



New dual 5-HT_{1A} and 5-HT₇ receptor ligands derived from SYA16263

Edward Ofori ^{a,1}, Edem K. Onyameh ^a, Uma M. Gonela ^a, Chandrashekhar Voshavar ^a, Barbara Bricker ^a, Tracy L. Swanson ^b, Amy J. Eshleman ^b, Jennifer L. Schmachtenberg ^b, Shelley H. Bloom ^b, Aaron J. Janowsky ^b, Seth Y. Ablordeppey ^{a,*}

^a Division of Basic Pharmaceutical Sciences, College of Pharmacy and Pharmaceutical Sciences, Institute of Public Health, Florida A&M University, Tallahassee, FL, 32307, USA

^b Research Service, VA Portland Health Care System, And Department of Psychiatry, Oregon Health and Science University, Portland, OR, 97239, USA

ARTICLE INFO

Article history:

Received 15 October 2020

Received in revised form

23 January 2021

Accepted 25 January 2021

Available online 3 February 2021

Keywords:

Dopamine and serotonin receptors

Pyridinyl piperazine

Indanones

5-HT subtype Receptors

Dual 5-HT_{1A} and 5-HT₇ receptors

SYA16263

ABSTRACT

We have previously reported that dual 5-HT_{1A} and 5-HT₇ receptor ligands might find utility as treatment options for various CNS related conditions including cognitive and anxiolytic impairments. We have also more recently reported that SYA16263 has antipsychotic-like properties with an absence of catalepsy in animal models ascribed to its ability to recruit β-arrestin to the D₂ receptor. However, SYA16263 also binds with very high affinity to 5-HT_{1A}R (*K_i* = 1.1 nM) and a moderate affinity at 5-HT₇R (*K_i* = 90 nM). Thus, it was of interest to exploit its pharmacophore elements in designing new dual receptor ligands. Using SYA16263 as the lead molecule, we have conducted a limited structure-affinity relationship (SAFIR) study by modifying various structural elements in the arylalkyl moiety, resulting in the identification of a new dual 5-HT_{1A}R and 5-HT₇R ligand, 6-chloro-2-methyl-2-(3-(4-(pyridin-2-yl)piperazin-1-yl)propyl)-2,3-dihydro-1H-inden-1-one (21), which unlike SYA16263, has a sub-nanomolar (5-HT_{1A}R, *K_i* = 0.74 nM) and a low nanomolar (5-HT₇R, *K_i* = 8.4 nM) affinity for these receptors. Interestingly, 21 is a full agonist at 5-HT_{1A}R and antagonist at the 5-HT₇R, functional characteristics which point to its potential as an antidepressant agent.

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1. Introduction

The pharmacotherapy of major central nervous system (CNS) disorders relies on multi-CNS receptor targeting. In fact, atypical antipsychotic agents such as cariprazine, aripiprazole, clozapine, ziprasidone, and lurasidone owe their superior efficacy and tolerable side-effect profiles to their ability to modulate dopamine (DA), serotonin (5-HT), and even cholinergic (ACh) receptors simultaneously [1–3].

Serotonin receptors (5-HTRs) are found predominantly on neurons in the dorsal and median raphe nuclei of the brainstem that project into other brain regions such as the hypothalamus, thalamus, hippocampus, and cortex where they regulate mood, perception, reward, anger, aggression, appetite, memory, sexuality, and attention [4–6]. Not surprisingly, perturbation in serotonergic

neurotransmission is implicated in several neuropsychiatric illnesses including major depressive disorder (MDD), bipolar disorder, anxiety, attention deficit and hyperactivity disorder (ADHD) and schizophrenia [7,8]. Several lines of evidence have revealed that the 5-HT_{1A} receptor subtype (5-HT_{1A}R) is involved in the pathophysiology of the aforementioned mental disorders making it a tenable target for antidepressant, antianxiety and antipsychotic drug discovery. For instance, there is a growing body of evidence that supports the anti-negative symptom and pro-cognitive effects of ligands that activate 5-HT_{1A}R in schizophrenia [9–11]. In addition, 5-HT_{1A} knockout mice display increased anxiety and depressive behaviors, and have been used as animal models for these disorders, further highlighting the key roles that the 5-HT_{1A}R plays in the pathophysiology of CNS disorders [12,13]. In fact, antidepressants such as vilazodone that inhibit 5-HT reuptake and partially activate 5-HT_{1A}Rs are more efficacious, fast acting, and tolerable [14,15].

Another closely related 5-HTR, the serotonin-7 receptor (5-HT₇R), mediates key physiological functions that include sleep, mood, learning memory and cognition [16–19]. Indeed, the antidepressant effects of amisulpride are attributed to its antagonism at

* Corresponding author.

E-mail address: seth.ablordeppey@fam.u.edu (S.Y. Ablordeppey).

¹ Present Address: College of Pharmacy, Chicago State University, 9501 S. King Dr., Douglas Hall, Chicago, IL 60628, USA.

the 5-HT₇ receptor [20]. A growing wealth of evidence suggests that 5-HT₇ is co-localized with 5-HT_{1A}, and cross talks resulting from heterodimers of these receptors have been implicated in CNS diseases such as depression [21]. Not only do these receptors share over 40% sequence homology, ligands that activate these receptors tend to exhibit cross activity [22,23]. We posit that dual ligands that activate 5-HT_{1A} and antagonize 5-HT₇ receptors may serve as potential treatment options for depression, anxiety and other diseases characterized by cognitive deficits.

N-Aryl/heteroaryl piperazines are of interest due in part to their unique ability to impart desirable pharmacological properties to clinically useful CNS drugs including aripiprazole and cariprazine (Fig. 1) [24]. In fact, evidence from pharmacophore models suggests that the pyridinyl-piperazine moiety may provide key structural elements necessary for binding to both 5-HT_{1A} and 5-HT₇ [25]. In our previous publications [26,27] we identified the deoxygenated azaperone SYA 16263 (2) (Fig. 1), as a ligand that displayed a desirable *in-vitro* binding affinity profile to clinically relevant CNS receptors. In animal studies, SYA 16263 possesses moderate antipsychotic-like activity with low propensity to cause motoric side effects [28]. We reported in a recent patent [29] that, as a possible mechanism of action, 2 displayed a biased β -arrestin functionality at the D₂ receptor. However, compound 2 also displayed a very high affinity at the 5-HT_{1A}R, sparking our interest in exploring its structure-affinity relationship (SAFIR) at relevant CNS receptors and further expand and probe the potential utility of its analogs in CNS diseases. To that end, we have designed, synthesized and evaluated several *N*-alkyl substituted pyridinyl-piperazines to identify new leads for further development.

2. Chemistry

Azaperone (1) is a commercially available compound and we previously reported SYA16263 (2) [27]. In general, the final compounds evaluated in this manuscript were obtained by refluxing or carrying out a microwave-assisted synthesis (MWAS) reaction of the amine portion, 1-(pyridin-2-yl)piperazine, with various alkylating agents in DME, CH₃CN or toluene in the presence of K₂CO₃ or NaHCO₃ as a base and a catalytic amount of KI. Final target compounds, where applicable, were converted to the HCl or oxalate salts. Comprehensive descriptions of the synthetic methods and accompanying data are provided in the experimental section. Briefly, in order to produce the alkylating agent 1c and 1d, aniline (1a) was *N*-acetylated under basic conditions and the resulting acetanilide (1b) acylated with 4-chlorobutyryl chloride under Friedel-Crafts conditions to produce intermediate 1c (Scheme 1). The keto group in 1c was removed under a modified phenyl-keto reduction reaction described by West and colleagues [30] to afford the intermediate 1d (Scheme 1). Alkylating agents 1c and 1d were then separately reacted with 1-(pyridin-2-yl)piperazine to produce acetamides 3 and 4 respectively. Deacetylation of 3 and 4 under acidic conditions following a modified method described by

Kilbourn et al. [31] afforded 5 and 6 respectively (Scheme 1).

Compounds 7 and 8 were prepared by first acylating dihydrocarbostyryl 2a under Friedel-Crafts acylation conditions as described in Scheme 1 to produce ketone 2b which was subsequently reduced, similar to 1c, to produce the alkyl halide 2c (Scheme 2). The alkyl halides 2b and 2c were separately reacted with 1-(pyridin-2-yl)piperazine leading to compounds 7 and 8 respectively (Scheme 2).

Commercially available 6-hydroxy-dihydrocarbostyryl 3a was *O*-alkylated with 1-bromo-3-chloropropane to give intermediate 3b which was then used to react separately with 1-(pyridin-2-yl)piperazine and 1-(pyridin-2-yl)-1,4-diazepane under the general alkylation procedure to produce compounds 9 and 10 respectively (Scheme 3).

To access the alkylating agent 4b, 5-hydroxy-2,3-dihydro-1H-inden-1-one 4a was *O*-alkylated under similar condition as 3a (Scheme 3). The resulting alkylating agent was subsequently reacted with 1-(pyridin-2-yl)piperazine to afford compound 11 (Scheme 4).

To prepare compound 12, the indane 5a was similarly acylated as 1c and 2b (Schemes 1 and 2) to produce the ketone 5b which was then used to react with 1-(pyridin-2-yl)piperazine under the general alkylation conditions to produce compound 12 (Scheme 5).

In order to obtain compound 13, the alkylating agent 5-chloro-2-(2-chloroethyl)-2,3-dihydro-1H-inden-1-one 6a previously reported by us [27,32,33], was reacted with 1-pyridin-2-yl-piperazine under general alkylation reaction conditions (Scheme 6).

Ketone 6a was reduced under Luche reduction [34] conditions to yield alcohol 7a that was subsequently used to *N*-alkylate 1-pyridin-2-yl-piperazine to produce compound 14 (Scheme 7).

Alkylating agent 8a, previously reported by us [27,33], was reduced to produce indane 8b under Clemmensen reduction conditions. Under the general alkylation conditions, alkylating agents 8a and 8b were separately reacted with 1-pyridin-2-yl-piperazine to produce compounds 15 and 16 respectively (Scheme 8).

To synthesize compounds 17 and 18, a five-step procedure (Scheme 9) similar to that reported by us [32] was followed. Briefly, commercially available chloroindanones 9a and 9b were separately refluxed with glyoxylic acid in an aqueous sulfuric acid under a cross-aldol condensation reaction to afford the α,β -unsaturated ketones 9c and 9d respectively. Next, the obtained ketones were reduced under the palladium-carbon catalyzed hydrogenation reaction to afford the γ -keto-carboxylic acids 9e and 9f respectively. Ethylene ketal protection of the keto groups in each intermediate and subsequent reduction of the carboxylic function to an alcohol using LiAlH₄ produced the corresponding alcohols 9g and 9h. The alcohol functions were separately converted to the tosylate to form alkylating agents 9i and 9j respectively, and were used to react with 1-pyridin-2-yl-piperazine followed by deprotection to make the final compounds 17 and 18 (Scheme 9) respectively.

Synthesis of 5-chloro-1-(4-chlorophenyl)pentan-1-one 10a followed a similar procedure as reported by Komissarov et al. [35] and

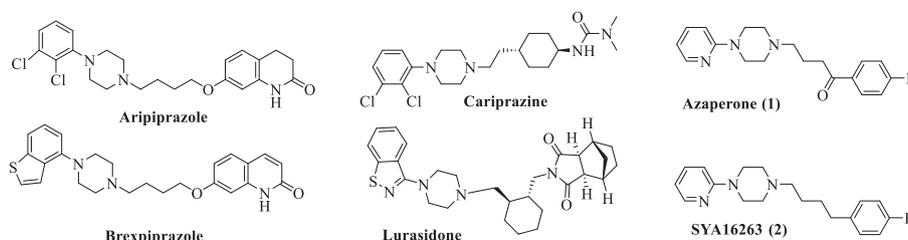
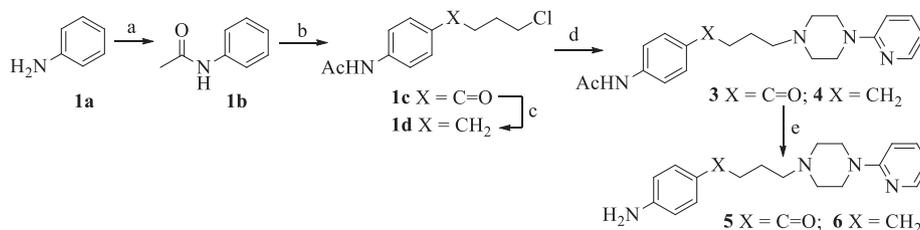
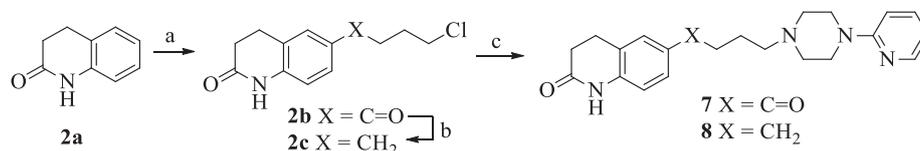


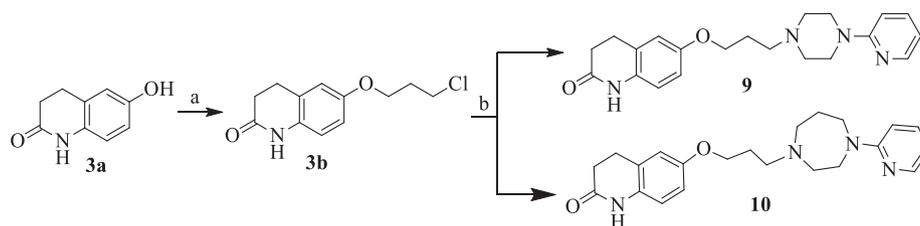
Fig. 1. Structures of some FDA approved CNS drugs containing *N*-Aryl/heteroaryl piperazine moieties along with Azaperone (1) and SYA16263 (2).



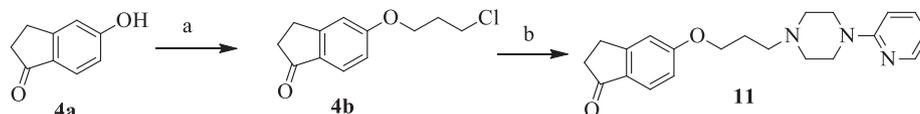
Scheme 1. Synthesis of compounds 3, 4, 5 and 6. Reagents and conditions: (a) acetyl chloride, DCM, Et₃N; (b) 4-chlorobutyl chloride, AlCl₃, CS₂, reflux; (c) triethylsilane (TES), TFA, 0 °C-rt, 15 min; (d) 1-(pyridin-2-yl)piperazine, K₂CO₃/KI, DME, MWAS; (e) conc. HCl/AcOH, MWAS, 30 min.



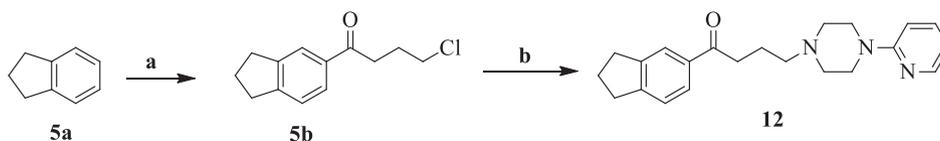
Scheme 2. Synthesis of compounds 7 and 8. Reagents and conditions: (a) 4-chlorobutyl chloride, AlCl₃, CS₂, reflux; (b) triethylsilane, TFA, 0 °C-rt, 15 min s; (c) 1-(pyridin-2-yl)piperazine, K₂CO₃/KI, DME, MWAS.



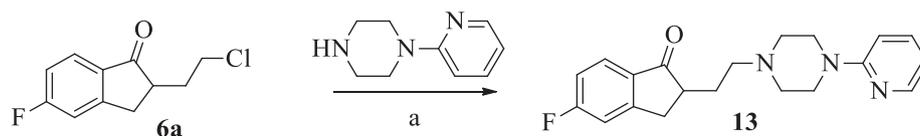
Scheme 3. Synthesis of compounds 9 and 10. Reagents and conditions: (a) 1-bromo-3-chloropropane, K₂CO₃, KI, DME, MWAS; (b) amine [(9, amine = 1-(pyridin-2-yl)piperazine; 10, amine = 1-(pyridin-2-yl)-1,4-diazepane], K₂CO₃, KI, DME, MWAS.



Scheme 4. Synthesis of compound 11. Reagents and conditions: (a) 1-bromo-3-chloropropane, K₂CO₃, KI, toluene, MWAS; (b) 1-(pyridin-2-yl)piperazine, K₂CO₃, KI, toluene, MWAS.



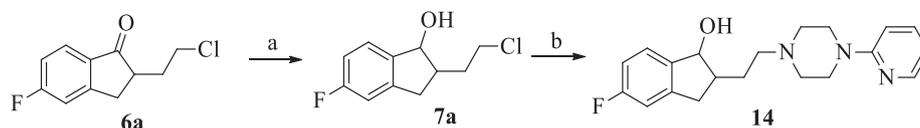
Scheme 5. Synthesis of compound 12. Reagents and conditions: (a) 4-chlorobutyl chloride, AlCl₃, DCM, rt; (b) 1-(pyridin-2-yl)piperazine, K₂CO₃/KI, DME, MWAS.



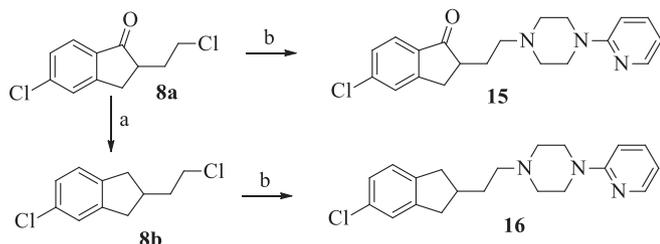
Scheme 6. Synthesis of compound 13. Reagents and conditions: (a) NaHCO₃, KI, toluene, reflux.

the resulting alkyl halide was subjected to the same two-step reaction reported by us [27,33] to prepare the indanone 10b. Following same general alkylation procedure compound 19 was prepared in good yield (Scheme 10).

Claisen condensation of the 5-substituted-indanone 11a with diethylcarbonate (DEC) under basic conditions afforded the key β -keto ester 11b. Subsequent alkylation of the β -keto ester with the 1-bromo-4-chlorobutane produced the C-disubstituted β -keto esters



Scheme 7. Synthesis of compound 14. Reagents and conditions: (a) CeCl_3 , NaBH_4 , MeOH , rt , 1 h; (b) 1-(pyridin-2-yl)piperazine, KI , K_2CO_3 , DME , reflux.



Scheme 8. Synthesis of compounds 15 and 16. Reagents and conditions: (a) Zn amalgam, conc. HCl , toluene, reflux; (b) 1-(pyridin-2-yl)piperazine, K_2CO_3 , KI , DME , reflux.

11c. An acid catalyzed decarboxylation of 11c under microwave heating conditions produced the alkylating agent 11d in good to moderate yield (Scheme 11). This decarboxylation method was a variant of what was described by Ulrich, Jordis, and colleagues [36]. The obtained alkylating agent 11d was reacted with 1-(pyridin-2-yl)piperazine under the general alkylation conditions to produce compound 20 (Scheme 11).

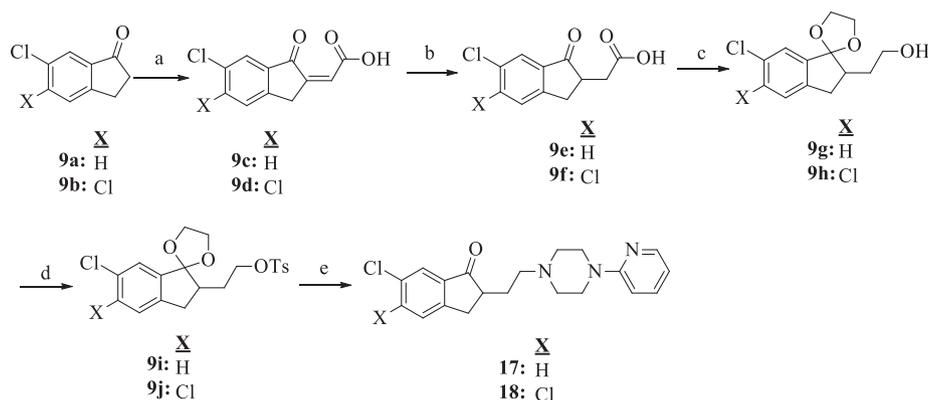
To prepare the α -methylated 5-chloroindanone 12b, the β -keto ester 11a obtained from Scheme 11 was deprotonated and methylated under basic conditions to produce intermediate 12a (Scheme 12) which was decarboxylated to yield 12b. The alkylation step was repeated using 1-bromo-3-chloropropane and 1-bromo-4-chlorobutane to produce alkylating agents 12c and 12d

respectively which were reacted separately with 1-(pyridin-2-yl)piperazine to afford compounds 21 and 22 respectively.

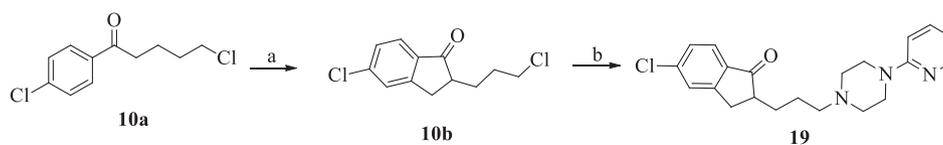
3. Results and discussion

Our previous campaign expanded the antipsychotic potential of butyrophenones, and we reported that 1-(4-(4-fluorophenyl)butyl)-4-(pyridin-2-yl)piperazine (SYA16263 (2)) displays desirable multi-receptor binding affinity for D_2 -like receptors (K_i : $\text{D}_2 = 124 \text{ nM}$, $\text{D}_3 = 86 \text{ nM}$, $\text{D}_4 = 3.5 \text{ nM}$), and 5-HTs (K_i : 5-HT $_2\text{A}$, = 50 nM, 5-HT $_7 = 90 \text{ nM}$), particularly the 5-HT $_{1\text{A}}$ receptor (K_i 5-HT $_{1\text{A}} = 1.1 \text{ nM}$), (Table 1). Previous functional and *in vivo* behavioral studies indicated that compound 2 is a β -arrestin biased ligand at D_2R and has low propensity to cause catalepsy in rats, a key early test to screen antipsychotic agents with the potential to cause motoric side effects [29]. Inspired by these seminal results, we embarked on SAFIR studies to explore the effects of structural changes in compound 2 on binding affinity at key CNS receptors. Due to the metabolic side effects associated with ligands that interact with 5-HT $_{2\text{C}}\text{R}$, all compounds in this study were evaluated for binding affinity at this receptor.

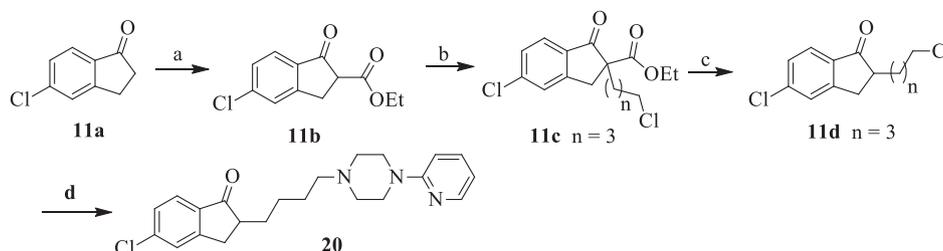
To probe the effects of electronic features of the *para* position of the phenyl ring in compounds 1 and 2, we introduced acetanilide ($-\text{NHC}(\text{O})\text{CH}_3$) and amino ($-\text{NH}_2$) groups to produce compounds 3–6 and the binding affinity constants are reported in Table 1. Generally, these compounds showed no improvement in affinity for the clinically relevant DA and 5-HT receptors, compared to SYA16263. In particular, these changes were not tolerated at the D_4



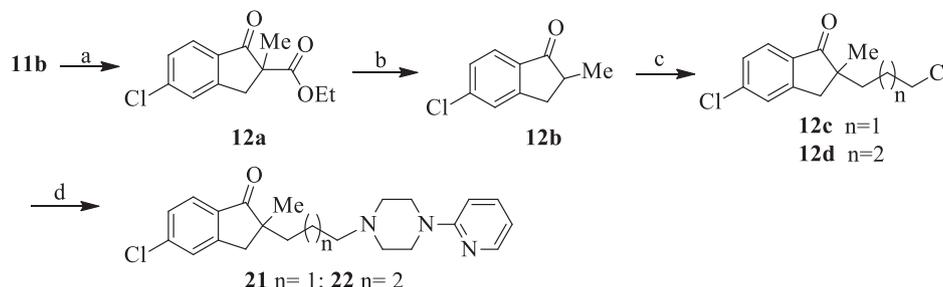
Scheme 9. Synthesis of compounds 17 and 18. Reagents and conditions: (a) glyoxylic acid, H_2SO_4 , H_2O , reflux; (b) Pd/C (10%) H_2 , MeOH/Dioxane 48 h; (c) i) ethylene glycol, $p\text{TSA}$, reflux, ii) LiAlH_4 dry THF , $0 \text{ }^\circ\text{C}$; (d) $p\text{-TsCl}$, Et_3N , CH_2Cl_2 ; (e) i) 1-(pyridin-2-yl)piperazine, K_2CO_3 , KI , DME , reflux, ii) TsOH , MeOH , rt , 12 h.



Scheme 10. Synthesis of compound 19. Reagents and conditions: (a) i) hexamethylenetetramine, Ac_2O , $105 \text{ }^\circ\text{C}$, 18 h, ii) H_2SO_4 , $60 \text{ }^\circ\text{C}$ [33]; (b) 1-(pyridin-2-yl)piperazine NaHCO_3 , KI , toluene, reflux.



Scheme 11. Synthesis of compound 20. Reagents and conditions: (a) NaH, DEC, 0 °C-rt, 12 h; (b) NaH, 1-bromo-4-chlorobutane, DMF, rt, 18–24 h; (c) Conc. HCl in AcOH (glacial), MWAS; (d) i) 1-(pyridin-2-yl)piperazine, K₂CO₃/KI, toluene, MWAS, ii) ethereal HCl.



Scheme 12. Synthesis of compounds 21 and 22. Reagents and conditions: (a) NaH, DMF, MeI, rt, 24 h; (b) Conc. HCl in AcOH (glacial), MWAS; (c) NaH, DMF, 1-bromo-3-chloropropane (for 12c) or 1-bromo-4-chlorobutane (for 12d), rt, 24 h; (d) 1-(pyridin-2-yl)piperazine, K₂CO₃/KI, toluene, MWAS.

receptor. However, these compounds maintained moderate binding affinity at 5-HT_{1A}, 5-HT_{2A} and 5-HT₇ receptors.

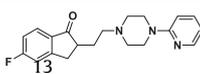
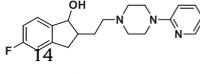
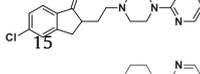
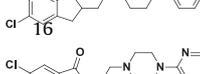
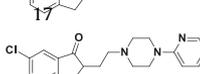
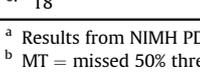
Replacing the *p*-fluoro-phenyl group in azaperone 1 with dihydrocarbostyryl moiety to form compound 7 led to a 25-fold increase in potency at the 5-HT₇R ($K_i = 4.8$ nM), moderate binding affinity at 5-HT_{1A}R ($K_i = 47.3$ nM) and loss of activity at D₂R. Deoxygenating the keto function in compound 7 to obtain 8 restored the high binding potency at 5-HT_{1A}R ($K_i = 4.5$ nM) with moderate to low affinity at all other receptors as reported in Table 1. Replacing the benzylic carbon at position 6 of the dihydrocarbostyryl moiety found in 8 with an oxygen atom to give compound 9 did not produce any significant improvement in binding affinity at all receptors evaluated. Similarly, expanding the piperazine ring in compound 9 to a homopiperazine in 10 resulted in diminished affinity for all receptors under consideration, especially at 5-HT_{1A}R where there was a 20-fold decrease in potency compared to compound 9 (9: K_i (5-HT_{1A}) = 20.3 nM vs 10: K_i (5-HT_{1A}R) = 411 nM) (Table 1). This suggests that the six-membered piperazine ring is essential for maintaining activity at 5-HT_{1A}R. Removing the anilino-nitrogen in the dihydrocarbostyryl moiety in 9 gave 11, which displayed no apparent improvement in affinity across all evaluated receptors. Further modification by replacing *p*-fluoro-phenyl in azaperone with dihydro-indene to obtain compound 12 restored the high binding potency at 5-HT_{1A}R ($K_i = 5.2$ nM) and D₄R ($K_i = 14$ nM) while binding affinity for other receptors was either moderate or diminished.

To further explore the SAR of the lead compounds (1 and 2), we synthesized partially restrained butyl spacer analogs 13–18, and their binding affinity constants are reported in Table 2. Restraining the keto group in azaperone (1) into an indanone to produce 13 resulted in complete loss of binding affinity at D₂R. However, we observed a 20-fold improvement in binding affinity at the 5-HT₇R (1: $K_i = 120.5$ nM vs 13: $K_i = 11.91$ nM) for 13, with no significant change in activity at other 5-HTRs. Reduction of the keto group in 13 to produce alcohol 14 led to restoration of moderate binding affinity for the D₂R ($K_i = 510$ nM) while maintaining a similar trend,

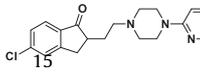
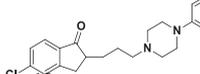
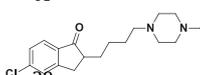
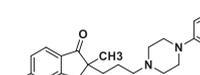
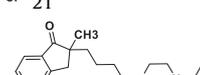
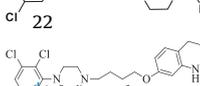
but with reduced affinity, at other receptors in comparison to SYA16263 (2). Compounds 15 and 16, chloro-indanone analogs of 1 and 2 respectively, were synthesized to explore the effects of isosteric replacement of the fluoro atom with a chloro atom. Interestingly, 15 displayed significant selectivity for the D₄R ($K_i = 65$ nM) as affinities at D₂R and D₃R were lost while maintaining moderate binding activity at other 5-HTRs (Table 2). Compound 16 displayed high binding affinity for 5-HT_{1A}R ($K_i = 6.1$ nM) and moderate affinity for 5-HT₇R ($K_i = 45$ nM). Moving the chloro atom (*para* to the keto group) in compound 15 to position 6 (*meta* to the keto group) of the indanone moiety produced 17, which resulted in little or no improvement in binding affinity at the 5-HTRs (except at 5-HT_{1A} where it displayed high affinity ($K_i = 5.1$ nM)) with no apparent gain in binding affinity for the D₂R. Further, we introduced an additional chloro group into 15 to obtain compound 18 which can be viewed as a merge of 15 and 17. Again, this resulted in no significant gain in affinity at either DA or 5-HT receptors (Table 2).

Inspired by the interesting binding affinity profile of the chloro-indanones in Table 2, and using 15 as the new lead compound, we further explored the effect of chain length between the indanone moiety and the pyridinyl-piperazine group on binding affinity. To this end, we synthesized compounds 19–22 and their binding affinities are reported in Table 3. Increasing the chain length from two (ethylenes) in 15 to three (propylenes) in 19 produced the much desired dual 5-HT_{1A} and 5-HT₇ ligands with low nanomolar binding affinity constants (19: K_i (5-HT_{1A}R) = 2.3 nM, K_i (5-HT₇R) = 7.8 nM). Further elongation of the chain length to four in compound 20 resulted in a similar binding affinity profile as observed with 19, particularly at the 5-HT_{1A}R and 5-HT₇R targets. Next, we explored the effects of introducing a methyl (CH₃) group at the alpha (α)-position of the indanone segment to replace the labile α -hydrogen in 19 and 20 to obtain compounds 21 and 22 respectively. In addition, this modification served as a perfect handle to mitigate keto-enol tautomerism that occurs in indanones, as reported by us [37]. Interestingly, 5-chloro-2-methyl-2-(3-(4-(pyridin-2-yl))

Table 2
Binding affinity Constants^a [mean *K_i*, nM & (p*K_i* ± SEM)^{b,c}] at relevant CNS receptors and SERT transporter.

Compound	D ₂	D ₃	D ₄	5-HT _{1A}	5-HT _{2A}	5-HT _{2C}	5-HT ₇	SERT
 13	MT ^b	1469.8	41.39	11.39	75.09	MT	11.91	MT
 14	510 ± 37	347 ± 24	7.9 ± 0.5	8 ± 0.5	580 ± 37	MT	62 ± 3	392 ± 33
 15	MT	MT	65 (7.19 ± 0.08)	32 (7.5 ± 0.09)	113 (6.95 ± 0.04)	1980 (5.7 ± 0.05)	14 (7.84 ± 0.06)	MT
 16	1234 (5.91 ± 0.07)	247 (6.61 ± 0.09)	44 (7.36 ± 0.06)	6.1 (8.21 ± 0.07)	110 (6.96 ± 0.04)	2729 (5.56 ± 0.08)	45 (7.34 ± 0.05)	MT
 17	4513 (5.35 ± 0.06)	407 (6.39 ± 0.04)	10 (7.99 ± 0.05)	5.1 (8.3 ± 0.04)	122 (6.91 ± 0.09)	1680 (5.77 ± 0.06)	63 (7.2 ± 0.06)	MT
 18	3071 (5.51 ± 0.06)	707 (6.15 ± 0.04)	45.5 (7.35 ± 0.06)	46 (7.33 ± 0.04)	159 (6.8 ± 0.09)	1644 (5.78 ± 0.06)	23 (7.64 ± 0.06)	MT

^a Results from NIMH PDSP.^b MT = missed 50% threshold inhibition at 10 μM concentration.^c Data points without standard error (SE) have errors below 20% of the mean value.**Table 3**
Binding affinities^a [mean *K_i*, nM & (p*K_i* ± SEM)^{b,c}] at relevant CNS receptors and transporters.

Compound	D ₂	D ₃	D ₄	5-HT _{1A}	5-HT _{2A}	5-HT _{2C}	5-HT ₇	SERT
 15	MT ^b	MT	65 (7.19 ± 0.08)	32 (7.5 ± 0.09)	113 (6.95 ± 0.04)	1980 (5.7 ± 0.05)	14 (7.84 ± 0.06)	MT
 19	MT	80 (7.1 ± 0.09)	31 (7.51 ± 0.04)	2.3 (8.63 ± 0.08)	533 (6.27 ± 0.05)	1875 (5.73 ± 0.04)	78 (8.11 ± 0.06)	MT
 20	1056 (5.98 ± 0.08)	1167.5 (6.01 ± 0.06)	64 (7.19 ± 0.05)	4.4 (8.39 ± 0.08)	440.3 (6.4 ± 0.07)	367 (6.44 ± 0.07)	15 (7.83 ± 0.05)	2184 (5.72 ± 0.1)
 21	1096 (5.96 ± 0.05)	>10,000 (<5)	48 (7.32 ± 0.09)	0.74 (9.13 ± 0.08)	200 (6.7 ± 0.07)	1448 (5.84 ± 0.07)	8.4 (8.07 ± 0.08)	>10,000 (<5)
 22	3260 (5.49 ± 0.09)	>10,000 (<5)	143 (6.8 ± 0.1)	2.45 (8.62 ± 0.09)	578 (6.24 ± 0.07)	1100 (5.96 ± 0.07)	15 (7.82 ± 0.08)	>10,000 (<5)
 Aripiprazole	3.3 ± 0.1	9.7 ± 5.4	510 ± 93	5.6 ± 0.8	8.7 ± 2.0	180 ± 37	10.3 ± 3.7	1080 ± 180

^a Results from NIMH PDSP.^b MT = missed 50% threshold inhibition at 10 μM concentration.^c Data points without standard error (SE) have errors below 20% of the mean value.^d Aripiprazole data was obtained from reference 43.**Table 4**
^aSerotonin receptors functional assays, agonist effects.

Compound	5-HT _{1A} R GTPγS binding					5-HT _{7A} R adenylate cyclase				
	EC ₅₀ (nM)	SEM	n	% max	SEM	EC ₅₀ (nM)	SEM	n	%5-HT	SEM
21	2.74	0.91	4	100.5	3.6	>10 μM		1	0	
Serotonin	2.03	0.56	6	101.5	1.5	3.66	0.52	2	96.9	1.9

^a Experimental results yielding EC₅₀s greater than 10 μM were not repeated and so no SEM was generated.

and 100.0% adenylate cyclase inhibition (Table 5 & Fig. 3). Thus, compound 21 acts as a full agonist at the 5-HT_{1A}R but acts as an antagonist at the 5-HT_{7A}R. This functional characteristic is consistent with characteristics for treating cognitive impairments and for developing antidepressant drugs.

In conclusion, using SYA16263 (2), which displayed high selectivity (>80 fold) for 5-HT_{1A}R compared to 5-HT_{7R} as the lead molecule, we have now identified very high affinity dual acting ligands for 5-HT_{1A} and 5-HT₇ receptors. Notably, the chloroindanone pyridinyl-piperazine compound, 21 showed sub-

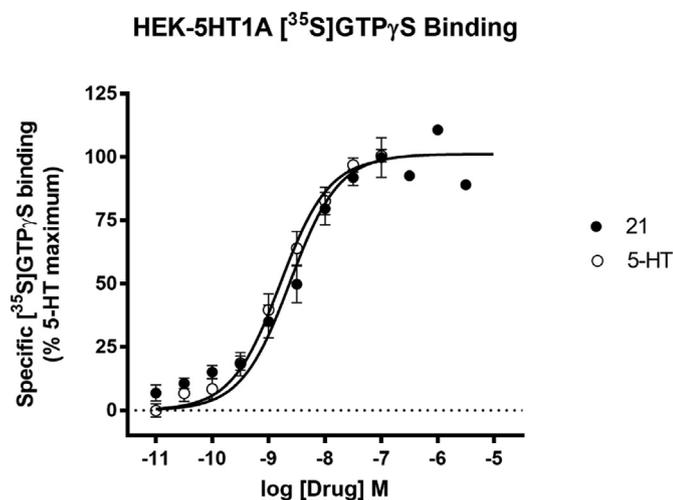


Fig. 2. *In vitro* serotonin receptor, HEK-5-HT_{1A} [³⁵S]GTP_γS binding functional assay results showing the agonistic activity for Compound 21 in comparison with serotonin.

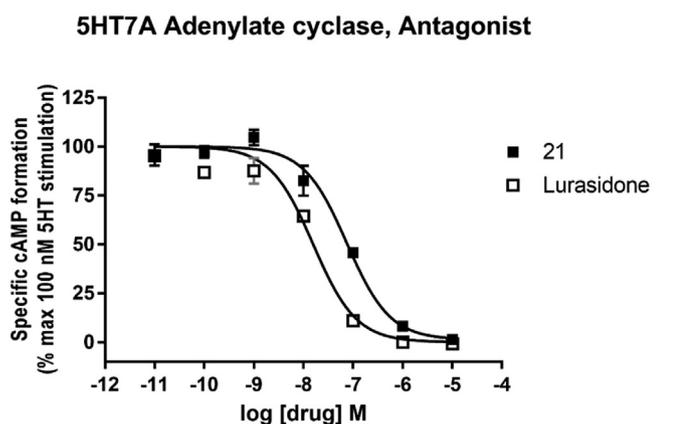
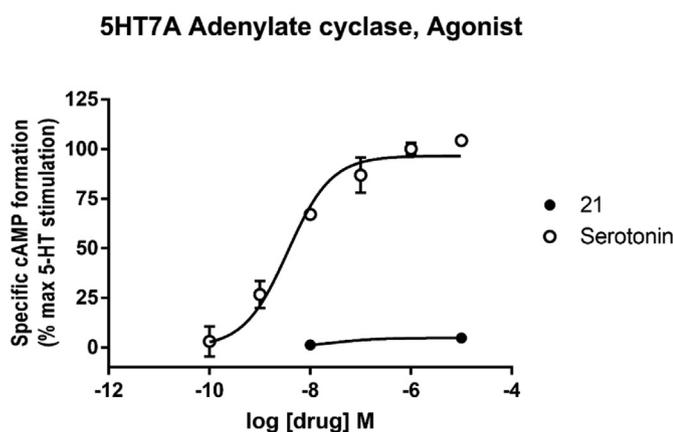


Fig. 3. *In vitro* serotonin receptor, 5-HT_{7A} adenylate cyclase functional assay results for compound 21. Graphs showing the specific cAMP formation for agonist activity of compound 21 in comparison to Serotonin (top graph) and antagonist activity of Compound 21 in comparison to Lurasidone (bottom graph).

nanomolar and low nanomolar binding affinities at the 5-HT_{1A}R and 5-HT_{7R} and with full agonist and antagonist effects at the respective receptors. Compound 21 is thus a valuable addition to the rather scarce group of reported dual 5-HT_{1A}R and 5-HT_{7R} selective ligands. It is anticipated that 21 may serve as a chemical probe for further exploitation of the SAR of multi-target ligands for CNS drug discovery.

4. Experimental section

4.1. Chemistry

Melting points were determined on a Gallenkamp (UK) apparatus and are uncorrected. All NMR spectra were obtained on a Varian 300 MHz Mercury Spectrometer and the free induction decay (FID) data were processed using Mestrelab's Mnova NMR software (version 8.1) to obtain the reported NMR data. Elemental analyses were carried out by Atlantic Microlab, Inc., Norcross, GA, and are within 0.4% of theory unless otherwise noted. Flash chromatography was performed using a Teledyne CombiFlash® with Davisil grade 634 silica gel. Starting materials were obtained from Sigma–Aldrich and were used without further purification. All microwave assisted syntheses (MWAS) were carried out using a Biotage Initiator®.

4.1.1. Acetanilide (1b)

Acetanilide 1b was prepared by acetylating aniline following a simple method described by Zhang and co-workers [39]. Briefly, to a mixture of aniline (3.7 mL, 40 mmol) and triethylamine (7.3 mL) in dry CH₂Cl₂ (50 mL) was added acetyl chloride (3.2 mL, 44 mmol) dropwise while stirring. After addition, the mixture was allowed to stir for 4 h at room temperature. The reaction was quenched by adding water (20 mL) and the organic layer washed further with water (2 × 20 mL), brine (50 mL), dried using Na₂SO₄ and concentrated *in-vacuo* to obtain acetanilide (5 g, 93%) as solid needle-like crystals. ¹H NMR (300 MHz, CDCl₃) δ 8.77 (1H, s), 7.59–7.48 (2H, m), 7.33–7.20 (2H, m), 7.13–7.01 (1H, m), 2.12 (3H, s). ¹³C NMR (75 MHz, CDCl₃) δ 169.50, 138.22, 128.83, 124.25, 120.38, 24.29.

4.1.2. *N*-(4-(4-chlorobutanoyl)phenyl)acetamide (1c)

A modified acylation reaction described by Lackey et al. [40] was followed to access intermediate 1c. Briefly, to a dry 100 mL round-bottomed flask equipped with a stirrer was added AlCl₃ (5 g, 37.5 mmol) carbon disulfide (CS₂) (30 mL) and 4-chlorobutyl chloride (2.5 mL, 22.5 mmol) at 0 °C with stirring. To the mixture obtained was added 1b (15 mmol) portionwise over 20 min. After addition is complete, the reaction mixture was allowed to warm to room temperature and stirred overnight. The content was dumped into a beaker containing 100 g of ice with 5 mL conc. HCl and stirred thoroughly. The brick red precipitate obtained was dissolved in methanol and loaded onto silica column and subsequently separated by flash chromatography (gradient elution up to 50% EtOAc in hexanes) to afford *N*-(4-(4-chlorobutanoyl)phenyl)acetamide (1c). Yellowish solid. Yield: 45%. ¹H NMR (300 MHz, DMSO-*d*₆) δ 10.26 (s, 1H), 7.92 (2H, dd, *J* = 6.5, 2.0 Hz), 7.71 (2H, dd, *J* = 6.5, 2.4 Hz), 3.73–3.60 (2H, m), 3.10 (2H, t, *J* = 6.8 Hz), 2.12–1.96 (5H, m). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 197.85, 169.36, 144.13, 131.49, 129.58, 118.62, 45.34, 35.22, 27.39, 24.61.

4.1.3. *N*-(4-(4-chlorobutyl)phenyl)acetamide (1d)

A modified phenyl-keto reduction reaction described by West and colleagues [30] was followed to access alkylating agent 1d. In brief, to a stirred trifluoroacetic acid (1.5 mL) solution of *N*-(4-(4-chlorobutanoyl)phenyl)acetamide 1c (0.33 g, 1.3 mmol) was added triethylsilane (0.5 mL) gradually over 10 min keeping the temperature at room temperature. After addition, the mixture was allowed to stir for 15 min. The reaction was then quenched by neutralizing the trifluoroacetic acid with saturated NaHCO₃ solution. The mixture was extracted with CH₂Cl₂ (3 × 25 mL) and the organic layer pulled together, washed with brine, dried over Na₂SO₄, and concentrated *in-vacuo* to afford *N*-(4-(4-chlorobutyl)phenyl)acetamide 1d as a pale yellow solid. Yield: 77%. ¹H NMR

Table 5#^aSerotonin receptors functional assays, antagonist effects.

5-HT _{1A} R GTPγS binding					5-HT _{7A} R adenylate cyclase					
Compound	IC ₅₀ (nM)	SEM	n	%max inhibition	SEM	IC ₅₀ (nM)	SEM	n	%max inhibition	SEM
21	ND					100	14	2	101.5	0.1
Lurasidone						15.72	0.26	2	100.0	0.0

^a #Compound 21 was a full agonist at the 5HT_{1A} receptor (figure 2, table 4) and no data was generated in the antagonist assay. ND = No data generated.

(300 MHz, DMSO-*d*₆) δ 9.83 (1H, s), 7.45 (2H, dd, *J* = 8.8, 2.4 Hz), 7.08 (2H, dd, *J* = 8.8, 2.4 Hz), 3.62 (2H, t, *J* = 6.1 Hz), 2.52 (2H, t, *J* = 7.0 Hz), 2.00 (3H, s), 1.78–1.50 (4H, m). ¹³C NMR (75 MHz, DMSO-*d*₆) 168.48, 137.61, 136.75, 128.85, 119.48, 45.66, 34.07, 32.01, 28.63, 24.38.

4.1.4. General alkylation procedure

A mixture of alkylating agent (1.1 equiv), base (1.0 equiv) K₂CO₃ (1.1 equiv), and KI (catalytic) in DME (10 mL) was placed in a 20 mL microwave vial with a stirrer and tightly sealed. The mixture was subjected to microwave heating (Biotage, 120–140 °C, 200–400 W, 15 ± 5 bar) for 30–60 min. The mixture was directly purified on silica by flash chromatography (Hexanes: EtOAc gradient up to 70% EtOAc) to afford the titled compound. The free base where necessary, was converted to the HCl or oxalate salt.

Following the general alkylation reaction described in 4.1.4, alkylating agents 1c and 1d were reacted separately with 1-(pyridin-2-yl)piperazine to afford compounds 3 and 4 respectively.

4.1.4.1. *N*-(4-(4-(4-(pyridin-2-yl)piperazin-1-yl)butanoyl)phenyl)acetamide oxalate (3). White amorphous powder. Yield: 48%. Mp: 140–142 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ 10.32 (1H, s), 8.18–8.10 (1H, m), 7.91 (2H, d, *J* = 8.4 Hz), 7.71 (2H, d, *J* = 8.5 Hz), 7.63–7.54 (1H, m), 6.92 (1H, d, *J* = 8.6 Hz), 6.72 (1H, dd, *J* = 7.1, 4.9 Hz), 3.86–3.62 (4H, m), 3.34–3.19 (4H, m), 3.17–3.07 (4H, m), 2.11–1.95 (5H, m). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 197.59, 169.42, 163.37, 158.44, 148.02, 144.20, 138.36, 131.39, 129.62 (2C), 118.62, 114.55, 108.12, 55.69, 51.02 (2C), 42.42 (2C), 35.04, 24.62, 18.53. Anal. C₂₁H₂₆N₄O₂·2.4(COOH)₂ (C, H, N).

4.1.4.2. *N*-(4-(4-(4-(pyridin-2-yl)piperazin-1-yl)butyl)phenyl)acetamide (4). White crystals. Yield: 50%. Mp: 135–136 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ 9.82 (1H, s), 8.13–8.03 (1H, m), 7.50–7.41 (2H, m), 7.08 (2H, d, *J* = 8.3 Hz), 6.76 (1H, d, *J* = 8.6 Hz), 6.64–6.54 (2H, m), 3.47–3.38 (4H, m), 2.51 (2H, t, *J* = 7.3 Hz), 2.42–2.33 (4H, m), 2.28 (2H, t, *J* = 7.2 Hz), 2.00 (3H, s), 1.61–1.36 (4H, m). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 168.44, 159.51, 147.96, 137.86, 137.49, 137.20, 128.82 (2C), 119.44 (2C), 113.34, 107.42, 58.17, 53.05 (2C), 45.09 (2C), 34.85, 29.33, 26.25, 24.38. Anal. C₂₁H₂₈N₄O·0.2H₂O (C, H, N).

4.1.5. Deacetylation of compounds 3 and 4

Under acidic conditions following a slightly modified method described by Kilbourn and co-workers [31] afforded 1-(4-aminophenyl)-4-(4-(pyridin-2-yl)piperazin-1-yl)butan-1-one, 5 and 4-(4-(4-(pyridin-2-yl)piperazin-1-yl)butyl)aniline 6 respectively. Briefly, to an acetic acid (glacial, 7 mL) solution of the acetamide 3 or 4 (1 mmol) in a 20-mL microwave vial was added conc. HCl (3 mL). The homogeneous solution obtained was sealed and subjected to microwave heating (Biotage, 145 °C, 200–400 W, 10 bar) for 15–30 min. The internal pressure in the vial was released prior to opening and the content neutralized with saturated NaHCO₃ until slightly alkaline (pH = 8). The aqueous mixture was extracted with CH₂Cl₂ (3 × 50 mL), organic layers pulled together, washed with brine (50 mL), dried over Na₂SO₄, and the excess solvent removed *in-vacuo* to afford the corresponding compounds 5 and 6 as free bases.

4.1.5.1. 1-(4-aminophenyl)-4-(4-(pyridin-2-yl)piperazin-1-yl)butan-1-one (5). White crystals. Yield: 78%. Mp: 203–204 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.07 (1H, s), 7.67 (2H, dd, *J* = 5.1, 1.8 Hz), 7.52–7.45 (1H, m), 6.77 (1H, d, *J* = 8.8 Hz), 6.62–6.56 (1H, m), 6.54 (2H, dd, *J* = 5.0, 1.8 Hz), 5.98 (2H, s), 3.43–3.37 (4H, m), 2.81 (2H, t, *J* = 7.1 Hz), 2.40 (4H, t, *J* = 5.1 Hz), 2.31 (2H, t, *J* = 7.2 Hz), 1.81–1.70 (2H, m). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 200.34, 161.63, 155.90, 150.05, 140.29, 132.98 (2C), 127.27, 115.70, 115.14 (2C), 109.81, 59.92, 54.98 (2C), 47.11 (2C), 37.38, 24.18. Anal. C₁₉H₂₄N₄O (C, H, N).

4.1.5.2. 4-(4-(4-(pyridin-2-yl)piperazin-1-yl)butyl)aniline (6). White solid. Yield: 69%. Mp: 90–91 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.07 (1H, d, *J* = 4.9 Hz), 7.48 (1H, d, *J* = 13.6 Hz), 6.87–6.72 (3H, m), 6.60 (1H, dd, *J* = 7.0, 4.9 Hz), 6.51–6.42 (2H, m), 4.79 (2H, s), 3.41 (4H, t, *J* = 5.1 Hz), 2.44–2.34 (6H, m), 2.27 (2H, t, *J* = 7.0 Hz), 1.55–1.34 (4H, m). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 159.51, 147.96, 146.76, 137.87, 129.57 (2C), 129.04, 114.39 (2C), 113.34, 107.43, 58.26, 53.05 (2C), 45.08 (2C), 34.70, 29.71, 26.25. Anal. C₁₉H₂₆N₄·0.3H₂O (C, H, N).

4.1.6. 6-(4-chlorobutanoyl)-3,4-dihydroquinolin-2(1H)-one (2b)

Commercially available 3,4-dihydroquinolin-2(1H)-one 2a was acylated similarly as 1a to produce the intermediate 6-(4-chlorobutanoyl)-3,4-dihydroquinolin-2(1H)-one 2b as yellow crystals. Yield: 68%. ¹H NMR (300 MHz, DMSO-*d*₆) δ 10.40 (1H, s), 7.78 (1H, s), 7.75 (1H, d, *J* = 2.0 Hz), 6.91 (1H, d, *J* = 8.2 Hz), 3.67 (2H, t, *J* = 6.7 Hz), 3.07 (2H, t, *J* = 7.1 Hz), 2.92 (2H, t, *J* = 7.6 Hz), 2.47 (2H, t, *J* = 6.5, 8.6 Hz), 2.04 (2H, q, *J* = 6.9 Hz). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 197.81, 170.88, 143.17, 130.86, 128.32, 128.16, 123.90, 115.22, 45.37, 35.20, 30.53, 27.43, 24.95.

4.1.7. 6-(4-chlorobutyl)-3,4-dihydroquinolin-2(1H)-one (2c)

Deoxygenation of intermediate 2b following the TES-mediated reaction described above for 1d afforded the alkylating agent 2c in 50% yield. ¹H NMR (300 MHz, DMSO-*d*₆) δ 9.99 (1H, s), 6.96–6.86 (2H, m), 6.77 (1H, d, *J* = 7.2 Hz), 3.57 (2H, t, *J* = 6.2 Hz), 2.78 (2H, t, *J* = 7.0 Hz), 2.50–2.36 (4H, m), 1.72–1.57 (4H, m). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 170.52, 136.60, 135.71, 127.94, 127.18, 123.80, 115.38, 45.53, 34.12, 32.05, 30.93, 28.74, 25.33.

4.1.8. The synthesis of compounds 7 and 8 followed the same alkylation general conditions as described in section 4.1.4

4.1.8.1. 6-(4-(4-(pyridin-2-yl)piperazin-1-yl)butanoyl)-3,4-dihydroquinolin-2(1H)-one (7). White crystalline solid. Yield: 71%. Mp: 192–193 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ 10.39 (1H, s), 8.07 (1H, d, *J* = 4.4 Hz), 7.79 (2H, s), 7.48 (1H, ddd, *J* = 2.1, 7.1, 8.9 Hz), 6.91 (1H, d, *J* = 8.1 Hz), 6.76 (1H, d, *J* = 8.6 Hz), 6.59 (1H, dd, *J* = 4.8, 7.1 Hz), 3.43–3.33 (4H, m), 2.98–2.86 (4H, m), 2.47 (2H, t, *J* = 7.7 Hz), 2.43–2.36 (4H, m), 2.32 (2H, t, *J* = 7.1 Hz), 1.79 (2H, p, *J* = 7.1 Hz). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 198.81, 170.87, 159.50, 147.96, 142.92, 137.86, 131.33, 128.34, 128.18, 123.82, 115.18, 113.33, 107.42, 57.65, 52.93 (2C), 45.04 (2C), 35.76, 30.56, 24.99, 21.89. Anal. C₂₂H₂₆N₄O₂ (C, H, N).

4.1.8.2. 6-(4-(4-(pyridin-2-yl)piperazin-1-yl)butyl)-3,4-dihydroquinolin-2(1H)-one (8). Solid white crystals. Yield: 62%. Mp:

144–145 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ 9.98 (1H, s), 8.07 (1H, d, *J* = 4.8 Hz), 7.49 (1H, dd, *J* = 1.8, 6.8 Hz), 7.00–6.88 (2H, m), 6.81–6.70 (2H, m), 6.65–6.54 (1H, m), 3.45–3.38 (4H, m), 3.37–3.32 (3H, m), 2.84–2.75 (2H, m), 2.48 (2H, t, *J* = 7.2 Hz), 2.44–2.33 (3H, m), 2.28 (2H, t, *J* = 7.0 Hz), 1.59–1.47 (2H, m), 1.47–1.32 (2H, m). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 170.51, 159.51, 147.96, 137.87, 136.50, 136.18, 128.01, 127.22, 123.79, 115.30, 113.34, 107.43, 58.19, 53.06 (2C), 45.10, 34.85, 30.95, 29.44, 26.31, 25.31, 25.22. Anal. C₂₂H₂₈N₄O · 0.8H₂O (C, H, N).

4.1.9. 6-(3-chloropropoxy)-3,4-dihydroquinolin-2(1H)-one (3b)

A modified O-alkylation method reported by Rampa and colleagues [41] was followed to access intermediate 3b. Succinctly, 6-hydroxy-3,4-dihydroquinolin-2(1H)-one 3a (0.82 g, 5 mmol), 1-bromo-3-chloropropane (1 mL, 10 mmol), K₂CO₃, and KI (catalytic) were suspended in DME (15 mL) placed in a 20-mL Biotage microwave vial equipped with a vial. The vial was sealed and subjected to microwave heating (Biotage, 130 °C, 200 W, 5 bar) for 1 h. After this period, the vial was removed from the microwave, allowed to cool, internal pressure released and opened. The excess alkylating agent was removed *in-vacuo* and the residue purified directly on silica using a Combiflash® (gradient elution: up to 40% EtOAc in hexanes) to afford 6-(3-chloropropoxy)-3,4-dihydroquinolin-2(1H)-one, 3b (0.98 g, 82%) as white crystals. ¹H NMR (300 MHz, DMSO-*d*₆) δ 9.88 (1H, s), 6.82–6.71 (3H, m), 4.00 (2H, t, *J* = 6.2 Hz), 3.75 (2H, t, *J* = 6.8 Hz), 2.81 (2H, t, *J* = 7.7 Hz), 2.38 (2H, t, *J* = 7.7 Hz), 2.16–2.06 (2H, m). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 170.18, 153.94, 132.37, 125.32, 116.21, 114.53, 113.47, 64.92, 42.46, 32.20, 30.78, 25.52.

4.1.10. Synthesis of compounds 9 and 10 used the same alkylation conditions described in section 3.1.4

4.1.10.1. 6-(3-(4-(pyridin-2-yl)piperazin-1-yl)propoxy)-3,4-dihydroquinolin-2(1H)-one (9). Using alkylating agent 3b and 1-(pyridin-2-yl)piperazine, compound 9 was obtained as its free base as white solid. Yield: 54%. Mp: 154–155 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ 9.88 (1H, s), 8.08 (1H, d, *J* = 5.0 Hz), 7.49 (1H, ddd, *J* = 8.9, 7.1, 2.0 Hz), 6.80–6.66 (4H, m), 6.60 (1H, dd, *J* = 7.0, 4.9 Hz), 3.93 (2H, t, *J* = 6.3 Hz), 3.44 (4H, t, *J* = 5.0 Hz), 2.80 (2H, t, *J* = 7.3 Hz), 2.47–2.41 (6H, m), 2.40–2.39 (1H, m), 2.38–2.35 (1H, m), 1.93–1.77 (2H, m). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 170.17, 159.51, 154.25, 147.97, 137.87, 132.12, 125.26, 116.20, 114.46, 113.43, 113.36, 107.45, 66.51, 54.99, 53.07, 45.11, 30.81, 26.73, 25.55. Anal. C₂₁H₂₆N₄O₂ (C, H, N).

4.1.10.2. 6-(3-(4-(pyridin-2-yl)-1,4-diazepan-1-yl)propoxy)-3,4-dihydroquinolin-2(1H)-one oxalate (10). Using alkylating agent 3b and 1-(pyridin-2-yl)-1,4-diazepane as the amine, compound 10 was obtained as an oxalate salt. Yield: 30%. Mp: 190–192 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ 9.90 (1H, s), 8.08 (1H, d, *J* = 4.8 Hz), 7.62–7.46 (1H, m), 6.86–6.51 (5H, m), 4.11–3.78 (4H, m), 3.64–3.47 (2H, m), 3.41–3.06 (6H, m), 2.80 (2H, t, *J* = 7.4 Hz), 2.37 (2H, t, *J* = 7.4 Hz), 2.26–1.96 (4H, m). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 170.17, 163.94, 157.89, 153.79, 147.94, 138.13, 132.44, 125.30, 116.20, 114.55, 113.52, 112.66, 106.29, 65.67, 54.96, 54.05, 53.93, 45.61, 30.78, 25.52, 24.49, 24.19. Anal. C₂₂H₂₈N₄O₂ · 1.7(COOH)₂ (C, H, N).

4.1.11. 5-(3-chloropropoxy)-2,3-dihydro-1H-inden-1-one (4b)

Using similar O-alkylation reaction method described for 3b above, intermediate 4b was obtained as white solid crystal by reacting commercially available 5-hydroxy-2,3-dihydro-1H-inden-1-one, 4a with 1-bromo-3-chloropropane. Yield: 73%. ¹H NMR (300 MHz, CDCl₃) δ 7.64 (1H, d, *J* = 8.3 Hz), 6.92–6.82 (2H, m), 4.16 (2H, t, *J* = 5.8 Hz), 3.72 (2H, t, *J* = 6.3 Hz), 3.04 (2H, t, *J* = 5.7 Hz),

2.67–2.58 (2H, m), 2.31–2.17 (2H, m). ¹³C NMR (75 MHz, CDCl₃) δ 205.17, 164.29, 158.13, 130.51, 125.29, 115.58, 110.29, 64.62, 41.25, 36.39, 31.97, 25.84.

4.1.12. 5-(3-(4-(pyridin-2-yl)piperazin-1-yl)propoxy)-2,3-dihydro-1H-inden-1-one (11)

Following the general alkylation reaction 4.1.4, intermediate 4b was reacted with 1-(pyridin-2-yl)piperazine to produce compound 11 as a white solid crystal. Yield: 68% Mp: 103–104 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.08 (1H, s), 7.59–7.42 (2H, m), 7.04 (1H, s), 6.92 (1H, d, *J* = 8.0 Hz), 6.76 (1H, d, *J* = 8.6 Hz), 6.66–6.55 (1H, m), 4.10 (2H, t, *J* = 6.3 Hz), 3.52–3.38 (4H, m), 3.05–2.95 (2H, m), 2.59–2.50 (4H, m), 2.00–1.80 (2H, m), 1.63–1.22 (4H, m). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 204.58, 164.60, 159.49, 158.66, 147.98, 137.85, 130.21, 124.97, 116.02, 113.36, 110.99, 107.42, 66.86, 54.79, 53.05 (2C), 45.10 (2C), 36.44, 26.48, 25.90. Anal. C₂₁H₂₅N₃O₂ · 0.1H₂O (C, H, N).

4.1.13. 1-(2,3-Dihydro-1H-inden-5-yl)-4-(4-(pyridin-2-yl)piperazin-1-yl)butan-1-one (12)

The commercially available indane, 5a was acylated under Friedel-Crafts acylation conditions and the intermediate 5b obtained was subsequently reacted with 1-(pyridin-2-yl)piperazine under general N-alkylation conditions to afford the final compound 12 as white solid crystals. Yield: 59%, Mp: 74–75 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.07 (1H, s), 7.78 (1H, s), 7.72 (1H, d, *J* = 8.0 Hz), 7.52–7.42 (1H, m), 7.29 (1H, d, *J* = 7.8 Hz), 6.73 (1H, d, *J* = 8.6 Hz), 6.63–6.52 (1H, m), 3.44–3.30 (4H, m), 2.96 (2H, t, *J* = 7.0 Hz), 2.91–2.79 (4H, m), 2.44–2.26 (6H, m), 2.06–1.92 (2H, m), 1.87–1.69 (2H, m). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 199.95, 159.49, 149.82, 147.95, 144.70, 137.80, 135.89, 126.78, 124.69, 124.14, 113.29, 107.37, 57.63, 52.93 (2C), 45.03 (2C), 36.15, 32.85, 32.47, 25.42, 21.83. Anal. C₂₂H₂₇N₃O (C, H, N).

4.1.14. 5-Fluoro-2-(2-(4-(pyridin-2-yl)piperazin-1-yl)ethyl)-2,3-dihydro-1H-inden-1-one (13)

A mixture of 2-(2-chloroethyl)-5-fluoro-2,3-dihydro-1H-inden-1-one, 6a (1.1 g, 5.2 mmol), 1-(pyridin-2-yl)piperazine (0.90 g, 5.5 mmol), KI (100 mg), NaHCO₃ (1.0 g, 11.9 mmol) in toluene (10 mL) was heated to reflux under N₂ for 12 h. After cooling to rt, the mixture was diluted with EtOAc (500 mL) and followed by washing with water (2 × 300 mL). The organic layer was dried over Na₂SO₄, and filtered. The filtrate was concentrated in *vacuo* to dryness and followed by column chromatography on silica gel afforded 5-fluoro-2-(2-(4-(pyridin-2-yl)piperazin-1-yl)ethyl)-2,3-dihydro-1H-inden-1-one, 13. The crystals were obtained by crystallization from EtOAc/Hexane in a yield of 26%. ¹H NMR (300 MHz, CDCl₃): 8.16 (1H, m), 7.73 (1H, dd, *J* = 5.1, 8.1 Hz), 7.46 (1H, ddd, *J* = 1.8, 6.9, 8.7 Hz), 7.02–7.13 (2H, m), 6.58–6.63 (2H, m), 3.38–2.90 (4H, m), 3.32 (1H, dd, *J* = 7.5, 16.8 Hz), 2.86 (1H, dd, *J* = 4.2, 16.8 Hz), 2.75–2.81 (1H, m), 2.45–2.60 (6H, m), 2.12–2.21 (1H, m), 1.78–1.88 (1H, m). ¹³C NMR (300 MHz, CDCl₃): 206.4, 167.0 (d, *J* = 254.2 Hz), 159.5, 156.2 (d, *J* = 10.4 Hz), 147.9, 137.4, 133.4, 126.0 (d, *J* = 10.4 Hz), 115.6 (d, *J* = 24.0 Hz), 113.2 (d, *J* = 3.4 Hz), 112.9, 107.0, 55.7, 53.0 (2C), 45.6, 45.0 (2C), 32.2, 28.3. Anal. C₂₀H₂₂FN₃O (C, H, N).

4.1.15. 2-(2-Chloro-ethyl)-5-fluoro-indan-1-ol (7a)

To a solution of 2-(2-chloro-ethyl)-5-fluoro-indan-1-one (2 g, 9.4 mmol) and CeCl₃ (160 mg, 0.65 mmol) in MeOH (10 mL) was added with stirring NaBH₄ (0.7 g, 18.5 mmol) at rt. After stirring at rt for 1 h, the mixture was diluted with EtOAc (200 mL) and washed with sat NaHCO₃ (50 mL). The organic layer was dried over Na₂SO₄, and filtered. The filtrate was concentrated in *vacuo* and followed by

column chromatography on silica gel afforded 2-(2-Chloro-ethyl)-5-fluoro-indan-1-ol, 1.85 g, yield = 92%. ¹H NMR (CDCl₃): 7.33 (1H, m), 6.91 (2H, m), 4.82 (1H, brs), 3.73 (2H, m), 3.15 (1H, dd, *J* = 7.2, 15.3 Hz), 2.50 (1H, m), 2.42 (1H, m), 2.34 (1H, m), 2.02 (1H, m).

4.1.16. 5-Fluoro-2-[2-(4-pyridin-2-yl-piperazin-1-yl)-ethyl]-indan-1-ol (14)

A mixture of 2-(2-Chloro-ethyl)-5-fluoro-indan-1-ol (0.60 g, 2.8 mmol) 7a, 1-pyridin-2-yl-piperazine dihydrochloride (0.67 g, 2.84 mmol), KI (0.48 g), K₂CO₃ (1.2 g, 8.7 mmol) in DME (10 mL) was heated to reflux under N₂ for 12 h. Then the mixture was directly purified through column chromatography on silica gel afforded 5-fluoro-2-[2-(4-pyridin-2-yl-piperazin-1-yl)-ethyl]-indan-1-ol (14) which was converted to the hydroiodide salt. Crystallization from MeOH–Et₂O gave a solid, 0.6 g, yield 46%. Mp: 174–175 °C. ¹H NMR (DMSO-*d*₆): 9.39 (1H, brs), 8.15 (1H, dd, *J* = 1.5, 4.5 Hz), 7.60 (1H, dt, *J* = 2.1, 8.7 Hz), 7.29 (1H, dd, *J* = 5.4, 7.5 Hz), 6.97 (3H, m), 6.74 (1H, dd, *J* = 4.8, 6.6 Hz), 5.52 (1H, brs), 4.68 (1H, brs), 4.38 (2H, brs), 3.59 (2H, brs), 3.04 (6H, m), 2.44 (1H, m), 2.08 (3H, m), 1.89 (1H, m). ¹³C NMR (150 MHz, CDCl₃): ¹³C NMR (151 MHz, DMSO) δ 162.47 (d, *J* = 241.6 Hz, C–F), 158.33, 148.02, 143.64, 142.35, 138.45, 125.74, 114.75, 113.81, 111.74, 108.25, 79.02, 55.06, 48.01 (2C), 42.49 (2C), 40.57, 35.39, 27.38. Anal. C₂₀H₂₅FN₃O (C, H, N).

4.1.17. 5-chloro-2-(2-(4-(pyridin-2-yl)piperazin-1-yl)ethyl)-2,3-dihydro-1H-inden-1-one (15)

Compound 15 was prepared similarly to 14 by reacting previously reported alkylating agent 5-chloro-2-(2-chloroethyl)-2,3-dihydro-1H-inden-1-one, 8a with 1-pyridin-2-yl-piperazine under same alkylation reaction conditions. The title compound was obtained as solid white crystal. Mp: 132–135 °C. ¹H NMR (300 MHz, DMSO-*d*₆): 8.05 (1H, dd, *J* = 1.8, 4.8 Hz), 7.66 (1H, s), 7.59 (1H, d, *J* = 8.4 Hz), 7.48 (1H, dt, *J* = 1.8, 8.4 Hz), 7.42 (1H, dd, *J* = 1.8, 8.4 Hz), 6.75 (1H, d, *J* = 8.1 Hz), 6.59 (1H, dd, *J* = 5.4, 7.2 Hz), 3.18–3.28 (4H, m), 2.88 (1H, dd, *J* = 4.2, 18.0 Hz), 2.70–2.79 (1H, m), 2.34–2.45 (4H, m), 2.25–2.33 (3H, m), 1.88–1.97 (1H, m), 1.74–1.86 (1H, m). ¹³C NMR (150 MHz, CDCl₃): 206.7, 159.4, 154.8, 147.9, 142.9, 137.4, 135.5, 128.1, 126.6, 124.9, 113.2, 107.0, 55.5, 52.9 (2C), 45.4, 45.0 (2C), 32.0, 28.2. Anal. C₂₀H₂₂ClN₃O (C, H, N).

4.1.18. 5-chloro-2-(2-chloroethyl)-2,3-dihydro-1H-indene (8b)

Amalgamated zinc was prepared by stirring a mixture of zinc (1.2 g), HgCl₂ (120 mg) in 5 mL water with conc HCl (0.1 mL) at room temperature. After stirring for 5 min, the mixture was decanted and followed by adding in order water (1 mL), conc HCl (1.75 mL), toluene (10 mL), and starting material 5-chloro-2-(2-chloroethyl)-2,3-dihydro-1H-inden-1-one, 8a (2 g, 8.73 mmol). The mixture was refluxed with stirring for 12 h. The solid was filtered off, aqueous layer was diluted with EtOAc (200 mL), washed with water, and sat. NaHCO₃ (50 mL). The organic layer was dried over Na₂SO₄, and filtered. The filtrate was concentrated in vacuo and used in the next step without further purification.

4.1.19. 1-(2-(5-chloro-2,3-dihydro-1H-inden-2-yl)ethyl)-4-(pyridin-2-yl)piperazine (16)

A mixture of 5-chloro-2-(2-chloroethyl)-2,3-dihydro-1H-indene, 8b (1 g, 4.6 mmol), 1-(pyridin-2-yl)piperazine (0.9 g, 5.6 mmol), KI (100 mg), K₂CO₃ (1.2 g, 9.2 mmol) in DME (10 mL) was heated to reflux under N₂ for 12 h. Then the mixture was directly purified through column chromatography on silica gel to afforded the titled compound 1-(2-(5-chloro-2,3-dihydro-1H-inden-2-yl)ethyl)-4-(pyridin-2-yl)piperazine, 16 as an HCl salt after crystallization from MeOH–Et₂O. Mp: 267–270 °C. ¹H NMR (300 MHz, CD₃OD) δ 8.16 (1H, ddd, *J* = 9.1, 7.1, 1.8 Hz), 8.12–8.04 (1H, m), 7.48 (1H, t, *J* = 10.4 Hz), 7.28–7.01 (4H, m), 3.79 (4H, s), 3.58 (1H, dd,

J = 17.5, 7.8 Hz), 3.46 (1H, d, *J* = 6.0 Hz), 3.30 (4H, s), 3.17–3.01 (2H, m), 2.77–2.60 (2H, m), 2.52 (1H, td, *J* = 15.0, 7.5 Hz), 2.14–1.96 (2H, m). ¹³C NMR (151 MHz, CD₃OD) δ 152.35, 145.01, 144.72, 141.15, 136.92, 131.66, 126.11, 125.29, 124.14, 114.48, 113.12, 55.73, 50.42 (2C), 43.35 (2C), 38.26, 37.78, 37.63, 29.03. Anal. C₂₀H₂₄ClN₃·2HCl (C, H, N).

4.1.20. (E)-2-(6-chloro-1-oxo-1,3-dihydro-2H-inden-2-ylidene)acetic acid (9c)

A mixture of 6-chloro-2,3-dihydro-1H-inden-1-one (5 g, 30 mmol), glyoxylic acid (50% aqueous solution, 6.6 g, 72 mmol), and conc. H₂SO₄ (1 mL) in dioxane (5 mL) were stirred at refluxing temperature for 12 h. The mixture was cooled, the product filtered off, washed with water and dried to give the acid (E)-2-(6-chloro-1-oxo-1,3-dihydro-2H-inden-2-ylidene)acetic acid, 9c as a white solid. ¹H NMR (600 MHz, DMSO-*d*₆): δ 13.8 (1H, s), 7.86–7.58 (3H, m), 6.54 (1H, t, *J* = 2.38 Hz) 4.05 (2H, s).

4.1.21. (E)-2-(5,6-dichloro-1-oxo-1,3-dihydro-2H-inden-2-ylidene)acetic acid (9d)

Preparation of intermediate 9d followed similar procedure as 9c to afford (E)-2-(5,6-dichloro-1-oxo-1,3-dihydro-2H-inden-2-ylidene)acetic acid (86%) as a white solid. ¹H NMR (300 MHz, CDCl₃): δ 7.97 (1H, s), 7.92 (1H, s), 6.50 (1H, s), 4.00 (2H, s).

4.1.22. 2-(6-chloro-1-oxo-2,3-dihydro-1H-inden-2-yl)acetic acid (9e)

(E)-2-(6-Chloro-1-oxo-1,3-dihydro-2H-inden-2-ylidene)acetic acid, 9c (3 g, 13.4 mmol) in MeOH (30 mL) and dioxane (120 mL) with Pd/C (10%, 1 g) was stirred under H₂ (40 psi) for 48 h. The mixture was filtered through Celite and the solvent evaporated to give 2-(6-chloro-1-oxo-2,3-dihydro-1H-inden-2-yl)acetic acid as an off-white solid. ¹H NMR (600 MHz, DMSO-*d*₆): δ 12.32 (1H, s), 7.75–7.51 (3H, m), 3.37–3.25 (1H, m), 2.94–2.83 (2H, m), 2.76–2.62 (2H, m).

4.1.23. 2-(5,6-Dichloro-1-oxo-2,3-dihydro-1H-inden-2-yl)acetic acid (9f)

Synthesis of 9f followed same procedure as 9e to give 2-(5,6-dichloro-1-oxo-2,3-dihydro-1H-inden-2-yl)acetic acid as an off-white solid. ¹H NMR (300 MHz, CDCl₃): δ 12.32 (s, 1H), 7.91 (s, 1H), 7.82 (s, 1H), 3.36–3.28 (m, 1H), 2.97–2.79 (m, 2H), 2.72–2.68 (m, 2H).

4.1.24. 2-(6-chloro-2,3-dihydrospiro[indene-1,2'-[1,3]dioxolan]-2-yl)ethan-1-ol (9 g)

A solution of 2-(6-chloro-1-oxo-2,3-dihydro-1H-inden-2-yl)acetic acid (1.1 g, 4.8 mmol), ethylene glycol (2 mL), TsOH (300 mg) in toluene (10 mL) was refluxed under N₂ for 48 h and H₂O was removed by azeotropic distillation. The reaction was monitored by ¹H NMR in a maximum conversion of 80% after which the reaction was quenched by addition of Et₃N (1 mL), diluted with EtOAc (250 mL), washed with sat NaHCO₃, (25 mL) and H₂O (25 mL). The organic layer was dried over anhydrous Na₂SO₄, and filtered. The filtrate was concentrated in vacuo to dryness and was used as such for the next reaction.

A solution of 2-(6-chloro-2,3-dihydrospiro [indene-1,2'- [1,3] dioxolan]-2-yl)acetic acid (1.3 g, 4.8 mmol) in dry THF (20 mL) was added dropwise to a suspension of LiAlH₄ (0.37 g, 9.7 mmol) in dry THF (15 mL) at 0 °C and the resulting mixture was stirred at refluxing temperature for 12 h. EtOAc was added to quench excess LiAlH₄ and then aqueous HCl solution (10%, 50 mL) was added and the organic fraction separated. The aqueous solution was extracted with EtOAc (3 × 50 mL), and the combined organic fraction dried and the solvent evaporated to give alcohol 2-(6-chloro-2,3-

dihydrospiro [indene-1,2'- [1,3]dioxolan]-2-yl)ethan-1-ol as a yellow oil which was used for the next step without further purification. ¹H NMR (300 MHz, CDCl₃): δ 7.26–7.22 (2H, m), 7.14 (1H, s), 4.29–4.13 (4H, m), 3.77–3.73 (2H, m), 3.05–2.95 (1H, m), 2.69–2.63 (2H, m), 1.99–1.91 (1H, m), 1.77–1.63 (1H, m).

4.1.25. 2-(5,6-Dichloro-2,3-dihydrospiro[indene-1,2'- [1,3]dioxolan]-2-yl)ethan-1-ol (9 h)

Preparation of 9 h followed the same procedure as 9 g to give the alcohol 2-(5,6-dichloro-2,3-dihydrospiro [indene-1,2'- [1,3]dioxolan]-2-yl)ethan-1-ol as a yellow oil which was used as such for the next step. ¹H NMR (300 MHz, CDCl₃): δ 7.36 (1H, s), 7.30 (1H, s), 4.26–4.22 (2H, m), 4.16–4.12 (4H, m), 3.05–2.95 (1H, m), 2.69–2.60 (2H, m), 1.99–1.91 (1H, m), 1.77–1.63 (1H, m).

4.1.26. 2-(6-chloro-2,3-dihydrospiro[indene-1,2'- [1,3]dioxolan]-2-yl)ethyl 4-methylbenzenesulfonate (9i)

To a solution of 2-(6-chloro-2,3-dihydrospiro [indene-1,2'- [1,3]dioxolan]-2-yl)ethan-1-ol (0.55 g, 2.1 mmol), Et₃N (0.66 mL, 6.3 mmol) in CH₂Cl₂ (10 mL) was added at room temperature *p*-TsCl (0.62 mg mL, 3.1 mmol). The mixture was stirred at room temperature for 12 h, and then followed by directly purification through column chromatography on silica gel, and provided 2-(6-chloro-2,3-dihydrospiro [indene-1,2'- [1,3]dioxolan]-2-yl)ethyl 4-methylbenzenesulfonate, yield 95%. ¹H NMR (300 MHz, CDCl₃): δ 7.81 (2H, d, *J* = 31.3 Hz), 7.37–7.32 (2H, d, *J* = 31.3 Hz), 7.26–7.23 (2H, m), 7.10–7.08 (1H, m), 4.24–3.99 (6H, m), 2.93–2.83 (1H, m), 2.59–2.51 (2H, m), 2.46 (3H, s), 1.99–1.91 (1H, m), 1.77–1.63 (1H, m).

4.1.27. 2-(5,6-Dichloro-2,3-dihydrospiro[indene-1,2'- [1,3]dioxolan]-2-yl)ethyl 4-methylbenzene sulfonate (9j)

Synthesis of alkylating agent 9j followed the same procedure as 9i. White solid. Yield 95%. ¹H NMR (300 MHz, CDCl₃): δ 7.81 (1H, d, *J* = 31.3 Hz), 7.37–7.32 (3H, m), 7.26 (1H, m), 4.23–3.97 (6H, m), 2.93–2.83 (1H, m), 2.59–2.51 (2H, m), 2.46 (3H, s), 1.99–1.91 (1H, m), 1.77–1.63 (1H, m).

4.1.28. 6-chloro-2-(2-(4-(pyridin-2-yl)piperazin-1-yl)ethyl)-2,3-dihydro-1H-inden-1-one (17)

A mixture of 2-(6-chloro-2,3-dihydrospiro [indene-1,2'- [1,3]dioxolan]-2-yl)ethyl 4-methylbenzene sulfonate (263 mg, 0.64 mmol), 1-(pyridin-2-yl)piperazine (104 mg, 0.64 mmol), KI (50 mg), K₂CO₃ (266 mg, 1.9 mmol) in DME (10 mL) was heated 90 °C N₂ for 12 h. The reaction was diluted with EtOAc (25 mL), washed with sat NaHCO₃, (10 mL), water (10 mL). The organic layer was dried over Na₂SO₄, and filtered. The filtrate was concentrated in vacuo to dry and proceeded for the further reaction. The crude product was dissolved in wet MeOH, TsOH (25 mg) was added with stirring at rt. After stirring at rt for 12 h, the solution was diluted with EtOAc (50 mL), and followed by washing with sat NaHCO₃ (10 mL). The organic layer was dried over Na₂SO₄, and filtered. The filtrate was concentrated in vacuo to dry and followed by column chromatography on silica gel afforded 6-chloro-2-(2-(4-(pyridin-2-yl)piperazin-1-yl)ethyl)-2,3-dihydro-1H-inden-1-one. The product was converted to a dihydrochloride salt, further crystallization from MeOH–Et₂O afforded the target product. Mp: 161–164 °C, ¹H NMR (600 MHz, CD₃OD) δ 8.23–8.06 (2H, m), 7.69 (2H, s), 7.59 (1H, d, *J* = 7.9 Hz), 7.48 (1H, d, *J* = 8.6 Hz), 7.15 (1H, s), 3.64 (4H, d, *J* = 31.1 Hz), 3.54–3.37 (3H, m), 3.32 (4H, s), 2.94 (2H, d, *J* = 14.9 Hz), 2.39 (1H, s), 2.10 (1H, s). ¹³C NMR (151 MHz, CD₃OD) δ 206.64, 152.90, 152.08, 144.49, 137.85, 137.40, 135.03, 133.66, 128.16, 122.91, 114.59, 112.73, 55.13, 50.67, 45.18 (2C), 43.32 (2C), 32.00, 25.03. Anal. C₂₀H₂₂ClN₃O·2HCl·1.2H₂O (C, H, N).

4.1.29. 5,6-Dichloro-2-(2-(4-(pyridin-2-yl)piperazin-1-yl)ethyl)-2,3-dihydro-1H-inden-1-one (18)

Following similar procedure as compound 17, the target compound 5,6-dichloro-2-(2-(3,4-dihydroisoquinolin-2(1H)-yl)ethyl)-2,3-dihydro-1H-inden-1-one, 18 was prepared in good yield, converted to hydrochloride salt and further crystallization from MeOH–Et₂O to afford a white solid. Mp: 246–248 °C. ¹H NMR (600 MHz, DMSO-*d*₆) δ 11.63 (1H, s), 8.13 (1H, dd, *J* = 5.6, 1.2 Hz), 7.97 (1H, s), 7.92 (1H, t, *J* = 7.7 Hz), 7.86 (1H, s), 7.28 (1H, d, *J* = 8.8 Hz), 6.95 (1H, t, *J* = 6.3 Hz), 4.48 (3H, d, *J* = 12.7 Hz), 3.45–3.29 (4H, m), 3.27–3.10 (4H, m), 3.01–2.89 (1H, m), 2.87 (1H, dd, *J* = 17.4, 4.2 Hz), 2.35–2.24 (1H, m), 1.97 (1H, td, *J* = 13.3, 4.4 Hz). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 205.22, 153.74, 142.32, 138.16, 136.49, 131.42, 129.60 (2C), 125.16 (2C), 114.43, 111.42, 53.97, 50.57, 45.26 (2C), 43.27 (2C), 32.09, 24.87. Anal. C₂₀H₂₁Cl₂N₃O·2HCl·H₂O (C, H, N).

4.1.30. 5-chloro-1-(4-chlorophenyl)pentan-1-one (10a)

The procedure reported by Komissarov, V. V. et al. [35] was followed to afford 5-chloro-1-(4-chlorophenyl)pentan-1-one, 10a. Yield 71%. ¹H NMR (300 MHz, CDCl₃) δ 7.90 (2H, d, *J* = 9.0 Hz), 7.45 (2H, d, *J* = 9.0 Hz), 3.59 (2H, t, *J* = 6.0 Hz), 2.99 (2H, d, *J* = 6.9 Hz), 1.85–1.91 (4H, m).

4.1.31. 5-chloro-2-(3-chloropropyl)-2,3-dihydro-1H-inden-1-one (10b)

Using 5-chloro-1-(4-chlorophenyl)pentan-1-one 10a, the synthesis of 5-chloro-2-(3-chloropropyl)-2,3-dihydro-1H-inden-1-one 10b followed similar procedure as 6a. Yield 100%. ¹H NMR (300 MHz, CDCl₃) δ 7.68 (1H, d, *J* = 8.1 Hz), 7.46 (1H, s), 7.35 (1H, d, *J* = 8.1 Hz), 3.56–3.61 (1H, m), 3.35 (1H, dd, *J* = 7.8, 17.4 Hz), 2.77–2.84 (1H, m), 2.68–2.75 (1H, m), 2.00–2.08 (1H, m), 1.90–1.96 (2H, m), 1.66–1.75 (2H, m).

4.1.32. 5-chloro-2-(3-(4-(pyridin-2-yl)piperazin-1-yl)propyl)-2,3-dihydro-1H-inden-1-one (19)

Following the general alkylation reaction described in 4.1.4, compound 19 was prepared in moderate yield (yield 37%) as the hydrobromide salt. ¹H NMR (DMSO-*d*₆): 9.70 (2H, brs), 8.14 (1H, d, *J* = 5.4 Hz), 7.80 (1H, dd, *J* = 6.9, 8.1 Hz), 7.71 (1H, s), 7.64 (1H, d, *J* = 8.1 Hz), 7.47 (1H, d, *J* = 8.1 Hz), 7.15 (1H, d, *J* = 8.7 Hz), 6.87 (1H, t, *J* = 6.0 Hz), 4.37 (2H, d, *J* = 14.7 Hz), 3.61 (2H, d, *J* = 11.7 Hz), 3.25–3.40 (3H, m), 3.06–3.19 (4H, m), 2.72–2.88 (2H, m), 1.76–1.82 (3H, m), 1.48–1.55 (1H, m). ¹³C NMR (151 MHz, CD₃OD) δ 207.52, 155.75, 153.11, 144.23, 141.34, 138.21, 134.77, 128.01, 126.70, 124.60, 114.61, 112.55, 56.54, 50.57 (2C), 46.53, 43.16 (2C), 32.14, 27.63, 21.40. Anal. C₂₁H₂₄ClN₃O·2HBr (C, H, N).

4.1.33. Ethyl 5-chloro-1-oxo-2,3-dihydro-1H-indene-2-carboxylate (11b)

A modified method described by Paccani and co-workers [42] was followed to access the 5-substituted β-keto ester 11b. In brief, a solution of the 5-chloro indanone 11a, (100 mmol, 1 equiv) in diethyl carbonate (50 mL) was added dropwise to a stirred suspension of NaH (200 mmol, 60% in mineral oil previously washed with hexanes) in diethyl carbonate (DEC) (25 mL) at 0 °C with stirring (note green coloration). When evolution of gas has ceased, the mixture was allowed to stir at room temperature overnight. The mixture was then diluted in CH₂Cl₂ and treated with aqueous acetic acid solution. The aqueous phase was separated and extracted with CH₂Cl₂. The combined organic extracts were dried (Na₂SO₄) and concentrated under reduced pressure to yield the brown thick oil crude. The crude was loaded onto a cartridge and purified using flash chromatography with a gradient elution (up to 10% EtOAc in hexanes) to afford ethyl 5-chloro-1-oxo-2,3-dihydro-1H-indene-2-

carboxylate, 11b. Needle-like white crystals. Yield: 89%. ^1H NMR (300 MHz, CDCl_3) δ 10.47–10.20 (1H, m), 7.65–7.40 (6H, m), 4.31–4.13 (4H, m), 3.71–3.64 (1H, m), 3.55–3.38 (2H, m), 3.37–3.22 (2H, m), 1.35–1.19 (6H, m). ^{13}C NMR (75 MHz, CDCl_3) δ 198.15, 168.59, 155.11, 144.83, 135.83, 134.08, 131.40, 130.78, 130.06, 129.88, 129.80, 127.98, 125.70, 123.71, 121.81, 102.72, 61.83, 60.23, 53.24, 32.40, 29.91, 14.42, 14.17.

4.1.34. Ethyl 5-chloro-2-(4-chlorobutyl)-1-oxo-2,3-dihydro-1H-indene-2-carboxylate (11c)

To a suspension of hexane washed NaH (60% in mineral oil, 12 mmol) in dry DMF was added a solution of ethyl 5-chloro-1-oxo-2,3-dihydro-1H-indene-2-carboxylate 11b (10 mmol) dropwise with stirring (note the green coloration) over 30 min. After the evolution of gas has ceased, the 1-bromo-4-chlorobutane (20 mmol) was added dropwise and allowed to stir for 18–24 h. After, the reaction was quenched in water (100 mL), shaken with CH_2Cl_2 (2×100 mL), the organic layers pulled together, washed with brine, and dried over Na_2SO_4 and concentrated *in-vacuo* to obtain the crude product which was then used directly in the next step without further purification.

4.1.35. 5-chloro-2-(4-chlorobutyl)-2,3-dihydro-1H-inden-1-one (11d)

Generally, an acid catalyzed decarboxylation of the tertiary β -keto ester was followed under microwave heating conditions to afford alkylation agent 11d in good to moderate yield. Briefly, the crude β -keto ester 11c was dissolved in 10 mL of glacial acetic acid and transferred into a 20 mL Biotage microwave vial equipped with a stirrer. To this homogenous solution was added 3 mL of conc. HCl. The vial was sealed and subjected to microwave heating (Biotage, 120 °C, 200 W, 18 bar) for 30 min. The vial was then allowed to cool to room temperature and internal pressure released prior to opening. The content was neutralized with saturated NaHCO_3 and extracted with CH_2Cl_2 (3×50 mL). The organic layers were pulled together, washed with brine, dried (Na_2SO_4), and solvent removed *in-vacuo* to produce a thick, oily crude. The crude was purified on flash column chromatography using gradient elution (up to 20% EtOAc in hexanes) to afford the corresponding alkylating agent 11d as a clear yellowish oil. Yield (overall over 2 steps): 51%. ^1H NMR (300 MHz, CDCl_3) δ 7.63 (1H, dd, $J = 8.1, 1.4$ Hz), 7.41 (1H, dd, $J = 1.9, 1.0$ Hz), 7.30 (1H, dd, $J = 8.2, 1.8$), 3.52 (2H, tt, $J = 6.6, 1.0$ Hz), 3.29 (1H, ddt, $J = 17.4, 7.9, 0.8$ Hz), 2.84–2.59 (2H, m), 1.99–1.85 (1H, m), 1.85–1.72 (2H, m), 1.63–1.50 (2H, m), 1.50–1.39 (1H, m). ^{13}C NMR (75 MHz, CDCl_3) δ 205.96, 154.61, 141.28, 134.76, 128.28, 126.65, 124.98, 45.00, 43.03, 34.06, 32.54.

4.1.36. 5-chloro-2-(4-(4-(pyridin-2-yl)piperazin-1-yl)butyl)-2,3-dihydro-1H-inden-1-one (20)

Using 11d as the alkylating agent, compound 20 was prepared under the same general alkylation procedure. The final compound 20 was converted to its HCl salt to obtain a white flaky solid. Yield: 72%, Mp: 233–234 °C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 11.63 (2H, s), 8.08 (1H, d, $J = 1.5, 5.9$ Hz), 7.99 (1H, d, $J = 8.0$ Hz), 7.66 (1H, s), 7.62 (1H, d, $J = 8.1$ Hz), 7.45 (1H, dd, $J = 1.6, 8.2$ Hz), 7.36 (1H, d, $J = 9.0$ Hz), 6.98 (1H, d, $J = 6.4$ Hz), 3.73–3.49 (4H, m), 3.31 (1H, dd, $J = 7.7, 17.5$ Hz), 3.10 (4H, q, $J = 8.9, 11.0$ Hz), 2.91–2.65 (2H, m), 1.93–1.63 (4H, m), 1.61–1.21 (4H, m). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ 207.25, 159.52, 156.23, 147.96, 140.13, 137.85, 135.50, 128.32, 127.35, 125.13, 113.33, 107.43, 58.21, 53.04 (2C), 47.30, 45.11 (2C), 32.53, 30.99, 26.68, 25.10. Anal. $\text{C}_{22}\text{H}_{26}\text{ClN}_3\text{O} \cdot 2\text{HCl}$ (C, H, N).

4.1.37. Ethyl 5-chloro-2-methyl-1-oxo-2,3-dihydro-1H-indene-2-carboxylate (12a)

To a stirred suspension of NaH (15 mmol, 60% in mineral oil

previously washed with hexanes) in dry DMF was added a solution of ethyl 5-chloro-1-oxo-2,3-dihydro-1H-indene-2-carboxylate 11b in dry DMF (7.5 mmol) portion-wise under N_2 . After stirring for 2 h, iodomethane (15 mmol) was added dropwise and the reaction stirred for 24 h. The reaction was quenched with water (50 mL), extracted with CH_2Cl_2 (2×50 mL), the organic layers pulled together was washed with brine, dried over Na_2SO_4 and the solvent removed under reduced pressure to obtain the crude product which was loaded onto a cartridge and purified by flash chromatography using a gradient of up to 5% EtOAc in hexanes to obtain compound the intermediate compound. Yield: 60%; ^1H NMR (300 MHz, CDCl_3) δ 7.54 (1H, d, $J = 8.1$ Hz), 7.34 (1H, s), 7.22 (1H, d, $J = 8.5$ Hz), 3.99 (2H, q, $J = 7.4$ Hz), 3.56 (1H, d, $J = 17.3$ Hz), 2.85 (1H, d, $J = 17.3$ Hz), 1.36 (3H, s), 1.13–0.99 (3H, m).

4.1.38. 5-chloro-2-methyl-2,3-dihydro-1H-inden-1-one (12b)

Ethyl 5-chloro-2-methyl-1-oxo-2,3-dihydro-1H-indene-2-carboxylate, 12a (2.2 mmol) was subsequently hydrolyzed and decarboxylated by dissolving it in acetic acid (5 mL) and concentrated HCl (2 mL) was added and transferred into a 20 mL-micro-wave vial with a stirrer and tightly sealed. The mixture was heated in the microwave at 80 °C for 1 h, the reaction was allowed to cool to room temperature, basified with NaHCO_3 solution and extracted with CH_2Cl_2 . The organic layer was washed with brine, dried over Na_2SO_4 and the solvent removed under reduced pressure. The crude was loaded onto a cartridge and purified by flash chromatography using a gradient of 5% EtOAc in hexanes to afford 12b. Yield: 79%; ^1H NMR (300 MHz, CDCl_3) δ 7.61 (1H, d, $J = 8.6$ Hz), 7.38 (1H, s), 7.28 (1H, d, $J = 8.0$ Hz), 3.40–3.25 (1H, m), 2.71–2.61 (2H, m), 1.26 (3H, d, $J = 7.3$ Hz).

4.1.39. 6-chloro-2-(3-chloropropyl)-2-methyl-2,3-dihydro-1H-inden-1-one, 12c and 6-chloro-2-(4-chlorobutyl)-2-methyl-2,3-dihydro-1H-inden-1-one (12d)

A solution of the 12b (3.32 mmol) in dry DMF was added dropwise to a stirred suspension of NaH (9.96 mmol, 60% in mineral oil previously washed with hexanes) in dry DMF. After the reaction mixture was stirred for an hour, dihalo-alkylhalide (1-bromo-3-chloropropane for 12c or 1-bromo-4-chlorobutane for 12d) (6.64 mmol) was added dropwise and stirred overnight. The reactions were quenched in water, extracted with CH_2Cl_2 , the organic layer washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to obtain the crude product which was used in the next step without further purification.

4.1.40. 6-chloro-2-methyl-2-(3-(4-(pyridin-2-yl)piperazin-1-yl)propyl)-2,3-dihydro-1H-inden-1-one (21)

The crude alkylating agent 12c was reacted with pyridinyl-piperazine under the general N-alkylation reaction conditions described above to afford compound 21 as a solid. Yield: 62%. Mp: 232–233.6 °C. ^1H NMR (300 MHz, CDCl_3) δ 8.17 (1H, d, $J = 4.7$ Hz), 7.67 (1H, d, $J = 8.2$ Hz), 7.45 (2H, d, $J = 8.7$ Hz), 7.35 (1H, d, $J = 7.9$ Hz), 6.62 (2H, d, $J = 8.1$ Hz), 3.51 (4H, s), 3.11 (1H, d, $J = 17.3$ Hz), 2.87 (1H, d, $J = 17.6$ Hz), 2.51 (4H, s), 2.33 (2H, d, $J = 7.3$ Hz), 2.07–1.99 (1H, m), 1.61 (3H, t, $J = 7.6$ Hz), 1.22 (3H, s). ^{13}C NMR (151 MHz, CD_3OD) δ 209.76, 154.72, 152.26, 145.04, 141.56, 136.75, 133.97, 128.15, 126.87, 125.00, 114.45, 113.15, 56.79, 50.37 (2C), 43.25, 43.23 (2C), 39.30, 34.19, 22.84, 19.15. Anal. $\text{C}_{22}\text{H}_{26}\text{ClN}_3\text{O} \cdot 2\text{HCl} \cdot \text{H}_2\text{O}$ (C, H, N).

4.1.41. 6-chloro-2-methyl-2-(4-(4-(pyridin-2-yl)piperazin-1-yl)butyl)-2,3-dihydro-1H-inden-1-one (22)

The crude alkylating agent 12d was reacted with pyridinyl-piperazine in a similar manner as described for 21 to afford under the general N-alkylation reaction conditions described 4.1.4 to

afford compound 22 as a solid. Yield: 52%. Mp: 214–215.9 °C; ^1H NMR (300 MHz, CDCl_3) δ 8.20–8.13 (1H, m), 7.70–7.62 (1H, m), 7.50–7.42 (2H, m), 7.38–7.22 (1H, m), 6.70–6.54 (2H, m), 3.52 (5H, t, $J = 4.8$ Hz), 3.09 (1H, d, $J = 17.5$ Hz), 2.85 (1H, d, $J = 17.5$ Hz), 2.50 (4H, t, $J = 5.2$ Hz), 2.39–2.27 (2H, m), 2.09–1.92 (1H, m), 1.68–1.47 (4H, m), 1.20 (3H, d, $J = 1.8$ Hz). ^{13}C NMR (151 MHz, CD_3OD) δ 210.38, 154.89, 145.04, 141.43, 136.76, 134.23 (2C), 128.06, 126.77, 124.85, 114.43, 113.15, 56.48, 50.32 (2C), 49.00, 43.24, 43.21.

4.2. Receptor binding affinity studies

Binding affinities reported in Tables 1–3 were conducted by the National Institute of Mental Health Psychoactive Drug Screening Program (NIMH-PDSP). Details of the methods and radioligands used for the binding assays were previously reported [43]. [^{35}S] GTP γ S binding.

The method from Newman-Tancredi et al. [44], was adapted for assessment of the functional status of compound 21 as follows: To prepare the membranes, five 15 cm plates of HEK-h5HT1A cells, 80–90% confluent, provided enough membranes for one assay plate (4 drug curves). GTP γ S assay buffer (20 mM HEPES, pH 7.4, 10 mM MgCl_2 , 100 mM NaCl, and 0.2 mM DTT) was used throughout the assay. The cells were scraped from the plates into buffer, centrifuged at 200 rpm for 15 min, the supernatant was removed, and the pellet was homogenized in 10 mL buffer/plate of cells. The homogenate was centrifuged at 17,500 rpm for 15 min, and the resulting pellet was washed 2 times by homogenization in 10 mL buffer and centrifugation to remove serotonin that was present in the growth medium. The supernatant was removed, and the final pellet from 5 plates was resuspended in 10 mL of assay buffer.

Cell membranes (40–75 μg protein) were preincubated (10 min, room temperature) with test compound in duplicate in assay buffer. The reaction was initiated by the addition of GDP (3 μM) and [^{35}S]GTP γ S (0.1 nM, 1350 Ci/mmol, PerkinElmer, ~150,000 cpm) in a final volume of 1 mL. The reaction was incubated for 60 min at room temperature on a rotating platform. Non-specific binding is defined with 1 μM WAY-100635. Agonist efficacy is expressed relative to that of 5-HT tested at a maximally effective concentration (100 nM) in each experiment. A dose response curve with the prototypical full agonist serotonin was conducted in each experiment to identify full and partial agonist compounds. Experiments were terminated by rapid filtration over Filtermat A with ice-cold saline using a Tomtec harvester and radioactivity determined using a PerkinElmer microbeta-plate counter. EC_{50} values are calculated with GraphPad Prism and maximal effect for a given drug each day is normalized to the maximal effect of serotonin.

4.3. HEK-5-HT $_{7A}$ /cAMP functional assay

Human embryonic kidney cells were transfected with 10 μg h5-HT $_{7A}$ cDNA using PEI in unsupplemented DMEM. After 5 h, cells were plated at a density of 200,000 cells per well in 48 well plates in DMEM supplemented with 10% charcoal-stripped FetalClone and pen-strep. The medium was removed about 18 h later. For agonist assays, 0.9 mL EBSS (116 mM NaCl, 22 mM glucose, 15 mM HEPES, 8.7 mM NaH_2PO_4 , 5.4 mM KCl, 1.3 mM CaCl_2 , 1.2 mM MgSO_4 , 1 mM ascorbic acid, 0.5 mM IBMX [3-isobutyl-1-methyl-xanthine] without BCS, pH 7.4 at 37 °C) was added. After 20 min, the compound was added in a final volume of 1 mL, and incubated for 20 min. For antagonists, 0.8 mL EBSS is added, cells were incubated for 10 min, the compound was added, and incubated for 10 min after which serotonin (100 nM) was added. For all conditions, after 20 min incubation with agonist, the reaction was terminated by aspiration of the buffer, and 0.1 mL trichloroacetic acid was added.

Plates were incubated for 2 h on a rotator. Adenylate cyclase activity was measured using a cyclic AMP EIA kit (Cayman). Aliquots (40 μl) of each well were diluted to 200 μl with EIA buffer from the kit, and 50 μl of the dilution was added to the EIA plate. After addition of tracer and monoclonal antibody, the EIA plates were incubated for 18 h at 4 °C. The reaction was aspirated, plates were washed $5 \times 300 \mu\text{l}$ with wash buffer, and Ellman's reagent was added. After a 2-h incubation in the dark on a rotator, the plates were read at 410 nm. Basal cAMP is subtracted from all values. 5-HT $_{7A}$ agonists stimulate cAMP formation, maximal stimulation was defined with 10 μM serotonin. The maximal drug effect is normalized to maximal serotonin effect in the tables. For antagonists, maximal inhibition of cAMP formation is defined with 10 μM lurasidone.

Data analysis

For functional assays, GraphPAD Prism was used to calculate either EC_{50} (agonists) or IC_{50} (antagonists) values using data expressed as pg cAMP for adenylate cyclase activity, % serotonin-stimulation for GTP γ S binding.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was financially supported by an NIH/NIGMS SCORE grant number 2SC1GM116724 and a Title III Grant to Florida A&M University. We also acknowledge the financial support from Anxiolytech toward the ongoing studies. The work was also supported in part by the NIH Biomedical Endowment Eminent Scholar Chair in Biomedical Sciences research grant to S.Y.A. Ki determinations and receptor binding assays were generously carried out by the National Institute of Mental Health's Psychoactive Drug Screening Program, Contract # HHSN-271-2013-00017-C (NIMH PDSP). The NIMH PDSP is directed by Bryan L. Roth MD, PhD at the University of North Carolina at Chapel Hill and Project Officer Jamie Driscoll at NIMH, Bethesda MD, USA. Funding sources acknowledged had no involvement in the study design, data collection, interpretation, article preparation and submission of this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ejmech.2021.113243>.

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