

Structure-activity profiling of alkaloid natural product pharmacophores against a *Schistosoma* serotonin receptor

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ABSTRACT

Serotonin (5-HT) is an important regulator of numerous aspects of flatworm biology, ranging from neuromuscular function to sexual maturation and egg laying. In the parasitic blood fluke *Schistosoma mansoni*, 5-HT targets several G-protein coupled receptors (GPCRs), one of which has been demonstrated to couple to cAMP and regulate parasite movement. This receptor, Sm.5HTR_L, has been successfully co-expressed in mammalian cells alongside a luminescent cAMP-biosensor, enabling pharmacological profiling for candidate anti-schistosomal drugs. Here, we have utilized this assay to perform structure-activity investigations of 143 compounds containing previously identified alkaloid natural product pharmacophores (tryptamines, aporphines and protoberberines) shown to regulate Sm.5HTR_L. These experiments mapped regions of the tryptamine pharmacophore amenable and intolerant to substitution, highlighting differences relative to orthologous mammalian 5-HT receptors. Potent Sm.5HTR_L antagonists were identified, and the efficacy of these compounds were evaluated against live adult parasites cultured *ex vivo*. Such structure-activity profiling, characterizing the effect of various modifications to these core ring systems on Sm.5HTR_L responses, provides greater understanding of pharmacophores selective for this target to aid future drug development efforts.

1. Introduction

Flatworm G-protein coupled receptors (GPCRs) are promising anthelmintic drug targets, given their role regulating movement, sexual maturation and egg laying (Patocka et al., 2014; Saberi et al., 2016; Chan et al., 2018; Hahnel et al., 2018). Over 200 million people worldwide are infected with the parasitic *Schistosoma* flatworms that cause the disease schistosomiasis (Hotez and Fenwick, 2009). This disease is largely treated by just one broad spectrum anti-schistosomal drug, praziquantel (PZQ) (Andrews et al., 1983). PZQ has been enormously beneficial in mitigating morbidity due to schistosomiasis and moving towards control of this disease, but drawbacks include PZQ's ineffectiveness against immature parasites (Sabah et al., 1986), frequent side effects (Coulibaly et al., 2017), and cure rates that are rarely 100% effective (Olliaro et al., 2011; Coulibaly et al., 2017). Reliance on a single compound also raises the concern of emerging drug resistance. Therefore, there is a need for new anti-schistosomal drugs, either to supplement PZQ or to serve as an alternative in the event of treatment failure.

In the past several years, several studies have prioritized flatworm GPCRs as targets for drug development. RNAi and pharmacological approaches have identified serotonin (5-HT) GPCRs that control flatworm movement (Patocka et al., 2014; Chan et al., 2015), and GPCRs have also been implicated in flatworm sexual maturation and egg laying (Lu et al., 2016; Saberi et al., 2016; Wang et al., 2017; Chan et al., 2018). Additionally, while the parasite target(s) of PZQ remains undefined, our recent work has shown that the active R-PZQ enantiomer acts as a GPCR ligand, engaging human serotonergic GPCRs that regulate mesenteric vessel tone (Chan et al., 2017). Precedent for engagement of GPCRs by PZQ should prioritize development of scalable functional assays to study flatworm GPCRs in more detail. One such approach centers upon co-expression of G_{as}/G_{ai} coupled GPCRs with GloSensor, a modified luciferase reporter which detects changes in cellular cAMP levels. This assay provides a high sensitivity and real-time readout of GPCR activity that can be scaled to miniaturized format. Previously, we optimized this methodology to enable pharmacological profiling of a schistosome serotonergic GPCR (Sm.5HTR_L) that controls worm movement (Patocka et al., 2014; Chan et al., 2016c).

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This approach implicated several classes of natural product heterocyclic alkaloid scaffolds as regulators of Sm.5HTR_L activity (Chan et al., 2016b, 2016c, 2018). As natural products have a proven track record as leads for drug development (Newman and Cragg, 2012), we subsequently performed a more extensive analysis of structure-activity relationships for ergot alkaloids (Chan et al., 2018), and here in this study, tryptamine, aporphine and protoberberine scaffolds at this parasite GPCR. As these compounds are known regulators of mammalian 5-HT receptors (Cabedo et al., 2009; Harding, 2016), the goal is to understand pharmacophore features that determine selectivity and potency for Sm.5HTR_L to identify effective small molecule tools useful for probing schistosome biology as well as potential leads for anthelmintic development.

2. Materials and methods

Chemicals. A complete list of chemical structures and vendors for the compounds used in this work is provided in Supplemental Table 1. Compounds were selected from libraries sourced from the National Cancer Institute (NCI Natural Products Set IV) as well as commercial vendors (TimTec Natural Product Library and NDL-3000 Natural Derivatives Library). Individual compounds were also sourced from various vendors (Tocris, Sigma Aldrich, Santa Cruz, Pharmeks and Abcam, see Supplemental Table 1 for catalog numbers) identified through searches of the ZINC and PubChem databases. Finally, several aporphine compounds were kindly provided by Wayne Harding (CUNY). The synthesis and activity profile of these compounds against mammalian bioaminergic receptors has been reported elsewhere (Chaudhary et al., 2009, 2011; Kapadia and Harding, 2015).

Cell culture. HEK-293 cells (ATCC CRL-1573, authenticated by STR profiling) were cultured in growth media consisting of DMEM supplemented with GlutaMAX (Gibco cat # 10566016), 10% heat inactivated fetal bovine serum and penicillin-streptomycin (100 units/mL, ThermoFisher). GPCR functional assays were performed as described in (Chan et al., 2018). The coding sequence for Sm.5HTR_L (GenBank accession number KX150867) was codon optimized for mammalian expression and sub-cloned into pcDNA3.1 (–) between *NotI* and *EcoRI*. Stable cell lines for the GloSensor-22F cAMP Plasmid (Promega) and schistosome 5HTRs were selected with hygromycin (200 µg/mL) and G418 (400 µg/mL). These lines were then cultured in DMEM supplemented with GlutaMAX, Pen-Strep, and 10% dialyzed FBS.

cAMP luminescence assays. Stable cell lines expressing the GloSensor-22F construct or both GloSensor-22F and Sm.5HTR_L were cultured in T-75 flasks. The day prior to performing assays, cells were trypsinized (TrypLE Express, Gibco) and plated in solid white 96 well plates (Costar

cat # 3917) at a density of 2.5×10^4 cells/well. One day later media was removed and replaced with assay buffer (HBSS buffered with HEPES (20 mM, pH 7.4) + BSA (0.1% w/v) and D-luciferin sodium salt (1 mg/mL, Gold Biotechnology). Plates were equilibrated at room temperature for 1 h, the phosphodiesterase inhibitor 3-Isobutyl-1-methylxanthine (IBMX 200 µM, Sigma Aldrich) was added, and plates were equilibrated for a further 30 min prior to test compound addition. Test compounds were added and luminescence was read for 45 min on a GloMax[®]-Multi Detection System plate reader (Promega) to identify agonist responses. Plates were removed, and a maximal concentration of serotonin (500 nM) was added to each well. Plates were immediately returned to the plate reader and read for a further 45 min to identify compounds with antagonist activity.

Adult schistosome mobility assays. Female Swiss Webster mice infected with *S. mansoni* cercariae (NMRI strain) were sacrificed 49 days post infection by CO₂ euthanasia. Adult schistosomes were recovered by dissection of the mesenteric vasculature. Harvested schistosomes were washed in RPMI 1640 supplemented with HEPES (25 mM), 5% heat inactivated FBS (Gibco) and penicillin-streptomycin (100 units/mL). Worms were cultured overnight (37 °C/5% CO₂) in vented 100 × 25 mm petri dishes (ThermoFisher cat # 4031) containing 50 mL of media. Movement assays were performed the next day. Male worms were transferred to a six well dish (4–5 individual worms per well) containing 3 mL media. For agonist assays, worms were incubated for 5 min in various concentrations of drug prior to acquiring videos of worm movement. For antagonist assays, worms were incubated for 1 h in test compound, and then a maximal concentration of serotonin (200 µM) was added before videos were acquired. Imaging was performed using a Zeiss Discovery v20 stereomicroscope and a QiCAM 12-bit cooled color CCD camera controlled by Metamorph imaging software (version 7.8.1.0). Recordings (1 min) were acquired at 4 frames per second, saved as an image stack, and movement analyzed using ImageJ software as described in (Chan et al., 2016c). Data represents mean ± standard error for ≥ 3 independent experiments. Animal work was carried out with the oversight and approval of the Laboratory Animal Resources facility at the Medical College of Wisconsin. All animal experiments followed ethical regulations approved by the Medical College of Wisconsin IACUC committee.

3. Results

The schistosome serotonin (5-HT) receptor Sm.5HTR_L was expressed in mammalian HEK-293 cells alongside the GloSensor 22-F cAMP luminescent reporter (Chan et al., 2016a, 2016c). Addition of 5-HT caused a rapid increase in cAMP-dependent luminescence in cells

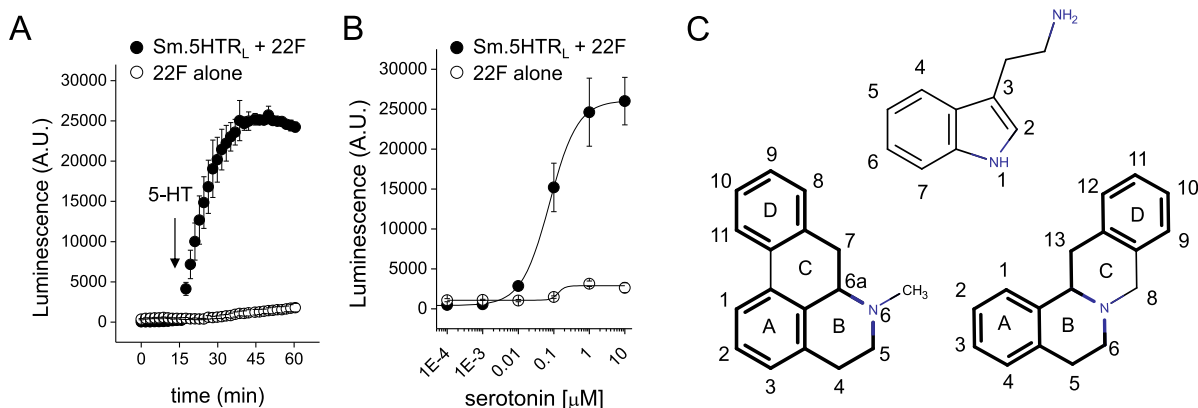


Fig. 1. Functional expression of Sm.5HTR_L. (A) Kinetics and (B) dose-response curves for 5-HT evoked luminescence in Sm.5HTR_L expressing cells (solid symbols) and a negative control cell line lacking Sm.5HTR_L (open symbols). (C) Pharmacophores assayed for activity against Sm.5HTR_L. Ring systems are shown with numbering referred to throughout this manuscript. Clockwise from top - tryptamine, protoberberine, aporphine (benzylisoquinoline scaffold highlighted in bold).

expressing Sm.5HTR_L (Fig. 1A). This increase was dose-dependent (EC₅₀ 94 ± 18 nM) and not observed in cells expressing the GloSensor 22-F reporter alone (Fig. 1B). The sensitivity and selectivity of this response was explored for three classes of heterocyclic scaffolds – tryptamine derivatives, aporphines and protoberberines. The core structures of these groups, and scaffold numbering nomenclature, are shown in Fig. 1C.

3.1. Modifications of the tryptaminergic pharmacophore impair agonist activity Sm.5HTR_L

First, various tryptamine derivatives were selected based on their activity at mammalian serotonergic GPCRs: the majority of which were substituted at the terminal amine and/or the C-5 position. For example, 5-carboxamidotryptamine (5-CT) and 5-methoxytryptamine (5-MeOT) bind to and are potent full agonists at the mammalian 5-HT₇ receptors (Bard et al., 1993; Shen et al., 1993) that are orthologous to Sm.5HTR_L (Patocka et al., 2014; Chan et al., 2016c). N-methylserotonin, methylated at the terminal nitrogen, is also a full agonist at mammalian 5-HT₇ receptors (Powell et al., 2008).

In the Sm.5HTR_L activity assay, the terminal amine of 5-HT accommodated the addition of methyl groups: both N-methylserotonin (N-MS) and bufotenine (5-OH-DMT) elevated cAMP with an E_{max} of 113 ± 28% and 99 ± 34% relative to 5-HT, respectively (Fig. 2A). However, while the single methyl substitution on N-MS had little effect on potency (EC₅₀ 154 ± 11 nM, compared to 94 ± 18 nM for 5-HT), the dimethyl substitution on 5-OH-DMT decreased potency by an order of magnitude (EC₅₀ 2.4 ± 0.2 μM). α-methylserotonin (αMS), methylated at the α position of the ethylamine side chain, was a low potency, partial agonist (E_{max} of 59 ± 2% relative to 5-HT; Fig. 2A).

In contrast, the 5-OH group present on serotonin was poorly tolerant to modification. Tryptamine ('T', Fig. 2B), which is unsubstituted at the C-5 position, displayed negligible activity at Sm.5HTR_L. Substitutions at the C-5 position with amide or methoxy groups to produce 5-carboxamidotryptamine (5-CT) and 5-methoxytryptamine (5-MeOT) respectively, were poorly tolerated. Both compounds were inactive at Sm.5HTR_L (Fig. 2B). Smaller C-5 substitutions, as seen in 5-methyltryptamine (5-MT) or 5-bromotryptamine (5-BrT), were better tolerated as these compounds displayed partial agonist activity (Fig. 2B). All compounds tested harboring dual substitutions at both the C-5 and terminal amine positions compared with 5-HT were inactive at Sm.5HTR_L. These analogs included the psychedelic tryptamine derivatives N,N-diallyl-5-methoxy tryptamine (5-MeO-DALT), 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT), N-methyltryptamine (N-MT) and N,N-dimethyltryptamine (DMT, Fig. 2C). None of the compounds identified as Sm.5HTR_L agonists displayed activity against HEK-293 cells expressing the GloSensor-22F reporter alone (Supplemental Fig. 1).

3.2. Functional characteristics of the aporphine alkaloid scaffold at Sm.5HTR_L

The aporphine alkaloids are tetracyclic benzyloquinolines that exhibit a range of activities at mammalian bioaminergic GPCRs (Cabedo et al., 2009; Harding, 2016). Numerous naturally occurring (Indra et al., 2002; Farrell et al., 2016) and synthetic (Linnanen et al., 2001; Chaudhary et al., 2009, 2011; Kapadia and Harding, 2015) aporphines are potent antagonists of mammalian serotonergic receptors, and preliminary structure-activity profiling of this chemical series has been performed on Sm.5HTR_L (Chan et al., 2016a). Based on these data, we expanded our study to encompass 89 aporphine structures.

Compounds containing the aporphine core ring structure were curated from the NCI and TimTec natural product libraries (Supplemental Table 1) and screened at an initial concentration of 10 μM. None of the tested aporphines showed agonist activity. Full concentration-

inhibition curves were generated from hits that blocked 5-HT evoked cAMP generation, as well as related analogs with substitutions to provide insight into structure-activity relationships at Sm.5HTR_L. In total, concentration-response curves were generated for 83 aporphines, and this compound series demonstrated a gradation of inhibitory potencies at Sm.5HTR_L (Fig. 3A). A subset of 9 ligands (Fig. 3A) were identified with an IC₅₀ ≤ 500 nM (inhibition of 500 nM 5-HT, a maximal concentration) and calculated affinity, pK_i ≥ 7.0, estimated using the Cheng-Prusoff equation. Another 20 aporphines displayed pK_i values between 6.9 and 6.0 (IC₅₀ < 5.5 μM, Fig. 3A).

Potent compounds clustered in several chemical series (Fig. 3A, inset). These analogs typically possessed methoxy modifications on aporphine rings A (C-1, C-2 or C-3 position) or D (C-9 and/or C-10 position) – either as “open” (as seen with nuciferine, compound 4b) or “closed” dioxolane ring groupings (as seen with nantenine, compound 2a). Other substitutions were identified that were poorly tolerated. These included, (i) addition of bulky substituents to the aporphine nitrogen on ring B (Fig. 3B), (ii) unsaturation of the ring C, C-6a – C-7 bond: D-glaucine and L-nuciferine were antagonists with respective pK_is of 6.8 and 7.1, but dehydroglaucine and dehydronuciferine were both inactive (Fig. 3C) and (iii) substitution of the C-3 position on ring A (Fig. 3D).

3.3. Functional characteristics of the protoberberine scaffold at Sm.5HTR_L

Protoberberines are another class of tetracyclic benzyloquinolines that display antagonism at Sm.5HTR_L. Rotundine is a constituent of traditional Chinese herbal medicine preparations, with activity against both cestodes (Das et al., 2009; Gogoi and Yadav, 2017) and schistosomes (Chan et al., 2016c). We previously identified racemic D/L-rotundine as an antagonist at Sm.5HTR_L and showed this compound inhibited movement of adult worms (Chan et al., 2016c). Given these observations, we explored the structure-activity profile of protoberberines against Sm.5HTR_L in greater detail, screening 28 molecules (Supplemental Table 1), with a focus on structures sharing a high degree of similarity with rotundine (Fig. 4A).

Racemic D/L-rotundine was a potent antagonist at Sm.5HTR_L (pK_i 7.2). This activity was due to the L-rotundine enantiomer, as D-rotundine was inactive (Fig. 4B&C). Palmatine, which resembles rotundine but contains a quaternary amine (Fig. 4A), was also inactive (Fig. 4B&C). Closure of ring substituent methoxy groups into dioxolane rings also resulted in a loss of activity, as observed comparing rotundine to canadine or tetrahydrocoptisine. Shifting the placement of the D-ring methoxy groups (rotundine versus xylopinine) also decreased potency at Sm.5HTR_L. Several compounds screened possessed an open protoberberine C-ring, retaining the benzyloquinoline core (laudanosine, papaverine, papaveraldine). None of these showed any inhibition at Sm.5HTR_L (Supplemental Table 1). Again, none of the benzyloquinoline compounds that inhibited 5-HT evoked luminescence in Sm.5HTR_L expressing cells blocked forskolin-evoked signals, indicating that drug action was not due to toxicity or other mechanisms inhibiting cAMP generation (Supplemental Fig. 1).

3.4. Sm.5HTR_L ligands alter the mobility of adult schistosomes cultured *ex vivo*

Sm.5HTR_L is required for worm movement (Patocka et al., 2014) and Sm.5HTR_L ligands can stimulate or block movement in larval schistosomes and adult worms (Chan et al., 2016a, 2016c). Therefore, we examined the effects of several substituted tryptamines (Sm.5HTR_L agonists) and benzyloquinolines (Sm.5HTR_L antagonists) against adult schistosomes to assess whether these hits were active and whether they recapitulated structure-activity profiles observed against Sm.5HTR_L *in vitro*.

Tryptaminergic Sm.5HTR_L agonists stimulated worm movement, although with decreased potency relative to results from *in vitro* cAMP

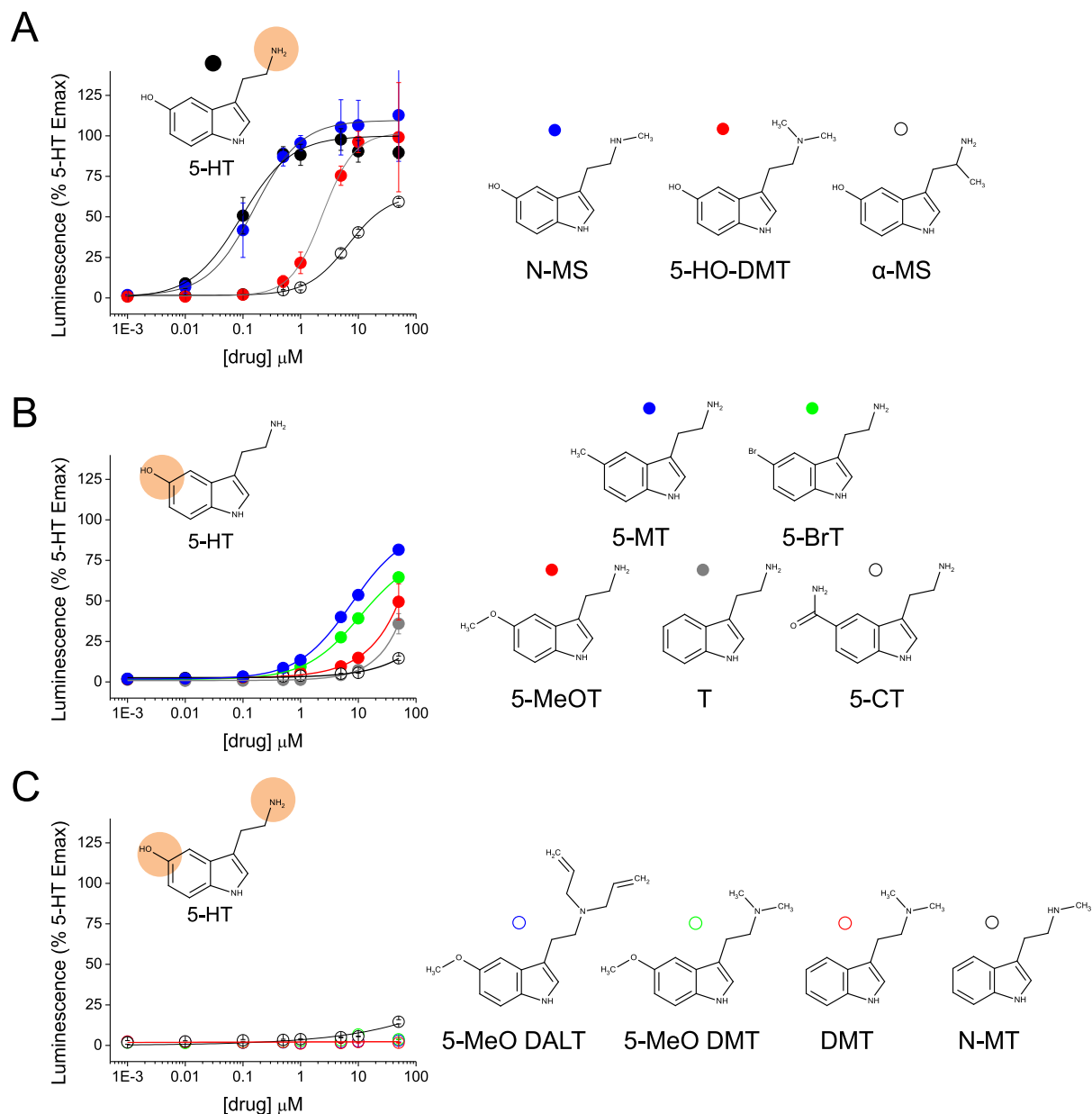


Fig. 2. Structure-activity relationship of tryptamine derivatives at Sm.5HTR_L. (A) Action of tryptamine analogs with modifications to the ethylamine group highlighted on the inset structure of serotonin (5-HT). N-MS = N-methylserotonin, 5-HO-DMT = bufotenine, α MS = α -methylserotonin. (B) Effect of analogs with modifications to the highlighted tryptamine C-5 position of 5-HT (5-MT = 5-methyltryptamine, 5-BrT = 5-bromotryptamine, 5-MeOT = 5-methoxytryptamine, T = tryptamine, 5-CT = 5-carboxamidotryptamine), or (C) both the C-5 and terminal amine groups (5-MeO DALT = N,N-diallyl-5-methoxytryptamine, 5-MeO DMT = 5-methoxy-N,N-dimethyltryptamine, DMT = N,N-Dimethyltryptamine, N-MT = N-Methyltryptamine). Values expressed relative to the maximal 5-HT response (scaled to 100% on all Y-axes).

assays (Fig. 5A). Consistent with assays on Sm.5HTR_L, substitution of the terminal amine was tolerated. N-methylserotonin (N-MS) was a full agonist increasing movement by $252 \pm 9\%$, comparable to the 5-HT evoked-increase of $224 \pm 17\%$. The indole C-5 position was amenable to slight modifications. 5-methyltryptamine (5-MT) stimulated movement, but only at high concentrations in the hundreds of micromolar. 5-methoxytryptamine (5-MeOT) stimulated movement, which contrasts with a lack of agonist activity at Sm.5HTR_L *in vitro* (Figs. 5A and 2B). 5-Carboxamidotryptamine (5-CT) did not stimulate movement, consistent with this compound being inactive at Sm.5HTR_L. DMT, which is modified at both the indole C-5 and terminal amine, also had no effect on worm movement, mirroring the observed lack of activity at Sm.5HTR_L.

Next, the aporphine and protoberberine Sm.5HTR_L antagonists were

tested on live worms to assess whether these compounds inhibited 5-HT evoked movement. Worms were incubated in compound for 1 h, after which a maximal dose of serotonin (200 μM) was added to stimulate motility. Several aporphines resembling nuciferine were studied. Nuciferine was the most potent antagonist of worm movement, inhibiting 5-HT evoked movement at concentrations as low as 100 nM (Fig. 5B). Compound phar025462, a nuciferine derivative with a charged nitrogen and a closed dioxolane ring in place of methoxy groups, and compound 785177, which substitutes the nuciferine A-1 methoxy with an hydroxy group, both inhibited 5-HT evoked movement, albeit with lower potency in the 1–10 μM range (Fig. 5B). The compound MDO-NPA, which potently inhibited Sm.5HTR_L ($\text{pK}_i = 7.0$), was relatively inactive on adult worms with inhibition of 5-HT not

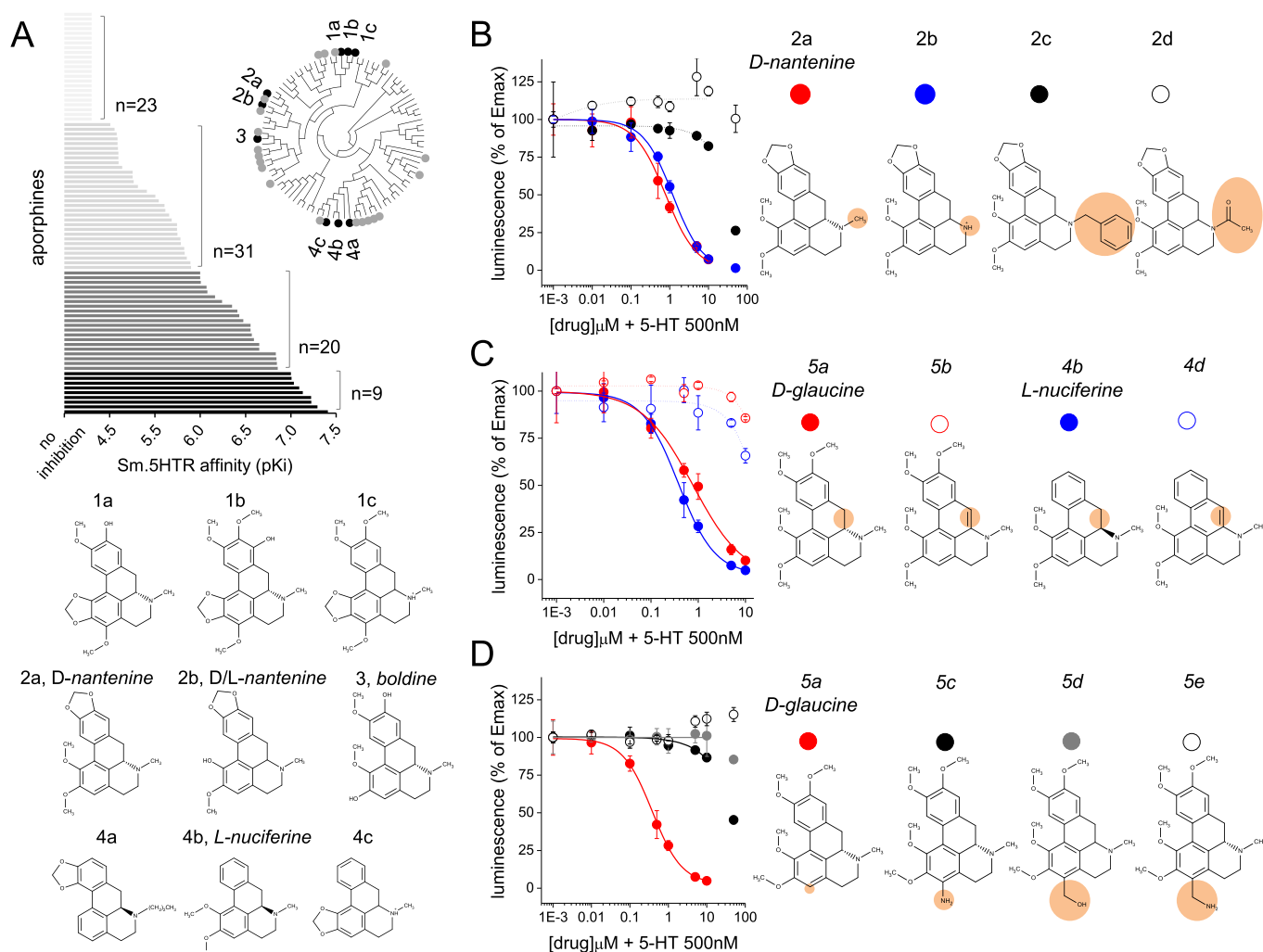


Fig. 3. Structure-activity relationship of aporphine alkaloids at Sm.5HTR_L. (A) Affinity of 84 aporphines screened against Sm.5HTR_L, with pKi estimated using the Cheng-Prusoff equation. Inset - tree resulting from hierarchical clustering of structures, with most potent hits (pKi > 7.0) denoted with black symbols, and hits with pKi between 6.0 and 6.9 denoted with gray circles. Bottom – structures of the nine most potent compounds. (B) Structure activity profile of a nantenine-like aporphine series with modifications to the basic B-6 nitrogen. (C) Effect of dehydrogenation of the C-6a/C-7 bond of either D-glucaine or L-nuciferine. (D) Effect of various modifications to the D-glucaine A4 position resulting in a loss of activity at Sm.5HTR_L.

surpassing 50% even at 10 μ M (Fig. 5B).

Of the protoberberines, the Sm.5HTR_L antagonist L-rotundine, the inactive analog D-rotundine and the closely related palmatine were screened on live worms. As expected, L-rotundine inhibited 5-HT evoked movement at concentrations ≥ 1 μ M, while D-rotundine and palmatine showed no inhibition of 5-HT evoked movement at concentrations as high as 50 μ M (Fig. 5C).

4. Discussion

These data highlight the structure-activity profile of tryptamine- and benzyloquinoline-based analogs on a schistosome 5-HT receptor (Sm.5HTR_L) that controls worm movement. 5-HT has long been recognized as an important regulator of schistosome neuromusculature (Hillman et al., 1974; Tomosky et al., 1974; Patocka et al., 2014), and 5-HT receptors have been advanced as appealing targets for anthelmintic drug development (Mansour, 1979, 1984; Geary et al., 1992). The successful functional expression and miniaturization of a Sm.5HTR_L profiling assay has enabled high-throughput compound screens to identify ligands with potential anti-schistosomal activity (Chan et al., 2018). Given these data validating Sm.5HTR_L as a viable anti-schistosomal drug target, and the precedent of both tryptaminergic

and benzyloquinoline natural products as serotonergic ligands (Cabedo et al., 2009; Nichols, 2012; Harding, 2016) and antiparasitic compounds (Bonne, 1919; Iwasa et al., 1999; Nkwengoua et al., 2009; Das et al., 2009; Shahinas et al., 2012; Gogoi and Yadav, 2017), we performed a more detailed exploration of the properties of these pharmacophores that determine their efficacy as Sm.5HTR_L ligands and activity against *ex vivo* cultured parasites.

4.1. Tryptaminergic ligand SAR at Sm.5HTR_L

Substituted tryptamines with modifications to the C-5 and terminal amine often display agonist activity against mammalian serotonergic receptors (Plassat et al., 1993; Shen et al., 1993; Shulgin, 1997; Powell et al., 2008; Nichols, 2012). Compounds with subtle modifications to the terminal amine retained agonist activity at Sm.5HTR_L (Fig. 2A), but the pharmacophore was poorly tolerant to substitution at the C-5 position (Fig. 2B). The poor activity of 5-CT and 5-MeOT at Sm.5HTR_L contrasts with the action of these ligands as full agonists at the orthologous human 5-HT₇ receptor (Plassat et al., 1993). Given that the amino acid sequence of Sm.5HTR_L shares just 40% similarity with the human ortholog, it is not surprising that the two GPCRs exhibit unique pharmacological sensitivities. A possible reason for the inactivity of the

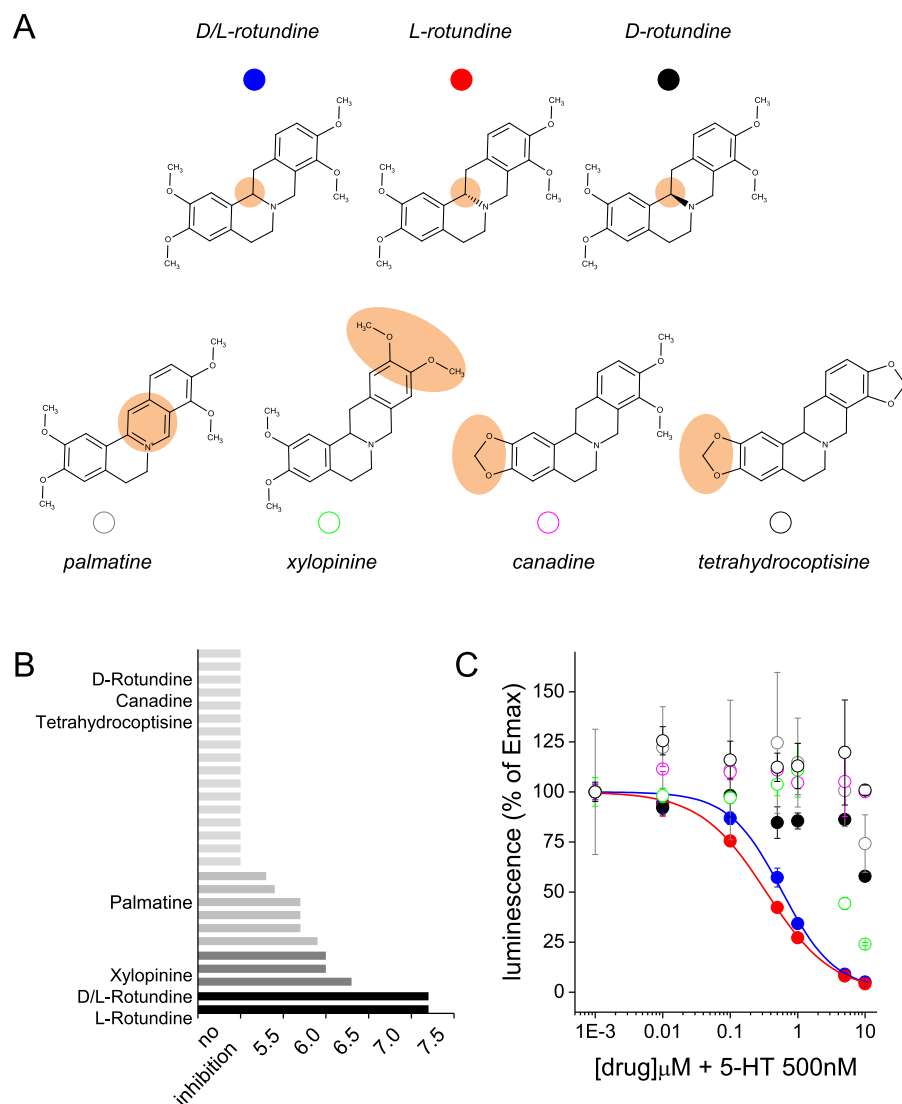


Fig. 4. Structure-activity relationship of protoberberine alkaloids at Sm.5HTR_L. (A) Structures of various protoberberines with structural similarity to the Sm.5HTR_L antagonist D/L-rotundine. (B) Affinity of protoberberine ligands at Sm.5HTR_L, pK_i estimated using the Cheng-Prusoff equation (structures and pK_i for all protoberberines screened are listed in Supplemental Table 1). (C) Concentration response curves for rotundine (racemate = solid blue symbol, L-enantiomer = solid red symbol, D-enantiomer = solid black symbol) and related compounds (symbols pair with structures shown in A.). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

C-5 substituted tryptamines at Sm.5HTR_L is that mutation of the glutamic acid at the 7.35 position of the human 5-HT₇ receptor abrogates 5-CT binding and function (Impellizzeri et al., 2015). This site varies in the schistosome receptors (phenylalanine), as well as the invertebrate *C. elegans* SER-7 receptor (aspartic acid) which is also refractory to 5-CT (Hobson et al., 2006). This difference, as well as the finding that neither DMT or related derivatives were active on Sm.5HTR_L (Fig. 2C), despite their activity on mammalian serotonergic receptors (Shen et al., 1993; Keiser et al., 2009; Ray, 2010; Cozzi and Daley, 2016), advances our pharmacological understanding of the unique pharmacological signatures of host and parasite receptors. Other examples include the ergot alkaloid ergotamine, a potent antagonist at the human 5-HT₇ receptor (Wacker et al., 2013) but full agonist at Sm.5HTR_L (Chan et al., 2018), as well as the commonly used 5-HT₇ chemical probe SB-269970, which is inactive at Sm.5HTR_L (Chan et al., 2016c). These data emphasize the need for considerable caution when using pharmacological probes developed against mammalian targets to study invertebrate GPCRs, while revealing opportunities for developing parasite-selective ligands.

4.2. Determinants of benzyloquinoline antagonism of schistosome serotonin receptors

Our previous work identified the benzyloquinolines rotundine (Chan et al., 2016c) and nuciferine (Chan et al., 2016a) as potent

antagonists at Sm.5HTR_L and inhibitors of schistosome movement prompting exploration of compounds containing these ring systems in further detail. A thorough analysis of 117 benzyloquinoline structures identified only antagonist hits. The strictly antagonist profile associated with this scaffold (Figs. 3 and 4) contrasts with the agonist/partial agonist profile of the tested tryptamine derivatives (Fig. 2) and the mixed agonist/antagonist profile of ergot alkaloids reported elsewhere (Chan et al., 2018).

Of the aporphine compounds tested, the most potent antagonists had a 1,2,9,10-substituted pattern, with methoxy groups or a dioxolane ring attached to the A and D rings of the aporphine core (nantenine, boldine, D-glaucine). The stereochemistry of the B6-B6a bond did not seem crucial –the *levo* structure L-nuciferine and *dextro* structures D-glaucine, boldine and nantenine were all potent antagonists (Fig. 3). However, saturation of the C ring at this position was important; dehydrogenation of the C-6a – C-7 bond of either D-glaucine or L-nuciferine resulted in a loss of activity (Fig. 3C). In addition to nuciferine, boldine and D-glaucine, which were previously identified as Sm.5HTR_L antagonists (Chan et al., 2016a), we have shown that nantenine and ocoteine-like aporphines are also potent Sm.5HTR_L antagonists (pK_i > 7.0, Fig. 3).

The protoberberine skeleton is similar to the aporphine skeleton – both share a benzyloquinoline core. Consequently, it is unsurprising that like the aporphines, all active protoberberine compounds were

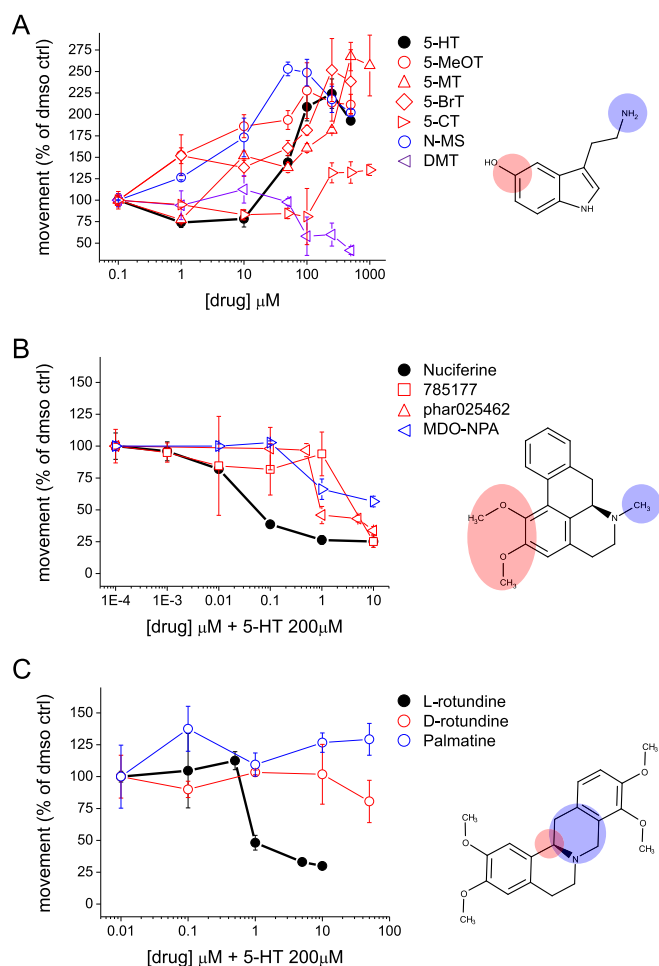


Fig. 5. Efficacy of tryptamine and benzyloquinoline ligands on adult *S. mansoni* cultured *ex vivo*. (A) Quantified changes in movement of adult parasites exposed to escalating doses of 5-HT (black, bold) and various other substituted tryptamine derivatives. Right – structure of serotonin, with highlighted regions corresponding to substituted regions at the C-5 position (red; 5-MeOT, 5-MT, 5-BrT, 5-CT), or the terminal amine (blue, N-MS). (B) Effect of various concentrations of nuciferine-like aporphines on 5-HT evoked movement. Right – structure of nuciferine, with highlighted regions corresponding to substituted regions at the 1,2 positions of the A ring (red, compound 785177 and phar025462) and methyl group on the B ring (blue, MDO-NPA). (C) Effect of various concentrations of protoberberines on 5-HT evoked movement. Right – structure of L-rotundine, with highlighted regions corresponding to the stereocenter in the protoberberine core and C-ring substitutions on D-rotundine and palmatine. SMILES for all compounds are provided in [Supplementary Table 1](#). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

antagonists. However, unlike the aporphines, the chirality of the stereocenter adjacent to the nitrogen atom seems crucial. For the antagonist D/L-rotundine, the L-rotundine enantiomer was active and D-rotundine displayed no activity at Sm.5HTR_L (Fig. 4). This stereochemistry mirrors rotundine's antagonism of mammalian bioaminergic GPCRs, where L-rotundine is the active enantiomer antagonizing mammalian dopamine D₁ (Mantsch et al., 2007) and serotonin 5HT_{1A} receptors (Mi et al., 2017). Like the active aporphines, L-rotundine possesses methoxy groups on the A and D rings. However, in the case of the protoberberines, formation of dioxolane rings – closing the methoxy positions on the A ring to produce canadine, or both the A and D rings to produce tetrahydrocopsitine – resulted in inactive compounds. The structure of xylopinine is identical to rotundine except for the movement of one methoxy from the D9 to the D11 position, but xylopinine

was ~10 times less potent than rotundine, indicating that the stereochemistry of the methoxy groups are critical. This again differs from the aporphines, which accommodated various methoxy group placement around the A and D rings.

4.3. Correlation of Sm.5HTR_L pharmacology with worm movement *ex vivo*

Schistosome movement is regulated by serotonergic signaling through Sm.5HTR_L (Patocka et al., 2014), and there is a correlation between drug activity at this receptor and movement of parasites cultured *ex vivo*, with Sm.5HTR_L agonists stimulating movement and Sm.5HTR_L antagonists inhibiting movement (Chan et al., 2016a, 2016c, 2018). Tryptaminergic ligands were agonists at Sm.5HTR_L, and substitutions that eliminated activity at Sm.5HTR_L also eliminated the ability of the same compounds to stimulate worm movement (5-CT and DMT, Fig. 2B&C and Fig. 5A). Similarly, substitutions that were tolerated in Sm.5HTR_L cAMP assays were also generally tolerated in movement assays. For example, N-MS (differing from 5-HT by addition of a methyl group to the terminal amine) mirrored 5-HT in both assays. Modification of the tryptamine C-5 position resulted in compounds that were displayed impaired activity relative to 5-HT. Several compounds stimulated movement of worms *ex vivo*, but were inactive against Sm.5HTR_L. For example, 5-MeOT did not potently activate Sm.5HTR_L (Fig. 2B), but potently stimulated movement in adult worms (Fig. 5A). While these differences may reflect differences between Sm.5HTR_L function in live worms versus a mammalian expression system, an alternative interpretation is that 5-MeOT stimulates movement through other serotonin/bioaminergic GPCRs (Zamanian et al., 2011). This explanation can be tested as other schistosome GPCRs become amenable to functional profiling.

Reciprocally some compounds that are active against Sm.5HTR_L show poor efficacy in *ex vivo* worm movement assays, perhaps due to pharmacokinetic variables such as whether the ligand is free to diffuse through the parasite tegument, is a substrate for active transporters such as SERT (Patocka and Ribeiro, 2007), or is degraded by worm enzymes such as monoamine oxidase (Nimmo-Smith and Raison, 1968). These variables may account for the right shifted dose response curves of serotonin in cell based cAMP assays (Fig. 1A) relative to worm movement assays (Fig. 5A). Similarly, benzyloquinoline Sm.5HTR_L antagonists were less potent on worms (Fig. 5B&C) than in cell-based cAMP assays (Figs. 3 and 4).

Human and parasite bioaminergic GPCRs are proven druggable targets (Evans and Gee, 1980; Hauser et al., 2017), and exploration of bioaminergic targets is a promising route for novel anti-schistosomal drug development (Wang et al., 2017; Camicia et al., 2018; Chan et al., 2018; Hahnel et al., 2018). One barrier to this work has been the functional expression of schistosome GPCRs in scalable assays that permit their thorough pharmacological characterization. While part of this difficulty may stem from the divergence of schistosome GPCRs relative to established model organisms (Sm.5HTR_L has a 25% and 24% amino acid identity to human 5-HT₇ and *C. elegans* SER-7, respectively), this lack of conservation may also offer the opportunity to achieving a degree of pharmacological selectivity. This is supported by the structure-activity profiles outlined here, which highlight specific modifications that confer affinity as well as selectivity between worm and human orthologs. Expanding this approach to other schistosome GPCRs and other bioactive pharmacophores will be informative for future anthelmintic pharmacophore development and drug discovery initiatives.

Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijpddr.2018.09.001>.

References

- Andrews, P., Thomas, H., Pohlke, R., Seubert, J., 1983. Praziquantel. *Med. Res. Rev.* 3, 147–200.
- Bard, J.A., Zgombick, J., Adham, N., Vaysse, P., Branchek, T.A., Weinshank, R.L., 1993. Cloning of a novel human serotonin receptor (5-HT7) positively linked to adenylate cyclase. *J. Biol. Chem.* 268, 23422–23426.
- Bonne, C., 1919. Has emetine any influence on the schistosomes? *Trans. R. Soc. Trop. Med. Hyg.* 12, 82–84.
- Cabedo, N., Berenguer, I., Figadere, B., Cortes, D., 2009. An overview on benzylisoquinoline derivatives with dopaminergic and serotonergic activities. *Curr. Med. Chem.* 16, 2441–2467.
- Camicia, F., Celentano, A.M., Johns, M.E., Chan, J.D., Maldonado, L., Vaca, H., Di Siervi, N., Kamentezky, L., Gamo, A.M., Ortega-Gutierrez, S., Martin-Fontecha, M., Davio, C., Marchant, J.S., Rosenzvit, M.C., 2018. Unique pharmacological properties of serotonergic G-protein coupled receptors from cestodes. *PLoS Neglected Trop. Dis.* 12, e0006267.
- Chan, J.D., Agbedanu, P.N., Grab, T., Zamanian, M., Dosa, P.I., Day, T.A., Marchant, J.S., 2015. Ergot alkaloids (Re)generate new leads as antiparasitics. *PLoS Neglected Trop. Dis.* 9, e0004063.
- Chan, J.D., Acharya, S., Day, T.A., Marchant, J.S., 2016a. Pharmacological profiling an abundantly expressed schistosome serotonergic GPCR identifies nuciferine as a potent antagonist. *Int. J. Parasitol. Drugs Drug Resist.* 6, 364–370.
- Chan, J.D., Grab, T., Marchant, J.S., 2016b. Kinetic profiling an abundantly expressed planarian serotonergic GPCR identifies bromocriptine as a perdurant antagonist. *Int. J. Parasitol. Drugs Drug Resist.* 6, 356–363.
- Chan, J.D., McCorvy, J.D., Acharya, S., Johns, M.E., Day, T.A., Roth, B.L., Marchant, J.S., 2016c. A miniaturized screen of a schistosome mansoni serotonergic G protein-coupled receptor identifies novel classes of parasite-selective inhibitors. *PLoS Pathog.* 12, e1005651.
- Chan, J.D., Cupit, P.M., Gunaratne, G.S., McCorvy, J.D., Yang, Y., Stoltz, K., Webb, T.R., Dosa, P.I., Roth, B.L., Abagyan, R., Cunningham, C., Marchant, J.S., 2017. The anthelmintic praziquantel is a human serotonergic G-protein-coupled receptor ligand. *Nat. Commun.* 8, 1910.
- Chan, J.D., Day, T.A., Marchant, J.S., 2018. Coalescing Beneficial Host and Deleterious Antiparasitic Actions as an Antischistosomal Strategy. *eLife* In Press.
- Chaudhary, S., Pecic, S., Legendre, O., Navarro, H.A., Harding, W.W., 2009. (+/-)-Nantenine analogs as antagonists at human 5-HT(2A) receptors: C1 and flexible congeners. *Bioorg. Med. Chem. Lett.* 19, 2530–2532.
- Chaudhary, S., Ponnala, S., Legendre, O., Gonzales, J.A., Navarro, H.A., Harding, W.W., 2011. New aporphinoid 5-HT2A and alpha1A antagonists via structural manipulations of nantenine. *Bioorg. Med. Chem.* 19, 5861–5868.
- Coulibaly, J.T., Panic, G., Silue, K.D., Kovac, J., Hattendorf, J., Keiser, J., 2017. Efficacy and safety of praziquantel in preschool-aged and school-aged children infected with *Schistosoma mansoni*: a randomised controlled, parallel-group, dose-ranging, phase 2 trial. *Lancet Glob Health* 5, e688–e698.
- Cozzi, N.V., Daley, P.F., 2016. Receptor binding profiles and quantitative structure-affinity relationships of some 5-substituted-N,N-dialkyltryptamines. *Bioorg. Med. Chem. Lett.* 26, 959–964.
- Das, B., Tandon, V., Lyndem, L.M., Gray, A.I., Ferro, V.A., 2009. Phytochemicals from *Flemingia vestita* (Fabaceae) and *Stephania glabra* (Menispermaceae) alter cGMP concentration in the cestode *Raillietina echinobothrida*. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* 149, 397–403.
- Evans, P.D., Gee, J.D., 1980. Action of formamidin pesticides on octopamine receptors. *Nature* 287, 60–62.
- Farrell, M.S., McCorvy, J.D., Huang, X.P., Urban, D.J., White, K.L., Giguere, P.M., Doak, A.K., Bernstein, A.I., Stout, K.A., Park, S.M., Rodriguez, R.M., Gray, B.W., Hyatt, W.S., Norwood, A.P., Webster, K.A., Gannon, B.M., Miller, G.W., Porter, J.H., Shoichet, B.K., Pantegrossi, W.E., Wetsel, W.C., Roth, B.L., 2016. In vitro and in vivo characterization of the alkaloid nuciferine. *PLoS One* 11, e0150602.
- Geary, T.G., Klein, R.D., Vanover, L., Bowman, J.W., Thompson, D.P., 1992. The nervous systems of helminths as targets for drugs. *J. Parasitol.* 78, 215–230.
- Gogoi, S., Yadav, A.K., 2017. Therapeutic efficacy of the leaf extract of *Croton joufra* Roxb. against experimental cestodiasis in rats. *J. Parasit. Dis.* 41, 417–422.
- Hahnel, S., Wheeler, N., Lu, Z., Wangwiwatsin, A., McVeigh, P., Maule, A., Berriman, M., Day, T., Ribeiro, P., Grevelding, C.G., 2018. Tissue-specific transcriptome analyses provide new insights into GPCR signalling in adult *Schistosoma mansoni*. *PLoS Pathog.* 14, e1006718.
- Harding, N.K.A.W., 2016. Aporphine alkaloids as ligands for serotonin receptors. *Med. Chem.* 6, 241–249.
- Hauser, A.S., Attwood, M.M., Rask-Andersen, M., Schioth, H.B., Gloriam, D.E., 2017. Trends in GPCR drug discovery: new agents, targets and indications. *Nat. Rev. Drug Discov.* 16, 829–842.
- Hillman, G.R., Olsen, N.J., Senft, A.W., 1974. Effect of methysergide and dihydroergotamine on *Schistosoma mansoni*. *J. Pharmacol. Exp. Therapeut.* 188, 529–535.
- Hobson, R.J., Hapiak, V.M., Xiao, H., Buehrer, K.L., Komuniecki, P.R., Komuniecki, R.W., 2006. SER-7, a *Caenorhabditis elegans* 5-HT7-like receptor, is essential for the 5-HT stimulation of pharyngeal pumping and egg laying. *Genetics* 172, 159–169.
- Hotez, P.J., Fenwick, A., 2009. Schistosomiasis in Africa: an emerging tragedy in our new global health decade. *PLoS NTD* 3, e485.
- Impellizzeri, A.A., Pappalardo, M., Basile, L., Manfra, O., Andressen, K.W., Krobort, K.A., Messina, A., Levy, F.O., Guccione, S., 2015. Identification of essential residues for binding and activation in the human 5-HT7(a) serotonin receptor by molecular modeling and site-directed mutagenesis. *Front. Behav. Neurosci.* 9, 92.
- Indra, B., Tadano, T., Nakagawasai, O., Arai, Y., Yasuhara, H., Ohizumi, Y., Kisara, K., 2002. Suppressive effect of nantenine, isolated from *Nandina domestica* Thunberg, on the 5-hydroxy-L-tryptophan plus clorgyline-induced head-twitch response in mice. *Life Sci.* 70, 2647–2656.
- Iwasa, K., Nishiyama, Y., Ichimaru, M., Moriyasu, M., Kim, H., Wataya, Y., Yamori, T., Takashi, T., Lee, D., 1999. Structure-activity relationships of quaternary protoberberine alkaloids having an antimalarial activity. *Eur. J. Med. Chem.* 34, 1077–1083.
- Kapadia, N., Harding, W.W., 2015. C4 phenyl aporphines with selective h5-HT(2B) receptor affinity. *Bioorg. Med. Chem. Lett.* 25, 3451–3454.
- Keiser, M.J., Setola, V., Irwin, J.J., Laggner, C., Abbas, A.I., Hufeisen, S.J., Jensen, N.H., Kuijter, M.B., Matos, R.C., Tran, T.B., Whaley, R., Glennon, R.A., Hert, J., Thomas, K.L., Edwards, D.D., Shoichet, B.K., Roth, B.L., 2009. Predicting new molecular targets for known drugs. *Nature* 462, 175–181.
- Linnanen, T., Brisander, M., Unelius, L., Rosqvist, S., Nordvall, G., Hacksell, U., Johansson, A.M., 2001. Atropisomeric derivatives of 2',6'-disubstituted (R)-11-phenylaporphine: selective serotonin 5-HT(7) receptor antagonists. *J. Med. Chem.* 44, 1337–1340.
- Lu, Z., Sessler, F., Holroyd, N., Hahnel, S., Quack, T., Berriman, M., Grevelding, C.G., 2016. Schistosome sex matters: a deep view into gonad-specific and pairing-dependent transcriptomes reveals a complex gender interplay. *Sci. Rep.* 6, 31150.
- Mansour, T.E., 1979. Chemotherapy of parasitic worms: new biochemical strategies. *Science* 205, 462–469.
- Mansour, T.E., 1984. Serotonin receptors in parasitic worms. *Adv. Parasitol.* 23, 1–36.
- Mantsch, J.R., Li, S.J., Risinger, R., Awad, S., Katz, E., Baker, D.A., Yang, Z., 2007. Levotetrahydropalmatine attenuates cocaine self-administration and cocaine-induced reinstatement in rats. *Psychopharmacology (Berl)* 192, 581–591.
- Mi, G., Liu, S., Zhang, J., Liang, H., Gao, Y., Li, N., Yu, B., Yang, H., Yang, Z., 2017. Levotetrahydroberberine produces anxiolytic-like effects in mice through the 5-HT1A receptor. *PLoS One* 12, e0168964.
- Newman, D.J., Cragg, G.M., 2012. Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J. Nat. Prod.* 75, 311–335.
- Nichols, D.E., 2012. Structure-activity relationships of serotonin 5-HT2A agonists. In: *Wiley Interdisciplinary Reviews: Membrane Transport and Signaling* 1, pp. 559–579.
- Nimmo-Smith, R.H., Raison, C.G., 1968. Monoamine oxidase activity of *Schistosoma mansoni*. *Comp. Biochem. Physiol.* 24, 403–416.
- Nkwengoua, E.T., Ngantcho, I., Nyasse, B., Denier, C., Blonski, C., Schneider, B., 2009. In vitro inhibitory effects of palmatine from *Enantia chlorantra* on *Trypanosoma cruzi* and *Leishmania infantum*. *Nat. Prod. Res.* 23, 1144–1150.
- Oliaro, P.L., Vaillant, M.T., Belizario, V.J., Lwambo, N.J., Ouldaballah, M., Pieri, O.S., Amarillo, M.L., Kaatano, G.M., Diaw, M., Dominguez, A.C., Favre, T.C., Lapujade, O., Alves, F., Chitsulo, L., 2011. A multicentre randomized controlled trial of the efficacy and safety of single-dose praziquantel at 40 mg/kg vs. 60 mg/kg for treating intestinal schistosomiasis in the Philippines, Mauritania, Tanzania and Brazil. *PLoS Neglected Trop. Dis.* 5, e1165.
- Patocka, N., Ribeiro, P., 2007. Characterization of a serotonin transporter in the parasitic flatworm, *Schistosoma mansoni*: cloning, expression and functional analysis. *Mol. Biochem. Parasitol.* 154, 125–133.
- Patocka, N., Sharma, N., Rashid, M., Ribeiro, P., 2014. Serotonin signaling in *Schistosoma mansoni*: a serotonin-activated G protein-coupled receptor controls parasite movement. *PLoS Pathog.* 10, e1003878.
- Plassat, J.L., Amlaiki, N., Hen, R., 1993. Molecular cloning of a mammalian serotonin receptor that activates adenylate cyclase. *Mol. Pharmacol.* 44, 229–236.
- Powell, S.L., Godecke, T., Nikolic, D., Chen, S.N., Ahn, S., Dietz, B., Farnsworth, N.R., van Breemen, R.B., Lankin, D.C., Pauli, G.F., Bolton, J.L., 2008. In vitro serotonergic activity of black cohosh and identification of N(omega)-methylserotonin as a potential active constituent. *J. Agric. Food Chem.* 56, 11718–11726.
- Ray, T.S., 2010. Psychedelics and the human receptorome. *PLoS One* 5, e9019.
- Sabah, A.A., Fletcher, C., Webbe, G., Doenhoff, M.J., 1986. *Schistosoma mansoni*: chemotherapy of infections of different ages. *Exp. Parasitol.* 61, 294–303.
- Saber, A., Jamal, A., Beets, I., Schoofs, L., Newmark, P.A., 2016. GPCRs direct germline development and somatic gonad function in planarians. *PLoS Biol.* 14, e1002457.
- Shahinas, D., Macmullin, G., Benedict, C., Crandall, I., Pillai, D.R., 2012. Harmine is a potent antimalarial targeting Hsp90 and synergizes with chloroquine and artemisinin. *Antimicrob. Agents Chemother.* 56, 4207–4213.
- Shen, Y., Monsma Jr, F.J., Metcalf, M.A., Jose, P.A., Hamblin, M.W., Sibley, D.R., 1993.

- Molecular cloning and expression of a 5-hydroxytryptamine₇ serotonin receptor subtype. *J. Biol. Chem.* 268, 18200–18204.
- Shulgin, A.S.a.A., 1997. *TIHKAL: the Continuation*. Transform Press.
- Tomosky, T.K., Bennett, J.L., Bueding, E., 1974. Tryptaminergic and dopaminergic responses of *Schistosoma mansoni*. *J. Pharmacol. Exp. Therapeut.* 190, 260–271.
- Wacker, D., Wang, C., Katritch, V., Han, G.W., Huang, X.P., Vardy, E., McCorvy, J.D., Jiang, Y., Chu, M., Siu, F.Y., Liu, W., Xu, H.E., Cherezov, V., Roth, B.L., Stevens, R.C., 2013. Structural features for functional selectivity at serotonin receptors. *Science* 340, 615–619.
- Wang, J., Yu, Y., Shen, H., Qing, T., Zheng, Y., Li, Q., Mo, X., Wang, S., Li, N., Chai, R., Xu, B., Liu, M., Brindley, P.J., McManus, D.P., Feng, Z., Shi, L., Hu, W., 2017. Dynamic transcriptomes identify biogenic amines and insect-like hormonal regulation for mediating reproduction in *Schistosoma japonicum*. *Nat. Commun.* 8, 14693.
- Zamanian, M., Kimber, M.J., McVeigh, P., Carlson, S.A., Maule, A.G., Day, T.A., 2011. The repertoire of G protein-coupled receptors in the human parasite *Schistosoma mansoni* and the model organism *Schmidtea mediterranea*. *BMC Genomics* 12, 596.