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New analogs of SYA013 as sigma-2 ligands with anticancer activity

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Abstract

Our previous study has revealed 4-(4-(4-chlorophenyl)-1,4-diazepan-1-yl)-1-(4-fluorophenyl)butan-1-one-2HCI (SYA013) **1** as a sigma ligand with moderate selectivity for the sigma-2 receptor. Given the overexpression of sigma receptors in solid tumors and reports of sigma ligands with anticancer activities, we selected **1** for evaluation in several solid tumor cell lines. In addition, we have synthesized new analogs of **1** and now report that several of them bind preferentially at the sigma-2 receptor and have shown inhibition of several cancer cell lines including MDA-MB-231, MDA-MB-486, A549, PC-3, MIA PaCa-2 and Panc-1 cells. In particular, compounds **1** and **12** have demonstrated sub-micromolar activity against the Panc-1 cell line. It has also been observed that several of these compounds demonstrate selective toxicity toward cancer cells, when compared to normal cells.

Keywords

Sigma receptors; sigma-2 receptor; indanone; homopiperazine; oxime; anticancer activity

1. Introduction

Sigma (σ) receptors are distinct proteins incorporated in plasma, mitochondrial and endoplasmic reticulum membranes of tissues in the brain, kidneys, endocrine, liver, immune system, and reproductive organs [1]. Currently, there are two well-defined sigma receptor subtypes; sigma-1 (σ_1) and sigma-2 (σ_2) [2]. The σ_1 receptor has a molecular weight of 25 kDa and the (σ_2) receptor has a molecular weight of 18 – 21.5 kDa and both have been cloned [3]. There are several recent reviews that address the importance of sigma receptors as targets for drug development [4]. Until recently, interest in sigma receptor ligands was focused on their use as CNS agents however, research has shown that both σ_1 and σ_2

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receptors are widely expressed in various cell lines [5], but non-malignant cells from the same tissue displayed less sigma receptor expression than malignant cells [6]. It has also been shown that there is an expression of σ_1 and σ_2 receptors in breast cancer and other cell lines, with increased levels of σ_2 receptors versus the σ_1 receptor subtype, [7]. In addition, several lines of evidence have suggested that the proportion of σ_2 receptors in fast multiplying cells is higher than in dormant cells with fast multiplying cells expressing up to ten times more σ_2 receptors than resting cells [8]. Furthermore, it has been noted that o receptors were abundant in human breast tumor biopsy tissue, but practically lacking in normal breast tissue [8],[9]. Thus, σ receptors, and in particular σ_2 receptors, have a great potential to serve as targets for anticancer drug development due to their high concentration in several tumor cell types with fast proliferation [10].

Three receptors are commonly targeted in anti-breast cancer drug design; these are estrogen receptors (ER), progesterone receptors (PR) and human epidermal growth factor receptor 2 (HER2). However, it has been shown that there is a subtype of breast cancers which is estrogen receptor-negative, progesterone receptor-negative and HER2-negative, designated “triple negative breast cancer” (TNBC) [11]. Depending on the stage of its diagnosis, TNBC can be particularly aggressive, and more likely to recur than other subtypes of breast cancers. Black women have the highest risk factor for TNBC. According to the Breastcancer.org website, an analysis has found that African American women are three times as likely as white women to be diagnosed with TNBC [12] which has a poorer prognosis. Given the absence of targeted receptors in TNBC, sigma ligands may serve as a useful target for anti-TNBC drug development [7].

We have previously demonstrated that SYA013, a homopiperazine analog of haloperidol, binds with moderate selectivity to σ_2 receptors (σ_1 = *Ki* = 24 and σ_2 *Ki* = 5.6 nM), and that its deoxygenated analog **2** (Fig. 1) resulted in increased affinity for the σ_1 receptor and a loss of selectivity for the σ_2 receptor [13]. Thus, we hypothesize that TNBC, which overexpresses sigma receptors, could be an interesting target for the design of new SYA013 analogs.

The aim of the current study therefore was to design and synthesize a limited number of new SYA013 analogs, investigate the structural elements that impact σ_2 selectivity and evaluate a select number of analogs for activity against TNBC and subsequently other cell lines. We now report the synthesis of several such analogs of SYA013, including selected oximes, that bind with high affinity to σ_2 receptors and display anticancer activity against TNBC and other cancer cell lines.

2. Results and discussion

2.1. Chemistry

Compound **3** was previously synthesized and reported by us [14]. The synthetic methods leading to alkylating agents **14, 15, 16** and **17** were also previously reported [14], [15], [16]. Compounds **4 – 8** were synthesized as described in Scheme 1a. The synthetic methods and conditions had been fairly well studied in order to identify conditions that could improve the yields of the products. The final synthesis employed a one-step coupling reaction of

alkylating agents **(14 – 17)** with amines **(18 – 20)** in toluene under refluxing conditions. Compound **9** was synthesized using the same method and conditions as described in Scheme 1b by coupling of alkylating agent **17** and 1-(4-chlorophenyl)piperazine.

Alkylating agents **21** and **22** were synthesized from the corresponding 2-(2-chloroethyl)-5-fluoro-2,3-dihydro-1 H-inden-1-one **14** and 5-chloroindanone counterpart **17** in a 2-step process, as depicted in Scheme 2.

Reaction of alkylating agents **21** and **22** with 1-(4-chlorophenyl)-1,4-diazepane **18** afforded analogs **10, 11 (**Scheme 3). A crucial step is the use of toluene as the solvent for alkylation, since isopropanol and other polar solvents yielded multiple spots with low yields which may be due to the competing Michael addition to the indenone [17].

Oxime **12**, **13** were prepared by refluxing ketone **1** and indanone **3** with hydroxylamine hydrochloride under a basic condition in ethanol (Scheme 4).

2.2. Biological evaluation

2.2.1. Radioligand binding assays—In our previous article [13], we reported that the carbonyl group in SYA013: 4-(4-(4-chlorophenyl)-1,4-diazepan-1-yl)-1-(4-fluorophenyl)butan-1-one **(1)** appears to play a significant role in the binding affinity of the homopiperazine analogs to sigma receptors. Thus, we wondered what effect restricting the carbonyl group into an indanone moiety as in **3,** might have on binding affinity and selectivity for the σRs. It was also of interest to probe the structure-affinity relationships in the SYA013 scaffold, and so changes were made at four locations on the scaffold of the restricted analog including dehydrogenated analogs or indenones to yield compounds **3 – 11.** Subsequently, the oxime analogs of **1** and **3** were synthesized for screening at the σRs and then evaluated along with several carbonyl analogs for anticancer activity against MDA-MB-231 and the compounds with activity against TNBC cells were screened against other cancer cell lines. The structures of the analogs are depicted in Fig. 2 and the binding affinity data are reported in Table 1. It is important to note that the screening activities were carried out on racemic mixtures of the compounds since the separated enantiomers were unstable under ambient conditions and reverted to the racemic mixtures thereafter.

Compound **3,** the carbonyl restricted analog of **1,** binds with more than 2-fold lower affinity for the σ_1 receptor but a greater than 9-fold higher affinity for σ_2 receptor compared to compound **1.** Compound **3** is thus, 105-fold more selective for the σ_2 receptor compared to σ_1 Compound **4** is the desfluoro analog of **3** and binds with 3-fold higher affinity than compound **3** at the σ_1 receptor but over 7-fold lower affinity at the σ_2 receptor, suggesting the fluorinated analog is preferred at the σ_2 receptor. Compound **5,** the pyridine analog of **3** binds with a higher affinity at the σ_1 receptor but maintains similar binding affinity at the σ_2 receptor while compounds **6** and **7** display similar binding profiles as **5.** It should however be noted that unlike the deoxygenated analog **2,** compound **7** did not display reversal of selectivity for the σ_2 receptor. Compound **8** is the dichloro analog of **3** with similar affinity at the σ_1 receptor but over 31-fold reduction in affinity for the σ_2 receptor. On the other hand, **4** is the deschloro analog of **8**, suggesting that the para chloro has no effect on the phenone moiety in binding to σ_1R but makes a significant contribution to binding to the

σ_2R. In compound **9,** the homopiperazine ring was replaced by a piperazine ring in order to explore another isostere of the homopiperazine. Interestingly, there was no significant change in their binding affinities at both sigma receptors suggesting that the piperazine could serve as a bioisostere for the homopiperazine moiety. Compounds **10** and **11** are the indenone analogs of indanones **3** and **8** respectively. While binding affinity was similar at the σ_1 receptor for **10**, affinity decreased significantly at the σ_2 receptor. On the other hand, affinity was enhanced at both sigma receptors when the fluorine **(8)** was replaced with chlorine **(11).** With limited data, it is unclear why the trend of binding affinities in the indanone and indenone pairs are inconsistent and we speculate that the binding modes could have changed. It should also be noted that separation of the enantiomers of the indanones was not possible because the enantiomers quickly racemize on standing producing the racemic mixture. Overall, the indanone analogs of SYA013 (compounds **3, 5, 6** and **7)** display high affinity and selectivity (>10 fold) for the σ_2 receptor. In fact, compound **3** has the highest binding affinity and is the most selective ligand for the σ_2 receptor ($\sigma_2 K_i$ = 0.6 nM and $\sigma_1 K_i/\sigma_2 K_i$ = 105) in this study.

Given the observation that oximes may enhance anticancer properties [18], [19], the oximes of compounds **1** and **3,** that is **12** and **13** respectively, were synthesized and evaluated for affinity at the sigma receptors. Compound **12** binds with high affinity at both sigma receptors but compound **13** resulted in a significantly lower affinity for both receptors. This significant change in binding affinity may be associated with a change in topology of the indanone and the indenone ring systems.

2.2.2. Cell viability studies against TNBC cells—With the radioligand binding screening results of the analogs at the σRs at hand, we turned our attention to their activity against a TNBC cell line (MDA-MB-231). The original lead compound **1** and the highest affinity ligands for the σ_2 receptor, i.e., compounds **3** and **5,** and the oximes of compounds **1** and **3,** i.e., **12** and **13,** were evaluated for their anti-TNBC activity. Cisplatin, a standard anti-TNBC drug, was included in the assay as a positive control. The cell viability IC_{50} values are reported in Table 2. In general, the compounds were effective in suppressing the viability of the MDA-MB-231 cells, and their potencies increased with increasing treatment time as indicated by the decrease in the IC_{50}S except for Cisplatin (Table 2).

As shown in Table 2 and Fig. 3, compound **3** exhibited both concentration-dependent as well as time-dependent suppression of cell viability and inhibits MDA-MB-231 cells with an IC_{50} of 23.5 μM compared to cisplatin (Table 2, Fig. 4), with an IC_{50} of 50.0 μM at 48 hours. Thus **3** is at least two times more potent than cisplatin in this assay. Among the five compounds evaluated, compounds **12** and **13** were the most potent against the TNBC cells which is consistent with reports by others [18], [19] indicating that the oxime analog displayed a much higher anticancer activity than the carbonyl counterpart. Among the carbonyl analogs, compound **1** has the highest anti-TNBC activity with an IC_{50} value of 15.0 μM in 72 h compared to that of cisplatin (64.2 μM) while compound **5,** a higher affinity ligand for σ_2R, had the lowest anti-TNBC activity. Thus, the available data suggest that the σ_2R binding affinity of the compounds in general, did not directly correlate with their anticancer activities.

2.2.3. Cell viability studies against normal cells (HEK293), comparisons—In addition, the IC_{50} value of **3** against MDA-MB-231 cells was compared to its effect against normal embryonic kidney cell lines, (Human Embryonic Kidney 293 cells or HEK 293 cells) in order to evaluate its selective toxicity (Fig. 5 and Table 3). As shown in Fig 3, compound **3** demonstrated a two-fold selectivity for inhibiting the cancer cells (IC_{50} = 20.1 μM) compared to HEK293 cells with an IC_{50} value of 41.3 μM (Table 3).

2.2.4. Cell viability studies against MDA-MB-468, A549, PC-3, MIA PaCa-2, and Panc-1 cell lines—Based on the initial results, we conducted further cell viability studies on other solid tumor cell lines known to overexpress σRs including the other TNBC cell line MDA-MB-468 [20], human alveolar basal epithelial adenocarcinoma A549 cells [21], prostate cancer PC-3 cells [22], the pancreatic cancer cell lines, MIA PaCa-2 [23], and Panc-1 [23]. The results are shown in Fig. 6 and Table 4.

Overall, the results demonstrate that **1** and **12** display marked selective toxicity against MDA-MB-468 when compared to the non-tumorigenic epithelial MCF-10A cells. In particular, while compounds **1** and **12** have activity against all the cell lines evaluated, they are especially effective against the pancreatic cancer cell lines, (MIA PaCa-2 and Panc-1 cell lines) as shown in Table 4. These two compounds exhibited sub-micromolar IC_{50} values against Panc-1 cells, which therefore suggest a need to take a closer look at them.

It is important to note that there are suggestions that pan-SR ligands might be more effective as anticancer agents [24]. This however, has not been the focus of this manuscript. In addition, the functional status of the current agents have not been evaluated and may very well explain why there was no correlation between the sigma-2 binding affinity of the reported compounds and inhibition of cancer cells.

3. Conclusions

In summary, we have shown in this study that several of the sigma ligands screened displayed some selectivity for the σ_2 receptor verses the sigma-1 receptor. Preliminary cell viability studies have demonstrated that not only do these compounds inhibit TNBC cells but they appear to show selective toxicity toward cancer cells when compared to normal cell lines. In particular, compounds **1** and **12** have shown significant inhibition of pancreatic cancer Panc-1 cells with sub-micromolar IC_{50} values of 0.4 and 0.14 μM, respectively. These encouraging results will be further evaluated in future studies.

4. Experimental

4.1. Chemistry

4.1.1. General—Melting points were determined on a Gallenkamp (UK) apparatus and are uncorrected. All NMR spectra were obtained on either a Varian 300 MHz Mercury Spectrometer or Bruker 600 MHz Spectrometer. The TopSpin 3.5 software running on the Bruker NMR has a multiplet analysis tool (MANA) that identifies and defines multiplets automatically or interactively. 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 on Isolera I (Biotage) instrument on silica gel (Davisil grade 634) with gradient elution varying from 100% Hexane to 100% EtOAc as a standard procedure except where otherwise indicated. Starting materials were obtained from Sigma–Aldrich and were used without further purification. Yields reported in the manuscript are not optimized yields.

4.1.2. Synthesis of indenones 21 and 22)

4.1.2.1. 1 2-(2-chloroethyl)-5-fluoro-1 H-inden-1-one (21): To a solution of 2-(2-chloroethyl)-5-fluoro-2,3-dihydro-1H-inden-1-one **14** (1 g, 4.7 mmol) in CH_2CI_2 (15 mL) was added dropwise a solution of Br_2 (800 mg, 5 mmol) in CH_2CI_2 (5 mL). After stirring for 3 h, the solvent was removed and 2-bromo-2-(2-chloroethyl)-5-fluoro-2,3-dihydro-1H-inden-1-one was obtained. The solid was dried under vacuum for 12 h and used as such for the next step. 2-Bromo-2-(2-chloroethyl)-5-fluoro-2,3-dihydro-1H-inden-1-one (1.2 g, 4.1 mmol), Li_2CO_3 (2 g, 27 mmol) in decane (30 mL) was heated at 180 ºC overnight. After cooling to room temperature, the mixture was purified by flash chromatography on silica gel using gradient elution from Hexane (100%) to EtOAc (100%) to give 2-(2-chloroethyl)-5-fluoro-1H-inden-1-one **21,** 250 mg, in a yield of 25.3%.

^{1}H NMR (300 MHz, $CDCI_3$): 8.30 (1H, dd, *J* = 5.7, 8.7 Hz), 7.19 (1H, dt, *J* = 2.7, 8.4 Hz), 7.06 (1H, dd, *J* = 2.7, 8.7 Hz), 6.37 (1H, s), 3.86 (2H, t, *J* = 6.6 Hz), 2.98 (2H, t, *J* = 6.6 Hz).

4.1.2.2. 2-(2-chloroethyl)-5-chloro-1H-inden-1-one (22): Using the above procedure, **22** was obtained in 21% yield. ^{1}H NMR (300 MHz, $CDCl_3$): 8.20 (1H, d, *J* = 8.4 Hz), 7.45 (1H, d, *J* = 8.4 Hz), 7.38-7.40 (1H, m), 6.32 (1H, d, *J* =3.2 Hz), 3.85 (2H, t, *J* = 6.6 Hz), 3.69 (2H, t, *J* = 6.6 Hz), 3.08 (1H, t, *J* = 6.6 Hz), 2.98 (1H, t, *J* = 6.6 Hz).

4.1.3. Synthesis of analogs (4 – 11)

4.1.3.1. General Procedure I: A mixture of 2-(2-chloroethyl)-5-fluoro-2,3-dihydro-1H-inden-1-one, **14** (or **15-17)** (5.2 mmol), 1-(5-chloropyridin-2-yl)-1,4-diazepane, **18** (or **19-20**) (5.7 mmol), Kl (150 mg), $NaHCO_3$ (11.9 mmol) in Toluene (10 mL) was heated to reflux under N_2 for 12 h. After cooling to rt, the mixture was diluted with EtOAc (500 mL) followed by washing with H_2O (2x300 mL). The organic layer was pooled, dried over Na_2SO_4 and filtered. The filtrate was concentrated in vacuo to dryness followed by column chromatography on silica gel to afford the final product.

4.1.3.2. 2-(2-(4-(4-chlorophenyl)-1,4-diazepan-1-yl)ethyl)-2,3-dihydro-1H-inden-1-one (4): Using **14** and **18,** resulted in the free base **4,** which was converted to the HCI salt, and recrystallized from MeOH-Et_2O to afford the pure HCI salt, in a yield of 36%, mp 209-210 °C. ^{1}H NMR (300 MHz, DMSO-d^6): 10.96 (1H, brs), 7.69 (1H, d, *J* = 7.2 Hz) (**indanone**), 7.64 (1H, d, *J* = 7.2 Hz) (**indanone**), 7.57 (1H, d, *J* = 7.5 Hz) (**indanone**), 7.42 (1H, m) (**indanone**), 7.19 (2H, d, *J* = 9.0 Hz), 6.74 (2H, d, *J* = 9.0 HZ), 3.76 (2H, m), 3.49 (2H, m), 3.37 (5H, m), 3.10 (2H, m), 2.82 (1H, m), 2.74 (1H, m), 2.34 (1H, m), 2.23 (1H, m), 2.13 (1H, m), 1.86 (1H, m).

^{13}C NMR (150 MHz, DMSO-d^6): 207.2, 154.0, 147.7, 136.3, 135.6, 129.2 (2), 128.3, 127.3, 120.5, 113.9 (2), 54.7, 54.3, 53.5, 47.2, 44.7, 43.9, 32.5, 25.6, 23.7.

Calcd for $C_{22}H_{25}ClN_2O$•HCl: C 65.19, H 6.46, N 6.91; *Found*: C 65.18, H 6.55, N 6.90.

4.1.3.3. 2-(2-(4-(5-chloropyridin-2-yl)-1,4-diazepan-1-yl)ethyl)-5-fluoro-2,3-dihydro-1H-inden-1-one (5): Using **14** and **19,** resulted in the free base **5,** which was converted into HCI salt, followed by crystallization from MeOH-Et_2O to afford pure HCI salt in a yield of 28%, mp 195-197 °C.

^{1}H NMR (300 MHz, DMSO-d^6): 11.04 (1H, s), 8.28 (1H, brs), 8.08 (1H, d, *J* = 2.4 Hz), 7.70 (1H, dd, *J* = 5.4 **(H-F coupling),** 8.4 Hz) (**indanone**), 7.64 (1H, dd, *J* = 2.7 **(H-F coupling),** 9.0 Hz) (**indanone**), 7.43 (1H, dd, *J* = 1.8, 9.0 Hz), 7.26 (1H, dt, *J* = 2.4, 9.0 Hz), 6.78 (1H, d, *J* = 9.0 Hz) (**indanone**), 4.21-4.28 (1H, m), 3.68-3.78 (1H, m), 3.45-3.58 (4H, m), 3.24-3.37 (2H, m), 3.04-3.19 (3H, m), 2.76-2.86 (2H, m), 2.36-2.41 (1H, m), 2.10-2.26 (2H, m), 1.82-1.92 (1H, m).

^{13}C NMR (75 MHz DMSO-d^6): 205.5, 167.1 (d, *J* = 255 Hz), 157.2 (d, *J* = 10.5 Hz), 155.0, 142.4,139.6, 132.9 (d, *J* = 1.6 Hz), 126.3 (d, *J* = 11.2 Hz), 118.7, 116.2 (d, *J* = 23.2 Hz), 113.9 (d, *J* = 22 Hz), 110.0, 54.6, 53.9, 53.3, 46.7, 45.0, 42.1, 32.5, 25.4, 23.4.

Calcd for $C_{21}H_{23}ClFN_3O$•2HCI: C 54.74, H 5.47, N 9.12; *Found*: C 54.96, H 5.51, N 9.14.

4.1.3.4. 5-fluoro-2-(2-(4-(pyridin-2-yl)-1,4-diazepan-1-yl)ethyl)-2,3-dihydro-1H-inden-1-one (6): Using **14** and **20,** resulted in the free base **6,** which was converted into the HCI salt, followed by crystallization from MeOH-Et_2O to afford the pure salt in a yield of 18.5 %, mp 242-243 °C.

^{1}H NMR (300 MHz, DMSO-d^6): 14.36 (1H, brs), 11.45 (1H, s), 8.04 (1H, d, *J* = 6.3 Hz), 7.99 (1H, t, *J* = 8.1 Hz), 7.75 (1H, dd, *J* = 5.4 **(H-F coupling),** 8.7 Hz) (**indanone**), 7.47 (1H, d, *J* = 8.7 Hz) (**indanone**), 7.27 (2H, m), 6.96 (1H, t, *J* = 6.6 Hz) **(indnaone),** 4.23 (1H, m), 4.06 (1H, m), 3.64 (5H, m), 3.30 (4H, m), 2.86 (2H, m), 2.53 (1H, m), 2.22 (2H, m), 1.88 (1H, m).

^{13}C NMR (150 MHz, DMSO-d^6): 205.4, 167.0 (d, *J* = 251.9 Hz), 157.2 (d, *J* = 10.1 Hz), 152.8, 143.4, 138.3, 132.9, 126.3 (d, *J* = 10.8 Hz), 116.1 (d, *J* = 23.9 Hz), 113.8 (d, *J* = 22.1 Hz), 112.9, 112.1, 54.6, 53.3, 52.7, 47.7, 44.9, 43.1, 32.4, 25.2, 23.0.

Calcd for $C_{21}H_{24}FN_3O$•2H$_2$)O:C 58.66, H 6.19, N 9.77; *Found*: C 58.69, H 6.23, N 9.67.

4.1.3.5. 1-(2-(5-fluoro-2,3-dihydro-1H-inden-2-yl)ethyl)-4-(pyridin-2-yl)-1,4-diazepane (7): Using **16** and **20** resulted in the free base **7,** which was converted into the HCI salt followed by crystallization from MeOH-Et_2O to afford the pure salt, in a yield of 27.5%, mp 172-173 °C.

^{1}H NMR (300 MHz, DMSO-d^6): 11.39 (1H, brs), 8.03 (1H, m), 7.99 (1H, m), 7.26 (1H, m), 7.17 (1H, dd, *J* = 5.8 **(H-F coupling),** 8.4 Hz) (**indanone**), 6.91 (3H, m) **(overlap, 2 of 3**

indanone), 4.21 (1H, m), 4.06 (1H, m), 3.70 (3H, m), 3.57 (2H, m), 3,21 (2H, m), 3.14 (2H, m), 2.99 (2H, m), 2.56 (2H, m), 2.41 (1H, m), 2.18 (1H, m), 1.90 (2H, m).

^{13}C NMR (75 MHz, $CDCI_3$) for free base **7**: 161.9 (d, J = 241.6 Hz), 158.2, 147.9, 145.5 (d, J = 8.0 Hz), 138.6, 137.2, 125.0 (d, J = 8.0 Hz), 112.7 (d, J = 23.0 Hz), 111.4 (d, J = 20.5 Hz), 111.3, 105.4, 56.7, 55.2, 55.0, 46.6, 46.5, 39.4, 39.0, 38.4, 33.3, 27.7.

Calcd for $C_{21}H_{26}FN_3$•*2HCl*•*0.7* H_2O*:* C 59.35, H 6.64, N 9.89; *Found*: C 59.35, H 6.96, N 9.72.

4.1.3.6. 5-chloro-2-(2-(4-(4-chlorophenyl)-1,4-diazepan-1-yl)ethyl)-2,3-dihydro-1H-inden-1-one (8): Using **17** and **18** resulted in the free base **8,** which was converted into the HCI salt followed by crystallization from MeOH-Et_2O to afford the pure salt in a yield of 27%, mp 189-191 °C.

^{1}H NMR (600 MHz, DMSO-d^6): 10.78 (1H, brs), 7.73 (1H, s) (**indanone**), 7.67 (1H, d, J = 8.1 Hz) (**indanone**), 7.51 (1H, dd, J = 1.7, 8.1 Hz) (**indanone**), 7.21 (2H, d, J = 9.0 Hz), 6.77 (2H, d, J = 9.0 Hz), 3.72-3.82 (2H, m), 3.41-3.53 (3H, m), 3.31-3.39 (3H, m), 3.17-3.29 (2H, m), 3.07-3.11 (1H, m), 2.80-2.88 (2H, m), 2.32-2.35 (1H, m), 2.23-2.27 (1H, m), 22.14-2.17 (1H, m), 1.84-1.93 (1H, m).

^{13}C NMR (75, MHz DMSO-d^6): 205.9, 155.9, 147.7, 140.4, 135.0, 129.2 (2), 128.5, 127.3, 125.3, 120.4, 113.9 (2), 54.6, 54.5, 47.1, 44.8, 44.0, 32.3, 32.2, 25.4, 23.8.

Calcd for $C_{22}H_{24}Cl_2N_2O$•*HCl*•*0.2* H_2O C 59.59, H 5.77, N 6.32; *Found*: C 59.41, H 5.69, N 6.24.

4.1.3.7. 5-chloro-2-(2-(4-(4-chlorophenyl)piperazin-1-yl)ethyl)-2,3-dihydro-1H-inden-1-one (9): Using **17** and 1-(4-chlorophenyl)piperazine resulted in the free base **9,** which was converted into the HCI salt followed by crystallization from MeOH-Et_2O to afford the pure salt in a yield of 34%, mp 211-213 °C.

^{1}H NMR (600 MHz, DMSO-d^6): 11.00 (1H, brs), 7.75 (1H, s) (**indanone**), 7.68 (1H, d, J = 8.0 Hz) (**indanone**), 7.51 (1H, d, J = 8.0 Hz) (**indanone**), 7.29 (2H, d, J = 8.9 Hz), 7.02 (2H, d, J = 8.9 Hz), 3.81 (2H, brs), 3.57 (2H, brs), 3.31-3.41 (2H, m), 3.21-3.24 (1H, m), 3.12-3.16 (4H, m), 2.84-2.89 (2H, m), 2.26-2.29 (1H, m), 1.91-1.94 (1H, m).

^{13}C NMR (150 MHz, DMSO-d^6): 206.0, 156.0, 148.9, 140.5, 135.1, 129.3 (2), 128.6, 127.4, 124.0, 117.9 (2), 54.0, 51.1, 45.7 (2), 44.9 (2), 32.4, 25.1.

Calcd for $C_{21}H_{22}Cl_2N_2O$•HCl: C 59.24, H 5.45, N 6.58; *Found*: C 59.24, H 5.26, N 6.65.

4.1.3.8. 2-(2-(4-(4-chlorophenyl)-1,4-diazepan-1-yl)ethyl)-5-fluoro-1 H-inden-1-one (10): Using **21** and **18** resulted in the free base **10,** which was converted into the HCI salt followed by crystallization from MeOH-Et_2O to afford the pure salt in a yield of 32%, mp 234-235 •C.

^{1}H NMR (300 MHz, DMSO-d^{6}): 11.11 (1H, s), 8.17 (1H, dd, *J* = 5.7 **(H-F coupling),** 8.7 Hz) (**indanone**), 7.36-7.48 (2H, m) (**indanone**), 7.19 (2H, d, *J* = 9.0 Hz), 6.75 (2H, d, *J* = 9.0 Hz), 6.69 (1H, s), 3,68-3.84 (2H, m), 3.54-3.59 (2H, m), 3.33-3.47 (3H, m), 3.19-3.25 (1H, m), 3.01-3.14 (4H, m), 2.31-2.41 (1H, m), 2.11-2.20 (1H, m).

^{13}C NMR (150 MHz, DMSO-d^{6}): 166.5 (d, *J* = 252.2 Hz), 161.1, 154.8, 147.7, 140.2 (d, *J* = 11.4 Hz), 132.9 (d, *J* = 10.4 Hz), 129.2 (2), 120.6, 117.1, 116.9, 113.9 (2), 111.9 (d, *J* = 22.8 Hz), 104.6, 54.6, 53.7, 53.3, 47.2, 44.0, 28.4, 23.8.

Calcd for $C_{22}H_{22}ClFN_2O$•*2HCl:* C 57.72, H 5.28, N 6.12; *Found*: C 57.68, H 5.21, N 6.20.

4.1.3.9. 5-chloro-2-(2-(4-(4-chlorophenyl)-1,4-diazepan-1-yl)ethyl)-1H-inden-1-one (11): Using **22** and **18** resulted in the free base **11**, which was converted into the HCI salt followed by crystallization from MeOH-Et_2O to afford the pure salt in a yield of 28%, mp 242-243 °C.

^{1}H NMR (300 MHz, DMSO-d^{6}): 10.93 (1H, s), 8.10 (1H, d, *J* = 8.7 Hz) (**indanone**), 7.72 (1H, s) (**indanone**), 7.61(1H, dd, *J* = 2.7, 8.7 Hz) (**indanone**), 7.21 (2H, d, *J* = 9.0 Hz), 6.77 (2H, d, *J* = 9.0 Hz), 6.69 (1H, s), 3.74-3.80 (2H, m), 3.54-3.60 (2H, m), 3.38-3.48 (4H, m), 3.21-3.27 (1H, m), 3.07-3.14 (3H, m), 2.28-2.35 (1H, m), 2.15-2.21 (1H, m).

^{13}C NMR (150 MHz, DMSO-d^{6}): 161.3, 154.9, 147.7, 140.7, 139.0, 131.4, 129.2 (2), 129.0, 125.6, 120.5, 118.9, 113.9 (2), 104.2, 54.6, 53.7, 53.2, 47.2, 44.0, 28.4, 23.7.

Calcd for $C_{22}H_{22}CI_2N_2O'HCl$ •*1.0*H_2O C 57.97, H 5.53, N 6.15; *Found*: C 57.86, H 5.18, N 6.16.

4.1.4. Synthesis of oximes (12 and 13)

4.1.4.1. General procedure II: To a solution of 4-(4-(4-chlorophenyl)-1,4-diazepan-1-yl)-1-(4-fluorophenyl)butan-1-one (1.33 mmol), hydroxylamine hydrochloride (2.0 mmol) in EtOH (10 ml) was added a solution of NaOH (5 mL, 1M). The resulting mixture was refluxed overnight. Water (100 mL) was added which resulted in the formation of a solid. The solid was dried under vacuum overnight and subsequently recrystallized from MeOH to give, 4-(4-(4-chlorophenyl)-1,4-diazepan-1-yl)-1-(4-fluorophenyl)butan-1-one oxime.

4.1.4.2. 4-(4-(4-chlorophenyl)-1,4-diazepan-1-yl)-1-(4-fluorophenyl)butan-1-one oxime (12): Using procedure II, compound **1** was converted to the oxime, **12** in a yield of 42%, mp 203-204 °C.

^{1}H NMR (300 MHz, DMSO-d^{6}): 11.42 (1H, s), 7.68-7.73 (2H, m) (**indanone**), 7.16-7.25 (4H, m) **(overlap, 2 of 4 indanone),** 6.73 (2H, d, *J* = 9.0 Hz), 3.68-3.74 (2H, m), 3.36- 3.48 (4H, m), 3.04-3.18 (4H, m), 2.69-2.74 (2H, m), 2.26-2.32 (1H, m), 2.05-2.13 (1H, m), 1.82-1.92 (2H, m).

^{13}C NMR (75 MHz, DMSO-d^{6}): 162.9 (d, *J* = 244.6 Hz), 154.9, 147.7, 132.4 (d, *J* = 3.3 Hz), 129.2 (2), 128.3 (2, d, *J* = 8.2 Hz), 120.4, 115.8 (2, d, *J* = 21.5 Hz), 113.8 (2), 56.2, 54.4, 53.6, 47.1, 43.8, 23.7, 22.6, 21.1.

Calcd for $C_{21}H_{25}ClFN_3O$: C 64.69, H 6.46, N 10.78. *Found:* C 64.70, H 6.38, N 10.66.

4.1.4.3. 2-(2-(4-(4-chlorophenyl)-1,4-diazepan-1-yl)ethyl)-5-fluoro-2,3-dihydro-1H-inden-1-one oxime (13): Using procedure II, compound **3** was converted to the oxime **13,** in a 34% yield, mp 185-186 °C.

^{1}H NMR (300 MHz, DMSO-d^6): 10.97 (1H, s), 8.28 (1H, dd, *J* = 5.4 **(H-F coupling),** 8.4 Hz) (**indanone**), 7.02-7.16 (4H, m) **(overlap, 2 of 4 indanone),** 6.64 (2H, d, *J* = 8.4 Hz), 3.37-3.44 (4H, m), 3.10 (1H, dd, *J* = 8.4, 16.8 Hz), 2.93-3.00 (1H, m), 2.63-2.70 (3H, m), 2.49-2.53 (4H, m), 1.76-1.89 (3H, m), 1.46-1.54 (1H, m).

^{13}C NMR (150 MHz, DMSO-d^6): 163.6 (d, *J* = 246 Hz), 159.2, 150.6 (d, *J* = 8.8 Hz), 147.9, 130.8 (d, *J* = 9.4 Hz), 130.6 (d, *J* = 2.2 Hz), 129.1 (2), 118.9, 114.2 (d, *J* = 22 Hz), 113.2 (2), 112.7 (d, *J* = 21.5 Hz), 54.8, 54.6, 54.1, 49.2, 48.1, 39.6, 35.7, 32.4, 27.2.

Calcd for $C_{22}H_{25}ClFN_3O$ 0.4H_2O: C 64.59, H 6.36, N 10.27; *Found:* C 64.79, H 6.09, N 10.24.

4.2. Biological testing

4.2.1. Receptor binding studies—Binding affinities (*Ki*, nM) reported in Tables 1 were conducted by the National Institute of Mental Health Psychoactive Drug Screening Program (NIMH-PDSP). Details of the methods and the radioligands used for the binding assays at each receptor were previously reported [25].

4.2.2. Material and methods for cell culture and cell viability studies—Human adenocarcinoma MDA-MB-231, MIAPaCa-2, Panc-1, A549, MDA-MB-468 and HEK293, MCF-10A cell lines were purchased from American Type Culture Collection (Manassas,VA. USA). Dulbecco's modified eagle medium (DMEM), high glucose, GlutaMax, Ham's F-12K, fetal bovine serum (FBS), penicillin-streptomycin-neomycin antibiotic mixture (PSN) were obtained from Life Technologies; Thermo Fisher (Grand Island, NY. USA). DMEM/F-12 was from Gibco. Hank's balanced salt solution was from (Sigma Aldrich, St. Louis, MO. USA); and PBS was purchased from Genesee Scientific (San Diego, CA. USA).

4.2.2.1. Cell culture: MDA-MB-231, MIAPaCa-2, Panc-1, MDA-MB-468 and HEK 293 were cultured in DMEM, high glucose, GlutaMax; MCF-10A cells were maintained in DMEM/F-12; and A549 cells were cultured in Ham's F-12K medium. The DMEM/F-12 was supplemented with insulin (10 μg/mL), epidermal growth factor (EGF, 20ng/ml), cholera toxin (100 ng/ml), hydrocortisone (0.5 μg/mL), horse serum (5%), and penicillin/streptomycin (100 U/ml/100 μg/mL); while the other media were supplemented with 100 U/ml penicillin, 100 U/ml streptomycin, and 10% heat inactivated FBS. The cells were incubated in a humidified incubator with atmosphere of 95% CO_2 set at 37 °C and sub-cultured when approximately 80-90% confluent. Unless stated otherwise, assays were performed with experimental media containing 5% FBS.

4.2.2.2. Cell viability studies: Cells were seeded into 96-well plates at 20,000 cells per well and incubated overnight in experimental media. Upon confirmation of cells being

attached, they were treated for 24 h, 48 h, and 72 h with varying concentrations of the σ_2R ligands (σ2RL) in a 1:9 mixture of DMSO/acetone or Cisplatin dissolved in 0.9% sodium chloride (normal saline). Control cells for σ2RL were treated with equivalent volumes of the 1:9 mixture of DMSO/acetone, while the controls of Cisplatin were treated with normal saline. The treatments were repeated at the 24th h for the 48-h assay and at the 24th and 48th h for the 72-h assay.

To determine and quantify the effect of the compounds on cell viability, resazurin reagent that is metabolized by live cells to the fluorescent resorufin product was used. Briefly, 20 μL of 0.02% resazurin reagent prepared in PBS was added to each well and incubated for 2-3 h. The fluorescence was measured at 560 nm excitation wavelength and a detection wavelength of 590 nm using the FLx 800 Microplate Fluorescence Reader from BioTek (Winooski, VT. USA). Using GraphPad Prism 5 software (San Diego, CA. USA), cell viability was expressed as the percentage of the fluorescence in the treated cells relative to that of the controls and the IC_{50} values were determined from the plots of the non-linear regression of the logs of σ2RL or Cisplatin concentrations.

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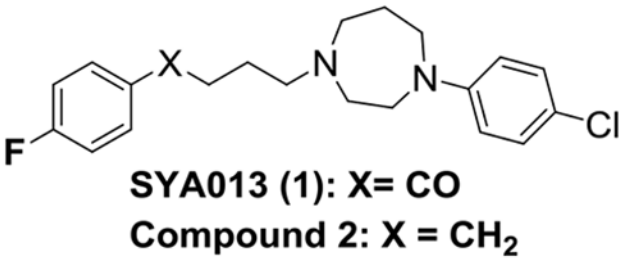
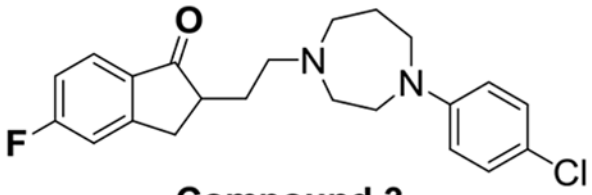


SYA013 (1): X= CO

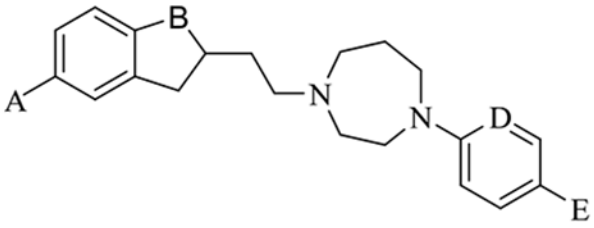
Compound 2: X = CH_2



Compound 3

Fig. 1.

Structures of SYA013, analog **2** and **3**



3. A = F; B = CO; D = CH; E = Cl
4. A = H; B = CO; D = CH; E = Cl
5. A = F; B = CO; D = N; E = Cl
6. A = F; B = CO; D = N; E = H
7. A = F; B = CH_2; D = N; E = H
8. A = Cl; B = CO; D = CH; E = Cl

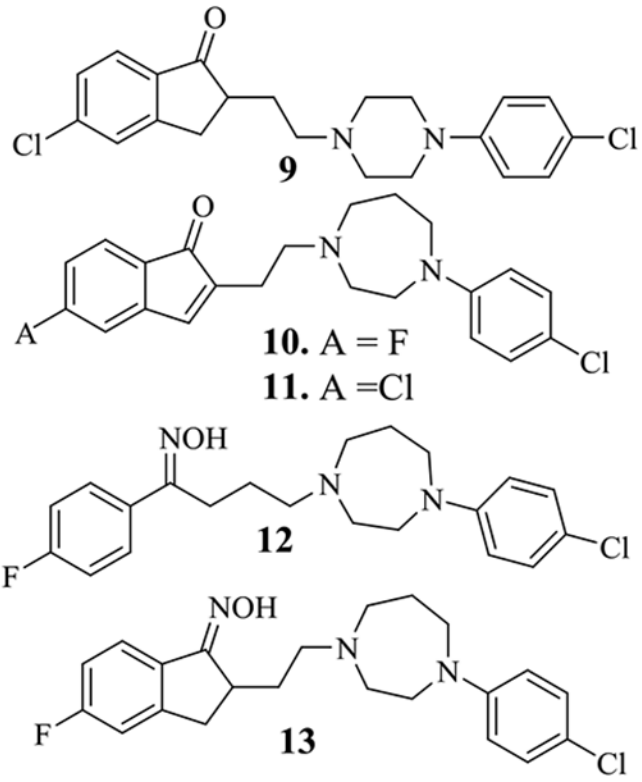


Fig 2.
Indanone and oxime analogs of SYA013.

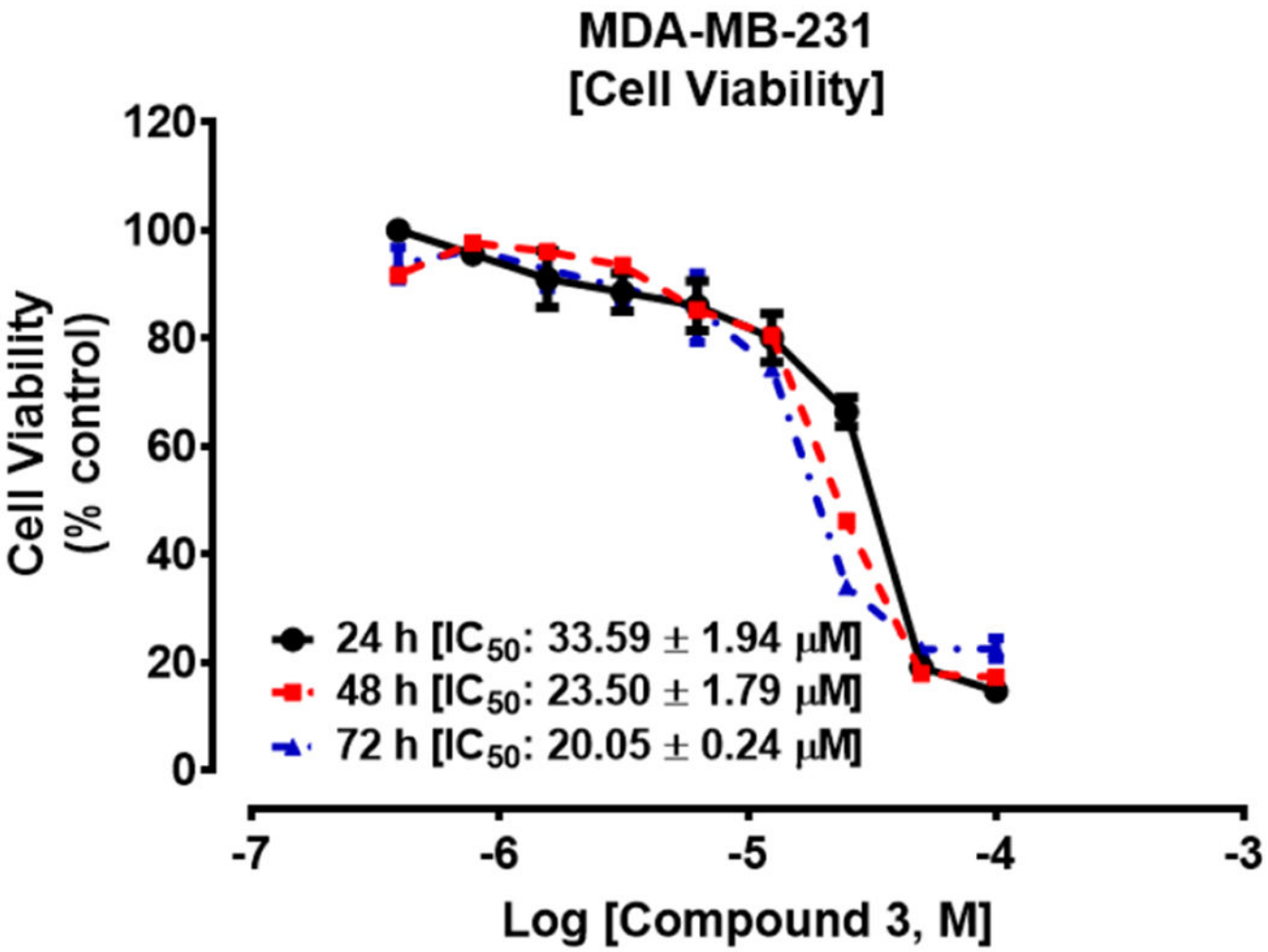


Fig 3:

Compound 3 suppressed the viability of MDA-MB-231 cells. Cells were cultured and seeded in 96-well plates at a density of 2×10^4 per well and allowed to attach overnight at 37°C in 5% CO_2/95% humidified air. Cells were then treated with compound 3 (0 – 200 μM) for 24 h, 48 h and 72 h as described in the methods. Cell viability was determined after the final treatment by fluorescence using the resazurin reduction assay.

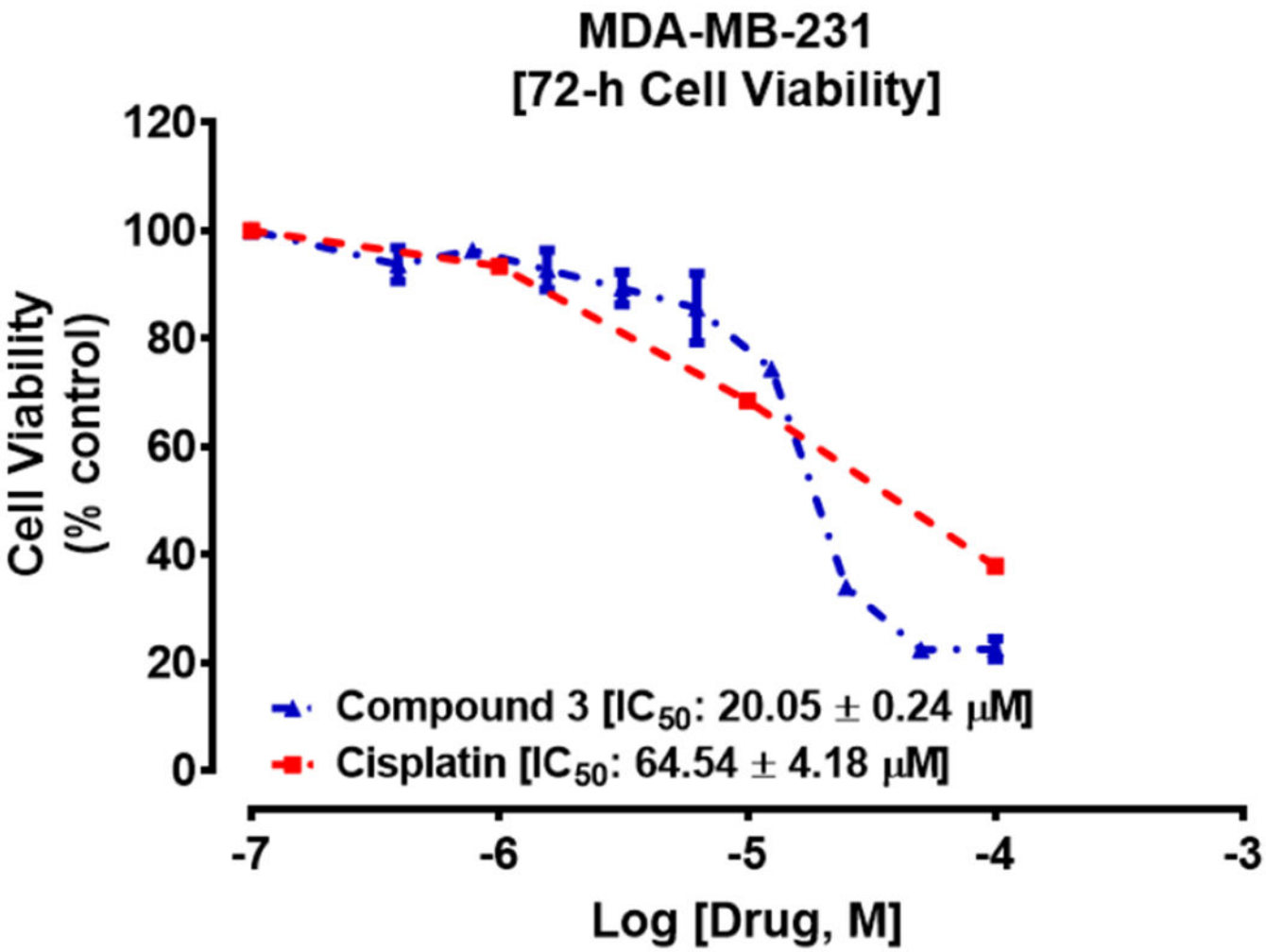


Fig 4:

Cisplatin (IC_{50} 64.2±4.2 μM) appears to be relatively less potent compared to compound 3 (IC_{50} 20.1±0.2 μM) on MDA-MB-231 cells after treatment for 72 h. Each point represents the mean ± SEM relative to the control untreated cells.

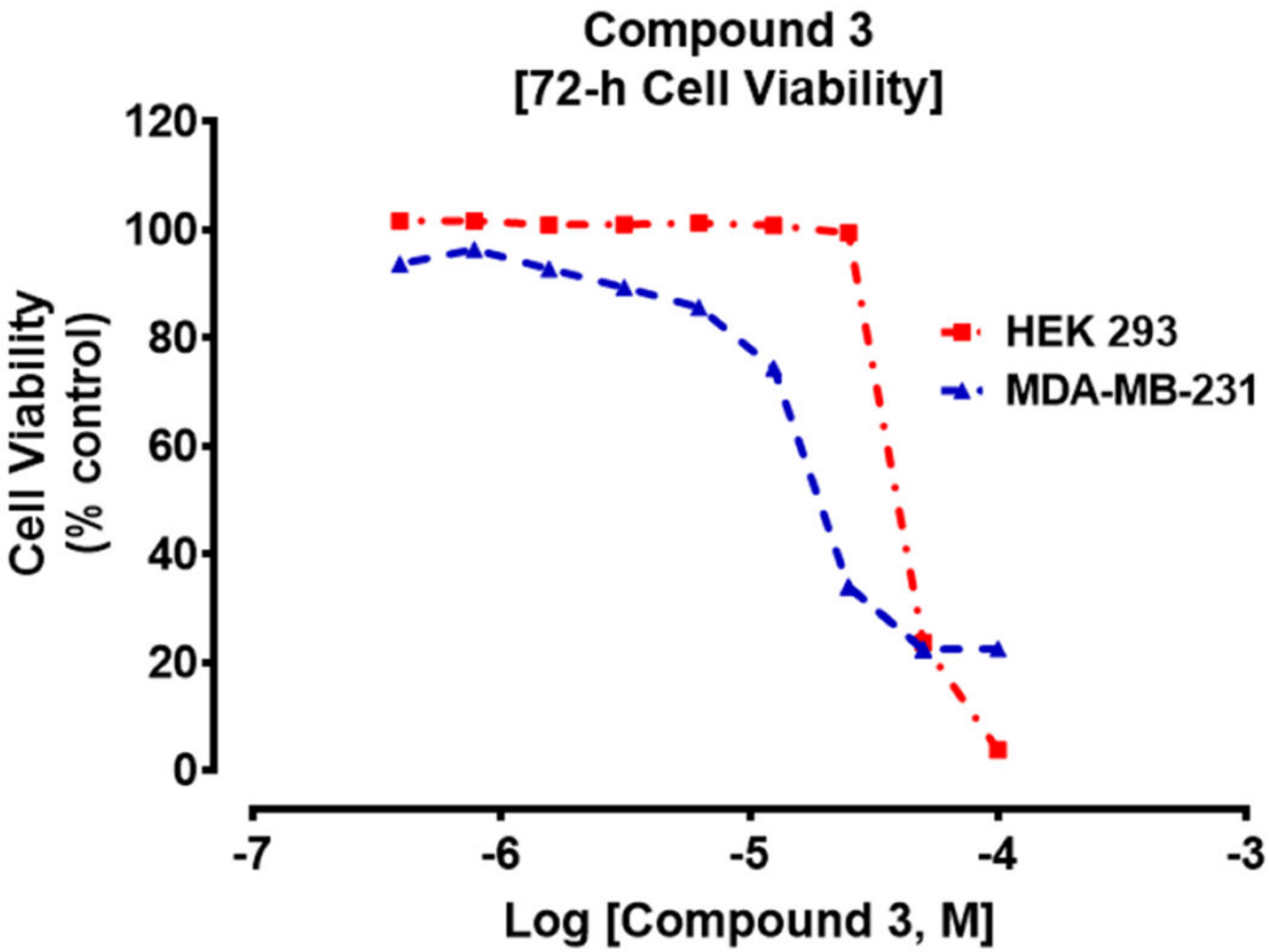


Fig 5.
Effect of **3** on TNBC or HEK 293 cells.

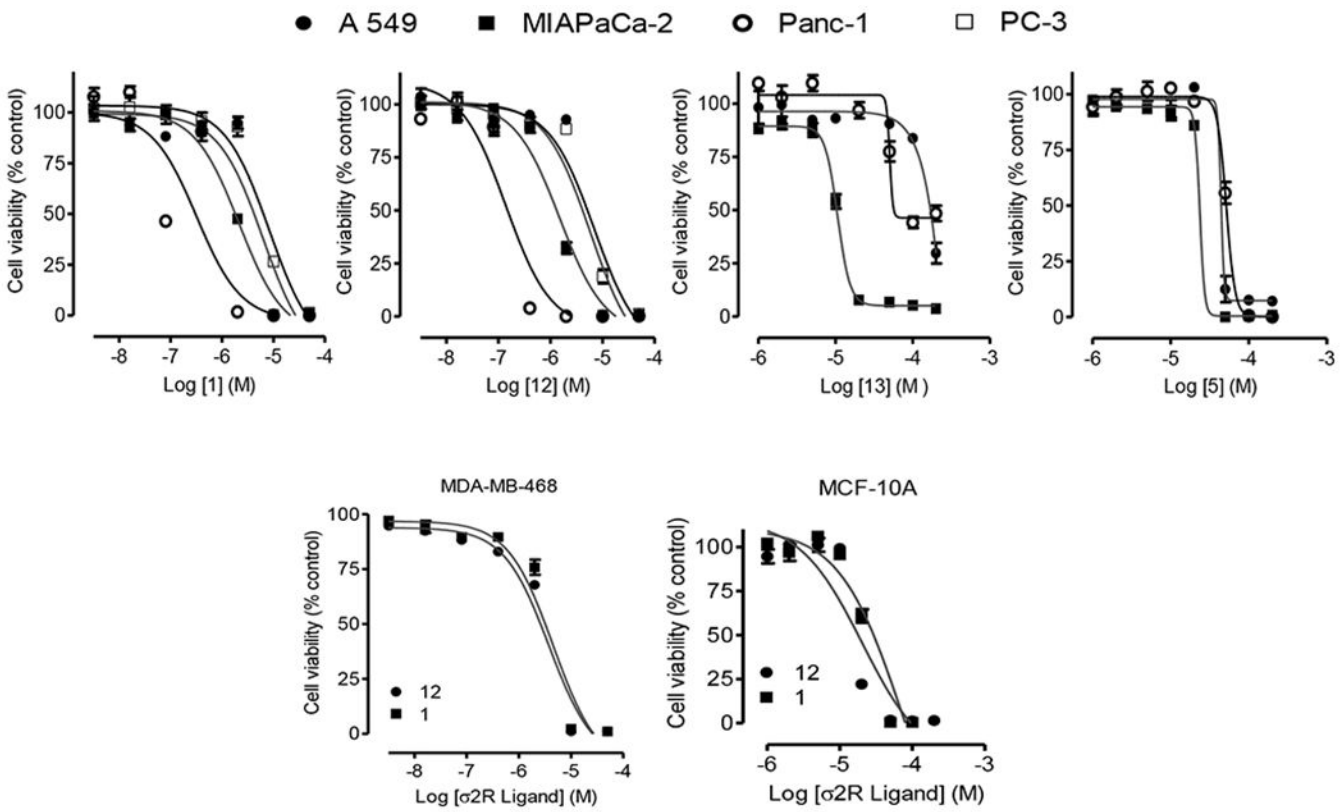
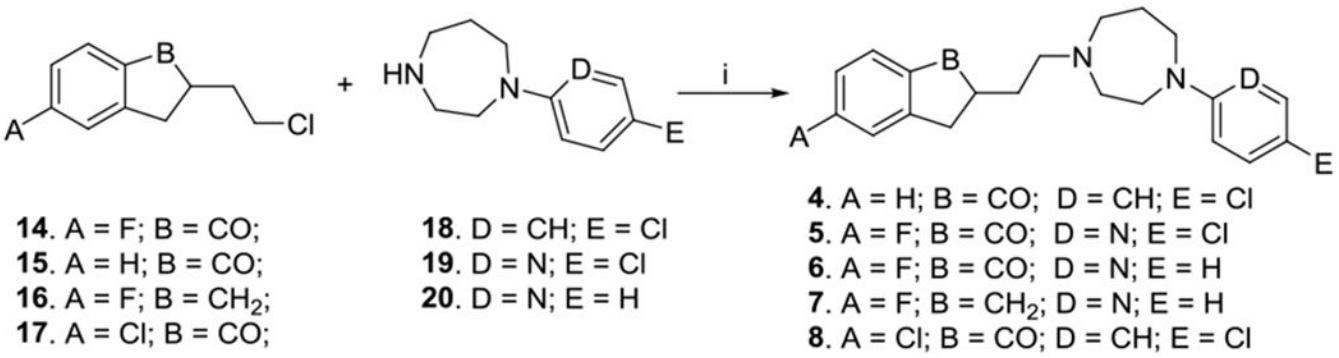


Fig. 6.

Sigma-2 ligands inhibit diverse cancer cell viability. (Top panel) Human lung cancer A549 cells (●), human prostate cancer PC-3 cells (□), human pancreatic cancer MIAPaCa-2 (■) and Panc-1 (○) cells were seeded into 96-wells tissue culture plates at 20,000 cells per well overnight in experimental media. Cells were then treated with the indicated σ_2R ligands as described in the methods. (Bottom panel) Similar tests were conducted on human breast cancer, MDA-MB-468 cells and the non-tumorigenic epithelial cell line, MCF-10A using compounds **1** and **12.** Cell viability was determined after the final treatments by fluorescence using the resazurin reduction assay. Each point represents the mean ± SEM relative to the control untreated cells.



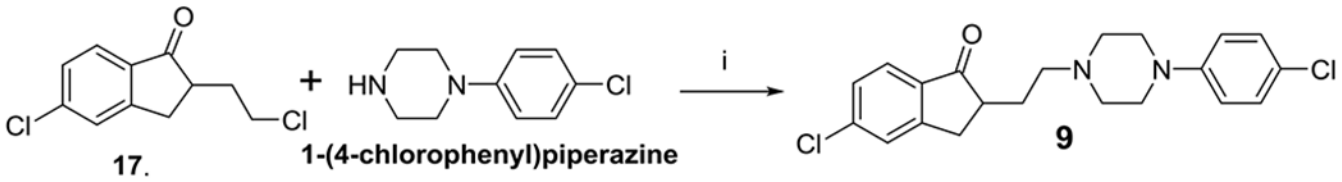
14. A = F; B = CO;
15. A = H; B = CO;
16. A = F; B = CH_2;
17. A = Cl; B = CO;

18. D = CH; E = Cl
19. D = N; E = Cl
20. D = N; E = H

4. A = H; B = CO; D = CH; E = Cl
5. A = F; B = CO; D = N; E = Cl
6. A = F; B = CO; D = N; E = H
7. A = F; B = CH_2; D = N; E = H
8. A = Cl; B = CO; D = CH; E = Cl

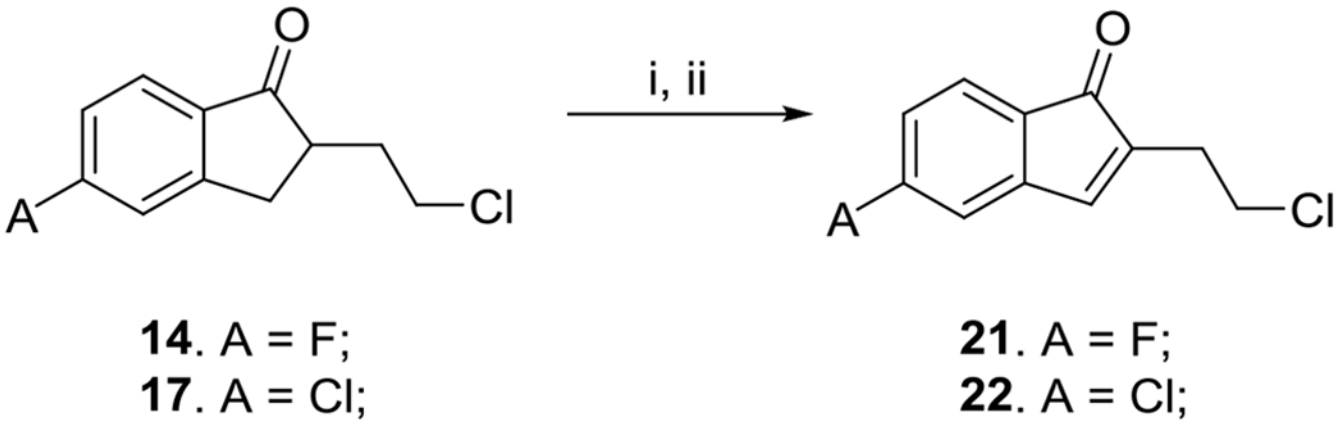
Scheme 1a.

Synthesis of indanone analogs of SYA013. Reagents and conditions: i) Kl, $NaHCO_3$, toluene, reflux.



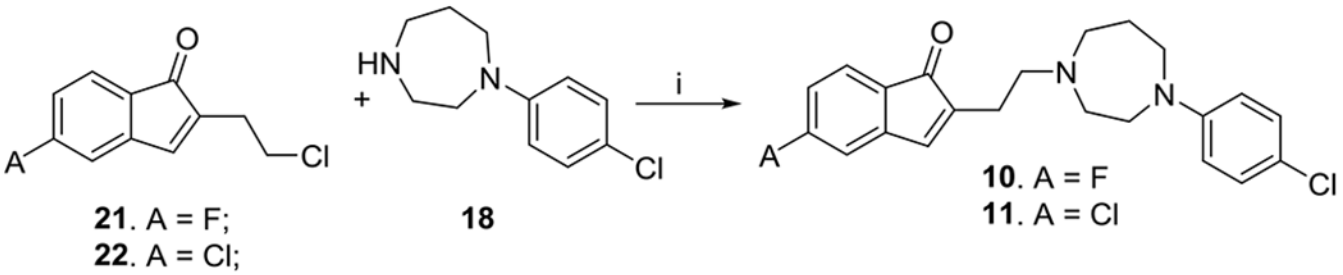
Scheme 1b.

Synthesis of compound **9.** Reagents and conditions: i) Kl, $NaHCO_3$, toluene, reflux.



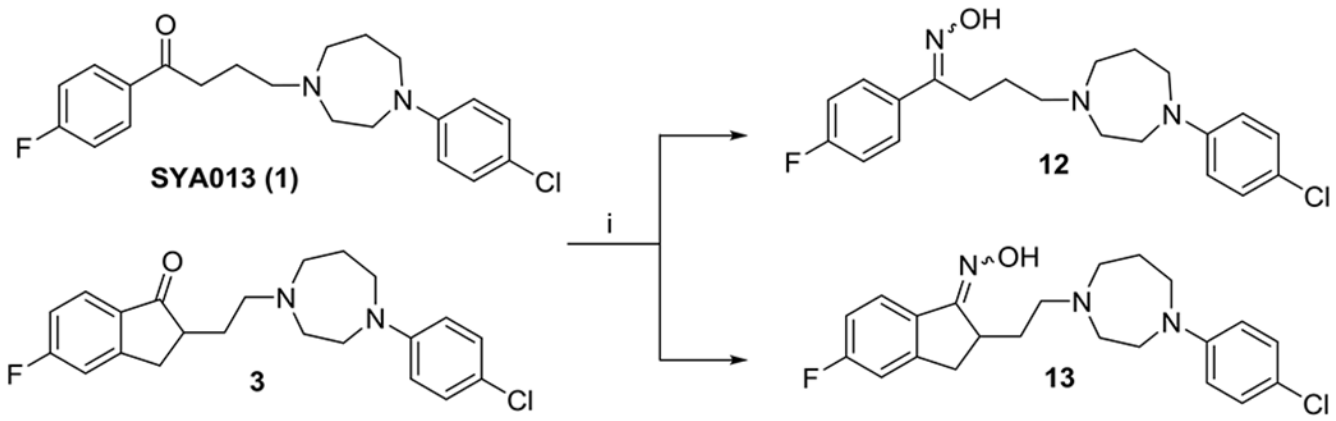
Scheme 2.

Preparation of alkylating agents **21, 22.** Reagents and conditions: i) Br_2, CH_2Cl_2, rt; ii) Li_2CO_3, decane, 180 °C, 12 hr.



Scheme 3.

Synthesis of analogs **10**, **11**. Reagents and conditions: i) Kl, $NaHCO_3$, toluene, reflux.



Scheme 4.

Synthesis of oximes **12** and **13.** Reagents and conditions: i) hydroxylamine hydrochloride, NaOH, EtOH-H_2O, reflux.

Table 1

*Binding affinity constants of indanone and oxime analogs of **(1).**

Compds	σ1, p*Ki* ± SEM (K_i, nM)	σ2, p*Ki* ± SEM (K_i, nM)	K_i(σ1)/*Ki*(σ2)
%**1**	7.63±0.07 (24.0)	8.29±0.07 (5.6)	4.3
%**2.**	8.44±0.05 (3.6)	8.07±0.06 (8.5)	0.4
3.	7.2±0.04 (63.0)	9.22±0.07 (0.6)	105
4.	(21.0±3.4)	(4.4±0.8)	4.8
5.	7.79±0.05 (16.0)	9.13±0.07 (0.7)	22.9
6.	7.36±0.06 (44)	8.75±0.05 (1.8)	24.4
7.	7.65±0.08 (22.0)	8.85±0.05 (1.4)	15.7
8.	7.59±0.04 (26.0)	7.72±0.04 (19.0)	1.4
9.	7.68±0.04 (21.0)	7.74±0.04 (18.0)	1.2
10.	7.11±0.06 (78.0)	7.7±0.1 (20.0)	3.9
11.	8.30 ± 0.1 (4.9)	7.9±0.1 (13.0)	0.4
12.	8.29±0.03 (5.1)	8.46±0.04 (3.5)	1.5
13.	6.60 ± 0.1 (277)	6.4±0.1 (407)	0.7
Haloperidol	8.61±0.1 (2.46)	8.32±0.1 (4.8)	0.51
#**PB 28**	(0.38)	(0.68)	0.56

*p*Ki* data are recorded as the Mean ± SEM from three independent experiments, each performed at least in triplicates. *Ki* data are within 20% of the mean value.

%Previously reported in Ref 13.

#*Ki* data is obtained from Ref 26.

Table 2

IC_{50} (μM) values of synthetic compounds against MDA-MB-231 cell line as determined by cell viability studies.

Compound	1	3	5	12	13	Cisplatin
IC_{50} μM; (24 h)	36.0±7.0	33.6±1.9	ND	17.0±0.4	ND	ND
IC_{50} μM; (48 h)	18.0±0.4	23.5±1.8	58.0±16.0	8.0±0.2	8.0±0.2	50.0±9.8
IC_{50} μM; (72 h)	15.0±1.1	20.1±0.2	ND	7.0±0.3	ND	64.2±4.3

ND = Not determined.

Table 3

IC_{50} (μM) values for TNBC and HEK293 cells

σ_2R Ligand	IC_{50} (μM)	
	MDA-MB-231	HEK 293
3	20.1±0.2	41,3±0.2

Table 4

IC_{50} (μM) values of synthetic compounds against various cancer cell lines and the non-tumorigenic epithelial cell line, MCF-10A

σ_2R Ligands	IC_{50} (μM)					
	MDA-MB-468	MCF-10A	A549	PC-3	MIA PaCa-2	Panc-1
12	4.0±0.8	20.0 ± 7.0	5.8 ± 2.0	6.9 ± 2.0	1.5 ± 0.24	0.14 ± 0.1
1	5.0±1.5	22 ± 1.3	6.2 ± 2.0	8.2 ± 2.0	2.0 ± 0.3	0.4 ± 0.2
13	ND	ND	>200	ND	10.0 ± 0.3	76.0 ± 0.3
5	ND	ND	52.0 ± 1.2	ND	42.0 ± 15.0	51.0 ± 35.0

ND: Not determined