacher C, Graefe KH, Schomig E. Pharmacokinetic and alpha 1-adrenoceptor antagonistic properties of two cyanine-type inhibitors of extraneuronal monoamine transport. *Naunyn Schmiedeberg's Arch Pharmacol.* 1996; 354:268–274. [PubMed: 8878056]
11. Buettner AV. Radiationless transitions in cyanine dyes. *J Chem Phys.* 1967; 46:1398–1401.
12. Russ H, Engel W, Schömig E. Isocyanines and Pseudoisocyanines as a Novel Class of Potent Noradrenaline Transport Inhibitors: Synthesis, Detection and Biological Activity. *J Med Chem.* 1993; 36:4208–4123. [PubMed: 8277503]
13. Tan L, Lin W, Zhu S, Yuan L, Zheng K. A coumarin-quinolinium-based fluorescent probe for ratiometric sensing of sulfite in living cells. *Org Biomol Chem.* 2014; 12:4637–4643. [PubMed: 24849005]
14. Orlova NA, Zhuravlev FA, Shelkovnikov VV, Gerasimova TN. Synthesis of pseudocyanines containing unsaturated groups at the nitrogen atom. *Izv Akad Nauk Ser Khim.* 1995:1122–1124.
15. Pardal AC, Ramos SS, Santos PF, Reis LV, Almeida P. Synthesis and spectroscopic characterization of N-alkyl quaternary ammonium salts, typical precursors of cyanines. *Molecules.* 2002; 7:320–330.
16. Khoroshilov G, Demchak I, Saraeva T. Stereoselective synthesis of 2-[1-methylpyridin-2(1H)-ylidene]malononitrile derivatives. *Synthesis.* 2008:1541–1544.
17. Wahl H. The polymethine dyes. *Teintex.* 1948; 13:77.
18. Brown HC, Cahn A. Steric Effects in Displacement Reactions. II. The Rates of Reaction of Alkyl Iodides with the Monoalkylpyridines. Steric Strain in the Activated Complex. *Journal of the American Chemical Society.* 1955; 77:1715–1723.
19. Joule, JA., Smith, G., Mills, K. *Heterocyclic Chemistry.* Third. Chapman & Hall; 1994.
20. Meier KE, Snively MD, Brown SL, Brown JH, Insel PA. alpha 1- and beta 2-adrenergic receptor expression in the Madin-Darby canine kidney epithelial cell line. *J Cell Biol.* 1983; 97:405–415. [PubMed: 6309860]

21. Herr DW, Mailman RB. Buffer effects on high affinity [3H]-prazosin binding in brain and spinal cord. *Pharmacol Biochem Behav.* 1989; 32:831–834. [PubMed: 2740434]
22. Hayer-Zillgen M, Bruss M, Bonisch H. Expression and pharmacological profile of the human organic cation transporters hOCT1, hOCT2 and hOCT3. *Br J Pharmacol.* 2002; 136:829–836. [PubMed: 12110607]
23. Cryan JF, Mombereau C, Vassout A. The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. *Neurosci Biobehav Rev.* 2005; 29:571–625. [PubMed: 15890404]
24. Kostowski W. Possible relationship of the locus coeruleus–hippocampal noradrenergic neurons to depression and mode of action of antidepressant drugs. *Pol J Pharmacol Pharm.* 1985; 37:727–743.

Highlights

- Analogues of the organic cation decynium-22 have been evaluated for the first time as antidepressants.
- Analogues **5** and **6** show antidepressant-like activity without co-administration of an SSRI.
- Analogues **2**, **5**, **6** and **7** have significantly reduced α -1 adrenoceptor activity.
- Analogues **2**, **5**, **6** and **7** have significant potential for reduced adverse side effects.
- Analogues **5** and **6** show significant potential for further development as stand-alone antidepressants.

Figure 1.
Decynium-22 and related analogues **1–7**

Figure 2.
Crystal structure ORTEP diagram of coupling reaction intermediate 1-ethyl-2-iodoquinolinium iodide (**24**)

Figure 3. Crystal structure ORTEP diagram of (*E*)-6-chloro-*N*-ethyl-2-((*N*'-ethylquinolin-2(1*H*)-ylidene)methyl)quinolinium iodide (**6**)

Figure 4. Concentration effect curve for [3H]prazosin binding displacement by competing ligands, including corticosterone, D-22, tamsulosin, and analogues **2**, **5**, **6** and **7**. Curve fit determined by non-linear regression analysis with GraphPad Prism 6 software.

Figure 5. Effects of D-22 and analogues **1–7** on immobility time in the tail suspension test. * $P < 0.05$, one-way ANOVA with Dunnett's post-hoc comparisons versus saline.

Figure 6.

Dose-effect of D-22 and analogues **1–7** on locomotor activity. * $P < 0.05$ compared with the same dose of D22. *The number adjacent to symbols indicates the number of animals tested in each condition.

Reagents and conditions: (a) **8**, MeI, DCM, rt, 16 h, 81% (**10**); (b) **8**, 2-iodopropane, sealed tube 90 °C, 48 h, 75% (**11**); (c) **9**, MeI, DCM, reflux, 48 h, 92% (**12**); (d) (i) **10**, **12**, KOH, EtOH, rt, 18 h; (ii) TFA, 80 °C, 18 h, prep. HPLC, 54% (**1**); (e) (i) **11**, **12**, KOH, EtOH, rt, 18 h; (ii) TFA, 18 h, prep. HPLC, 48% (**2**).

Scheme 1.

Synthesis of *N*-alkylated quinoline precursors (**10–12**) and isocyanines (**1–2**)

Reagents and conditions: (a) **13**, EtI, neat, 90 °C, 3 days, 94% (**19**); (b) **14**, EtI, neat, 90 °C, 7 days, 78% (**20**); (c) **15**, EtI, neat, 90 °C, 5 days, 73% (**21**); (d) **16**, EtI, neat, 90 °C, 4 days, 98% (**22**); (e) **17**, EtI, neat, 90 °C, 5 days, 64% (**23**); (f) **18**, EtI, neat, 90 °C, 5 days, 58% (**24**); (g) **19**, **24**, KOH, EtOH, 35 °C, 2 days, 48% (**3**); (h) **20**, **24**, KOH, EtOH, 35 °C, 2 days, 57% (**4**); (i) **21**, **24**, KOH, EtOH, 35 °C, 2 days, 45% (**5**); (j) **22**, **24**, KOH, EtOH, 35 °C, 2 days, 38% (**6**); (k) **23**, **24**, KOH, EtOH, 35 °C, 2 days, 64% (**7**).

Scheme 2.

Synthesis of *N*-alkylated quinoline precursors (**19–24**) and pseudoisocyanines (**3–7**)

Table 1

Summary of IC₅₀ values (nM) for ligand displacement of 1.5 nM [³H]prazosin binding in mouse hippocampal homogenate. Estimations and statistical comparisons determined by non-linear regression analysis with GraphPad Prism.

Ligand	IC ₅₀ (nM) [95% CI], (n)
D22	60 [18.7–190], (5)
2	1066 ^{†*} [526–2160], (3)
5	348 ^{†*} [229–528], (3)
6	577 ^{†*} [286–1166], (3)
7	711 ^{†*} [326–1549], (5)
Corticosterone	Ambiguous fit
Tamsulosin	1.8 [0.7–5.0], (3)

Ligand curve fit comparison to tamsulosin

[†] P values < 0.0001 or D22

* P values as given (analogue **2**, $p = 0.0007$; analogue **5**, $p = 0.015$; analogue **6**, $p = 0.025$; analogue **7**, $p = 0.0005$). CI, confidence intervals.