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Synthesis and pharmacological evaluation of carbamic acid 1-phenyl-3-(4-phenyl-piperazine-1-yl)-propyl ester derivatives as new analgesic agents

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ABSTRACT

A series of carbamic acid 1-phenyl-3-(4-phenyl-piperazine-1-yl)-propyl ester derivatives were synthesized through discovery strategies for balancing target-based in vitro screening and phenotypic in vivo screening. All the newly synthesized compounds were screened for their analgesic activities and compared with standard drug morphine. Among them, compound **44r**, a potent analgesic agent that has favorable pharmacokinetic properties in rats and most importantly, has a wide safety margin. We demonstrated with in vitro and in vivo functional assays that its analgesic activity might be through 5-HT_{2A} antagonism to some extent. Hence, it is concluded that there is ample scope for further study in developing compound **44r** as a good lead candidate for an analgesic agent.

Among the centrally acting analgesic drugs, opioids are the most widely used in pain treatment because of their strong analgesic efficacy.[1] However, their use is limited by serious side-effects including tolerance, physical dependence, respiratory depression and constipation.[2] Therefore, investigations for new analgesic drugs with safer and more effective pharmacological actions have become a great deal of interest. In the past few decades, various targets have been identified as being involved in normal sensory, analgesic perception such as noradrenergic, dopaminergic, cholinergic, adenosinergic and serotonergic pathways,[3] which have the potential to provide multiple opportunities for development of novel analgesic drugs. In the search for new analgesic drugs lack of side effects, we have focused our efforts on the development of analgesic agents through serotonergic mechanisms. Actually, considerable evidences exist for the implication of serotonin (5-HT) in the modulation of nociceptive transmission elicited from several antidepressants such as amitriptyline and duloxetine.[4] Among serotonergic ligands, arylpiperazines are the most common as pharmacophore moieties and several new arylpiperazine derivatives already have been reported to exert potent and efficacious analgesic activity without displaying the behavioral properties associated with morphine and its congeners.[5]

Few truly innovative central nervous system (CNS) drugs have been approved despite of tremendous advances in pharmaceutical industry, which raises questions about the limitation of target-based approach to drug discovery by David C. Swinney et al.[6] Indeed, before the introduction of target-based approaches, drug discovery was driven primarily by phenotypic assays, often was limited knowledge of the molecular mechanisms of disease. In view of these observations coupling with this environmental challenges, we explored to utilize the arylpiperazine as a core template and proposed the combination of in vitro receptor binding assays (5-HT_{1A}, 5-HT_{2A}) and in vivo phenotypic assays in confirmatory mice models (i.e., acetic acid-induced writhing test as a prescreening tool) to accelerate the drug discovery of novel analgesics. Initially we have identified several novel chemicals not yet reported as arylpiperazine chemotype from our in-house library collection. Among them, we have identified our initial hit **1** keeping in the view of the structural requirements suggested in the

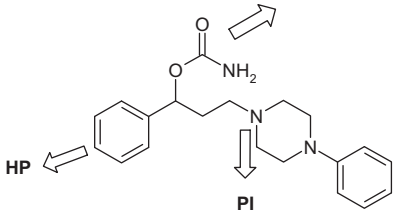

Figure 1. Structures of initial hit compound **1** showing the required pharmacophoric elements (HP, hydrophobic moiety; HBA, hydrogen bond acceptor; PI, positively ionizable group).

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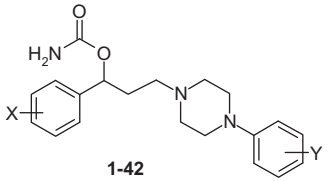
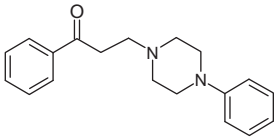
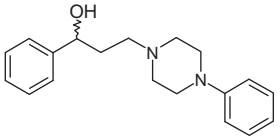
Table 1
In vitro receptor binding potencies and *in vivo* analgesic efficacies of derivatives **1**–**42**



1-42

Entry	X	Y	Receptor binding assays[a]		Acetic acid-induced writhing test in mice[b]		Rotarod test in mice[c]	
			5-HT_{1A}	5-HT_{2A}	IP	PO	IP	PO
Morphine					0.96ip	5.57po		
1C			47.5 nM	1.07 μM	37.2%			
1D			174 nM[d] 495 nM[e]	452 nM[d] 289 nM[e]	70.1%[d] 66.7%[e]			
1	H	H	434 nM	139 nM	0.49ip	6.31po	52.3ip	124.1po
2	4-Cl	H	64.6%	84.6%		2.1po	2/4(50ip)	
3	3-NO_2	H	74.6%	86.4%	100%	48.0%	2/4(50ip)	
4	4-NO_2	H	81.4%	88.0%	100%	91.2%	4/4(50ip)	53.4po
5	4-Tertbutyl	H	44.4%	98.8%		62.0%		3/4(120po)
6	4-F	H	65.5%	84.7%	99%	2.79po	0/4(50ip)	140.7po
7	3-Cl	H	76.4%	93.7%	100%	59.0%	1/4(50ip)	
8	4-OMe	H	67.7%	82.0%	100%	43.0%	4/4(50ip)	
9	4-Me	H	59.6%	85.6%	100%		3/4(50ip)	1/4(120po)
10	H	2-Cl	52.5 μM	>100 μM				
11	H	2-F	160 nM	110 nM	0.34ip		44.7ip	
12	H	2-Me	149 nM	78.3 nM	1.96ip			
13	H	2-OMe	97.6 nM	164 nM	0.67ip	7.80po	8/8(30ip)	
14	H	3-OMe	80.8 nM	502 nM	55.0%			
15	H	3-Cl	93.8 nM	61.5 nM	0.57ip	17.5po	4/4(60ip)	
16	H	3-CF_3	12.6 nM	660 nM	14.6%		4/4(100ip)	
17	H	3-Me	59.6 nM	92.5 nM	0.81ip		2/4(30ip)	
18	H	4-NO_2	6.57 μM	2.05 μM	2.92ip			
19	H	4-OMe	1.98 μM	5.85 μM	0.14ip	72.0% 3.12po		122.1po
20	H	4-F	10.5 μM	64.5 nM	0.55ip	88.0%	4/4(20ip)	7/8(60po)
21	H	4-Cl	1.86 μM	264 nM	52.0%			
22	H	4-CF_3	7.22 μM	2.11 μM	1.64ip			
23	H	4-Me	1.28 μM	369 nM				
24	H	2,4-Me_2				34.0%		
25	H	2,4-OMe_2				19.0%		
26	H	2-OH				37.0%		
27	H	2-NO_2,4-CF_3				50.0%		
28	H	4-Cl,3-CF_3				52.0%		
29	H	3,5-OMe_2				13.0%		
30	H	2,6-Me_2				48.0%		
31	H	4-OCF_3				18.0%		

(*continued on next page*)

Table 1 (*continued*)

Entry	X	Y	Receptor binding assays[a]		Acetic acid-induced writhing test in mice[b]		Rotarod test in mice[c]	
			5-HT_{1A}	5-HT_{2A}	IP	PO	IP	PO
32	4-F	4-F	60.9%	93.9%		52.0%		3/4(120po)
33	4-F	4-OMe				12.4po		
34	4-F	4-NO_2	29.6%	29.6%		70.0%		97.8po
35	4-F	4-OH				7.24po		
36	4-Cl	4-OMe				51.0%		
37	3,4-Cl_2	4-OMe				61.0%		
38	4-OMe	4-OMe				53.0%		
39	3,4-Me_2	4-OMe				58.0%		
40	2,4-Cl_2	4-OMe				16.0%		
41	4-Cl,3-CF_3	4-OMe				46.0%		
42	2,4-Me_2	4-OMe				62.0%		

[a] % inhibition at 1 μM or IC_{50}, receptor binding assays were carried out in duplicate using homogenates of rat brain.
[b] % inhibition at 10 mg/kg ip or 10 mg/kg po or ED_{50} (mg/kg, the median effective dose), each value represents the mean ± SEM (n = 8).
[c] Falling-off ratio at the specified dose (ip or po) or NTD_{50} (mg/kg, the median neurotoxic dose), each value represents the mean ± SEM (n = 4–8).
[d] *R*-enantiomer.
[e] *S*-enantiomer.

pharmacophore model of arylpiperazines.[7] The corresponding pharmacophore of hit compound **1** could be identified as following: the N-1 atom at the arylpiperazine corresponds to the PI, the phenyl moiety serve as HP portion, respectively, and the carbamate group as the HBA as described in Figure 1.[8]

And also, hit compound **1** showed preliminary evidence of balanced in vitro target profile and in vivo analgesic activity, with a potential for scaffold optimization. (See Table 1)

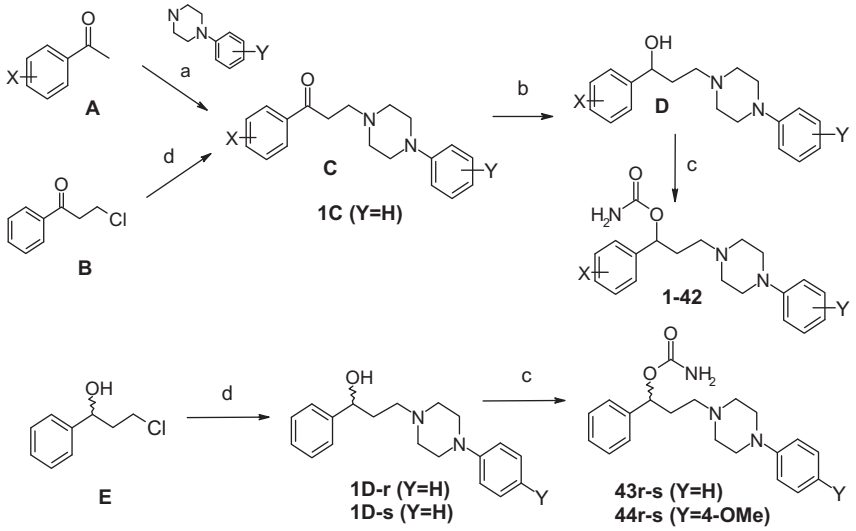
We report herein the syntheses of some new arylpiperazine derivatives outlined in Scheme 1. Various substituted acetophenone (A) were treated with formaldehyde and arylpiperazines to give the Mannich products **1–9** and **36–42** in 50–60% yield. Analogues **10–35** were prepared by nucleophilic substitution with arylpiperazines from commercially available 3-chloro-1-phenylpropan-1-one (B). Thus the intermediate ketones (C) were reduced with $NaBH_4$ to give the intermediate alcohol (D) which were readily converted to the corresponding carbamates using 1,1-carbodimidazole and excess ammonium hydroxide as a described procedure.[9] The corresponding chiral enantiomers were readily prepared by the nucleophilic substitution with the corresponding arylpiperazines from commercially available *R*- or *S*-chloro-1-phenyl-1-propanol (*E*) as described in Scheme 1.

Arylpiperazine **1–42** were evaluated for their analgesic properties in the acetic acid-induced writhing test in mice[10] and compared to classical analgesic drug morphine which was known to possess potent anti-nociceptive effects. In this model, the compounds were administered intraperitoneal (ip) or orally (po) 0.5 or 1 h prior to the intraperitoneal administration of acetic acid. Their potencies were then measured as the inhibition of painful reactions (writhing). The affinity of compounds for 5-HT_{2A} receptors of the rat brain cortex and for 5-HT_{1A} receptors of the rat brain hippocampus was assessed on the basis of their ability to displace [^{3}H]-ketanserin and [^{3}H]-8-OH-DPAT, respectively, according to the published procedures.[11]

Taken all together, the promising compounds were scrutinized in order to assess their toxicity profile in the rotarod test[12] and their possible interactions with serotonergic system. Initial SAR (Structure–Activity Relationship) studies began with the investigation on the role of carbamate. Compounds **1C** and **1D** (prepared as *R*- and *S*-enantiomer, respectively) are key intermediates of hit compound **1**. In comparison, we tested their analgesic activities as well. As depicted in Table 1, the analogues with ketone and alcohol group (compounds **1C** and **1D**) also had 5-HT_{1A} and 5-HT_{2A} binding affinity as previously reported[13] but their in vivo analgesic activities in acetic acid-induced writhing test were moderate. While hit compound **1** only showed remarkable analgesic activity in the same model suggesting that the introduction of carbamoyl group may be favorable to enhance the analgesic activity in this series.

We moved on to exploration of the improvement of the 5-HT_{1A} or 5-HT_{2A} receptor binding affinity of compound **1** employing the Topliss 'decision tree' approach (Table 1)[14] which accounts for the electronic, lipophilic, and steric factors for substitution on a phenyl ring .

For optimization at the X site, we kept the un-substituted phenyl at the Y site. All the compounds **2–9** displayed moderate potency in vitro and we discovered that the optimization on the X site have less influential to receptor binding potency and in vivo efficacy. But, from the observation that compound **2**, **4** and **6** had slightly improvement on in vivo analgesic efficacy, it might be the optimal substituents on the X site which had positive π values and σ values at the *para*-position (4-NO_2, 4-Cl and 4-F). Next, the optimization at the Y site (compounds **10–31**) was performed with the unsubstituted phenyl at the X site. In general, when the substituent was moved to the *ortho* or *meta*-position, most analogues seemed to be potent in both 5-HT_{1A} and 5-HT_{2A} binding assay



Scheme 1. Formation of arylpiperazine carbamates **1**–**44r**–**s**. Reagents and conditions: (a) HCHO, conc HCl, EtOH, reflux; (b) $NaBH_4$, MeOH, 0 °C; (c) (i)1,1-carbodimidazole, THF, rt (ii) excess NH_4OH; (d) triethylamine, CH_3CN, reflux.

regardless of electronic or hydrophobic characteristics with the exception of 2-Cl. In particular, we found similar SAR pattern regarding to 5-HT_{1A} binding affinity as previously reported in N4-unsubstituted N1-arylpiperazines for 5-HT_{1A} receptor.[7,15] While the Topliss tree was followed at this site, it can be noticed that the analgesic activities of the compounds were somewhat related to 5-HT_{2A} binding affinity rather than 5-HT_{1A} binding affinity exemplified by compounds **11–13**, **15**, **17**. For instance, the analogues with 3-OMe and 3-CF_3 (compounds **14** and **16**) having most potent 5-HT_{1A} binding affinity (80.8, 12.6 nM, respectively) led to a dramatic loss of in vivo analgesic activity. The analogues with 4-NO_2, 4-OMe and 4-CF_3 (compounds **18, 19** and **22**) showed potent analgesic activities even though they lost significant both 5-HT_{1A} and 5-HT_{2A} binding affinities and also the analogue with 4-Cl (compound **21**) displayed weak in vivo activity in spite of its moderate 5-HT_{2A} binding affinity. It seemed that the correlation between in vitro binding affinity and in vivo efficacy was less distinct in these series.

Having these initial findings that while the Topliss approach was slightly accurate in predicting the most active substituent in vitro, the degree of improvement in vivo was in general weak, we quickly expanded our SAR to further optimize the in vivo efficacy and safety in this series. As depicted in Table 1, most analogues failed to improve neurotoxicity and hit compound **1** which showed potent analgesic activity with improved safety was proved to be rapidly metabolized by human liver microsomes ($HLM_{T1/2}$ = 19 min).[16] We found that the HLM stability was generally low in this series. Compounds **2** and **6** which had *para*-blocking substituent on the X site, albeit more potent and safe, did not improve microsomal stability (**2**, $HLM_{T\ 1/2}$ = 15 min; **6**, $HLM_{T1/2}$ = 18 min). Employing LC/MS/MS, in vitro metabolite identification studies in this series showed that one major metabolite was resulted from oxidation of the *para*-position on the arylpiperazine ring and we confirmed the compound **35** to be stable in microsomal stability ($HLM_{T1/2}$ = 149 min). On the basis of these results, we synthesized the analogues possessing dual *para*-blocking substituents on both side rings to improve the microsomal stability better, but the results revealed that introduction of *para*-blocking substituents at both side rings were detrimental to their in vivo efficacies exemplified by compounds **32–42**. As expected, they gave improvement in microsomal stability test (data not shown). From these observations, we performed the microsomal stability test of compound **19** which was *para*-blocking with 4-OMe on the arylpiperazine ring. The compound **19** was proved to be very metabolic stable ($HLM_{T1/2}$ = 124 min) demonstrating that it might be well-matched with our metabolite identification experiment. Although compound **19** was not active in our binding assays, its additional in vivo potential as novel analgesics was enough us to optimize further in accordance with our discovery strategy.

Table 2
The chiral effect of hit compound **1** and selected compound **19**

Entry	Stereochemistry	5-HT_{1A}[a]	5-HT_{2A}[a]	AA[b]
1	Racemate	434 nM	139 nM	0.49ip 6.31po
43r	R	534 nM	62 nM	0.18ip
43s	S	772 nM	1.02 μM	87.8%
19	Racemate	1.98 μM	5.85 μM	0.14ip 3.12po
44r	R	N.T[c] 8.82 μM[d]	N.T[c] 5.77 μM[e]	0.94ip 2.65po
44s	S	N.T[c]	N.T[c]	1.43ip

[a] IC_{50}.
[b] Acetic acid-induced writhing test in mice, ED_{50} (mg/kg, the median effective dose), each value represents the mean ± SEM (n = 8) or% inhibition at 10 mg/kg ip.
[c] N.T: not tested in our system.
[d] IC_{50} (MDS Pharma Services binding assay data).
[e] IC_{50} (Novascreen binding assay data).

With a set of these data in hand, we turned our attention toward chiral separation of selected compound **19** and our initial hit compound **1** for comparison as depicted in Figure 2.

In general, *R*-enantiomer was found to be more potent in vivo than *S*-enantiomer. Conversion of compound **1** to its corresponding enantiomers **43r–s** led to the finding that its analgesic potency was clearly dependent on 5-HT_{2A} binding affinity, but not in the case of **44r–s**. Differing from compound **43r**, the analgesic activity of **44r**

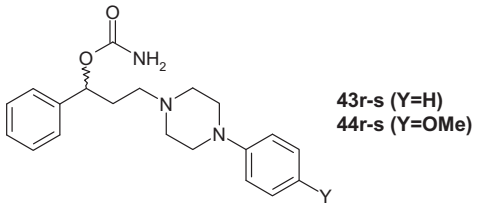


Figure 2. Selected chiral enantiomers **43r**–**s**, **44r**–**s**.

Table 3
The analgesic profiles of compound **44r**

In vivo pain models	**44r**	Morphine	Ketorolac
Acetic acid-induced writhing test in mice	0.94ip 2.65po	0.96ip 5.57po	0.59ip 39.1po
Hot plate test in mice	2.86po	13.5po	N.E[#] up to 100ip
Formalin test in mice (late phase)	4.77po	9.15po	12.9ip 17.9po
Carrageenan-induced mechanical hyperalgesia in rats	0.68po	0.60po	36.4%* [a]

NE#: Not effective /All data were represented as ED_{50} (mg/kg).
[a] % inhibition at 100 mg/kg po (*p <0.05).

Table 4
Pharmacokinetic data of compound **44r** after single administration in rats

PK parameters	iv[a] (15 mg/kg, n = 3)	po (15 mg/kg, n = 3)
T_{max}, h		1.1
C_{max}, ng/mL	9953	2200
AUC_{all}, ng h/mL	17825	13159
CL, mL/kg/h	846	
V_{ss}, mL/kg	1769	
$T_{1/2}$, h	1.8	2.6
Bioavailability,%		73.8

[a] Intravenous.

might be notably from other different mechanism as well as 5-HT_{2A} mechanism. The compound **44r** showed better metabolic stability in both species compared to compound **43r** (**44r**, $HLM_{T1/2}$ = 319 min, $RLM_{T\ 1/2}$ = 91 min; **43r**, $HLM_{T\ 1/2}$ = 62 min; $RLM_{T\ 1/2}$ = 8 min, respectively).

Considering all these data, although compound **44r** was weakly active in receptor binding assays as depicted in Table 2, the analgesic potential of the compound **44r** was encouraged us to further evaluate it for analgesic profiles in both anti-nociceptive and anti-inflammatory pain models using reference drugs (Morphine and Ketorolac, respectively).

As depicted in Table 3, compound **44r** emerged as the most active analgesic agent. The anti-nociceptive activities of compound **44r** in acetic acid-induced writhing test, hot plate test and formalin test[17] in mice were orally more potent when compared to the reference compound morphine. Moreover, its activity was not blocked in the hot plate test after the subcutaneous administration of the opioid antagonist naloxone (0.1–10 mg/kg) demonstrating it might act through a non-opioid mechanism of action (data not shown). And also compound **44r** reversed carrageenan-induced mechanical hyperalgesia in a dose-dependent manner in a rat model of persistent inflammatory pain.

In addition to its potent analgesic activities, the median neurotoxic doses of compound **44r** in the rotarod test were determined at 52.7 mg/kg (ip) and 139.0 mg/kg (po), suggesting no significant drug-related side effects such as myorelexation or sedation at the effective doses. These observation could be of relevance in terms of the side effect profile of the compound **44r** indicating a wide margin of safety. With the promising in vivo efficacy results in hand, the pharmacokinetic properties of **44r** were evaluated, which in summarized in Table 4.

After oral administration of a 15 mg/kg dose of **44r** to rats, C_{max} of 2200 ng/mL was observed at 1.1 h. The elimination half-life for **44r** following oral administration was 2.6 h in rats. And compound **44r** showed good oral bioavailability (73.8%).

To furnish additional evidences concerning the mechanism of its action, compound **44r** was subjected to a broad receptor binding assay (MDS Pharma Services/Novascreen, USA). At a concentration of 10 μM, significant binding (>80%) was observed to 3 out of 45 receptors, most notably the 5-HT_{2B} receptor (K_i = 164 nM). Moderate binding was observed to Sigma 1 receptor (K_i = 392 nM), α_1-receptor (K_i = 863 nM). At 10 μM concentration, no significant binding was observed to most receptors tested including opioids, ion channels, COXs, 5-HT/NE transporters, and other 5-HT receptors. However, to explain mechanism of action of **44r** more in detail, in vitro functional assays were necessary and it was determined that compound **44r** only exhibit weak 5-HT_{2A} functional antagonist activity (IC_{50} = 5.8 μM). To determine the 5-HT_{2A} antagonistic effects of the compound **44r** in vivo, its ability to inhibit the (+)-l-(4-iodo-2,5-dimethoxyphenyl)-2-propanamine (DOI)-induced head twitches and 5-hydroxytryptophan (5-HTP)-induced head twitches in mice were employed.[18] Compound **44r** dose-dependently blocked DOI-induced head twitches (40.0%**, 61.4%** and 89.3%** of the antagonism at 0.3, 1 and 3 mg/kg, ip, respectively, **p <0.01) and blocked the 5-HTP-induced head twitches (respectively, 46.2%** and 100%** of the antagonism at 3 and 10 mg/kg ip, **p <0.01) demonstrating that compound **44r** might exhibit its analgesic activities to some degree through 5-HT_{2A} antagonistic mechanism.

In summary, we have disclosed the development of a novel arylpiperazine series for analgesic agents. Starting from hit compound **1** which was moderate in vitro binding assay (5-HT_{1A} and 5-HT_{2A}) we were able to develop **44r** by combining the phenotypic in vivo screening. It is a potent analgesic agent that has favorable pharmacokinetic properties in rats and most importantly, has a wide safety margin. We demonstrated with in vitro and in vivo functional assays that its analgesic activity might be through 5-HT_{2A} antagonism to some extent. This work further prompted by discovery of other more promising lead series in this scaffold, which will be reported in due course.

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