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Novel malonamide derivatives as potent κ opioid receptor agonists

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Abstract—A novel series of malonamide derivatives was synthesized. These amides were shown to be potent and selective κ opioid receptor agonists.

Opioids are widely used in the treatment of moderate to severe pain. However, the clinical usefulness of the opioids such as morphine, which exert their analgesic effect through agonism of the μ-opioid receptor, is limited by significant side effects such as respiratory depression, constipation, development of tolerance, and physical dependence and addiction liabilities. One approach to limit or avoid μ-receptor mediated side effects is to selectively target κ and δ-opioid receptors. Receptor selective κ-agonists are of particular interest because they produce analgesia without the undesirable side effects of the μ-opioids.[1–4] The most important selective κ-agonists developed so far are the arylacetamide derivatives, such as U-50,488,[4] and ICI 199441.[5] However these centrally acting κ-agonists have been of limited therapeutic use owing to their own set of centrally mediated side effects such as sedation, dysphoria, and diuresis.[1–3,6] To avoid the side effects associated with CNS penetration, considerable attention has been focused in recent years on the development of peripherally acting κ-agonists as potential analgesic therapeutics.[1,6–14]

Recently, we reported the design and synthesis of two novel series of non-arylacetamide κ-agonists, the constrained aryloxyacetamides **1**,[12] and the phenylamino acetamide derivatives, exemplified by compound **2**.[13] The latter class of compounds are bioisosteres of the aryloxyacetamides with the replacement of oxygen with nitrogen to increase the polarity and hydrophilicity of the compounds, thus limiting CNS penetration and increasing peripheral selectivity. This may have the benefit of minimizing or eliminating the CNS side effects of the centrally active κ opioid receptor ligands while retaining antihyperalgesic activity.[1] The phenylamino acetamide **2** exhibited high κ receptor affinity (K_i = 0.17 nM, EC_{50} = 0.05 nM) and >500-fold selectivity versus both μ and δ receptors, and demonstrated potent analgesic effects in vivo.[13] In addition, as compared to the arylacetamide κ-agonist ICI 199441, compound **2** displayed a much weaker inhibitory activity against the cytochrome P_{450} 2D6 (CYP2D6): IC_{50} = 4.1 μM versus IC_{50} = 26 nM for ICI 199441.[13,14] Based on these results, we report here the synthesis and biological evaluation of a third novel series of malonamide derivatives with the general structure **I**. These agents are the analogs of the phenylamino acetamide **2** with insertion of a carbonyl group between the NH and CH_2. The rationale behind their design was to further increase the polar surface area (to reduce the CNS penetration), to avoid the potential toxicity of the anilinic structure of compound **2**, and to explore further the SAR of the non-arylacetamide κ-agonists. These novel malonamide derivatives possess high κ opioid receptor binding affinity, high selectivity versus μ, and moderate selectivity versus δ receptors. Compound **3**, the most potent malonamide-based κ-agonist, exhibited potent analgesic effects in the in vivo formalin-induced nociception and acetic acid-induced writhing assays.

Keywords: Opioid; κ Agonist; Analgesic.
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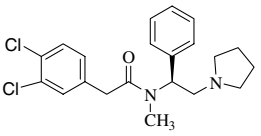
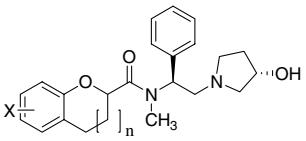
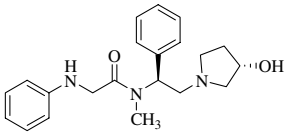


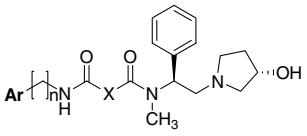
ICI 199441[5]
K_i (κ) = 0.043 nM
μ/κ: 1233; δ/κ: 558



1[12]
n = 0: 2,3-Dihydrobenzofuran class
n = 1: Chromane class



2[13]
K_i (κ) = 0.17 nM
μ/κ: 5900; δ/κ: 510



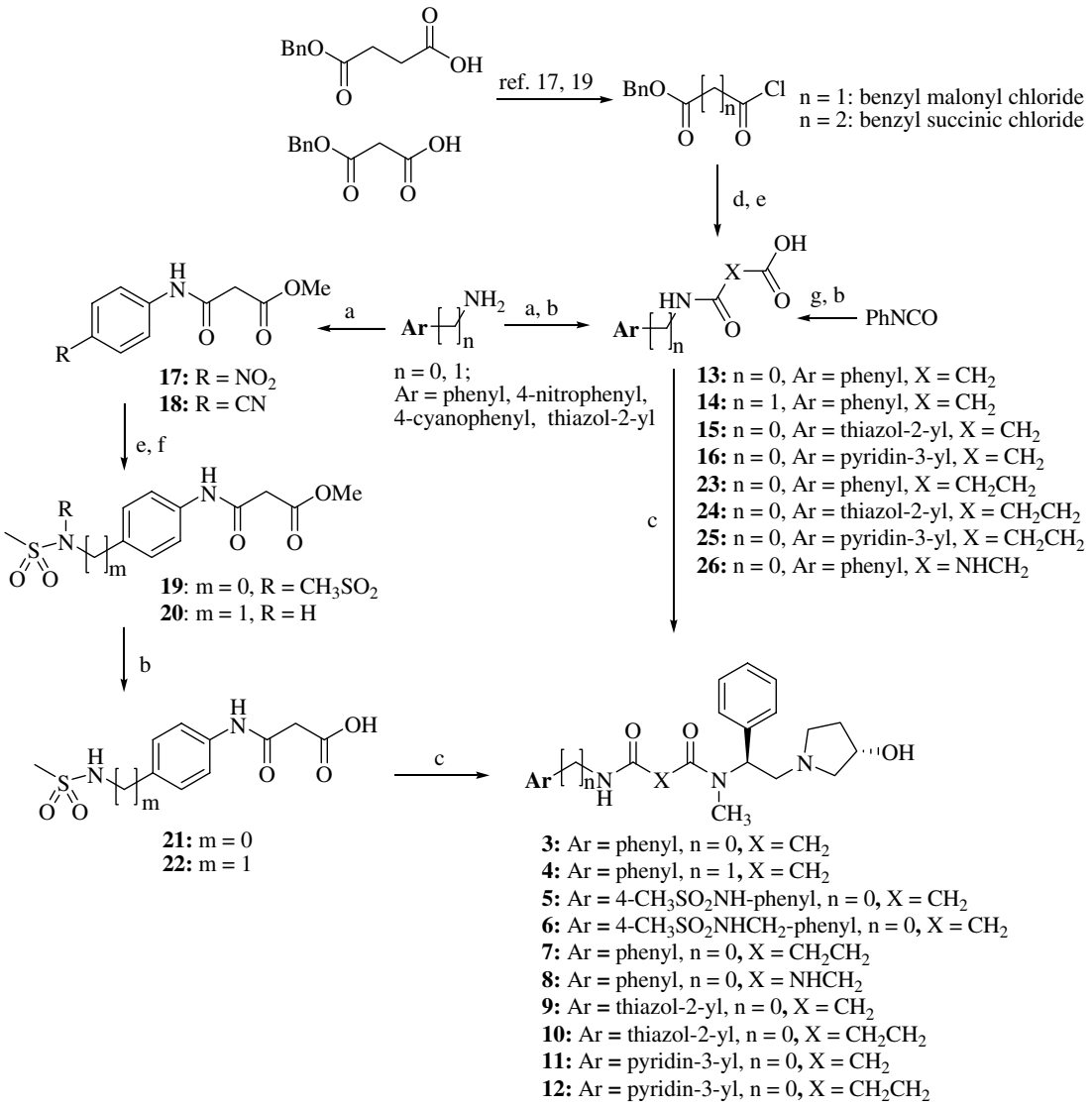
I
(this work)

The synthesis of the target compounds **3**–**12** is summarized in Scheme 1. Reaction of aniline and benzylamine with methyl malonyl chloride followed by hydrolysis of the resulting methyl esters with lithium hydroxide gave the corresponding *N*-phenyl and *N*-benzyl malonamic acids **13** (85%) and **14** (83%). These intermediates were coupled with 1-(2-methylamino-(*S*)-2-phenyl-ethyl)-pyrrolidin-(*S*)-3-ol[15] using Mukaiyama reagent[16] as the acylating reagent to furnish the target compounds **3** (79%) and **4** (79%). The analogs with the replacement of the phenyl group with the nitrogen-containing heteroaromatics to increase hydrophilicity and polarity were also prepared. By following the same reaction sequence, the thiazole analog **9** (64%) was synthesized using 2-aminothiazole as the starting material via the intermediate acid **15** (38%). For the synthesis of the pyridine analog **11**, the synthetic approach was modified using a benzyl ester instead of a methyl ester protection strategy for the carboxyl group. Reaction of 3-aminopyridine with benzyl malonyl chloride[17] gave the corresponding malonamic acid benzyl ester (71%), which was then converted to the *N*-pyridine-malonamic acid **16** (50%) by hydrogenation. This reaction sequence was convenient, avoiding an aqueous work-up of the water soluble zwitterionic pyridine carboxylic acid product. The resulting acid was converted to **11** (60%) using the above-mentioned method.

In our previous studies of the constrained chroman-2-carboxamides and 2,3-dihydrobenzofuran-2-carboxamides (compounds **1**)[12] as potent κ agonists, we found that the sulfonylamino groups, which are para to the oxygen, are the most preferred substituents at the phenyl ring for obtaining both high κ affinity and low inhibitory activity at CYP2D6.[12,18] In the phenylamino acetamide series,[13] sulfonylamino-bearing agonists were well tolerated by the κ receptor, and weak CYP2D6 inhibitors, with para-substitution having highest receptor selectivity. We incorporated this SAR information into the structure of our new chemical series of malonamide derivatives **I**. Thus, compounds **5** and **6** which contain para sulfonylamino groups were prepared. Reaction of 4-nitro- and 4-cyanoanilines with methyl malonyl chloride gave the corresponding malonamic acid methyl esters **17** (91%) and **18** (87%). The nitro compound **17** and the nitrile **18** were hydrogenated to afford the corresponding aniline and benzylamine derivatives, which were reacted without further purification with methanesulfonyl chloride using triethylamine as a base to give the disulfonated and monosulfonated products **19** (82%) and **20** (15%), respectively. The sulfonamides **19** and **20** were treated with lithium hydroxide to give the *N*-(4-methanesulfonylamino)- and *N*-(4-methanesulfonylaminomethyl)malonamic acids **21** (88%) and **22** (95%), which were converted to the sulfonylamino-bearing target compounds **5** (48%) and **6** (80%) in the same manner described above.

In order to investigate the importance of the chain length linking the two amide functional groups in the molecules for the κ affinity, homologs of the malonamides were prepared. The succinamide analogs **7** (76%) and **10** (37%) were prepared via the malonamic acids **23** (100%) and **24** (90%) by following the same reaction sequence as for the synthesis of **3** and **9** except that methyl succinyl chloride replaced methyl malonyl chloride in the first step. The pyridine succinamide analog **12** (25%) was synthesized via the intermediate acid **25** (66%) in the same manner as malonamide **11** except that benzyl succinic chloride[19] replaced benzyl malonyl chloride as the starting material. The urea analog **8** with one methylene group (CH_2) in succinamide **7** replaced by amino group (NH) was prepared via a three-step reaction sequence: reaction of the glycine methyl ester with phenyl isocyanate (100%) followed by treatment with lithium hydroxide (96%), and conversion of the resulting acid **26** to the target compound **8** (83%) using the standard procedure.

Malonamide derivatives **3**–**12**[20] were evaluated in the in vitro opioid receptor binding assays and the results are shown in Table 1.[21] Malonamide **3** showed subnanomolar κ binding affinity (K_i = 0.27 nM) comparable to the phenylamino acetamide **2** (K_i = 0.17 nM), high selectivity over μ, and moderate selectivity versus δ receptor. In contrast, compound **4** with the replacement of aniline with benzylamine showed more than a 50-fold loss of κ binding affinity. Compounds **9** and **11**, in which the phenyl was replaced with the heteroaromatic rings, thiazolyl and pyridinyl, had a slightly decreased κ binding affinity, but increased selectivity over the μ receptor compared with compound **3**. Compounds **5** and **6**, with sulfonylamino groups substituted on the phenyl ring, groups that were well tolerated in the previous series,[12,13] had a significantly decreased κ affinity and also lost μ opioid receptor selectivity. The sulfonylamino substitution was not tolerated in the malonamide series. The homologs of the malonamides with a two-carbon tether, succinamides **7**, **10**, **12**, were 20- to 200-fold less active at the κ receptor than their corresponding malonamides **3**, **9**, **11**. The urea analog **8** showed a similar 20-fold loss of κ affinity in comparison to the malonamide **3**. These data indicate that the chain length linking the two amide functionalities in the molecules is important for optimal κ binding, with a one-atom tether (CH_2) preferred over



Scheme 1. Synthesis of malonamide derivatives **3**–**12**. Reagents: (a) methyl 3-chloro-3-oxopropionate or methyl 4-chloro-4-oxobutyrate, Et_3N, DCM; (b) LiOH, MeOH–THF–H_2O; (c) 1-(2-methylamino-(*S*)-2-phenyl-ethyl)-pyrrolidin-(*S*)-3-ol dihydrochloride, 2-chloro-1-methylpyridinium iodide (Mukaiyama reagent), Et_3N, DCM; (d) 3-Aminopyridine, Et_3N, DCM; (e) H_2, 10% Pd/C, MeOH; (f) MsCl, Et_3N, DCM; (g) glycine methyl ester hydrochloride, Et_3N, DCM.

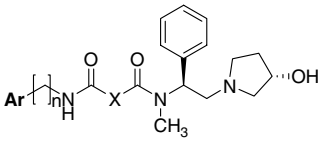
a two-atom tether (CH_2CH_2 and $NHCH_2$). This research of malonamide-based κ-agonists, and our previous works of the constrained aryloxyacetamides[12] and the phenylamino acetamide derivatives[13] as potent and selective κ-agonists, suggest that the methylene group (CH_2) linking the aromatic and the amide functional groups in the classic arylacetamide κ-agonists most likely acts as a spacer and could be replaced with other linkers with retention of high κ receptor affinity. Our discoveries of the non-arylacetamide κ-agonists significantly expand the SAR of the κ-agonists.

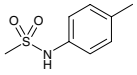
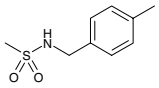
Agonists **3**–**12** were also evaluated against the cytochrome P450 2D6 enzyme using a fluorescent-based assay.[14] The reference compound, ICI 199441, a highly potent and selective arylacetamide κ-agonist, is a potent inhibitor (IC_{50} = 26 nM) of CYP2D6.[14] This is an undesirable property to have in a drug.[14,18] In general, κ agonists **3**–**12** showed low CYP2D6 inhibitory activity, that is, nine out of 10 compounds had IC_{50} values in the micromolar range (Table 1). The pyridine-containing agonists **11** and **12** showed the lowest level of enzyme inhibition with IC_{50} values >10 μM in this new chemical series.

Compound **3**, the most potent κ agonist (K_i = 0.27 nM) and a weak CYP2D6 inhibitor (IC_{50} = 5.3 μM), was tested in the in vivo nociceptive assays.[22] It displayed potent analgesic effects, producing 93% antinociception at 300 μg given intrapaw (sc, injection in the dorsal surface of the paw) in the late phase formalin-induced flinching assay. The compound also inhibited acetic acid-induced writhing when administered subcutaneously with an ED_{50} = 0.03 mg/kg.

In summary, a novel series of malonamides was synthesized and found to be highly potent κ receptor agonists. This work, together with our recent discoveries of the

Table 1. Opioid receptor binding results



Compound	Ar	*n*	X	κK_i (nM)	μ/κ	δ/κ	CYP2D6 IC_{50} (nM)
3		0	CH_2	0.27	120	25	5300
4		1	CH_2	15	120	40	3200
5		0	CH_2	29	4.8	39	10,000
6		0	CH_2	96	4.3	10	5400
7		0	CH_2CH_2	6.0	>830[a]	220	8600
8		0	$NHCH_2$	6.5	110	8.7	5100
9		0	CH_2	0.53	180	71	700
10		0	CH_2CH_2	23	>220[a]	14	3400
11		0	CH_2	1.3	450	15	>10 μM
12		0	CH_2CH_2	280	>18[a]	5	>10 μM

[a] μK_i is estimated to be >5 μM.

constrained chroman-2-carboxamides, 2,3-dihydrobenzofuran-2-carboxamides,[12] and phenylamino acetamides[13] as potent κ-agonists, broadens the structural diversity of the κ-agonists beyond the classic arylacetamides. In this new chemical series of κ-agonists, five compounds displayed single digit nanomolar κ binding affinity, >100-fold selectivity versus the μ receptor, and low CYP2D6 activity. Compound **3** with high κ affinity (K_i = 0.27 nM) and >100-fold selectivity versus μ and moderate selectivity versus δ receptors (25-fold) demonstrated potent analgesic effects in the in vivo formalin-induced nociception and acetic acid-induced writhing assays.

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22. (a) For a description of the in vivo nociceptive assays, see Ref. 6; (b) No data regarding brain penetration are available by now.