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Synthesis and characterization of *N,N*-dialkyl and *N*-alkyl-*N*-aralkyl fenpropimorph-derived compounds as high affinity ligands for sigma receptors

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ABSTRACT

The sigma-1 receptor is a unique non-opioid, non-PCP binding site that has been implicated in many different pathophysiological conditions including psychosis, drug addiction, retinal degeneration and cancer. Based on the structure of fenpropimorph, a high affinity (K_i = 0.005 nM)[1] sigma-1 receptor ligand and strong inhibitor of the yeast sterol isomerase (ERG2), we previously deduced a basic sigma-1 receptor pharmacophore or chemical backbone composed of a phenyl ring attached to a di-substituted nitrogen atom via an alkyl chain.[2] Here, we report the design and synthesis of various *N,N*-dialkyl or *N*-alkyl-*N*-aralkyl derivatives based on this pharmacophore as well as their binding affinities to the sigma-1 receptor. We introduce three high affinity sigma-1 receptor compounds, *N,N*-dibutyl-3-(4-fluorophenyl)propylamine (**9**), *N,N*-dibutyl-3-(4-nitrophenyl)propylamine (**3**), and *N*-propyl-*N*′-4-aminophenylethyl-3-(4-nitrophenyl)propylamine (**20**) with K_i values of 17.7 nM, 0.36 nM, and 6 nM, respectively. In addition to sigma receptor affinity, we show through cytotoxicity assays that growth inhibition of various tumor cell lines occurs with our high affinity *N,N*-dialkyl or *N*-alkyl-*N*-aralkyl derivatives.

1. Introduction

To date, two subtypes of the sigma receptor have been identified, the sigma-1 receptor and the sigma-2 receptor, which are distinguishable by their pharmacology, function, and molecular weight. The sigma-1 receptor was first cloned from guinea pig liver in 1996[3] and subsequently from other sources including human placental choriocarcinoma cells,[4] human brain,[5] rat brain,[6,7] and mouse brain.[8] The sigma-2 receptor, however, has yet to be cloned. For over a decade, the sigma-1 receptor, has been known to exclusively share significant amino acid sequence similarity with the yeast sterol C8–C7 isomerase (ERG2 protein) as demonstrated by the Glossman group in 1996.[3] A fundamental enzyme in ergosterol biosynthesis, which is the fungal counterpart of cholesterol in mammalians, the ERG2 protein is 30.3% identical and 66.4% similar to the sigma-1 receptor.[3] These amino acid sequence similarities were thought to provide a pharmacological and structural correlation between the yeast sterol isomerase and the sigma-1 receptor. Sigma-1 receptor function, however, has proven to be relatively unclear because unlike the yeast or mammalian sterol isomerases, it lacks sterol isomerase activity.[3] Recently, however, the sigma-1 receptor was discovered to possess chaperone activity as a Ca^{2+}-sensitive and ligand-operated chaperone complexed with another chaperone protein known as BiP.[9] The C-terminus of the sigma-1 receptor has also been implicated in the activation of IP_3 receptors by inducing its dissociation from ankyrin B 220.[10] In Chinese Hamster Ovary (CHO-K1) cells, ligand-activated sigma-1 receptors target to focal adhesion contacts (FAC) and colocalize with Talin and Kv1.4 potassium channels.[11] We have purified the recombinant guinea pig sigma-1 receptor to homogeneity[12] and shown that ligand binding sites on the sigma-1 receptors include regions of the receptor that have been identified as steroid binding domains (SBDLI and SBDLII) in the yeast sterol isomerase.[13,14]

In 1997, the Glossman lab investigated the ability of sterol C8-C7 isomerase inhibitors to compete with (+)-[^{3}H]-pentazocine labeled sigma-1 receptors.[1] Interestingly, they discovered that of all the inhibitors tested, an agricultural fungicide, fenpropimorph, bound with exceptionally high affinity to the guinea pig hepatic (K_i 0.011 nM),[1] cerebral (K_i 0.005 nM),[1] and yeast-expressed sigma-1 receptor (K_i 0.08 nM).[1] Other pharmacological studies have indicated that this receptor also binds a wide range of compounds including opiates, antipsychotics, antidepressants, anti-histamines, PCP-like compounds, beta-adrenergic receptor ligands, serotonergic compounds, cocaine and cocaine analogs, neurosteroids, and neuropeptides. Previously, we observed that these drugs have a

Abbreviations: ERG2, yeast sterol C8–C7 isomerase; CHO-K1, Chinese hamster ovary cells; FAC, focal adhesion contacts; SBDLI, steroid binding domain-like I; SBDLII, steroid binding domain-like II; BD1047, *N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(dimethylamino)ethylamine; BD1063, 1-[2-(3,4-dichlorophenyl)ethyl-4-methylpiperazine; DTG, ditolyl guanidine.

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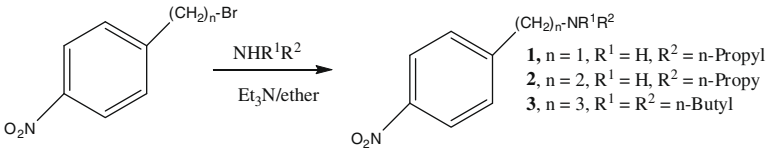
common pharmacophore, which can also be generated from the chemical structure of fenpropimorph.[2] This chemical backbone is composed of a phenyl ring attached to a di-substituted nitrogen atom by an alkyl chain. Further examination led to the observation that similar chemical backbones could be derived from other high affinity sigma-1 ligands such as haloperidol and cocaine,[2] resulting in a common *N*,*N*-dialkyl or *N*-alkyl-*N*-aralkyl product. A number of other sigma-1 ligands reported in the literature support this structural pharmacophore such as *N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(dimethylamino)ethylamine (BD1047),[15] 1-[2-(3,4-dichlorophenyl)ethyl-4-methylpiperazine (BD1063),[15] methamphetamine,[16] FN/C-1 to FN/C-4,[17] (piperazin-2-yl)methanol derivatives,[18] 1-aralkyl-4-benzylpiperazine derivatives,[19] dimemorfan,[20] (*R*/*S*)-4-(dimethylamino)-2-(napthalen-2-yl)butan-2-ol,[21] 1-methoxycarbonyl-1-phenyl-2-cyclopropylmethylamines[22] and [^{11}C]SA4503.[23]

One of the striking features of the sigma receptor is the discovery that sigma-1 and sigma-2 receptors are overexpressed in many human and non-human tumors[24,25] such as rodent C6 glioma, rodent N1E-115 neuroblastoma, human t47D breast ductal carcinoma, human MCF7 breast adenocarcinoma, human NCI-H727 lung carcinoid, human A375 melanoma, rodent PC12 pheochromocytoma cells,[26] and NCB-20 cells.[27,28] Consequently, the pharmacological study of small molecule sigma receptor ligands for potential clinical treatment and imaging applications has been a developing area of cancer research. In 2004, for example, it was demonstrated that the small molecule sigma-1 receptor ligands rimcazole, BD-1047, and BD-1063, inhibited tumor cell survival while SKF-10047 and pentazocine repressed these effects. In another study,[25] the sigma-1 receptor was found to be expressed in most neoplastic breast epithelial cells and cell lines. Furthermore, the sigma receptor ligands haloperidol and progesterone were found to inhibit growth of several breast cancer cell lines in a dose-dependent manner.[25] Sigma-1 antagonists have previously been shown to initiate tumor-selective and caspase-dependent apoptosis, which could be rescued by sigma-1 agonists.[29] In addition, the sigma-2 receptor is emerging as an important player in tumor imaging efforts since sigma-2 receptors are highly concentrated in tumor cells.[30] Sigma ligands inhibit proliferation and induce apoptosis in mammary and colon carcinoma cell lines,[31–33] which in some instances are attributed to their actions on the sigma-2 receptor.[33,34] It has also been shown that sigma-2 receptor activation by selective and non-selective ligands triggers cell death in various tumors through a pathway involving reactive oxygen species and lysosomal membrane leakage.[35] In addition to the sigma-2 selective compound, ibogaine, several high affinity sigma-2 ligands have been synthesized and generally contain N-alkylated piperazine or piperidine rings.[36–40]

Currently, we report the design, synthesis, and evaluation of the relative affinities of several *N*,*N*-dialkyl or *N*-alkyl-*N*-aralkyl compounds to the sigma-1 receptor by competition assays against (+)-[^{3}H]-pentazocine and to the sigma-2 receptor using [^{3}H]-ditolyl guanidine ([^{3}H]-DTG).[14] In addition we test our high affinity *N*,*N*-dialkyl or *N*-alkyl-*N*-aralkyl derivatives in cytotoxicity assays for their ability to inhibit the growth of various tumor cell lines.

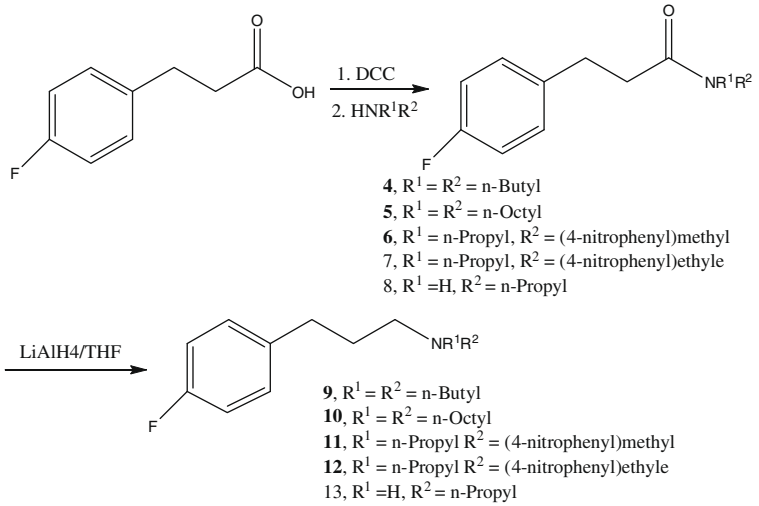
2. Results and discussion

3-(4-Nitrophenyl)propylbromide was prepared by reaction of 3-propylbromide with HNO_3 in the presence of P_2O_5/silica gel under solvent-free conditions.[36] The *N*,*N*-dialkyl derivatives **1–3** were prepared by reaction 2-(4-nitrophenyl)propylbromide or 3-(4-nitrophenyl)propylbromide[ref] with amines as demonstrated in Scheme 1. Amides **4–8** were synthesized employing 4-flurophenyl-propionic acid and appropriate amines in the presence of DCC and the isolated amide without further purification were reduced with $LiAlH_4$ in THF to the corresponding amines **9–13** in excellent yields (Scheme 2). As shown in Scheme 3 compounds **11** and **12** were reduced to corresponding amines **14** and **15** using H_2/Pd–C in methanol in quantitative yields and then the isolated amine **14** and **15** were converted to compounds **16–17** in high yields. Compounds



1, n = 1, R^1 = H, R^2 = n-Propyl
2, n = 2, R^1 = H, R^2 = n-Propy
3, n = 3, R^1 = R^2 = n-Butyl

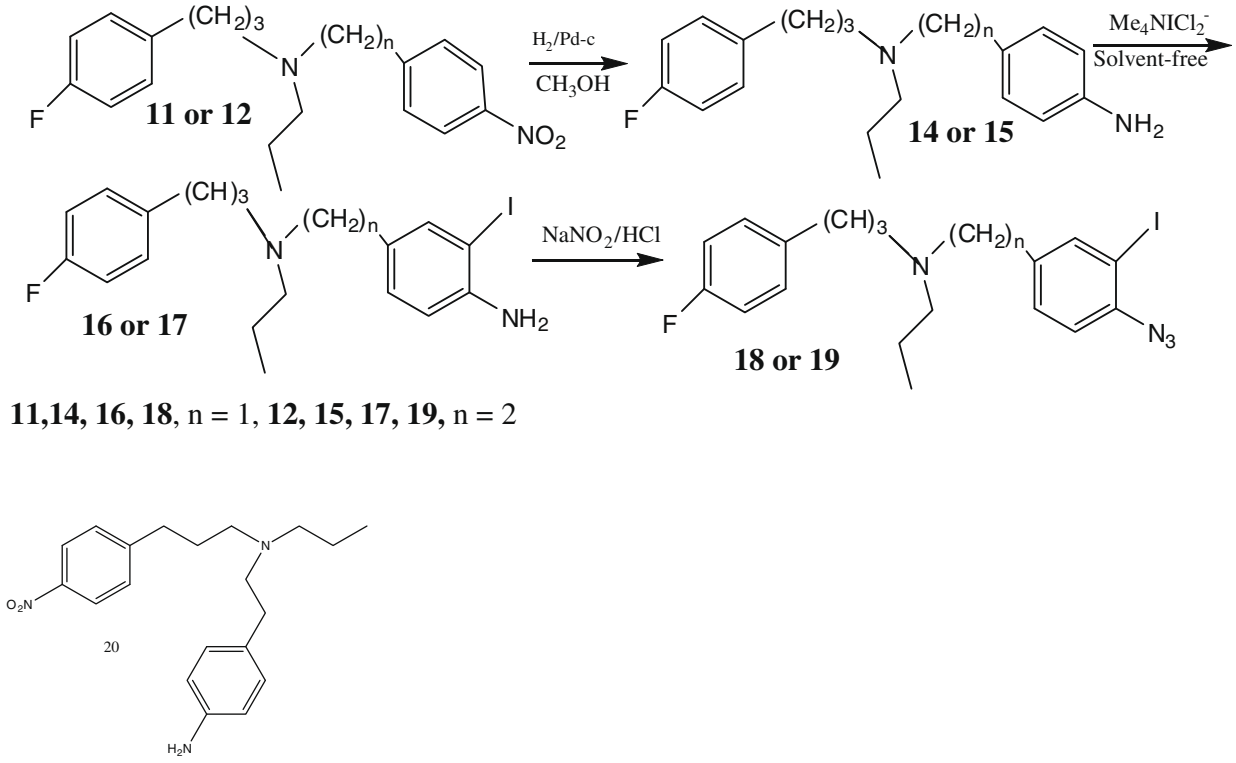
Scheme 1.



4, R^1 = R^2 = n-Butyl
5, R^1 = R^2 = n-Octyl
6, R^1 = n-Propyl, R^2 = (4-nitrophenyl)methyl
7, R^1 = n-Propyl, R^2 = (4-nitrophenyl)ethyle
8, R^1 =H, R^2 = n-Propyl

9, R^1 = R^2 = n-Butyl
10, R^1 = R^2 = n-Octyl
11, R^1 = n-Propyl R^2 = (4-nitrophenyl)methyl
12, R^1 = n-Propyl R^2 = (4-nitrophenyl)ethyle
13, R^1 =H, R^2 = n-Propyl

Scheme 2.



Scheme 3.

20 was synthesis by reaction of 3-(4-nitrophenyl)propylbromide and *N*-propyl-*N*-4-aminophenylethylmine in the presence of Et_3N in Et_2O in 94% yields.

The synthesized *N,N*-dialkyl (**1–3**,) or *N*-alkyl-*N*-aralkyl compounds (compounds **4–18**) were tested for their binding affinities to sigma-1 receptors in guinea pig liver membranes, and to sigma-2 receptors in rat liver membranes, as summarized in Table 1. The binding affinities of these compounds were determined by competitive displacement of [^{3}H]-(+)-pentazocine (10 nM) and showed high affinity and selectivity to the sigma-1 receptor. For determination of binding to the sigma-2 receptor, 3 nM [^{3}H]-ditolyl guanidine (DTG) was utilized in the presence of non-radioactive (+)-pentazocine (100 nM), which masked the sigma-1 receptor population from binding to [^{3}H]-DTG. Non-specific binding was determined by adding 5 mM Haloperidol as a control condition. Curve fitting using 'GraphPad Prism version 4.0C' indicated that all the compounds fit to a single binding site for the sigma-1 receptor with regression values (R^2) between 0.94 and 0.99 (Table 1). Selectivity ratios between the sigma-1 receptor and the sigma-2 receptor were also calculated to determine relative specificity and are summarized in Table 1.

Table 1
Binding affinities of *N,N′*-dialkyl and *N*-alkyl-*N′*-aralkyl derivatives

Ligand	Sigma 1 K_i values (nM) (±SEM, $n = 3$), R^2 value	Sigma 2 K_i values (nM) (±SEM, $n = 3$), R^2 value	Ratio $\sigma 2/\sigma 1$
2	2254 (±1.2, 0.95)	53,617	23.8
3	0.3 (±1.29, 0.96)	404 (±1.21, 0.97)	1347
4	32,063 (±2.16, 0.94)	126,333	3.94
5	53,579 (±3.70, 0.98)	[a]	nd[b]
6	[a]	236,000	nd[b]
9	17.7 (±1.07, 0.99)	685 (±1.17, 0.98)	38.7
10	665.3 (±1.13, 0.98)	1653 (±1.69, 0.96)	2.49
13	91 (±1.16, 0.97)	230 (±1.75, 0.90)	2.53
14	164 (±1.16, 0.97)	2150 (±1.79, 0.93)	13.11
15[c]	2590 (±0.63, 0.96)[c]	120 (±0.045, 0.91)[c]	0.046[c]
16	393,000	133,200	0.3389
18	89,000 (±3.94, 0.96)	>500,00,000	570.8
19[c]	7240 (±2.03, 0.98)[c]	1290 (±3.4, 0.96)[c]	0.178[c]
20	6 (±1.21, 0.96)	83.6 (±1.68, 0.85)	13.93

[a] Does not compete with [^{3}H]-pentazocine or [^{3}H]-DTG.
[b] nd—not determined.
[c] Fontanilla, D. et al. Biochemistry 2008, 47, 7205–7217.

With the exception of compound **16**, the compounds which were synthesized based on our proposed sigma-1 receptor ligand pharmacophore[2] generally had a higher affinity and specificity for the sigma-1 receptor than for the sigma-2 receptor. Specific binding of compounds **4**, **5**, and **6**, either could not be detected to bind to the sigma-1 receptor (K_i >100,000 nM) or possessed very low affinity, presumably because the amide group present in these compounds traps the nitrogen's lone pair, which is needed for optimal sigma receptor binding as previously reported by the Glennon group.[41] The importance of the nitrogen's lone pair is further illustrated by comparing the K_i values of compounds **4** (32,063 nM) with **9** (17.7 nM) and **5** (53,579 nM) with **10** (665.3 nM), showing a 2000-fold and 100-fold affinity difference, respectively. In contrast, compounds **9, 3,** and **20** were found to be exceptionally high affinity compounds for the sigma-1 receptor with K_i values of 17.7 nM, 0.3 nM, and 6 nM, respectively. The nitro substituent on the phenyl ring of compound **20** likely enhances binding to the sigma-1 receptor due to its greater electron withdrawing character[13] as demonstrated by the 400-fold difference in affinity between compound **20** (6 nM) and *N*-propyl-*N*-(4-aminophenyl-ethyl)-3-(4-fluorophenyl)propylamine (compound **15**, sigma-1 K_i = 2590 nM),[42] which contains a fluorine atom replacing the nitro group. In a similar manner, addition of a nitro group on the phenyl ring of the high affinity compound **9** (sigma-1 K_i = 17.7 nM), further improves binding to the sigma-1 receptor as demonstrated by compound **3** (sigma-1 K_i = 0.3 nM; sigma-1 vs sigma-2 selectivity is 1347-fold). Interestingly, **6** clearly demonstrates that compounds with amide groups, even in the presence of a nitro substituent on the phenyl ring, effectively prevented sigma-1 receptor binding, providing further

Carcinoma type	Cell Line	Compound 14 IC50 (uM) (SE)	Compound 9 IC50 (uM) (SE)	Compound 20 IC50 (uM) (SE)	Compound 15 IC50 (uM) (SE)	Compound 3 IC50 (uM) (SE)	Compound 19 IC50 (uM) (SE)
HU lung	NCI-H460	>100	>100	>100	44.77 (7.81)	40.52 (4.86)	40.32 (5.61)
HU Ovarian	SKOV-3	>100	56.18 (6.74)	>100	20.15 (5.34)	27.85 (5.13)	>100
HU Prostate	Du145	>100	>100	>100	>100	32.67 (1.62)	13.06 (0.58)
HU Breast	MCF7	>100	>100	88.1 (6.41)	41.34 (1.75)	22.36 (1.86)	16.75 (1.48)
HU CNS	SF-268	>100	>100	>100	>100	>100	38.8 (1.62)
HU lung	A549	>100	>100	>100	>100	>100	>100
HU Breast	MB-MDA-231	>100	>100	>100	68.12 (3.03)	57.12 (4.72)	21.6 (0.98)
HU Colorectal	HT-29	>100	>100	>100	>100	>100	36.42 (1.16)
HU Colorectal	HCT-15	>100	>100	>100	>100	>100	54.12 (7.84)
HU Breast	MCF10A	>100	>100	>100	>100	>100	88.63 (7.38)
HU Lung	H1299	>100	>100	>100	>100	>100	90.81 (4.44)

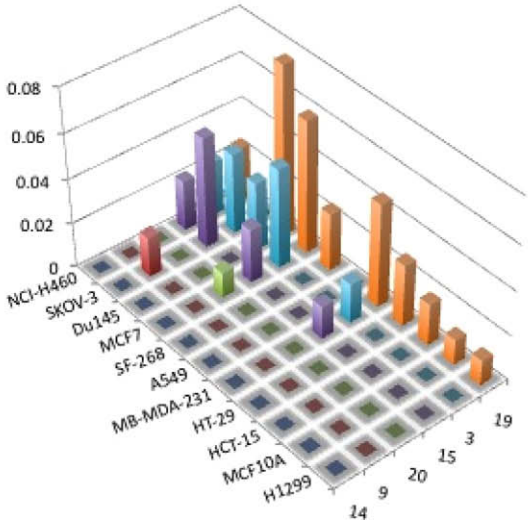


Figure 1. Growth inhibition of tumor cell lines. Compounds **3, 9, 14, 15, 19**, and **20** were used in cytotoxicity assays to measure their ability to inhibit growth of various tumor cells. IC_{50} values of the compounds are reported in tabular form. Also depicted is the graphical representation of $1/IC_{50}$ of the compounds plotted against the various tumor cell lines.

evidence that the nitrogen's lone pair is vital for optimal sigma-1 receptor binding.[2,41,42]

Since sigma-1 and sigma-2 receptors are overexpressed in numerous tumor cell lines, which include breast cancer, lung carcinoma, renal carcinoma, colon carcinoma, sarcoma, brain tumors, melanoma, glioblastoma, neuroblastoma, and prostate cancer, we tested the ability of our compounds to inhibit the growth of various tumor cell lines. Cytotoxicity assays revealed that our *N,N*-dialkyl or *N*-alkyl-*N*-aralkyl derivatives are cytotoxic against a number of cancer cells lines (Fig. 1) including breast, lung, prostate, ovarian, colorectal, and CNS, indicating their utility as potential anticancer or diagnostic agents. Specifically, we tested compounds **9**, **3**, **14**, **15**, **19**, and **20** (Schemes 2 and 3), based on their high affinities and specificities for the sigma-1 receptor. Except for compound **14**, the selected *N,N*-dialkyl or *N*-alkyl-*N*-aralkyl derivatives could inhibit cell growth in vitro (Fig. 1). Interestingly, as illustrated in Figure 1, compound **19** was non-specifically cytotoxic in almost all the cell lines tested whereas compound **14** lacked any cytotoxic properties. Furthermore, only specific cell lines were susceptible to compounds **3**, **9**, **15**, and **20** (Fig. 1). Cell lines that seemed to have the greatest susceptibility to the *N,N*-dialkyl or *N*-alkyl-*N*-aralkyl derivatives were NCI-H460 (human lung adenocarcinoma), SKOV-3 (human ovarian adenocarcinoma), MCF7 (human breast adenocarcinoma), and MB-MDA-231 (human breast adenocarcinoma). Correlation between the cytotoxicity growth inhibition levels and the sigma receptor binding affinities is less clear due to unknown involvement of the sigma-1 versus sigma-2 receptor with regard to these novel compounds. Though structurally similar except for a nitro substituent (Scheme 1), compound **3** has a higher affinity and higher selectively for the sigma-1 receptor than compound **9** (Table 1), and robustly inhibits growth in MB-MDA231, MCF7, Du145, and NCI-H460 cell lines in addition to SKOV-3 cells, the only cell line whose growth is inhibited by compound **9**. Compounds **15** and **20** are also structurally similar to one another, but do not follow the same trend as compounds **9** and **3**. Compound **15** has a higher binding affinity for the sigma-2 receptor than the sigma-1 receptor (Table 1) and is cytotoxic to more tumor cell lines than the structurally similar compound **20**, which has high affinity for sigma-1 (Fig. 1, Table 1). As previously mentioned, sigma-2 receptor ligands have been demonstrated to inhibit cell proliferation in mammary and colon carcinoma cells[33,34] and furthermore, cell death initiated by sigma-2 ligands seems to occur through pathways involving reactive oxygen species and lysosomal membrane leakage.[35] Interestingly, the observations reported here suggests that the cytotoxicity produced by compound **15** might be due to actions on sigma-2 receptors, while the reponses produced by compound **3** seem to occur through sigma-1 receptors. Although compounds **15** and **3** have a substantial amount of selectivity for their respective sigma receptor subtypes, it is unclear at this time whether these cytotoxicity responses are due to simultaneous actions on both sigma-1 and sigma-2 receptors.

In conclusion, the findings from this study show that *N,N*-dialkyl and *N*-alkyl-*N*-aralkyl fenpropimorph derivatives are sigma ligands that exhibit increased sigma-1 receptor affinity with the addition of electron withdrawing nitro substituents. Alternatively, sigma-1 receptor affinity is abolished when an amide group is introduced into the compound structure. Furthermore, these fenpropimorph

derivatives exhibit specific cytotoxic activity against numerous tumor cell lines, demonstrating their potential use as clinical anticancer, imaging, or diagnostic agents.

3. Methods

3.1. Chemistry

Yields refer to isolated pure products after column chromatography. The products were characterized by their spectral (IR, ^1H and ^{13}C NMR and CHN Analysis). All ^1H NMR spectra were recorded at 300 MHz in $CDCl_3$ relative to TMS (0.00 ppm) and IR spectra were recorded on a Shimadzu 435 IR spectrometer. Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are reported uncorrected. Chemicals were obtained from Aldrich Chemical Co. and utilized without further purification.

3.2. Preparation of 3-(4-nitrophenyl)-propylbromine

2 g of P_2O_5/silica gel (65% w/w) (10 mmol)[36] and 3-phenylpropylbromine (10 mmol, 1.98 g) was ground for 30 seconds, and then 5 ml of HNO_3 65% was added. The mixture was ground with a pestle at rt until a deep-yellow color appeared (2 min). When TLC (*n*-hexane/EtOAc 90:10) showed complete disappearance of 3-phenylpropylbromide (10 min), ether (100 ml) was added to the reaction mixture and the solid was separated through a short pad of silica gel and washed with ether (3 × 20 ml). The filtrate was washed with 10% $NaHCO_3$ (3x20 ml) and dried ($MgSO_4$). The solvent was evaporated under reduced pressure and the residue was purified by short column chromatography (*n*-hexane/EtOAc, 90:10). 3-(4-Nitrophenyl)-propylbromide was obtained (8.3 mmol, 2.02 g 83%) as a yellow oil. ^1H NMR ($CDCl_3$): δ 8.2 (d, J = 6.3), 7.38 (d, 2H, 6.3), 3.4 (t, 2H, J = 7.8), 2.90 (m, 2H, J = 7.8), 2.2 (m, 2H). Anal. Calcd for $C_9H_{10}BrNO_2$: C, 44.29: H, 4.13; N, 5.74. Found: C, 44.50; H, 48.30; N, 5.80.

3.3. General procedure for preparation of amines 1–3

To a stirring mixture of 3-(4-nitrophenyl)ethylbromide or 4-nitrobenzyl bromide (1 mmol), Et_3N (1.1 mmol, 0.11 g) in Et_2O (10 ml) was added the appropriate amines (1.0 mmol). The reaction mixture was stirred at room temperature for 10 h. After filtration, the solvent was removed to give a yellow residue. The crude products were purified by column chromatography (silica gel, toluene/Et_2NH, 20:1) to afford pure product.

3.3.1. *N*-(4-Nitrobenzyl)propan-1-amine (1)

Pale yellow oil, bp 120–122 °C (15 mm Hg). Yield 80% (0.15 g, 0.80 mmol). IR (KBr): 3268 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.14 (d, 2H, J = 6.8), 7.38 (d, 2H, J = 6.8), 3.99 (s, 2H), 2.60 (t, 2H, J = 7.8), 1.55 (m, 2H), 1.40 (s, 1H, NH), 0.96 (t, 3H, J = 7.8). Anal. Calcd for $C_{10}H_{14}N_2O_2$: C, 61.84; H, 7.27; N, 14.42. Actual: C, 61.50; H, 7.40; N, 14.20.

3.3.2. *N*-propyl-*N*-3-(4-nitrophenyl)-ethylamine (2)

Mp 142–144 °C. Yield 86% (0.18 g, 0.86 mmol). IR (KBr): 3261 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.18 (d, 2H, J = 6.8), 7.58 (d, 2H, J = 6.8), 2.60 (t, 2H, J = 7.8), 2.46 (m, 2H), 2.16 (s, 1H, NH), 1.86 (m, 2H), 1.45 (m, 2H), 0.96 (t, 3H, J = 7.8). Anal. Calcd for $C_{11}H_{16}N_2O_2$: C, 63.44; H, 7.74; N, 13.45. Actual: C, 63.30; H, 7.90; N, 13.20.

3.3.3. *N*-Dibutyl-3-(4-nitrophenyl)ethylamine (3)

^1H NMR ($CDCl_3$): δ 8.16 (d, 2H), 7.24 (d, 2H), 3.04 (t, 2H, J = 7.8), 2.8 (m, 4H), 1.55 (m, 2H), 1.72 (m, 2H), 1.2–0.98 (m, 8H), 0.80 (t, 6H, J = 7.8). Anal. Calcd for $C_{16}H_{26}N_2O_2$: C, 69.03; H, 9.41; N, 10.06. Found: C, 69.80; H, 9.20; N, 10.10.

3.4. General procedure for preparation of amides (4–8)

A mixture of DCC (1 mmol, 2.1 g) and 4-flurophenylpropionic acid (0.17 g, 1 mmol) was ground with a pestle in a mortar for 30 s and then the amine (1 mmol) was added to the reaction mixture. The reaction was ground with a pestle until TLC showed no remaining 4-flurophenylpropionic acid (*n*-hexane/EtOAc, 75:25) (20 min). Then to the reaction mixture was added a mixture of ether (20 mL) and H_2O (5 mL). The etheral layer was washed with saturated $NaHCO_3$, HCl 5% and water and the organic phase dried ($MgSO_4$), and evaporated by a rotary evaporator to give a residue. The residue was used without further purification for the next step.

3.4.1. *N,N*-Dibutyl-3-(4-fluorophenyl)propionamide (4)

Yield: (0.26 g, 93%), mp 162–164 °C. IR (KBr): 1658 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.18 (m, 2H), 6.98 (m, 2H), 3.17 (t, 4H, J = 7.8), 2.8 (t, 2H, J = 7.8), 2.59 (t, 2H, J = 7.8), 1.1–4-1.0 (m, 8H), 0.96 (t, 6H, J = 7.8). Anal. Calcd for $C_{17}H_{26}FNO$: C, 73.08; H, 9.38; N, 5.01. Actual: C, 72.90; H, 9.40; N, 5.20.

3.4.2. *N,N*-Dioctyl-3-(4-fluorophenyl)propionamide (5)

Yield: (0.37 g, 95%), mp 190–193 °C. IR (KBr): 1663 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.18 (m, 2H), 6.97 (m, 2H), 3.18 (t, 2H, J = 7.8), 2.80 (t, 2H, J = 7.8), 2.60 (t, 2H, J = 7.8), 1.50 (m, 4H), 1.26 (m, 22H), 0.90 (t, 6H, J = 7.8). Anal. Calcd for $C_{25}H_{42}FNO$: C, 76.68; H, 10.81; N, 3.58. Actual: C, 76.40; H, 10.90; N, 3.40.

3.4.3. 3-(4-Fluorophenyl)-*N*-(3-nitrobenzyl)-*N*-propylpropanamide (6)

Yield: (0.31 g, 90%), Yellow oil. IR (KBr): 1661 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.18 (d, 2H, J = 6.8), 7.46 (d, 2H, J = 6.8), 7.15 (m, 2H), 6.94 (m, 2H), 4.85 (s, 2H), 3.22 (t, 2H, J = 7.8), 2.79 (t, 2H, J = 7.8), 2.30 (m, 2H), 1.42 (m, 2H), 0.96 (t, 3H, J = 7.8). Anal. Calcd for $C_{19}H_{21}FN_2O_3$: C, 66.26; H, 6.15; N, 8.13. Actual: C, 66.10; H, 6.30; N, 8.00.

3.4.4. 3-(4-Fluorophenyl)-*N*-(3-nitrophenethyl)-*N*-propylpropanamide (7)

Yield: (0.32 g, 88%), Yellow oil. IR (KBr): 1663 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.18 (d, 2H, J = 6.8), 7.46 (d, 2H, J = 6.8), 7.15 (m, 2H), 6.94 (m, 2H), 3.22 (t, 2H, J = 7.8), 2.82 (m, 2H), 2.35 (m, 2H), 1.42 (m, 2H), 0.96 (t, 3H, J = 7.8). Anal. Calcd for $C_{20}H_{23}FN_2O_3$: C, 67.02; H, 6.47; N, 7.82. Actual: C, 67.13; H, 6.30; N, 7.70.

3.4.5. 3-(4-Fluorophenyl)-*N*-propylpropanamide (8)

Yield: (0.19 g, 90%), Yellow oil. IR (KBr): 1660 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.28 (s, 1H,), 7.18 (m, 2H), 6.94 (m, 2H), 3.60 (t, 2H, J = 7.8), 2.78 (t, 2H, J = 7.8), 2.39 (t, 2H, J = 7.8), 1.45 (m, 2H), 0.96 (t, 3H, J = 7.8). Anal. Calcd for $C_{12}H_{16}FNO$: C, 68.88; H, 7.71; N, 6.69. Actual: C, 68.90; H, 7.50; N, 6.80.

3.5. General procedure for reduction of amides (4–8) to the corresponding amines (9–13)

In a double-necked round bottomed flask equipped with septum and condenser, a solution of amides (1 mmol) in anhydrous THF (5 ml) was added via a syringe dropwise to a stirred solution of $LiAlH_4$ (0.74 g, 2 mmol) in anhydrous THF (5 ml) under argon. TLC indicated the reaction to be almost completed after 15 min at room temperature. The reaction mixture was driven to completion by brief refluxing (15 min) and when it was cooled to rt, it was diluted by adding 5 ml THF. The excess $LiAlH_4$ was destroyed by dropwise addition of water 1 ml. The reaction mixture was stirred

for 30 min at rt and then the solids were removed by filtration. The filtrate was dried ($MgSO_4$), and the solvent evaporated by a rotary evaporator to give pure products as yellow oils in quantitative yield.

3.5.1. *N,N*-Dibutyl-3-(4-fluorophenyl)propylamine (9)

^{1}H NMR ($CDCl_3$): δ 7.18 (m, 2H), 6.92 (m, 2H), 3.03 (t, 2H, J = 7.8), 2.40 (m, 6H), 1.72 (m, 2H), 1.33 (m, 8H), 0.95 (t, 6H, J = 7.8). Anal. Calcd for $C_{17}H_{28}FN$: C, 76.93; H, 10.63; N, 5.28. Actual: C, 77.10; H, 10.70; N, 5.20.

3.5.2. *N,N*-Dioctyl-3-(4-fluorophenyl)propylamine (10)

^{1}H NMR ($CDCl_3$): δ 7.18 (m, 2H), 3.64 (t, 2H, J = 7.8), 3.13 (t, 2H, J = 7.8), 2.67 (t, 2H, J = 7.8), 2.40 (m, 4H), 1.85 (m, 2H), 1.50–1.26 (m, 22H), 0.90 (t, 6H, J = 7.8). Anal. Calcd for $C_{25}H_{44}N$: C, 79.52; H, 11.74; N, 3.71. Actual: C, 79.60; H, 11.50; N, 3.80.

3.5.3. 3-(4-Fluorophenyl)-*N*-(4-nitrobenzyl)-*N*-propylpropan-1-amine (11)

IR (KBr): 3258 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 8.20 (d, 2H, J = 6.8), 7.58 (d, 2H, J = 6.8), δ 7.18 (m, 2H), 6.98 (m, 2H), 3.64 (s, 2), 2.62 (t, 2H, J = 7.8), 2.42 (m, 4H), 1.80 (m, 2H), 1.42 (m, 2H), 0.96 (t, 3H, J = 7.8). Anal. Calcd for $C_{19}H_{23}FN_2O_2$: C, 69.07; H, 7.02; N, 8.48. Actual: C, 69.21; H, 7.30; N, 8.30.

3.5.4. 3-(4-Fluorophenyl)-*N*-(4-nitrophenethyl)-*N*-propylpropan-1-amine (12)

^{1}H NMR ($CDCl_3$): δ 8.18 (d, 2H, J = 6.8), 7.55 (d, 2H, J = 6.8), δ 7.10 (m, 2H), 6.95 (m, 2H), 2.60 (t, 6H, J = 7.8), 2.42 (m, 4H), 1.80 (m, 2H), 1.42 (m, 2H), 0.96 (t, 3H, J = 7.8). Anal. Calcd for $C_{20}H_{25}FN_2O_2$: C, 69.74; H, 7.32; N, 8.13. Actual: C, 69.60; H, 7.50; N, 8.10.

3.5.5. 3-(4-Fluorophenyl)-*N*-propylpropan-1-amine (13)

IR (KBr): 3323 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.18 (m, 2H), 6.94 (m, 2H), 2.60 (m, 6H), 1.80 (m, 3H), 1.45 (m, 2H), 0.96 (t, 3H, J = 7.8). Anal. Calcd for $C_{12}H_{18}FN$: C, 73.81; H, 9.29; N, 7.17. Actual: C, 73.90; H, 9.50; N, 7.10.

3.6. General procedure for reduction of nitro groups of compounds 11 and 12 to the corresponding amino group 14 and 15

A mixture of nitro compounds (1 mmol) and 10 mg of Pd/C (10%) in methanol (10 ml) was reduced with H_2 at normal pressure. The mixture was stirred at room temperature over night. After filtration, solvent was removed to give a yellow residue. The crude products were purified by column chromatography (silica gel, toluene/Et_2NH, 20:1) to afford pure amine.

3.6.1. *N*-Propyl-*N*-(4-amino-benzyl)-3-(4-fluorophenyl)propylamine (14)

Yield: (0.28 g, 94%), Semisolid. IR (KBr): 3258, 1661 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 8.14 (d, 2H, J = 6.8), 7.38 (d, 2H, J = 6.8), 6.96 (d, 2H, J = 6.8), 6.62 (d, 2H, J = 6.8), 4.00 (s, 2H), 3.60 (s, 2H, NH_2), 2.78 (t, 4H, J = 7.8), 2.43 (m, 6H), 1.65 (m, 2H), 1.34 (m, 2H), 0.94 (t, 3H, J = 7.8). Anal. Calcd for $C_{19}H_{25}FN_2$: C, 75.96; H, 8.39; N, 9.32. Actual: C, 75.80; H, 8.50; N, 9.10.

3.6.2. *N*-Propyl-*N*-(4-amino-phenylethyl)-3-(4-fluorophenyl)propylamine (15)

Yield: (0.29 g, 94%), yellow oil. IR (KBr): 3258, 1661 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 8.14 (d, 2H, J = 6.8), 7.38 (d, 2H, J = 6.8), 6.93 (d, 2H, J = 6.8), 6.65 (d, 2H, J = 6.8), 3.60 (s, 2H, NH_2), 2.70 (t, 6H, J = 7.8), 2.45 (m, 6H), 1.79 (m, 2H), 1.43 (m, 2H), 0.94 (t, 3H, J = 7.8). Anal. Calcd for $C_{20}H_{27}FN_2O$: C, 76.39; H, 8.65; N, 8.91. Actual: C, 76.50; H, 8.80; N, 8.70.

3.7. General method for iodination of amine 14 and 15 to the corresponding amino iodoamino derivative 16 and 17

A mixture of amines **14** or **15** (0.5 mmol) and tetramethylammonium dichloroiodate (0.5 mmol, 0.14 g)[43] in a mortar was ground with a pestle to produce a homogenous paste and the mixture was left at room temperature until TLC (toluene/Et_2NH, 20:1) showed complete disappearance of amines. To the brown solid was added 5 ml sodium bisulfate (5%) and the reaction mixture was extracted with dichloromethane (3 × 5 ml). The combined extracts were dried with $MgSO_4$. Evaporation of the solvent gave the corresponding iodo derivatives (**16** or **17**). The product was purified by column chromatography (silica gel, toluene/Et_2NH, 20:1).

3.7.1. *N*-Propyl-*N*-(4-amino-3-iodo-benzyl)-3-(4-fluorophenyl)-propylamine (16)

Oil, 84% yield. IR (KBr): 3245 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 8.18 (m, 2H, J = 6.8), 7.98 (m, 2H, J = 6.8), 6.70–6.45 (m, 3H, J = 6.8), 4.00 (s, 2H), 3.45 (s, 2H, NH_2), 2.78 (t, 2H, J = 7.8), 2.45 (m, 4H), 1.66 (m, 2H), 1.30 (m, 2H), 0.96 (t, 2H, J = 7.8). Anal. Calcd for $C_{19}H_{24}IFN_2$: C, 53.53; H, 5.67; N, 6.57. Actual: C, 53.40; H, 6.70; N, 6.40.

3.7.2. *N*-Propyl-*N*-(4-amino-3-iodo-phenylethyl)-3-(4-fluorophenyl)propylamine (17)

Oil, 81% yield. IR (KBr): 3245 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 6.99–6.60 (m, 7H), 3.22 (s, 2H, NH_2), 2.62 (t, 6H, J = 7.8), 2.40 (m, 4H), 1.80 (m, 2H), 1.38 (m, 2H), 1.01 (t, 2H, J = 7.8). Anal. Calcd for $C_{20}H_{26}IFN_2$: C, 54.55; H, 5.95; N, 6.36. Actual: C, 54.40; H, 6.10; N, 6.20.

3.8. General procedure for conversion of amino iodo derivative 16–17 to the corresponding azido iodo derivative 18–19

To a cold mixture (0 °C) of **16** or **17** (0.1 mmol) in H_2O (2 ml), concentrated HCl (0.4 ml) was added an aqueous solution of $NaNO_2$ (0.30 mmol, 21 mg, in 0.5 ml H_20) in 5 min in a round bottomed flask. The reaction mixture stirred at room temperature for 30 min. Then to the reaction mixture at rt and darkness was added an aqueous solution of NaN_3 (0.36 mmol, 23 mg, in 0.5 ml H_20) dropwise. The reaction mixture was stirred at rt and darkness for 30 min and then extracted with EtOAc (3 × 3 ml). The combined EtOAc solution was dried with $MgSO_4$ and the solvent was evaporated with rotary evaporator to afford orange oil. The crude products were purified by column chromatography (silica gel, first toluene/Et_2NH, 20:1 and then toluene/Et_2NH, 4:1) to give the product as a yellow liquid.

3.8.1. *N*-Propyl-*N*-(3-iodo-4-azido-benzyl)-3-(4-fluorophenyl)-propylamine (18)

Oil, 98% yield. ^{1}H NMR: δ 6.99–6.80 (m, 7H), 4.10 (s, 2H, NH_2), 2.78 (t, 2H, J = 7.8), 2.49 (m, 4H), 1.82 (m, 2H), 1.48 (m, 2H), 0.99 (t, 2H, J = 7.8). Anal. Calcd for $C_{19}H_{22}IFN_4$: C, 50.45; H, 4.90; N, 12.39. Actual: C, 50.40; H, 5.00; N, 12.40.

3.8.2. *N*-Propyl-*N*-(3-iodo-4-azido-phenyethyl)-3-(4-fluorophenyl)propylamine (18)

Oil, 91% yield. ^{1}H NMR ($CDCl_3$): δ 6.99–6.80 (m, 7H), 2.60 (t, 6H, J = 7.8), 2.41 (m, 4H), 1.80 (m, 2H), 1.47 (m, 2H), 1.01 (t, 2H, J = 7.8). Anal. Calcd for $C_{20}H_{24}IFN_4$: C, 51.51; H, 5.19; N, 12.01. Actual: C, 51.40; H, 5.40; N, 12.10.

3.8.3. Synthesis of *N*-propyl-*N*-4-aminophenylethyl-3-(4-nitrophenyl)propylamine (20)

To a stirring mixture of 3-(4-nitrophenyl)propylbromide (1 mmol), Et_3N (1.1 mmol, 0.11 g) in Et_2O (10 ml) was added *N*-propyl-*N*-4-aminophenylethylmine (3 mmol). The reaction mixture was stirred at room temperature for 10 h. After filtration, solvent

was removed to give a yellow residue. The crude products were purified by column chromatography (silica gel, toluene/Et_2NH, 20:1) to afford pure product. Semisolid. Yield 94% (0.32 g, 0.94 mmol). IR (KBr): 3245 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.14 (d, 2H, J = 6.8), 7.38 (d, 2H, J = 6.8), 6.93 (d, 2H, J = 6.8), 6.65 (d, 2H, J = 6.8), 3.60 (s, 2H, NH_2), 2.80 (t, 6H, J = 7.8), 2.49 (m, 4H), 1.98 (m, 2H), 1.63 (m, 2H), 1.10 (t, 3H, J = 7.8). Anal. Calcd for $C_{20}H_{27}N_3O_2$: C, 70.35; H, 7.97; N, 12.31. Actual: C, 70.40; H, 8.10; N, 12.40.

3.9. Preparation of rat liver and guinea pig liver membranes

Minced frozen rat livers or guinea pig livers (65 g) were thawed in 100 ml homogenization buffer (10 mM phosphate buffer pH 7.4 containing 0.32 M sucrose, 1 M $MgSO_4$, 0.5 M EGTA, 1 mM phenylmethylsulphonyl fluoride (PMSF), 10 μg/ml leupeptin, 1 μg/ml pepstatin A, 10 μg/ml *p*-toluenesulfonyl-L-arginine methyl ester (TAME) and then homogenized on ice with a Brinkman polytron homogenizer (setting 6, 4 bursts of 10 s each) followed by a glass homogenizer (Teflon pestle by 6 slow passes at 3000 rpm). The homogenized tissues were then centrifuged at 17,000*g* for 10 min. The supernatants were re-centrifuged at 100,000*g* for 1 h. The microsomal pellets were resuspended in homogenization buffer, snap frozen with dry ice—ethanol, and stored at −80 °C at a final protein concentration of 20 mg/ml.

3.10. Sigma receptor binding assays

Competitive binding assays were performed to determine binding affinities of the compounds listed for the sigma-1 and sigma-2 receptors as previously described.[15,16] Assays for sigma-1 were performed using 10 nM (+)-[^{3}H]pentazocine in guinea pig liver homogenates (25 μg/well) incubated at 30 °C for 1 h with several concentrations of competing ligands reported in Figure 1B. After incubation, membranes were harvested on a 0.5% PEI-treated Whatman GF/B filters using a Brandel Cell Harvester. (+)-[^{3}H]pentazocine binding was determined by liquid scintillation counting. The assay for determining the sigma-2 binding property of IAF was performed using rat liver membranes (25 μg/well) and 3 nM [^{3}H]-DTG in the presence of 100 nM (+)-pentazocine. Serial concentrations of the compounds listed in Figure 1B were added to the reactions for 45 min at 30 °C and the samples vacuum filtered through 0.5% polyethyleneimine (PEI) treated Whatman GF/B as described above to measure displacement of the radioligands from the sigma receptor subtypes. Haloperidol (5 μM) was used to determine non-specific binding for both sigma-1 and sigma-2 receptor binding assays. Radioactivity on the filters was detected by liquid scintillation spectrometry using NEN formula 989 as scintillation cocktail. Values were fit to a non-linear regression curve using graphing software (Graphpad Prism) and reported inhibition constants, K_i, were calculated using the Cheng–Prussof equation.[44]

3.11. Cytotoxicity assays

Multi-plex cytotoxicity assays were performed by the Keck-UWCCC Small Molecule Screening Facility (Madison, WI). Specific methodology can be found online at http://hts.wisc.edu/Resources.htm#mpa.

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Supplementary data

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References and notes

1. Moebius, F. F.; Reiter, R. J.; Hanner, M.; Glossmann, H. *Br. J. Pharmacol.* **1997**, *121*, 1.
2. Fontanilla, D.; Johannessen, M.; Hajipour, A. R.; Cozzi, N. V.; Jackson, M. B.; Ruoho, A. E. *Science* **2009**, *323*, 934.
3. Hanner, M.; Moebius, F. F.; Flandorfer, A.; Knaus, H. G.; Striessnig, J.; Kempner, E.; Glossmann, H. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 8072.
4. Kekuda, R.; Prasad, P. D.; Fei, Y. J.; Leibach, F. H.; Ganapathy, V. *Biochem. Biophys. Res. Commun.* **1996**, *229*, 553.
5. Prasad, P. D.; Li, H. W.; Fei, Y. J.; Ganapathy, M. E.; Fujita, T.; Plumley, L. H.; Yang-Feng, T. L.; Leibach, F. H.; Ganapathy, V. *J. Neurochem.* **1998**, *70*, 443.
6. Seth, P.; Fei, Y. J.; Li, H. W.; Huang, W.; Leibach, F. H.; Ganapathy, V. *J. Neurochem.* **1998**, *70*, 922.
7. Mei, J.; Pasternak, G. W. *Biochem. Pharmacol.* **2001**, *62*, 349.
8. Pan, Y. X.; Mei, J.; Xu, J.; Wan, B. L.; Zuckerman, A.; Pasternak, G. W. *J. Neurochem.* **1998**, *70*, 2279.
9. Hayashi, T.; Su, T. P. *Cell* **2007**, *131*, 596.
10. Wu, Z.; Bowen, W. D. *J. Biol. Chem.* **2008**, *283*, 28198.
11. Mavlyutov, T. A.; Ruoho, A. E. *J. Mol. Signal.* **2007**, *2*, 8.
12. Ramachandran, S.; Lu, H.; Prabhu, U.; Ruoho, A. E. *Protein Expr. Purif.* **2007**, *51*, 283.
13. Chen, Y.; Hajipour, A. R.; Sievert, M. K.; Arbabian, M.; Ruoho, A. E. *Biochemistry* **2007**, *46*, 3532.
14. Pal, A.; Hajipour, A. R.; Fontanilla, D.; Ramachandran, S.; Chu, U.; Mavlyutov, T.; Ruoho, A. E. *Mol. Pharmacol.* **2007**.
15. Matsumoto, R. R.; Bowen, W. D.; Tom, M. A.; Vo, V. N.; Truong, D. D.; De Costa, B. R. *Eur. J. Pharmacol.* **1995**, *280*, 301.
16. Nguyen, E. C.; McCracken, K. A.; Liu, Y.; Pouw, B.; Matsumoto, R. R. *Neuropharmacology* **2005**, *49*, 638.
17. Barbieri, F.; Sparatore, A.; Alama, A.; Novelli, F.; Bruzzo, C.; Sparatore, F. *Oncol. Res.* **2003**, *13*, 455.
18. Bedurftig, S.; Wunsch, B. *Bioorg. Med. Chem.* **2004**, *12*, 3299.
19. Costantino, L.; Gandolfi, F.; Sorbi, C.; Franchini, S.; Prezzavento, O.; Vittorio, F.; Ronsisvalle, G.; Leonardi, A.; Poggesi, E.; Brasili, L. *J. Med. Chem.* **2005**, *48*, 266.
20. Shin, E. J.; Nah, S. Y.; Kim, W. K.; Ko, K. H.; Jhoo, W. K.; Lim, Y. K.; Cha, J. Y.; Chen, C. F.; Kim, H. C. *Br. J. Pharmacol.* **2005**, *144*, 908.
21. Collina, S.; Loddo, G.; Urbano, M.; Linati, L.; Callegari, A.; Ortuso, F.; Alcaro, S.; Laggner, C.; Langer, T.; Prezzavento, O.; Ronsisvalle, G.; Azzolina, O. *Bioorg. Med. Chem.* **2007**, *15*, 771.
22. Prezzavento, O.; Campisi, A.; Ronsisvalle, S.; Li Volti, G.; Marrazzo, A.; Bramanti, V.; Cannavo, G.; Vanella, L.; Cagnotto, A.; Mennini, T.; Ientile, R.; Ronsisvalle, G. *J. Med. Chem.* **2007**, *50*, 951.
23. Kawamura, K.; Ishiwata, K.; Tajima, H.; Ishii, S.; Matsuno, K.; Homma, Y.; Senda, M. *Nucl. Med. Biol.* **2000**, *27*, 255.
24. Vilner, B. J.; John, C. S.; Bowen, W. D. *Cancer Res.* **1995**, *55*, 408.
25. Wang, B.; Rouzier, R.; Albarracin, C. T.; Sahin, A.; Wagner, P.; Yang, Y.; Smith, T. L.; Meric-Bernstam, F.; Marcelo Aldaz, C.; Hortobagyi, G. N.; Pusztai, L. *Breast Cancer Res. Treat.* **2004**, *87*, 205.
26. Hellewell, S. B.; Bowen, W. D. *Brain Res.* **1990**, *527*, 244.
27. Wu, X. Z.; Bell, J. A.; Spivak, C. E.; London, E. D.; Su, T. P. *J. Pharmacol. Exp. Ther.* **1991**, *257*, 351.
28. Kushner, L.; Zukin, S. R.; Zukin, R. S. *Mol. Pharmacol.* **1988**, *34*, 689.
29. Spruce, B. A.; Campbell, L. A.; McTavish, N.; Cooper, M. A.; Appleyard, M. V.; O'Neill, M.; Howie, J.; Samson, J.; Watt, S.; Murray, K.; McLean, D.; Leslie, N. R.; Safrany, S. T.; Ferguson, M. J.; Peters, J. A.; Prescott, A. R.; Box, G.; Hayes, A.; Nutley, B.; Raynaud, F.; Downes, C. P.; Lambert, J. J.; Thompson, A. M.; Eccles, S. *Cancer Res.* **2004**, *64*, 4875.
30. Mach, R. H.; Wheeler, K. T. *Cent. Nerv. Syst. Agents Med. Chem.* **2009**, *9*, 230.
31. Brent, P. J.; Pang, G. T. *Eur. J. Pharmacol.* **1995**, *278*, 151.
32. Brent, P. J.; Pang, G.; Little, G.; Dosen, P. J.; Van Helden, D. F. *Biochem. Biophys. Res. Commun.* **1996**, *219*, 219.
33. Crawford, K. W.; Bowen, W. D. *Cancer Res.* **2002**, *62*, 313.
34. Crawford, K. W.; Coop, A.; Bowen, W. D. *Eur. J. Pharmacol.* **2002**, *443*, 207.
35. Ostenfeld, M. S.; Fehrenbacher, N.; Hoyer-Hansen, M.; Thomsen, C.; Farkas, T.; Jaattela, M. *Cancer Res.* **2005**, *65*, 8975.
36. Mach, R. H.; Huang, Y.; Freeman, R. A.; Wu, L.; Vangveravong, S.; Luedtke, R. R. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 195.
37. Mach, R. H.; Smith, C. R.; Childers, S. R. *Life Sci.* **1995**, *57*, PL57.
38. Berardi, F.; Ferorelli, S.; Abate, C.; Colabufo, N. A.; Contino, M.; Perrone, R.; Tortorella, V. *J. Med. Chem.* **2004**, *47*, 2308.
39. Mach, R. H.; Gage, H. D.; Buchheimer, N.; Huang, Y.; Kuhner, R.; Wu, L.; Morton, T. E.; Ehrenkaufer, R. L. *Synapse* **2005**, *58*, 267.

40. Mach, R. H.; Huang, Y.; Buchheimer, N.; Kuhner, R.; Wu, L.; Morton, T. E.; Wang, L.; Ehrenkaufer, R. L.; Wallen, C. A.; Wheeler, K. T. *Nucl. Med. Biol.* **2001**, *28*, 451.
41. Ablordeppey, S. Y.; Fischer, J. B.; Glennon, R. A. *Bioorg. Med. Chem.* **2000**, *8*, 2105.
42. Fontanilla, D.; Hajipour, A. R.; Pal, A.; Chu, U. B.; Arbabian, M.; Ruoho, A. E. *Biochemistry* **2008**, *47*, 7205.
43. Hajipour, A. R.; Arbabian, M.; Ruoho, A. E. *J. Org. Chem.* **2002**, *67*, 8622.
44. Cheng, Y.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.