Bioorganic & Medicinal Chemistry Letters 24 (2014) 1021–1025

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

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

Daniele Zampieri [a,*], Erik Laurini [b,*], Luciano Vio [a], Samuel Golob [a], Maurizio Fermeglia [b], Sabrina Pricl [b,*], Maria Grazia Mamolo [a]

[a] Department of Chemistry & Pharmaceutical Sciences, Piazzale Europa 1, University of Trieste, 34127 Trieste, Italy
[b] Molecular Simulation Engineering (MOSE) Laboratory, DI3, Piazzale Europa 1, University of Trieste, 34127 Trieste, Italy

ARTICLE INFO

*Article history:*
Received 9 December 2013
Revised 9 January 2014
Accepted 12 January 2014
Available online 20 January 2014

*Keywords:*
σ-Receptors
Arylcarboxamide derivatives
Radioligand binding assays

ABSTRACT

We describe here the synthesis and the binding interaction with $\sigma_1$ and $\sigma_2$ 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 $\sigma_1$ and $\sigma_2$ receptors. Two out of 16 derivatives showed an interesting $\sigma_1$ 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 ($\sigma_1$ and $\sigma_2$),[2,3] widely distributed in the central nervous system (CNS) and peripheral organs and tissues.[4,5]

So far, only the $\sigma_1$ subtype has been purified and cloned.[6] The $\sigma_1$ 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 $\sigma_1$ receptor is not endowed with sterol isomerase activity.[7] Several reports concurred to show that the $\sigma_1$ 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 $\sigma_1$ 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 $\sigma_1$-mediated neuromodulation. Far less is known about the $\sigma_2$ 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 $\sigma_1$ receptors has not been unequivocally identified to date. Progesterone[18,19] and *N,N* dimethyltryptamine (DMT)[20] were suggested as putative $\sigma_1$-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.

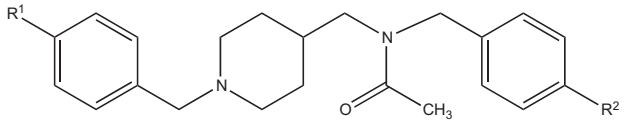
Some σ-Rs ligands displaying preferential affinity for the $\sigma_1$ receptor subtype are (+)-benzomorphans such as (+)-pentazocine and (+)-*N*-allylnormetazocine (NANM, SKF-10,047) whereas haloperidol and 1,3-di-(2-tolyl)guanidine (DTG) exhibit high affinity for both receptor subtypes.[21] (+)-Pentazocine shows a very low affinity for the $\sigma_2$-Rs and, as such, represents a prototypical selective agonist used (in its tritiated form) to label $\sigma_1$ 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 $\sigma_1$ receptor.

* Corresponding authors. Tel.: +39 0405587858 (D.Z.).
*E-mail addresses:* dzampieri@units.it (D. Zampieri), erik.laurini@di3.units.it (E. Laurini), sabrina.pricl@di3.units.it (S. Pricl).

0960-894X/$ - see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2014.01.032

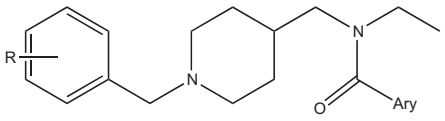


$R^1$= H, Cl
$R^2$= H, Cl, $CH_3$

**1**

The acetamide derivatives **1** were characterized by the presence of chemical features matching the requirement of a $\sigma_1$ 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 $\sigma_1$-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 $\sigma_1$ affinity.

On the spur of this favorable result, and with the twofold aim of (i) designing a second generation of stronger $\sigma_1$ binders and (ii) understanding the effect of the aromatic portions of the original molecular scaffold (compound **1**) on $\sigma_1$ 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-$CH_3$Ph, 4-$OCH_3$Ph, 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 $\sigma_1$-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 $\sigma_2$ affinities, with values ranging from 270 up to 3000 nM (Table 2).

The decrease in $\sigma_1$ 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 $\sigma_1$ receptor recently developed by our group. Although compounds **2a**–**p** possess the typical chemical functions required for binding the $\sigma_1$ 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 $\sigma_1$ 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 $\Delta G_{bind}$ = −9.09 ± 0.29 kcal/mol for **2f** and $\Delta G_{bind}$ = −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 $\sigma_1$ 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 ($\Delta G_{SOL}$) 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 $\sigma_1$ affinity between the two compounds hence stems mainly from the more favorable enthalpic contribution exhibited by the furyl-substituted **2p** ($\Delta H_{bind}$ = −36.06 kcal/mol) with respect to the phenyl derivative **2f** ($\Delta H_{bind}$ = −34.04 kcal/mol).

This difference in the enthalpically-driven affinity of **2f** and **2p** for the $\sigma_1$-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),