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Yohimbine as a Starting Point to Access Diverse Natural Product-Like Agents with Re-programmed Activities against Cancer-Relevant GPCR Targets

Nicholas G. Paciaroni, **Verrill M. Norwood IV**, **Ranjala Ratnayake**, **Hendrik Luesch**[*], **Robert W. Huigens III**[*]

University of Florida, Department of Medicinal Chemistry, Center for Natural Products, Drug Discovery and Development (CNPD3), College of Pharmacy, 1345 Center Dr., Gainesville, FL 32610, United States

Abstract

G protein-coupled receptors (GPCRs) constitute the largest protein superfamily in the human genome. GPCRs play key roles in mediating a wide variety of physiological events including proliferation and cancer metastasis. Given the major roles that GPCRs play in mediating cancer growth, they present promising targets for small molecule therapeutics. One of the principal goals of our lab is to identify complex natural products (NPs) suitable for ring distortion, or the dramatic altering of the inherently complex architectures of NPs, to rapidly generate an array of compounds with diverse molecular skeletal systems. The overarching goal of our ring distortion approach is to re-program the biological activity of select natural products and identify new compounds of importance to the treatment of disease, such as cancer. Described herein are the results from biological screens of diverse small molecules derived from the indole alkaloid yohimbine against a panel of GPCRs involved in various diseases. Several analogues displayed highly differential antagonistic activities across the GPCRs tested. We highlight the re-programmed profile of one analogue, **Y7g**, which exhibited selective antagonistic activities against AVPR2 (IC_{50} = 459 nM) and OXTR (IC_{50} = 1.16 μM). The activity profile of **Y7g** could correlate its HIF-dependent anti-cancer activity to its GPCR antagonism since these receptors are known to be upregulated in hypoxic cellular environments. Our findings demonstrate that the ring distortion of yohimbine can lead to the identification of new compounds capable of interacting with distinct cancer-relevant targets.

Keywords

yohimbine; indole alkaloids; ring distortion; drug discovery; GPCR drug targets; cancer

[*]Corresponding author: rhuigens@cop.ufl.edu, luesch@cop.ufl.edu.

Introduction

GPCRs are integral cell-surface proteins that serve as an interface between the intra- and extracellular environment.[1,2] These receptors respond to a plethora of external stimuli (e.g. small molecules, ions) that result in a wide variety of physiological events via downstream signaling cascades predominately activated by heterotrimeric G proteins. GPCRs function by releasing free Gα and Gβγ subunits that initiate intracellular signal transduction pathways. Various GPCRs operate through these heterotrimeric G proteins to induce uncontrolled cellular proliferation and cancer metastasis.[3–6] Over 30% of all FDA-approved drugs target GPCRs (implicated in various diseases).[7] Despite the successes in targeting certain GPCRs for chemotherapy, a large majority of these proteins have yet to be appropriately classified and successfully targeted with small molecules.[8,9] Therefore, success in targeting cancer-relevant GPCRs necessitates the discovery of novel and selective small molecule antagonists that can efficiently inhibit cellular signaling pathways that are critical to cancer.

Natural products have been the main source of therapeutic small molecules because of their exceptional ability to selectively bind and modulate numerous biological targets that play crucial roles in disease.[10,11] Architecturally complex natural products (e.g. paclitaxel, vancomycin, morphine) are able to form highly specific binding modes with their respective target due to their unique and multifaceted carbon skeletons, which are decorated with diverse functional groups. These aspects enable natural products to tightly bind their corresponding proteins and induce a desired biological response of therapeutic value. Despite the demonstrated utility of natural products for therapy, a paradigm shift to high-throughput screening (HTS) occurred in the mid-1990's, which heavily relies on small organic molecules to drive drug discovery efforts.[12–14] It has been well documented that HTS libraries are largely populated by structurally simple organic molecules that lack three-dimensionality when compared to natural product therapeutics. Although HTS efforts have served well in drugging certain biological targets (e.g. kinases) these libraries severely lack chemical diversity[15] and such compound collections have largely failed to produce viable leads for proteins that are more difficult to target (e.g. transcription factors[16,17]).

Several elegant strategies to address the lack of chemical diversity in screening have been employed. The most well established approaches are diversity-oriented synthesis (DOS)[18,19] and biology-oriented synthesis (BIOS)[20] that have been pioneered by Schreiber and Waldmann, respectively. These tactics aim at generating libraries of architecturally complex small molecules by building structural complexity in sequential synthetic reactions, starting from simple building blocks. Screening libraries derived from DOS and BIOS have led to discoveries of several small molecules that modulate diverse targets of therapeutic relevance.[21,22] Additionally, other complementary approaches to DOS and BIOS have been developed, which include complexity-to-diversity, also known as the "ring distortion" of natural products.[23–26] In a ring distortion approach, select natural products are subjected to a series of chemoselective reactions aimed to dramatically alter the inherently complex ring system to produce diverse and architecturally unique carbon scaffolds. A major goal of ring distortion is to identify complex small molecules that display biological activity that is distinct from the parent natural product.

We have recently reported efforts towards establishing a tryptoline-based ring distortion of the complex alkaloid natural product yohimbine (**Y**).[25,27,28] By exploiting the embedded tryptoline substructure of yohimbine, we were able to rapidly generate (two to six synthetic steps) an array of unique small molecules through a series of highly selective ring-cleavage and diastereoselective, oxidative indole rearrangement reactions that afforded cyanamide and spirooxindole products, respectively. Subsequent phenotypic screening of the yohimbine-derived library unveiled analogues that exhibited selective hypoxia-inducible factor (HIF)-dependent anti-cancer activities, and anti-inflammatory properties.[25] Additional screening was conducted on the yohimbine-derived compound library and identified new molecules that demonstrated antimalarial activities by preferentially inhibiting *Plasmodium falciparum* parasites without cytotoxicity toward human cells.[28] Several of these yohimbine ring distortion analogues were found to have sub-micromolar anti-plasmodial activity. Furthermore, the parent natural product yohimbine was completely inactive in these biological screening endeavors. Intrigued by the stark contrast in the cellular activity profiles of select ring distortion analogues to yohimbine, we hypothesized that new analogues could demonstrate re-programmed activities towards GPCR targets of relevance to cancer and other disease areas. The work presented here details the screening results of several yohimbine-derived analogues (and yohimbine itself) against a diverse panel of 168 GPCR targets that play important roles in various diseases. From the initial screen and subsequent hit validation, several yohimbine-derived analogues demonstrated differential antagonistic activity profiles against multiple GPCR targets, including several relevant to cancer.

Results and Discussion

From our initial ring distortion campaign, six analogues (one from each main scaffold class, Figure 1) and yohimbine itself, were chosen to screen for both agonist and antagonist activities against a panel of GPCRs. Our goal was to obtain a detailed activity profile for each analogue against 168 GPCRs using PathHunter β-arrestin cell-based assays.[6,29,30] From the primary screen (Figure 2A) at 20 μM, several analogues displayed significant antagonistic activities (≥80% inhibition) against GPCRs while agonist activity was mostly absent during these investigations. Most notably, yohimbine-derived compounds **Y6p**, **Y1f** and **Y7g** all exhibited significant inhibitory activities, whereas **Y4a**, **Y5a**, **Y2e** and **Y3h** were inactive and did not induce an antagonistic effect for any GPCR in the screen (see supporting information). The parent natural product yohimbine (**Y**, an adrenoceptor antagonist) unsurprisingly inhibited its native GPCR targets during these investigations, which include: α−2B adrenergic receptor (ADRA2B), dopamine D2b receptor (DRD2L) and hydroxytryptamine receptor (HTR1B).[31] A focused heat map of the most significantly inhibited GPCR targets showcases re-programmed activities of the different yohimbine-derived scaffolds when compared to yohimbine (Figure 2B). A loss of activity for **Y** and a gain of new activities against GPCRs for analogues **Y6p**, **Y1f** and **Y7g** demonstrates proof-of-concept that yohimbine's activity can be re-programmed to modulate different protein targets using a ring distortion approach.

During the course of these investigations, analogue **Y6p** demonstrated antagonistic activity against four disease-relevant GPCRs at ≥80% inhibition, three of which were advanced to dose-response experiments (Figure 2B, C). **Y6p** displayed low micromolar potencies for

follicle-stimulating hormone receptor (FSHR, IC_{50} = 8.03 μM), neuropeptide S receptor 1 (NPSR1B, IC_{50} = 10.3 μM) and prolactin releasing hormone receptor (PRLHR, IC_{50} = 4.89 μM) upon dose-dependent biochemical validation. FSHR regulates signaling of the follicle-stimulating hormone (FSH), which is essential to maturation and growth of the reproductive system.[32] FSHR has been shown to be expressed on most ovarian cancer subtypes and could serve as a promising immunotherapeutic target for chemotherapy.[33] NPSR1B, which is primarily found in the brain, is known to modulate anti-anxiety and arousal activities by enhancing dopamine production.[34] Selective antagonism of NPSR1B is being probed for its potential to treat cocaine abuse with promising preliminary results.[35] PRLHR is the receptor for prolactin releasing peptide (PrRP) and this ligand has been associated with digestive regulation and energy balance. Additionally, PRLHR is involved in the development of uterine fibroids (leiomyomas)[36] and has been reported to negatively modulate the effects of opioid stimulation.[37]

Y1f demonstrated antagonistic activities against five GPCRs (≥80% inhibition), including: the closely related chemokine receptors CCR3 (IC_{50} = 8.30 μM) and CCR8 (IC_{50} = 7.29 μM), CX3C (CX3CR1, IC_{50} = 8.47 μM), CX-C type 4 (CXCR4, IC_{50} = 7.94 μM) and 5-hydroxytryptamine (serotonin) receptor (HTR2C, IC_{50} = 180 nM) (Figure 2B, C). Several of these GPCRs are involved in cancer and new antagonists could play a critical role to those suffering from this disease. CCR8 is strongly expressed in malignant melanoma, and antagonism of this target inhibits tumor cell migration.[38] CXCR4 is a chemokine receptor that plays a critical role as a co-receptor with CCR5 to enable HIV-1 to enter into $CD4^+$ cells and is involved in leukocyte migration through signaling of its ligand SDF-1.[39] It is also known that tumor cell expression of CXCR4 promotes migration and homing to sites where non-malignant cells express SDF-1 (or CXCL12) and drives the metastasis of several cancer types (e.g., leukemia, other blood cancers, breast cancer).[40] In addition, HTR2C is a 5-hydroxytryptamine (serotonin) receptor and is involved in a wide range of functions in the central nervous system.[41,42] Antagonism of this specific serotonin receptor subtype has been implicated in managing migraine pain relief as well as inducing anxiolytic effects.[43–45]

Perhaps the most significant finding from these GPCR-focused investigations so far was the activity profile of **Y7g**, an analogue that was previously found to exhibit hypoxia-inducible factor (HIF)-dependent anti-cancer and anti-inflammatory activities.[25] From the GPCR screen, **Y7g** inhibited arginine vasopressin receptors (AVPR1A, AVPR1B, AVPR2) and the oxytocin receptor (OXTR), which share a high level of sequence homology (Figure 2B, C).[46] AVPRs and OXTR have shown involvement in tumor progression in cancers, including prostate[47] and small-cell lung cancer (SCLC).[48] The expression of OXTR in prostate cancer cell lines contributes to metastasis.[47] Interestingly, vasopressin receptor expression is a selective feature of breast cancers and is also associated with small-cell lung cancer.[49–52] **Y7g** inhibited AVPR2 (83% inhibition at 20 μM) during the initial screen and its antagonistic properties were found to be dose-dependent, reporting an IC_{50} = 459 nM (compared to AVPR2 antagonist tolvaptan, IC_{50} = 7.34 pM; see Supporting Information). In addition, **Y7g** demonstrated antagonistic activities against OXTR in the initial GPCR screen (97% inhibition at 20 μM). Subsequent dose-response studies revealed an IC_{50} value of 1.16

μM against this receptor (compared to OXTR antagonist L-368,899, IC_{50} = 4.62 nM; see Supporting Information).

Since AVPR2 is known to be induced by hypoxic cellular environments[53,54] and is expressed in a variety of cancers, the anti-cancer properties we previously reported[25] for **Y7g** could be due to its ability to inhibit AVPR2/OXTR. These findings demonstrate a complete re-programing of the innate biological activity of yohimbine through our ring distortion efforts. Yohimbine displayed ≤5% inhibition against AVPR2 and OXTR, while ring distortion compound **Y7g** gained significant antagonistic activities against these cancer-relevant targets (Figure 3). In addition to the "gain of function" activities demonstrated by **Y7g**, we also observed a "loss of function" against yohimbine's known targets ADRA2A, DRD2L and HTR1B as these targets were not inhibited by **Y7g**.

The GPCR profiles of active yohimbine-derived scaffolds **Y6p**, **Y1f** and **Y7g** indeed support our initial hypothesis that yohimbine's innate biological activity could be re-programmed through ring distortion and lead to the identification of new molecules that modulate different proteins of significance to human health. Not only did these analogues modulate GPCRs other than the target proteins yohimbine binds to, but they did so in a selective manner. From the 168 GPCRs tested, **Y6p**, **Y1f** and **Y7g** demonstrated hit rates of 2.4%, 3.0% and 2.4% (compared to yohimbine's hit rate of 4.2% in this screen, Figure 2B). Collectively, these findings serve as excellent proof-of-concept for the re-programing of yohimbine's biological activity.

Conclusions

The screening of ring-distorted yohimbine-derived compounds led to the identification of diverse small molecules that demonstrate re-programmed antagonistic activities against cancer-relevant GPCR targets. Analogues **Y6p**, **Y1f** and **Y7g** inhibited GPCRs that mediate cellular proliferation in breast, melanoma and prostate cancers. Additionally, a HIF-dependent anti-cancer agent we previously reported, **Y7g,** was found to antagonize arginine vasopressin receptors (AVPR1B, AVPR1A, AVPR2) and oxytocin receptor (OXTR), both of which are upregulated in multiple cancer types. Furthermore, hypoxic cellular environments are known to upregulate AVPR expression may be linked to our previous findings regarding **Y7g**'s antiproliferative activities against cancer cells.[25] We believe the results from these investigations could play an important role in the identification of new small molecules that may significantly impact future cancer treatments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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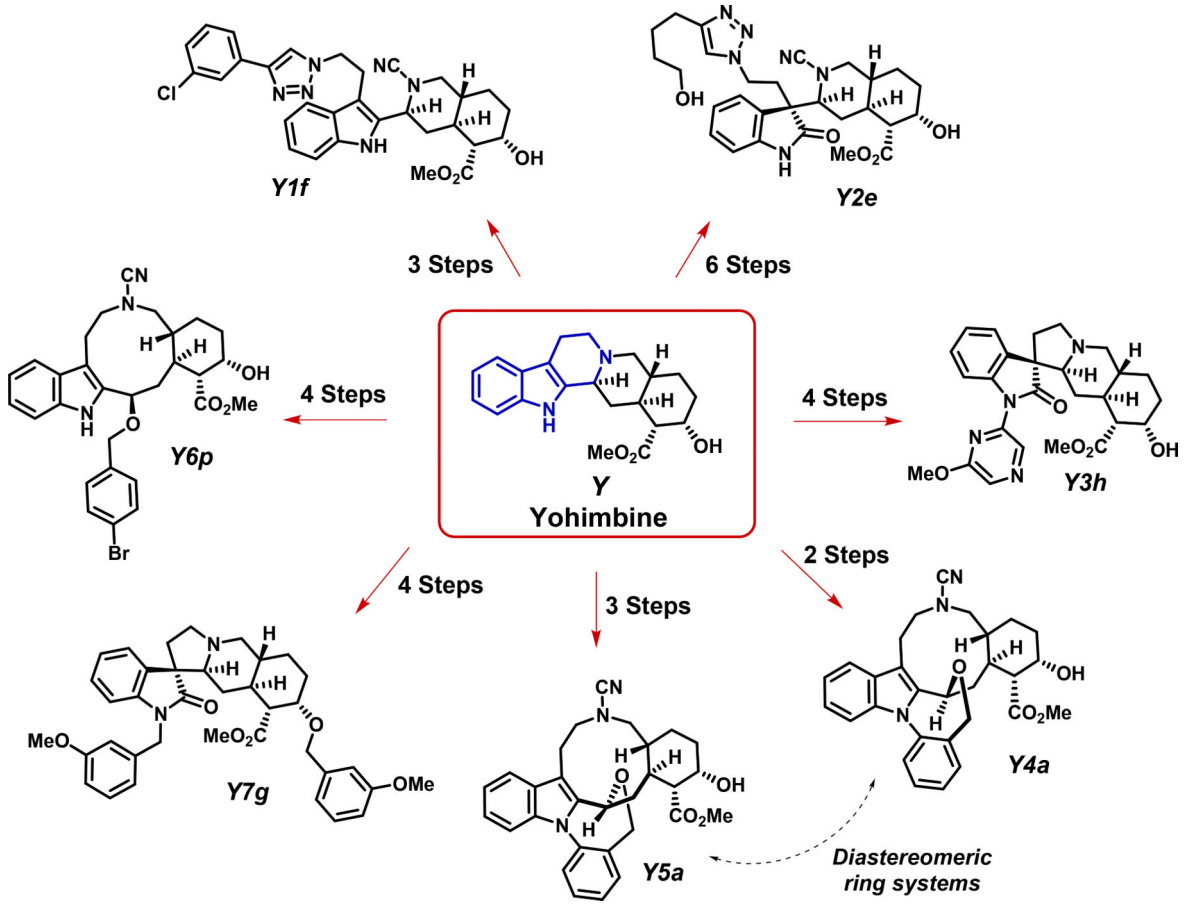
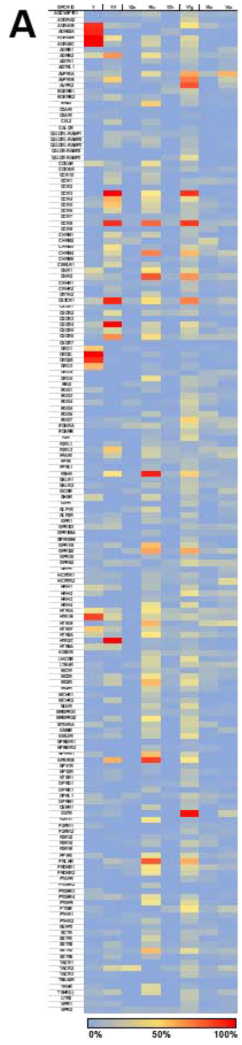


Figure 1.

Ring distortion of yohimbine (**Y**) to rapid synthetic access to architecturally diverse analogues for biological screens. These yohimbine-derived compounds were screened against a panel of GPCR drug targets. (Note: color should be used)



B

GPCR	Y	Y4a	Y5a	Y6p	Y1f	Y2e	Y3h	Y7g	Therapeutic Role
ADRA1B	90	5	16	-61	17	11	2	51	--
ADRA2B	107	-5	3	27	43	-11	-9	35	--
AVPR2	-1	16	-1	1	7	1	9	83	Breast Cancer & Metastasis
CCR3	-13	2	-2	50	112	-17	-7	89	Allergic Inflammation
CCR8	5	15	10	78	90	5	9	88	Melanoma & Tumor Cell Metastasis
CX3CR1	22	20	9	53	92	11	9	72	Multiple Sclerosis
CXCR4	21	-30	10	29	125	-37	-19	39	Leukemia & Other Cancers
DRD2L	102	3	-2	10	-16	-11	-8	9	--
FSHR	4	5	12	94	51	2	11	56	Ovarian Cancer
HTR1B	86	12	14	33	33	2	19	28	--
HTR2C	1	-1	2	8	108	-2	6	8	Migraine & Anxiety
NPSR1B	-3	-40	1	87	62	-22	-12	48	Anxiety & Addiction
OXTR	5	27	0	-7	9	0	-11	97	Breast & Prostate Cancers
PRLHR	1	5	-12	82	19	9	2	63	Gastric & Colorectal Cancers
Hits (≥80%)	7	0	0	4	5	0	0	4	Out of 168 GPCRs
Hit Rate	4.2%	0.0%	0.0%	2.4%	3.0%	0.0%	0.0%	2.4%	

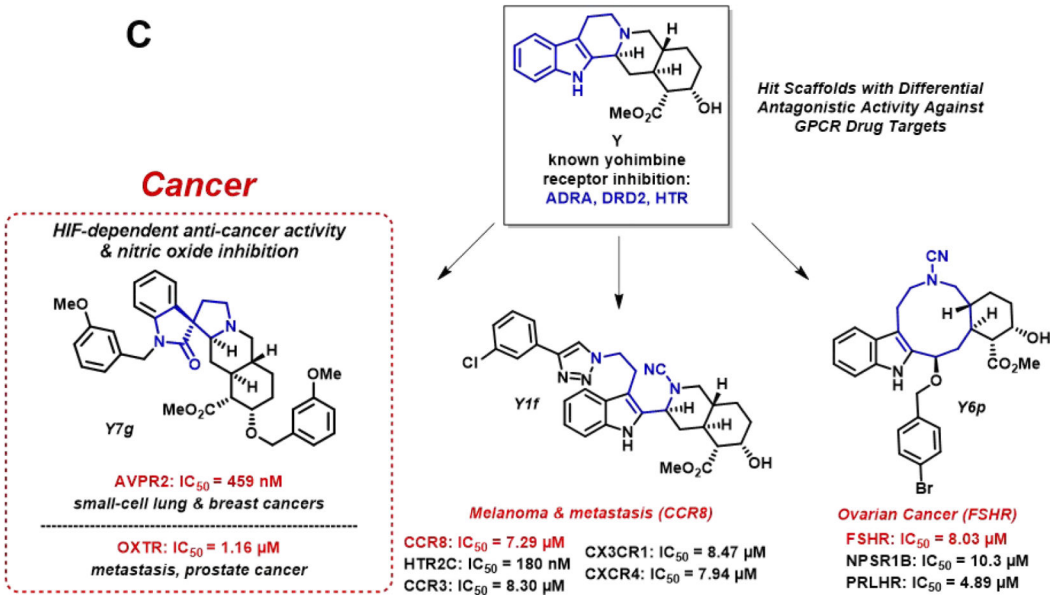
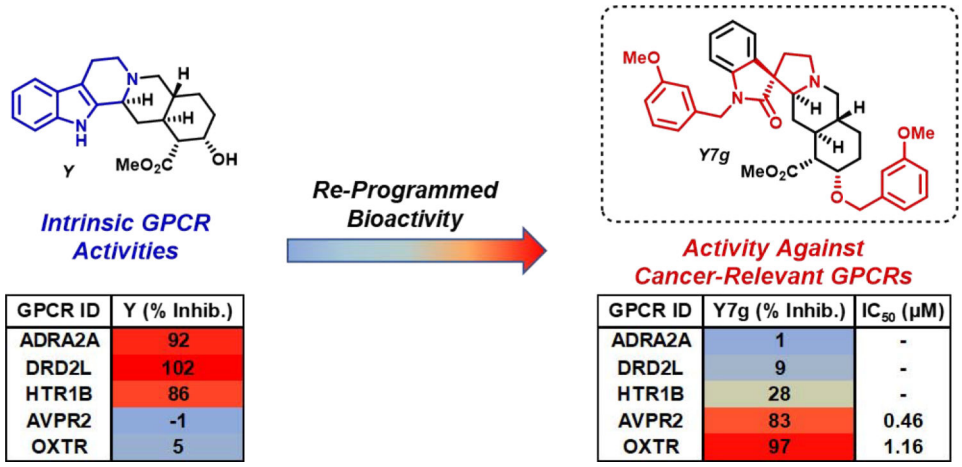


Figure 2.

A) Full heatmap of antagonist activity for all 168 GPCRs against **Y** and ring distortion analogues. **B)** Focused heatmap of disease-relevant GPCRs that were antagonized during these investigations. **C)** Chemical structures and IC_{50} values for yohimbine analogues with diverse “gain-of-function” activity profiles against GPCR targets. (Note: color should be used)



GPCR ID	Y (% Inhib.)
ADRA2A	92
DRD2L	102
HTR1B	86
AVPR2	-1
OXTR	5

GPCR ID	Y7g (% Inhib.)	IC_{50} (μM)
ADRA2A	1	-
DRD2L	9	-
HTR1B	28	-
AVPR2	83	0.46
OXTR	97	1.16

Figure 3.

Highlighted antagonistic activities for **Y** and **Y7g** demonstrate re-programing of activity profiles against select GPCR targets. (Note: color should be used)