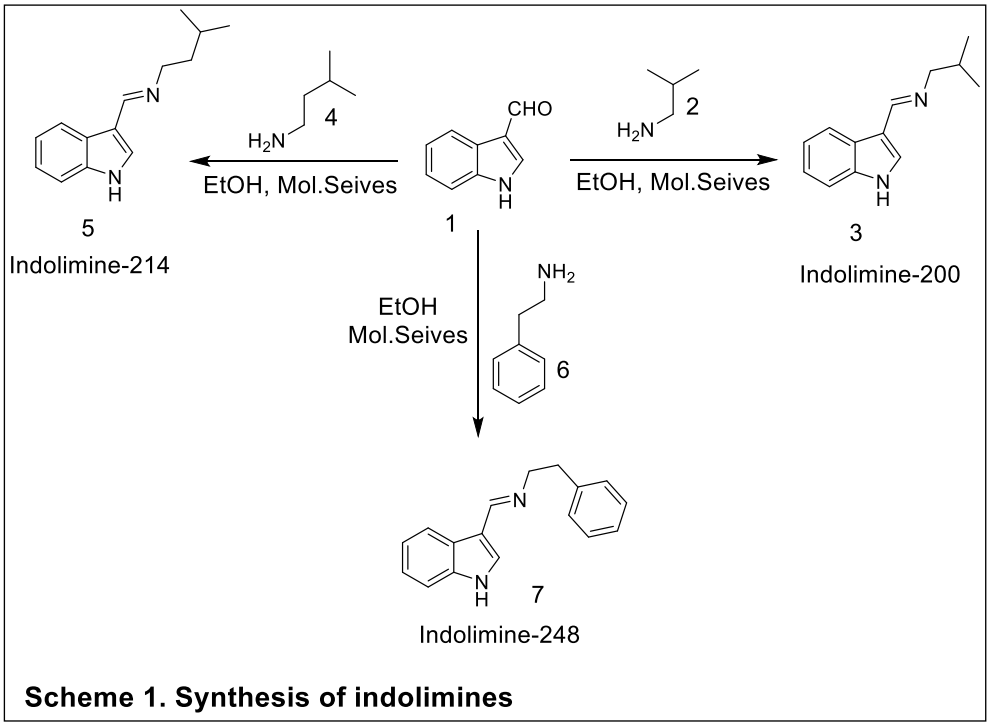
Supplemental Methods

General chemistry methods

LC/MS method section. Reagents and solvents were purchased from commercial vendors and were of the highest purity available and used without further purification unless otherwise noted. Qualitative/quantitative analysis was performed by reverse phase UHPLC using a Prominence 20 UFLCXR system (Shimadzu, Columbia, MD) with a Waters (Milford, MA) ACQUITY UPLC BEH C18 column (2.1 × 100 mm, 1.7 µm particle size) maintained at 55 °C and a 20 min aqueous acetonitrile gradient, at a flow rate of 250 µL/min. Solvent A was water with 0.1% formic acid and Solvent B was acetonitrile with 0.1% formic acid. The initial condition were 97% A and 3 % B, increasing to 45% B at 10 min, 75% B at 12 min where it was held at 75% B until 17.5 min before returning to the initial conditions. The eluate was delivered into an AB SCIEX TripleTOF™ 5600 System (QTOF) using a Duospray™ ion source (AB SCIEX, Framingham, MA). Purities of assayed compounds were in all cases greater than 95%, as determined by a Waters 2695 HPLC system (Waters, Milford, MA, U.S.A) with a Restek (Bellefonte, PA, U.S.A) HPLC C18 column (4.6 × 150 mm, 5 µm particle size) and a Viva C18 guard cartridge (10 × 4.0 mm, 5 µm particle size) with a 10 µL of injection volume. The mobile phase solvent A was 0.1% formic acid in water, and solvent B was 0.1% formic acid in acetonitrile. The gradient program was: 0-18 min, 5-45% B in A; 18-22 min, 45-90% B in A; 22-27 min, 90% B in A; 27-27.5 min, 90-5% B in A; 27.5-35 min, 5% B in A at a flow of 1 mL/min.

NMR method section. Each compound was dissolved in methanol-d4 or DMSO-d6. All NMR data were acquired at 298 K on Bruker Avance NEO 600 MHz spectrometer (Bruker Biospin, Rheinstetten, Germany) equipped with a 5 mm TCI cryoprobe. 1D ^{1}H NMR spectra and a series of 2D NMR spectra, including ^{1}H -^{1}H TOCSY, ^{1}H -^{1}H COSY, ^{1}H -^{1}H JRes, ^{1}H -^{13}C HSQC, and ^{1}H -^{13}C HMBC, were recorded and processed as previously described with some modifications.[56] The ^{1}H and ^{13}C chemical shifts were referenced to deuterated solvent residual proton signal at 3.31 ppm and ^{13}C signal at 49.5 ppm in methanol-d4, respectively and at 2.50 ppm and 39.5 ppm in DMSO-d6, respectively. Coupling constants (J value) are reported in Hz. Spin multiplicities are described as s (singlet), br (broad singlet), d (doublet), t (triplet), and m (multiplet).

Synthesis of Indolimines:



Scheme 1. Synthesis of indolimines

Indolimine-200, indolimine-214, and indolimine-248 were synthesized as shown in Scheme 1.

Synthesis of (*E*)-1-(1H-indol-3-yl)-*N*-isobutylmethanimine (Indolimine-200) (3):
Indol-3-carboxaldehyde **1** (500 mg, 3.44 mmol) and isobutylamine **2** (1.5 eq, 377 mg, 5.16 mmol) were dissolved in anhydrous ethanol (50 mL). Molecular sieves (5 g) were added, and the reaction mixture was refluxed for 7-8 hrs until the aldehyde completely disappeared (TLC). The reaction mixture was filtered through celite, and the excess isobutylamine was removed by rotavaporation, and the product was dried in a high vacuum pump to obtain (indolimine-200) **3** (660 mg, yield 95%) as a brown viscous oil. The identity of indolimine-200 was confirmed by nuclear magnetic resonance (NMR) and mass spectra analysis. ^{1}H-NMR (600 MHz, MeOD, 25 ºC): δ 8.46 (s, 1H, N=CH), 8.08 (m, 1H, =CH), 7.70 (s, 1H, =CH-N), 7.41 (m, 1H, =CH), 7.19 (m, 1H, =CH), 7.14 (m, 1H, =CH), 3.40 (dd, *J* = 6.72 Hz, 1.16 Hz, 2H, CH2), 1.99 (m, 1H, CH), 0.99 (d, *J* = 6.72 Hz, 6H, CH3); ^{13}C-NMR (150 MHz, MeOD, 25 ºC): δ 158.78, 139.2, 131.45, 127.07, 124.03, 121.97, 121.68, 115.5, 113.01, 70.7, 31.16, 21.1.

Synthesis of (*E*)-1-(1H-indol-3-yl)-*N*-isopentylmethanimine (Indolimine-214) (5):
Compound **5** was synthesized by following a similar experimental procedure as used above for compound **3,** starting from indol-3-carboxaldehyde **1** (500 mg, 3.44 mmol) and isopentylamine **4** (1.5 eq, 450 mg, 5.16 mmol). Indolimine-214 was characterized by NMR and mass spectra analysis (brown viscous oil, 690 mg, yield 93%). ^{1}H-NMR (600 MHz, DMSO-d6, 25 ºC): δ 11.43

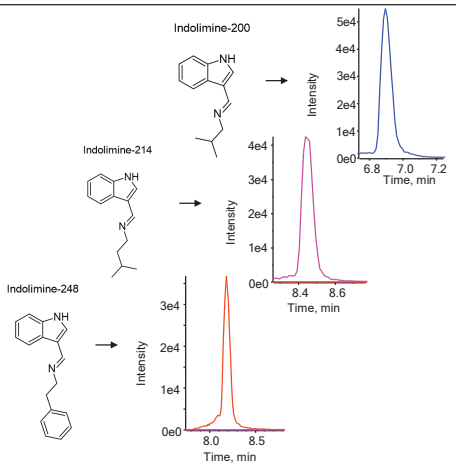
(br, 1H, NH), 8.45 (s, 1H, N=CH), 8.19 (m, 1H, =CH), 7.72 (s, 1H, =CH-N), 7.41 (m, 1H, =CH), 7.15 (m, 1H, =CH), 7.08 (m, 1H, =CH), 3.52 (dt, *J* = 7.15 Hz, 1.1 Hz, 2H, CH2), 1.71 (m, 2H, CH2), 1.51 (q, *J* = 7.05 Hz, 1H, CH), 0.93 (d, *J* = 6.66 Hz, 6H, CH3); ^{13}C-NMR (150 MHz, DMSO-d6, 25 ºC): δ 154.95, 136.83, 130.26, 124.94, 122.06, 121.4, 119.93, 114.4, 111.36, 59.24, 40.04, 25.09, 22.26.

Synthesis of (*E*)-1-(1H-indol-3-yl)-*N*-phenethylmethanimine (Indolimine-248) (7):
The experimental procedure used for synthesizing compound **7** was also the same as **3**. Compound **7** was prepared from indol-3-carboxaldehyde **1** (500 mg, 3.44 mmol) and phenethylamine **6** (1.5 eq, 625 mg, 5.16 mmol) in ethanol. The excess phenethylamine was removed by repeatedly washing the product with hexane and dried using a high vacuum pump to afford indolimine-248 as a brown viscous oil, and it was characterized by NMR and mass spectra analysis (760 mg, yield 88%). ^{1}H-NMR (600 MHz, MeOD, 25 ºC): δ 8.33 (s, 1H, N=CH), 8.05 (m, 1H, =CH), 7.64 (s, 1H, =CH-N), 7.4 (m, 1H, =CH), 7.248 (m, 2H, =CH), 7.247 (m, 2H, =CH), 7.19 (m, 1H, =CH), 7.157 (m, 1H, =CH), 7.13 (m, 1H, =CH), 3.80 (dt, *J* = 7.3 Hz, 1.1Hz, 2H, CH2), 2.99 (t, *J* = 7.3 Hz, 2H, CH2); ^{13}C-NMR (150 MHz, MeOD, 25 ºC): δ 159.16, 141.36, 139.03, 131.39, 130.3, 129.47, 127.2, 126.96, 123.9, 121.9, 121.61, 115.44, 112.89, 64.15, 39.01.

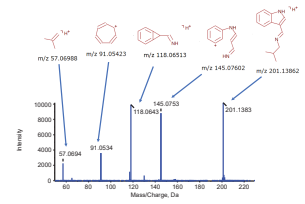
A

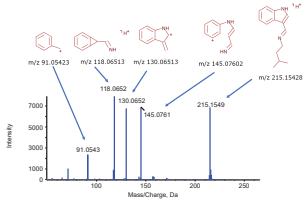
Compound name	Formula	Ionization mode	Exact mass	LOD (nM)
Indolimine-200	C13H16N2	Positive	200.1313	1
Indolimine-214	C14H18N2	Positive	214.1469	1
Indolimine-248	C17H16N2	Positive	248.1313	1

B



C





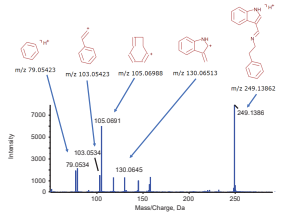


Figure S1. Identification of synthetic indolimines by LC-MS/MS. A, Overview of LC-MS/MS parameters of indolimines. B, Extracted ion chromatograms (EICs) of indolimines. C, Fragmentation annotations of the indolimines.

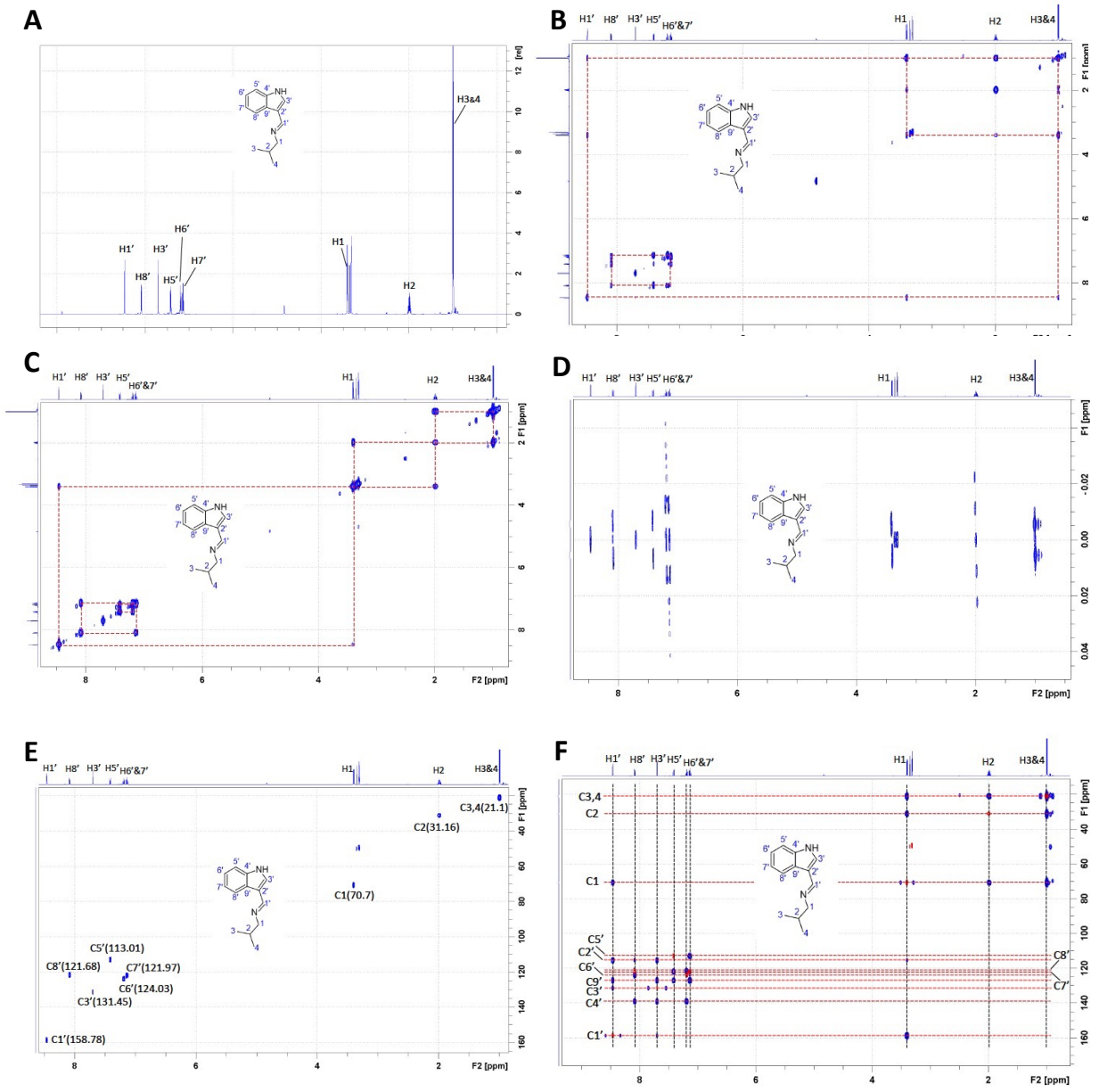


Figure S2. Conformation of the structure of indolimine-200 by NMR. (A) 1D ^{1}H spectrum of indolimine-200 with assignments. (B) ^{1}H-^{1}H TOCSY spectrum of indolimine-200 with signal assignments. (C) ^{1}H-^{1}H COSY spectrum of indolimine-200 with signal assignments. (D) ^{1}H-^{1}H JREs spectrum of indolimine-200 showing J-coupling patterns. (E) ^{1}H-^{13}C HSQC spectrum of indolimine-200 and signal assignments. (F) ^{1}H-^{13}C HMBC (blue) and HSQC (red) spectrum of indolimine-200 and signal assignments.

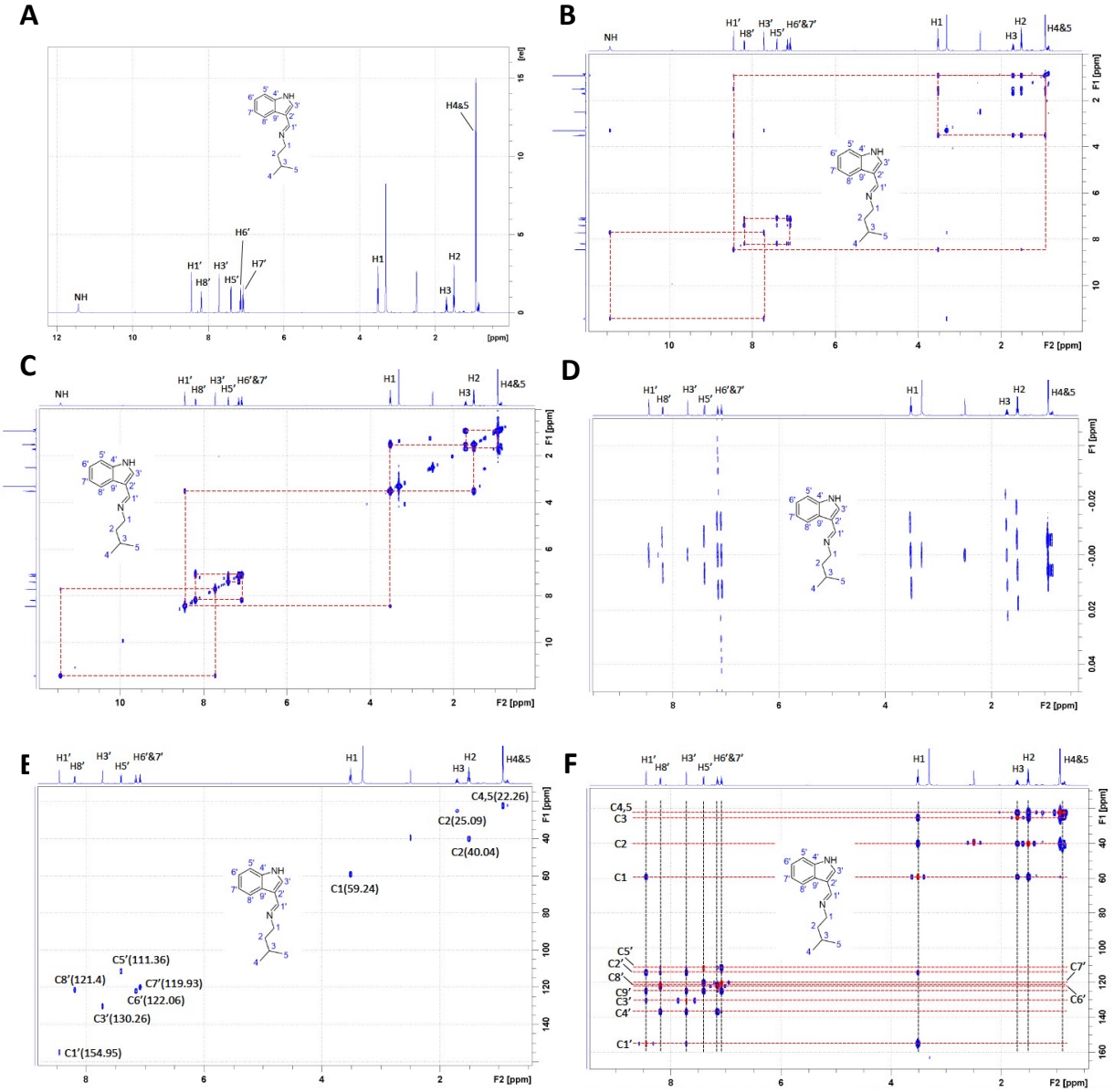


Figure S3. Conformation of the structure of indolimine-214 by NMR. (A) 1D ^{1}H spectrum of indolimine-214 with assignments. (B) ^{1}H-^{1}H TOCSY spectrum of indolimine-214 with signal assignments. (C) ^{1}H-^{1}H COSY spectrum of indolimine-214 with signal assignments. (D) ^{1}H-^{1}H JREs spectrum of indolimine-214 showing J-coupling patterns. (E) ^{1}H-^{13}C HSQC spectrum of indolimine-214 and signal assignments. (F) ^{1}H-^{13}C HMBC (blue) and HSQC (red) spectrum of indolimine-214 and signal assignments.

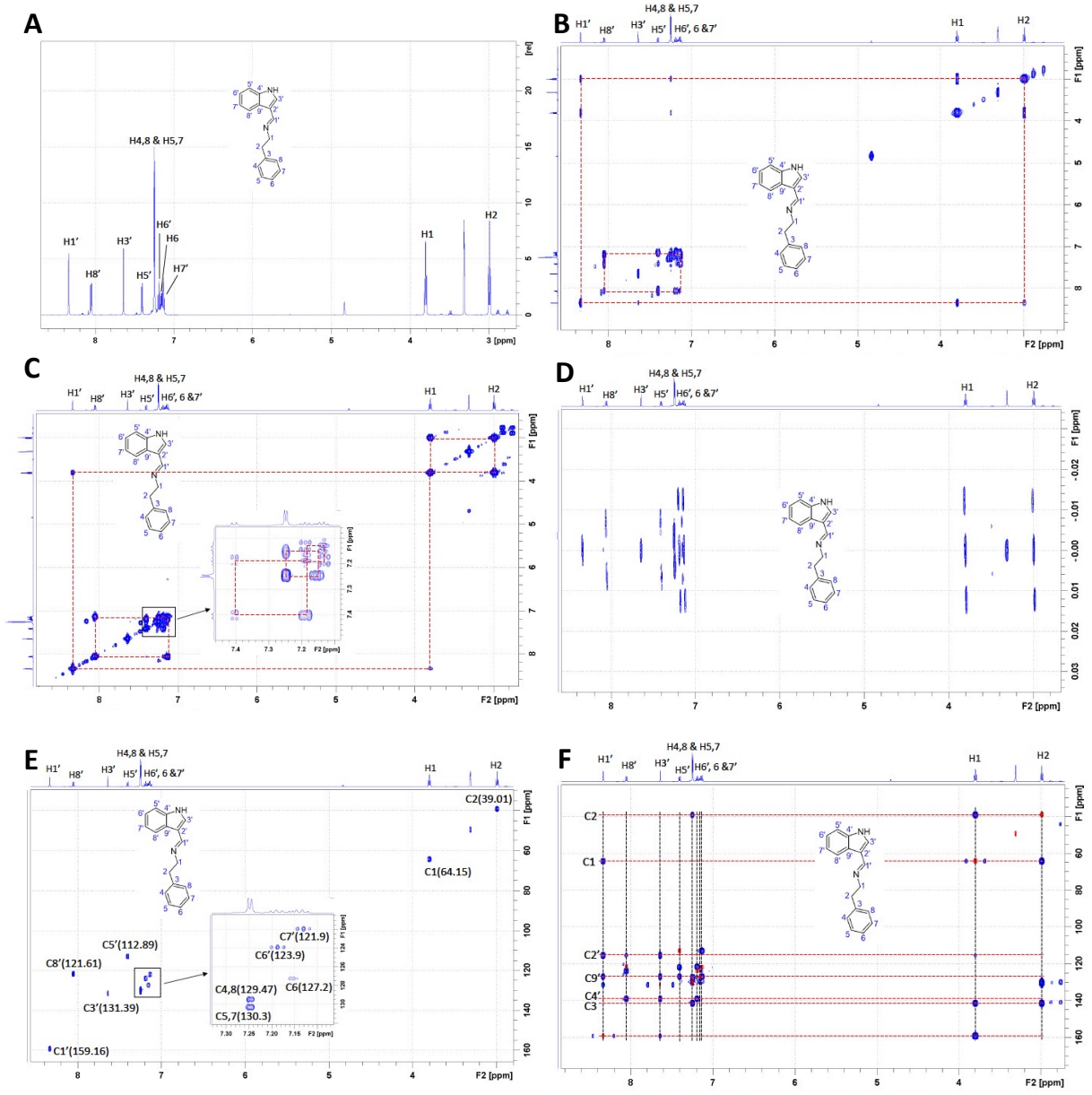


Figure S4. Conformation of the structure of indolimine-248 by NMR. (A) 1D ^{1}H spectrum of indolimine-248 with assignments. (B) ^{1}H-^{1}H TOCSY spectrum of indolimine-248 with signal assignments. (C) ^{1}H-^{1}H COSY spectrum of indolimine-248 with signal assignments. (D) ^{1}H-^{1}H JREs spectrum of indolimine-248 showing J-coupling patterns. (E) ^{1}H-^{13}C HSQC spectrum of indolimine-248 and signal assignments. (F) ^{1}H-^{13}C HMBC (blue) and HSQC (red) spectrum of indolimine-248 and signal assignments.

Supplemental Table S1. Primer sequences utilized in qRT-PCR analysis.

Gene	Forward sequence (5´→3´)	Reverse sequence (5´→3´)
ACTB	*caccattggcaatgagcgattc*	*aggtctttacggatatccacat*
CYP1A1	*acctcagcagccacctccaagat*	*gaggtcttgaggccctgat*
AHRR	*gtgcgaatcggaactgcatggaaa*	*tcagtctgttccctgagcaccaaa*
PARP7	*gattctcaggagcacttggaaag*	*tggtgtggacagccttgctagt*
Il6	*agacagccactcaggtcttca*	*ttctgccagtgcctctttactg*

Supplemental Table S2. Computational Validation of the human AHR PAS-B domain model.

Model	Active Site Cavity Volume (Å^3) [a]	Test Ligand	ANF Binding Energy (kcal/mol) [b]	ANF Re-Docking Error RMSD (Å) [c]
Human AHR PAS B domain model PDB: 7ZUB	830	Indirubin	-12.8	0.498

[a] Active site volume or cavity size (in angstroms) as calculated using the program Caver Web 1.0.

[b] Autodock Vina Binding Energy for Indirubin in a 30 Å^3 grid centered on the PAS-B domain of the AHR (7SUB).

[c] RMSD deviation for Indirubin re-docking solutions in structure-based models in Autodock Vina, as calculated using LigRMSD. Models with a ligand re-docking RMSD error of less than 2.0 Å are generally considered acceptable for our docking protocol.