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Synthesis and receptor binding studies of some new arylcarboxamide derivatives as sigma-1 ligands

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ABSTRACT

We describe here the synthesis and the binding interaction with σ_1 and σ_2 receptors of a series of new arylcarboxamide derivatives variously substituted on the aromatic portions. Maintaining a partial scaffold of a series of compounds previously synthesized by us, we evaluate the effect of the substitution on σ binding. The synthesized compounds have been tested to estimate their affinity and selectivity toward σ_1 and σ_2 receptors. Two out of 16 derivatives showed an interesting σ_1 affinity (21.2 and 13.6 nM—compounds **2m** and **2p**) and a good selectivity ($K_i(\sigma_2)/K_i(\sigma_1)$ >140 and >40, respectively).

More than thirty years ago Martin and co-workers introduced sigma receptors (σ-Rs) as a novel subtype of opioid receptors.[1] Currently, it has been conclusively ascertained that σ-Rs represent a unique binding site with two distinct subtypes (σ_1 and σ_2),[2,3] widely distributed in the central nervous system (CNS) and peripheral organs and tissues.[4,5]

So far, only the σ_1 subtype has been purified and cloned.[6] The σ_1 receptor shares 90% identity and 95% similarity across species, and the guinea pig receptor shares 30% identity and 67% similarity with the yeast enzyme C8-C7 sterol isomerase (ERG2) involved in postsqualene synthesis.[7] Despite this, however, the σ_1 receptor is not endowed with sterol isomerase activity.[7] Several reports concurred to show that the σ_1 receptor is a membrane protein of 25.3 kDa, recognized in recent year as a small-ligand operated chaperone essential for the regulation of the passage of Ca^{2+} form the endoplasmic reticulum (ER) to the mitochondria.[8] Additionally, the σ_1 protein can modulate voltage-gated K^+, Ca^{2+}, Na^+, and Cl^- channels,[9] while many transduction systems, such as the N-methyl-D-aspartate (NMDA), muscarinic, dopaminergic, and serotoninergic systems,[10] are sensitive to σ_1-mediated neuromodulation. Far less is known about the σ_2 receptor subtype, a 18–21.5 kDa protein not yet cloned.[6] It has been proposed that this σ receptor subtype is involved in cellular apoptotic response,[11,12] and in the release of Ca^{2+} though an IP3-independent manner.[13,14]

The elevated expression of both σ-R subtypes in cancer cell membranes has led to the speculation that these proteins may serve as markers for certain tumors[15] whilst, concomitantly, concerted efforts are currently being focused on the development of σ-R targeting anticancer agents and imaging tools.[16,17]

The endogenous ligand for σ_1 receptors has not been unequivocally identified to date. Progesterone[18,19] and *N,N* dimethyltryptamine (DMT)[20] were suggested as putative σ_1-R endogenous ligands; however, other steroids (e.g., pregnenolone, dehydropiandrosterone, and testosterone) show only moderate affinity for this receptor, thus making the attribution rather ambiguous.

Some σ-Rs ligands displaying preferential affinity for the σ_1 receptor subtype are (+)-benzomorphans such as (+)-pentazocine and (+)-*N*-allylnormetazocine (NANM, SKF-10,047) whereas haloperidol and 1,3-di-(2-tolyl)guanidine (DTG) exhibit high affinity for both receptor subtypes.[21] (+)-Pentazocine shows a very low affinity for the σ_2-Rs and, as such, represents a prototypical selective agonist used (in its tritiated form) to label σ_1 receptors.

More recently, new different structures endowed with sigma affinity and selectivity were identified by various research groups, such as arylalkylamines,[22a–f] benzooxazolones,[23] and spirocyclic pyranopyrazoles.[24]

In a previous work[25] we have synthesized the series of acetamide derivatives **1** showing an excellent affinity (K_i = 0.09 nM) toward the σ_1 receptor.

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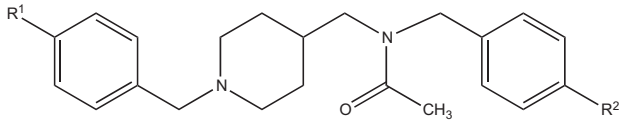
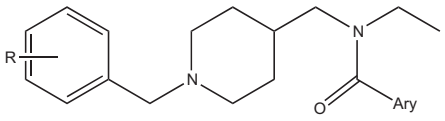


R^1= H, Cl
R^2= H, Cl, CH_3

1

The acetamide derivatives **1** were characterized by the presence of chemical features matching the requirement of a σ_1 receptor 3D pharmacophore model recently developed by our group.[26] Briefly, the two benzene rings map two hydrophobic aromatic pharmacophore features, the piperidine basic nitrogen fits the positive ionizable model site, the carbonyl oxygen of the acetamide group overlaps the hydrogen bond acceptor feature and, lastly, the small substituents at the *para* position of the benzyl moiety linked to the basic nitrogen atom (e.g., –Cl, $-CH_3$, or –H) match the last hydrophobic feature of the pharmacophore model. Accordingly, a 3D pharmacophore model mapping of compound **1** (R^1 = Cl, R^2 = H) resulted in predicted affinity for the σ_1-R of 1.04 nM, in excellent agreement with the corresponding experimental value ($K_i(\sigma_1)$ = 1.87 nM).[26] Based on this encouraging result, a number of other variously substituted derivatives **1** were synthesized, some of which were indeed found endowed with high σ_1 affinity.

On the spur of this favorable result, and with the twofold aim of (i) designing a second generation of stronger σ_1 binders and (ii) understanding the effect of the aromatic portions of the original molecular scaffold (compound **1**) on σ_1 affinity, we went further and replaced the substituted benzyl moiety linked to the acetamide group by a small ethyl chain while the acetyl residue was converted into a number of aroyl moieties, ultimately yielding the new derivatives **2a**–**p** (Table 1).



2a-p R= H, 4-Cl
Ary= Ph, 4-ClPh, 4-CH_3Ph, 4-OCH_3Ph, 1-Naphtyl, 2-Naphtyl, 2-Furyl

Table 1

Compds	R	Ary	Yield (%)	mp (°C)	C H N
2a	H	–Ph	77	80–84	$C_{22}H_{29}ClN_2O$
2b	H	4-Cl-Ph	73	76–80	$C_{22}H_{28}Cl_2N_2O$
2c	H	4-CH_3-Ph	85	108–112	$C_{23}H_{31}ClN_2O$
2d	H	1-Naphtyl	89	94–98	$C_{26}H_{31}ClN_2O$
2e	H	2-Naphtyl	96	90–94	$C_{26}H_{31}ClN_2O$
2f	4-Cl	–Ph	74	126–130	$C_{22}H_{28}Cl_2N_2O$
2g	4-Cl	4-Cl-Ph	47	140–144	$C_{22}H_{27}Cl_3N_2O$
2h	4-Cl	4-CH_3-Ph	70	120–124	$C_{23}H_{30}Cl_2N_2O$
2i	4-Cl	4-OCH_3-Ph	61	75–78	$C_{23}H_{30}Cl_2N_2O_2$
2j	4-Cl	1-Naphtyl	87	110–114	$C_{26}H_{30}Cl_2N_2O$
2k	4-Cl	2-Naphtyl	76	104–108	$C_{26}H_{30}Cl_2N_2O$
2l	H	–Ph-4-Ph	92	94–97	$C_{28}H_{33}ClN_2O$
2m	H	2-Furyl	64	86–90	$C_{20}H_{27}ClN_2O_2$
2n	H	4-OCH_3-Ph	86	88–92	$C_{23}H_{31}ClN_2O_2$
2o	4-Cl	–Ph-4-Ph	74	106–110	$C_{28}H_{32}Cl_2N_2O$
2p	4-Cl	2-Furyl	59	82–85	$C_{20}H_{26}Cl_2N_2O_2$

Characterization of derivatives **2a**–**p**.

All new arylcarboxamide derivatives **2a**–**p** have been synthesized starting from the commercially available 4-aminomethylpiperidine and acetaldehyde according to the pathway illustrated in Scheme 1. A typical Shiff reaction led to the imine derivatives **3** which were further alkylated to the piperidine nitrogen atom with benzyl chloride or 4-chlorobenzyl chloride to obtain intermediates **4** and **5**, respectively. Subsequently, the Shiff bases were reduced with $NaBH_4$ and the corresponding derivatives (**6**, **7**) acylated on the nitrogen atom of the secondary amine to afford the final arylcarboxamide compounds **2a**–**p**. All the derivatives were obtained as hydrochlorides.

Intriguingly, the presence of a furyl group and an unsubstituted benzene (or an aromatic group bearing a small substituent as chlorine atom) produced compounds **2m** and **2p**, gifted with the higher σ_1-R affinity ($K_i(\sigma_1)$ = 21.2 and 13.6 nM) and the best selectivity ($K_i(\sigma_2)/K_i(\sigma_1)$ >140 and >40, respectively) of the series.

In general, all compounds showed very low σ_2 affinities, with values ranging from 270 up to 3000 nM (Table 2).

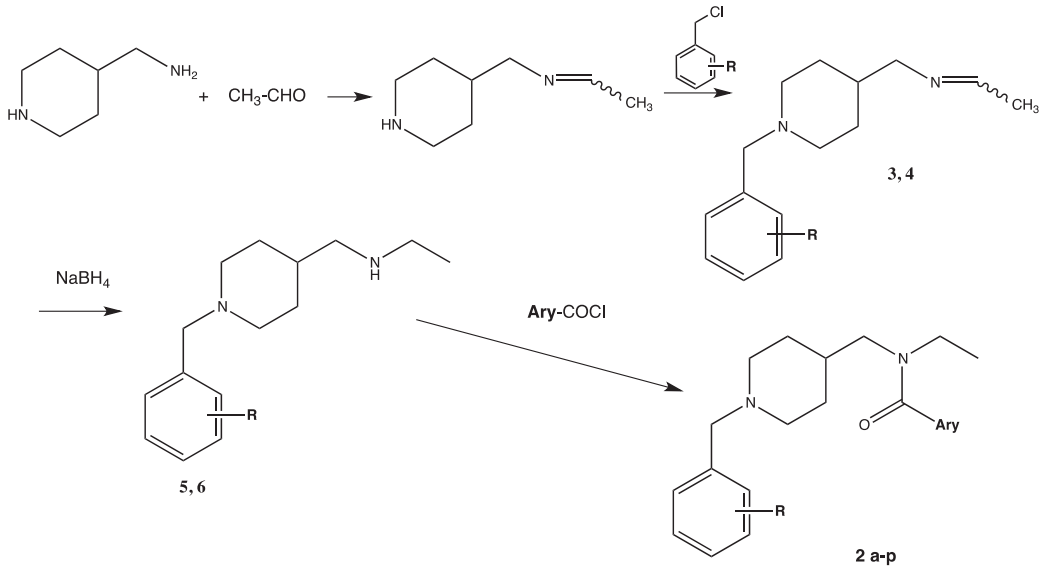
The decrease in σ_1 receptor affinity of the new arylcarboxamides **2a**–**p** with respect to the acetamide derivatives **1** was rationalized via a well-validated computational approach based on the 3D-pharmacophore model[26] and the 3D homology model[25] for σ_1 receptor recently developed by our group. Although compounds **2a**–**p** possess the typical chemical functions required for binding the σ_1 protein, the introduction of a bulkier substituent at the carboxamide moiety results in a suboptimal mapping of the pharmacophore features onto the 3D pharmacophore model in comparison with the lead compounds **1**, as showed in Figure 1.

We see that, while all chemical groups of **1** (R^1 = Cl, R^2 = H) perfectly overlay the corresponding features of our 3D pharmacophore model, the different orientation assumed by the oxygen atom of derivatives **2f** and **2p** results in an imperfect fit of the H-bond acceptor feature which, in turn, negatively influences the mapping of the hydrophobic features by their proximal aromatic portion. On the other hand, the original *N*-benzylpiperidine scaffold still assumes the conformation required for an apt positioning of the remaining chemical groups onto the corresponding pharmacophoric features (Fig. 1). Taking again compounds **2f** and **2p** as a proof-of-concept, further details of the interactions of compounds **2a**–**p** with the σ_1 receptor were gathered from extensive MM/PBSA molecular dynamics (MD) simulations[25,27] performed on the corresponding compound/protein complexes, as shown in Table 3.

According to our predictions the two molecules show quite different affinities towards the receptor, as ΔG_{bind} = −9.09 ± 0.29 kcal/mol for **2f** and ΔG_{bind} = −10.95 ± 0.31 kcal/mol for **2p**, respectively. Importantly, the corresponding $\sigma_1 K_{i,calc}$ values nicely compare with the affinity values experimentally tested toward the σ_1 receptor (220 nM vs 155 nM for **2f**, and 9.4 vs 13.6 nM for **2p**). The deconvolution of the total free energy of binding into its different contributions (Table 3) reveals that the solvation (ΔG_{SOL}) and the entropic ($-T\Delta S_{bind}$) terms for these two compounds are similarly unfavorable, a result somewhat expected since **2f** and **2p** are very similar from a structural viewpoint. The difference in σ_1 affinity between the two compounds hence stems mainly from the more favorable enthalpic contribution exhibited by the furyl-substituted **2p** (ΔH_{bind} = −36.06 kcal/mol) with respect to the phenyl derivative **2f** (ΔH_{bind} = −34.04 kcal/mol).

This difference in the enthalpically-driven affinity of **2f** and **2p** for the σ_1-R was further investigated by performing a per residue binding free energy decomposition, as detailed in Figure 2.

As well illustrated in Figure 2A, both molecules assume a similar binding pose within the receptor binding site. Similarly to the previously reported acetamide derivatives **1**, the *N*-chlorobenzylpiperidine moiety satisfies two important pharmacophore requirement: (1) a polar interaction via a salt bridge between the piperidine $-NH^+$ atom and the side chain of Asp126 (Fig. 2B),



Ary= Ph, 4-ClPh, 4CH_3Ph, 4-OCH_3Ph, 1-Naphtyl, 2-Naphtyl, 2-Furyl
R= H, 4-Cl

Scheme 1.

Table 2
Affinities and selectivities towards σ_1 and σ_2 receptors of compounds **2a–p**

Compds	$K_i\sigma_1$[a] (nM)	$K_i\sigma_2$[b] (nM)	Selectivity $K_i\sigma_2/K_i\sigma_1$
2a	66.8 ± 10[c]	n.d.	n.d.
2b	59.1 ± 7	>1000	>17
2c	160 ± 136	>500	>3
2d	161 ± 23	>1000	>6
2e	198 ± 35	270 ± 121	1.36
2f	155 ± 33	n.d.	n.d.
2g	91 ± 18	>1000	>11
2h	140 ± 9	>500	>3
2i	217 ± 14	283 ± 54	1.30
2j	215 ± 35	>1000	>4
2k	225 ± 55	n.d.	n.d.
2l	301 ± 65	>500	>1
2m	21.2 ± 11	>3000	>140
2n	192 ± 45	n.d	n.d.
2o	373 ± 57	n.d.	n.d.
2p	13.6 ± 1	>500	>40

[a] σ_1 Affinities were determined in rat liver homogenates using [3H](+)-pentazocine.
[b] σ_2 Affinities were determined in guinea pig brain using [3H]-DTG in the presence of (+)-pentazocine to block σ_1 receptors.
[c] The values are means ±SEM of three experiments performed in duplicate.

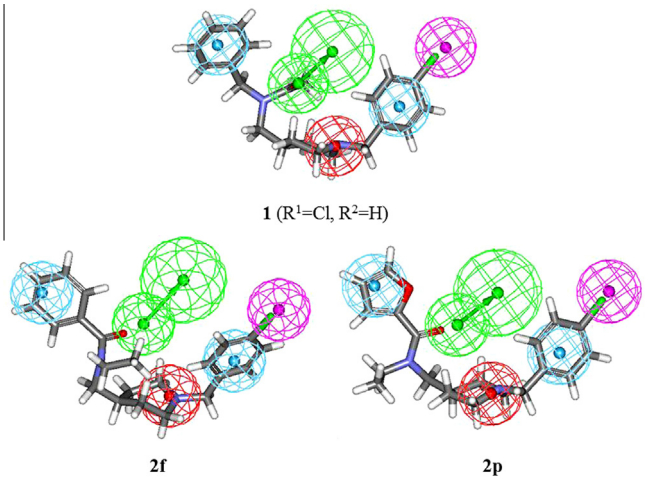


Figure 1. Mapping of compounds **1** (R^1 = Cl, R^2 = H), **2f**, and **2p** onto the 3D pharmacophore model developed for σ_1 ligands.

Table 3
Binding free energy (ΔG_{bind}) and its components for **2f** and **2p** in complex with the σ_1 receptor

Compds	**2f**	**2p**
ΔE_{VDW}	−43.21 ± 0.08	−44.65 ± 0.07
ΔE_{ELE}	−149.29 ± 0.11	−152.20 ± 0.12
ΔE_{MM}	−192.50 ± 0.14	−196.85 ± 0.14
ΔG_{PB}	165.45 ± 0.13	167.47 ± 0.14
ΔG_{NP}	−6.99 ± 0.02	−6.68 ± 0.01
ΔG_{SOL}	158.46 ± 0.13	160.79 ± 0.14
ΔH_{bind}	−34.04 ± 0.19	−36.06 ± 0.20
$-T\Delta S_{bind}$	24.95 ± 0.31	25.11 ± 0.29
ΔG_{bind}	−9.09 ± 0.36	−10.95 ± 0.35
$\sigma_1 K_{i,exp}$	155 ± 33	13.6 ± 1.3
$\sigma_1 K_{i,calc}$[a]	220	9.4

All energy values are in kcal/mol. The experimental and calculated K_i values (nM) are also reported for comparison.
[a] The $\sigma_1 K_{i,calc}$ values were obtained from the corresponding ΔG_{bind} values using the relationship $\Delta G_{bind} = -RT\ln(1/\sigma_1 K_i)$.

responsible for a favorable contribution to binding of −2.39 kcal/mol for **2f** and −2.47 kcal/mol for **2p** (Fig. 2C and D); and (2) the encasement of the aromatic phenyl ring of both derivatives by the side chains of the σ_1 receptor residues Arg119, Tyr120 and Trp121, contributing stabilizing van der Waals and hydrophobic interactions of −3.39 kcal/mol for **2f** and −3.72 kcal/mol for **2p**, respectively (Fig. 2C and D). Contrarily to **1**, however, the new aryl-carboxamide scaffold is only partially inserted into the protein binding cavity; consequently, this molecular portion establishes weaker interaction with the clustered residue Ile128, Glu172, and Tyr173 with respect to the best σ_1-R binder **1**. Interestingly, the binding conformation of the arylcarboxamide moiety is such that the carbonyl oxygen atom is hindered from establishing the topical, stabilizing H-bond interaction with any of the surrounding σ_1 residues (Fig. 2A and B). However, in the case of **2p**, the

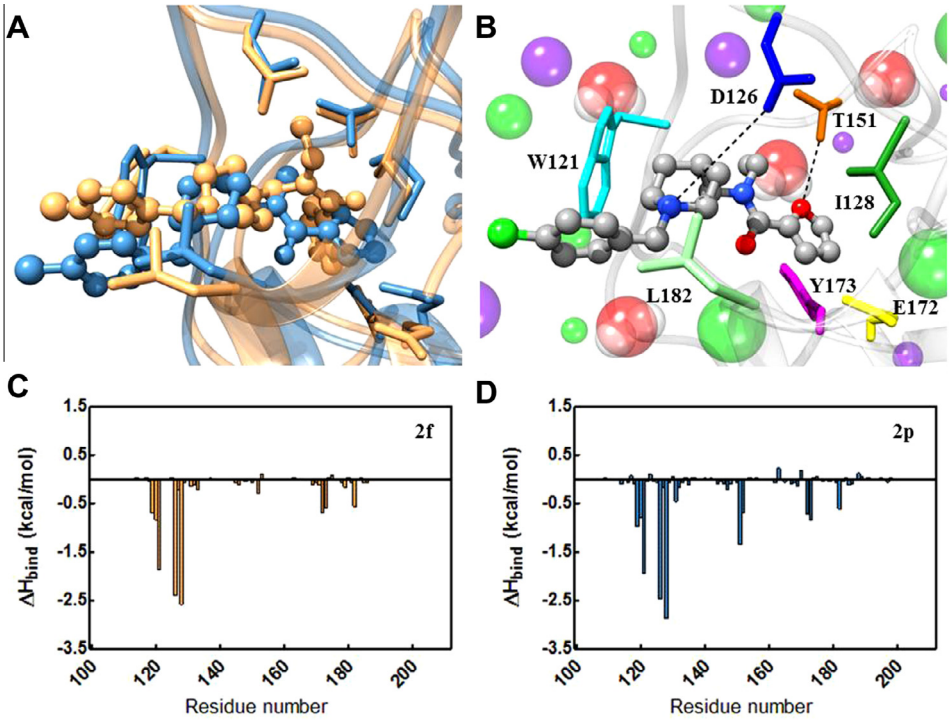


Figure 2. (A) Superposition of equilibrated MD snapshots of the σ_1 receptor in complex with **2f** (sandy brown) and **2p** (steel blue). The images are zoomed views of the receptor binding site. The ligands are portrayed in sticks-and-ball colored according to the protein in the corresponding complex. Water, ions and counterions are not shown for clarity. (B) Equilibrated MD snapshots of the σ_1 receptor in complex with **2p**. The image is a zoomed view of the receptor binding site. The ligand is portrayed in green sticks-and-balls and colored by element, while the protein residues mainly involved in the interaction with **2p** are highlighted as colored sticks and labelled. Salt bridge and H-bonds interactions are shown as dotted black lines. Some water molecules and ions are shown as sphere colored by element. (C) and (D) Per residue binding free energy decomposition for σ_1 in complex with **2f** and **2p**, respectively. Only σ_1 amino acids from position 100 to 200 are shown, as for all the remaining protein residues the contribution to ligand binding is irrelevant.

additional oxygen atom of the furyl ring is indeed engaged in a stable hydrogen bond with the side chain hydroxyl group of Thr151 (Fig. 2B), ultimately yielding a favorable contribution to binding enthalpy of −1.51 kcal/mol.

In conclusion, the results of our in vitro and in silico investigation confirm the role of the *N*-benzylpiperidine core as an excellent scaffold for σ_1 ligands. At the same time, this study highlights the following, specific moieties as beneficial to effective σ_1 binding: (i) a tertiary amide possibly substituted with an aromatic group, and (ii) a small heteroaromatic group like the furyl ring. Indeed, the presence of this latter substituent on the benzylpiperidine scaffold leads to a substantial increase of the σ_1 receptor affinity and selectivity towards the corresponding derivatives, resulting from optimized interactions between the compounds and some of the main residues of the σ_1 binding site.

Taken together, all this information might be exploited in the future to design and synthesize new molecules featuring a furyl group or an isoster thereof and characterized by strong binding contacts toward the σ_1 receptor subtype.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl. 2014. 01.032.

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