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Arylpiperazine-containing pyrimidine 4-carboxamide derivatives targeting serotonin 5-HT_{2A}, 5-HT_{2C}, and the serotonin transporter as a potential antidepressant

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ABSTRACT

Pyrimidine usually has good pharmacokinetic properties as a drug substance and considerable efforts have been devoted to develop pyrimidine derivatives into drug candidates. Arylpiperazine-containing pyrimidine 4-carboxamide derivatives were synthesized and evaluated for binding to serotonin receptors and transporter. Pyrimidine derivatives showed good antidepressant activity in FST (forced swimming test) animal model and also displayed no appreciable inhibitory activity against hERG channel blocking assay. Herein SAR studies of pyrimidine derivatives targeting serotonin receptors and transporter will be disclosed.

Major depressive disorder is a mental disorder characterized by an all-encompassing low mood accompanied by low self-esteem and loss of interest or pleasure in normally enjoyable activities. There is a growing awareness that patients with depressive disorders often also suffer cognitive impairment,[1] and some studies indicate that these deficits may persist even after remission.[2,3] The monoaminergic hypothesis of depression assumes that depression is caused by the dysfunction of the serotonin (5-HT, SER), noradrenaline (NE) and/or dopamine (DA) neurotransmitter systems.[4] This hypothesis has been used to explain the efficacy of existing antidepressant therapies. Among these available therapies, selective serotonin reuptake inhibitors (SSRIs) and more recently combined serotonin- and noradrenaline reuptake inhibitors (SNRIs) have become the standard treatment for depression.[5]

In recent years, SARI (serotonin antagonist/reuptake inhibitor) drugs that block both the serotonin 5-HT_2 receptors and the serotonin transporter have been developed.[6] YM-992 **1**,[7] LY367265 **2**,[8] Nefazodone **3**,[9] and aripiprazole **4** are the typical examples of that series (Fig. 1). Unlike most SSRIs, nefazodone is reported to have no negative effects on libido or sexual functioning. Nefazodone's claimed advantages over other antidepressants include reduced possibility of disturbed sleep or sexual dysfunction, and ability to treat some patients who did not respond to other antidepressant drugs.[10–13] In this regard, there are still urgent medical needs on

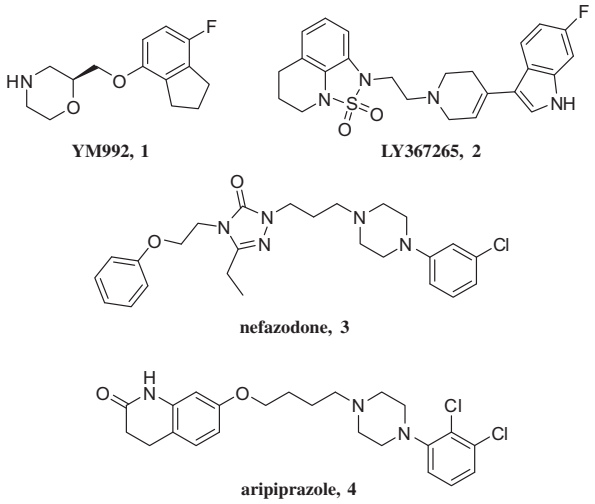

Figure 1. SARI drugs (serotonin antagonist/reuptake inhibitor).

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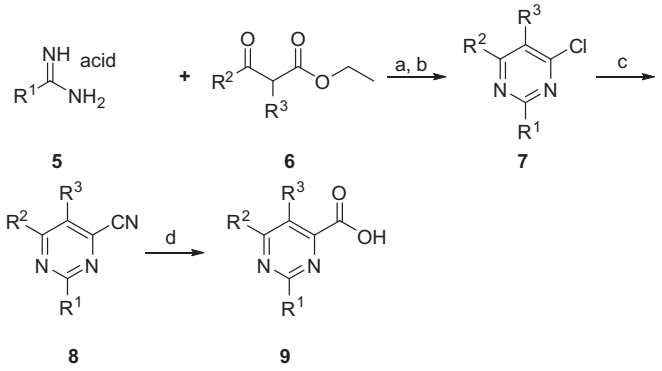


Scheme 1. Reagents and conditions: (a) NaOEt, EtOH, 100 °C; (b) $POCl_3$, 110 °C; (c) $Zn(CN)_2$, $Pd(PPh_3)_4$, THF, microwave reaction 165 °C, 30 min; and (d) NaOH, EtOH, water, reflux.

the development of novel drugs with better developability characteristics: improved pharmacologic properties and reduced side effects. Herein, we wish to describe the design, synthesis, and biological evaluation of novel arylpiperazine-containing pyrimidine 4-carboxamide derivatives targeting serotonin 5-HT_{2A}, 5-HT_{2C}, and the serotonin transporter as a potential antidepressant.

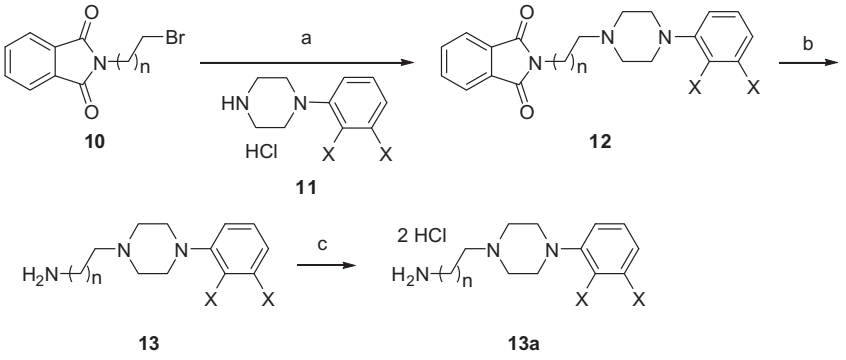
The carboxylic acid derivative **9** was prepared by a conventional method, for example, by reacting an amidine acid salt **5** with a keto-ester derivative **6** using sodium ethoxide, followed by chlorination using $POCl_3$ to produce a corresponding 4-chloropyrimidine **7**. Subsequent reaction of the resulting 4-chloropyrimidine **7** with zinc cyanide in the presence of $Pd(PPh_3)_4$ gave an intermediate 4-cyanopyrimidine **8**. An acid form **9** was transformed from the intermediate **8** using sodium hydroxide, followed by acidification, as shown in Scheme 1.

As shown in Scheme 2, preparation of aminoalkyl-arylpiperazine **13** started from the corresponding bromoalkyl-phthalimide **10** and arylpiperazine **11**.[14] Treatment of bromoalkyl-phthalimide **10** with arylpiperazine hydrochloride **11** in the presence of potassium carbonate in DMF at room temperature afforded N-protected amine **12**. Compound **12** was then treated with hydrazine in ethanol to give amine **13**. For the sake of convenience of handling on scale, liquid amine **13** was transformed to hydrochloride salt form **13a** with 4 N HCl in dioxane.

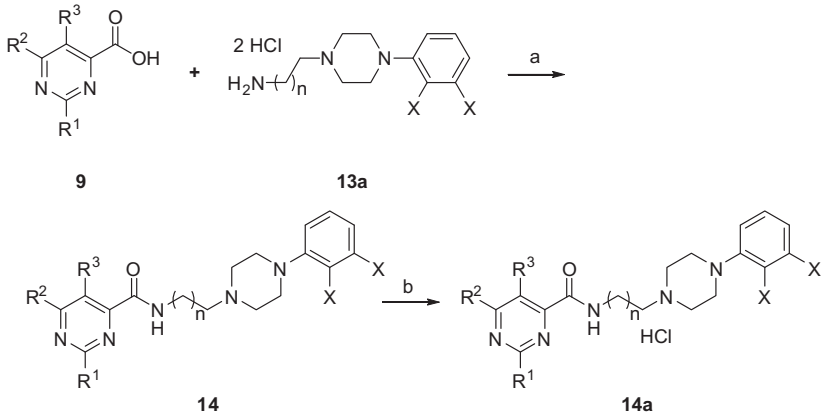
Finally, coupling reaction of acid **9** and aminoalkyl-arylpiperazine **13a** was conducted as shown in Scheme 3. With pyrimidine-4-carboxylic acid **9** and aminoalkyl-arylpiperazine dihydrochloride **13a** in hand, typical amide coupling was conducted under conditions involving EDCI, HOBT, and NMM in methylene chloride or DMF to produce amide **14**. As reaction was completed, purification was performed using preparative reverse-phase HPLC with 0.1% TFA mixture of acetonitrile and water solution. The neutral form of product **14** was converted to HCl salt form **14a** to increase the overall solubility for biological evaluation.

The binding affinity of current compounds against 5-HT_{2A}, 5-HT_{2C} receptor, and serotonin transporter, stably expressed in CHO-K1 cells, were evaluated by displacement binding using [^{3}H]Ketanserin, [^{3}H]Mesulergine, and [^{3}H]Imipramine, respectively, as radioligands.[15,16]

The work was focused on exploration of the substitution group of pyrimidine moiety. 2-Methylpyrimidine derivatives and 2,3-dichlorophenyl- or 2,3-dimethylphenylpiperazine were connected with propyl-carboxamide as a linker.



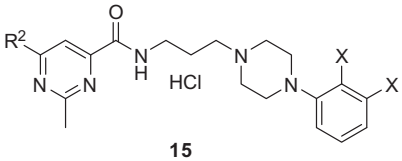
Scheme 2. Reagents and conditions: (a) **11**, K_2CO_3, DMF, rt; (b) $NH_2NH_2{\cdot}H_2O$, EtOH, rt; and (c) 4 N HCl in dioxane.



Scheme 3. Reagents and conditions: (a) EDCI, HOBt, NMM, DCM or DMF, rt; and (b) HCl, MeOH, 0 °C.

The binding affinities of prepared compounds against the 5-HT_{2A}, 5-HT_{2C}, and SERT are shown in Table 1. Because these compounds would interact with multiple targets, it is not easy to evaluate prepared compounds following SARI (serotonin antagonist and reuptake inhibitor) mechanism. Initially, most compounds tested displayed IC_{50} <1 μM, implying that this series of arylpiperazinyl pyrimidine 4-carboxamide might hold promises as a potential antidepressant. The substituted R^2 group of pyrimidine showed a tendency of preference for bulky group. Also, it showed considerable difference in binding affinity between 2,3-dichlorophenylpiperazinyl compounds and 2,3-dimethylphenylpiperazinyl compounds. In order to improve binding affinity against 5-HT_{2A}, 5-HT_{2C} receptors, and serotonin transporter, contraction of linker size was undertaken. For comparison, dimethylphenylpiperazinyl derivatives were synthesized and screened as shown in Table 2. In the case of 5-H pyrimidine (R^3 = H), 5-HT_{2A}, and 5-HT_{2C} receptor binding affinities were increased when the length of the linker was shorten from C3 to C2. On the contrary, SERT binding affinities were decreased at the same conditions. In the case of 5-OMe pyrimidine (R^3 = OMe), the effect of the linker size on the binding affinities was not obvious (see Table 2). To evaluate antidepressant activity of the interesting compounds, immobility in forced swimming test (FST) on mice were measured.[17,18] Zoloft (sertraline) was used as a reference compound for comparison. The results are shown in Figure 2.

Table 1
Binding affinities for 5-HT_{2A}, 5-HT_{2C} receptor, and serotonin transporter of arylpiperazinyl pyrimidine 4-carboxamide derivatives



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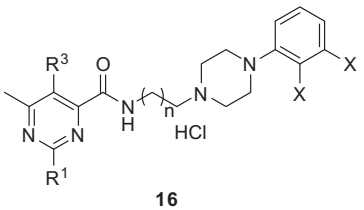
Compound	R^2	X	IC_{50} (nM)		
			5-HT_{2A}	5-HT_{2C}	SERT
15a	Me	Cl	166	610	289
15b	*i*Pr	Cl	113	227	644
15c	Ph	Cl	35	30	588
15d	*i*Pr	Me	490	1548	1213
15e	*t*Bu	Me	458	575	730

Table 2
Binding affinities for 5-HT_{2A}, 5-HT_{2C} receptor, and serotonin transporter of arylpipierazinyl pyrimidine 4-carboxamide derivatives



16

Compound	R^1	R^3	X	*n*	IC_{50} (nM)		
					5-HT_{2A}	5-HT_{2C}	SERT
16a	*t*Bu	H	Me	2	274	2688	537
16b	*t*Bu	H	Me	1	69	494	6374
16c	SMe	H	Me	2	54	1170	394
16d	SMe	H	Me	1	47	148	1014
16e	SMe	H	Cl	1	55	81	881
16f	*c*Pr	H	Me	2	256	1178	347
16g	Me	OMe	Me	2	744	2049	892
16h	Me	OMe	Me	1	842	1266	2096
16i	Me	OMe	Cl	1	263	135	646

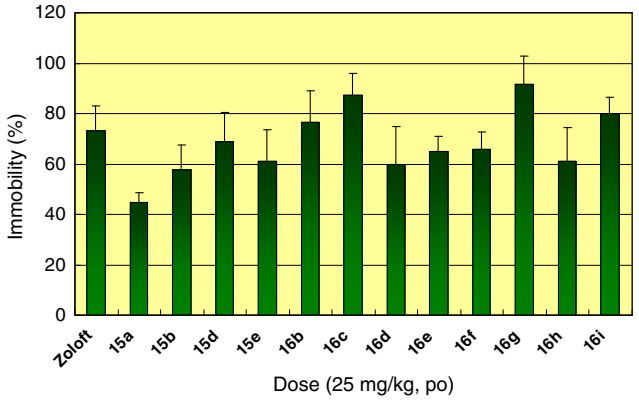


Figure 2. Effects of drugs on immobility in forced swimming test on mice. Drugs (25 mg/kg) were injected orally (po) 60 min before the testing, and total duration of immobility was recorded during the last 5 min of the 6-min testing period. Values are means ± SEM.

Compared with Zoloft, dichlorophenylpiperazine compounds with C3 linker size (**15a**, **15b**) showed more potent in vivo efficacy in animal model. In the case of 2-SMe series (R^1 = SMe), the C2 linker size compounds (**16d**,[19] **16e**) appeared to be more efficacious than C3 linker size compound (**16c**). 5-OMe substituted compounds (R^3 = OMe) also showed a similar trend of results in immobility test.

To distinguish antidepressant effect from hyperactivity of animal, spontaneous locomotor activity tests were conducted in mice. As a result, **15a** compound showed hyperactivity, while **16g** compound showed hypoactivity. Therefore, the efficacy in immobility of **15a** compound was caused by hyperactivity of animal, that is, false positive result. The results of the other compounds fell within the normal range (Fig. 3).

Compounds such as trazodone or nefazodone, are reported to be metabolized into *meta*-chlorophenylpiperazine (*m*-CPP), a serotonin receptor agonist with high affinity to 5-HT_{2A} and even higher to 5-HT_{2C} receptor. This compound has itself anxiogenic-like and hypolocomotor effects. Furthermore, SSRIs through the increase of extracellular 5-HT concentration, activate at least 5-HT_{2C} receptor to induce anxiogenic-like and hypolocomotion effects which could be antagonised by subtype-selective 5-HT_{2C} receptor antagonists.[13] Thus, compound **16d** should be further studied for in vivo metabolism. Off target activity of selected compounds was briefly evaluated. The activity of **16d** in the hERG potassium channel assay was determined to be greater than 10 μM IC_{50} (Table 3). Compound **16d** also showed no appreciable inhibition against CYP1A2, CYP2D6, CYP2C9, and CYP3A4, showing IC_{50} >20 μM.

In summary, we investigated a series of arylpiperazine containing pyrimidine 4-carboxamide derivatives for the treatment of depressive disorders. As an approach to overcome side effects of

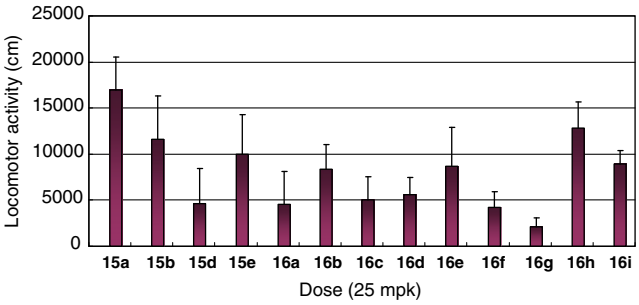


Figure 3. Locomotor activities of the mice treated with compounds were counted for 30 min by activity analyzer. Data were expressed as mean ± SEM of 6–7 mice.

Table 3
hERG channel binding assay

Compound	IC_{50} (μM)
15a	>10
15b	5.4
15e	5.9
16d	>10

known antidepressants and reach unmet needs in the field of antidepressants, novel pyrimidine-based small molecules which would work as 5-HT receptor antagonist and reuptake inhibitor (SARI) were designed and synthesized. Subsequent SAR studies were performed via substitution of pyrimidine, variation of the linker size by different number of carbons, and modification of aryl group. Based on the outcomes of in vitro SAR studies, forced swimming test, and locomotor activities, compound **16d** was identified as a lead compound in this series for this antidepressant program.[20] Its receptor binding affinity and in vivo test results are promising enough to warrant further studies around this pyrimidine scaffold.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.09.081.

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16. For serotonin 5-HT_{2A} receptor binding, an aliquot of frozen membrane from CHO-K1 cell line expressing the human recombinant 5-HT_{2A} receptor (PerkinElmer Life and Analytical Sciences, Boston, USA) and [^{3}H]Ketanserin 1 nM (PerkinElmer) were mixed in the presence of mianserin (20 μM) as nonspecific. The reaction mixture was incubated for 60 min at 27 °C using 50 mM Tris–HCl (pH 7.4) buffer containing 4 mM $CaCl_2$ and 0.1% ascorbic acid, and harvested through Filtermat A glass fiber filter presoaked in 0.5% PEI. The filter was covered with MeltiLex, sealed in a sample bag followed by drying in the microwave oven, and counted by MicroBeta Plus (Wallac, Finland). Competition binding studies were carried out with 5–6 varied concentrations of the test compounds run in duplicate tubes, and isotherms from three assays were calculated by computerized nonlinear regression analysis (GraphPad Prism, GraphPad Software, Inc., CA, USA) to yield IC_{50} values. For 5-HT_{2C} binding, frozen membranes from stable CHO-K1 cell line expressing the human recombinant 5-HT_{2C} receptor (PerkinElmer) were used. [^{3}H]Mesulergine (1.4 nM), receptor membrane and test compound were added into 50 mM Tris–HCl (pH 7.4) buffer containing 4 mM $CaCl_2$ and 0.1% ascorbic acid. Nonspecific binding was determined using 10 μM of methiothepin. The incubations were performed for 60 min at 27 °C, and these were terminated by rapid filtration through Filtermat A glass fiber filter presoaked in 0.5% PEI. Human serotonin transporter expressed in HEK293 (PerkinElmer) were used for serotonin transporter binding assays. For the binding, frozen membrane, 4 nM [^{3}H]Imipramine (PerkinElmer) and appropriate concentrations of test compounds were added to 0.25 mL assay buffer of 50 mM Tris–HCl (pH 7.4) containing 120 mM NaCl and 5 mM KCl. Incubations were carried out for 30 min at 27 °C, and these were terminated by rapid filtration through Filtermat A glass fiber filter presoaked in 0.5% PEI. Imipramine (100 μM) was used as the nonspecific ligand.
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18. The forced swimming test was performed according to the modified methods described by Porsolt et al. (1978).[17] Each mouse was placed in a 25-cm glass cylinder (10 cm diameter) containing 15 cm of water maintained at 23 ± 1 °C, and was forced to swim for 10 min. Later (24 h), the mouse was replaced 360 into the cylinder and the total duration of immobility was recorded during the last 5 min of the 6-min testing period. Mice are judged immobile when they float in an upright position and make only small movements to keep their head above water. Test compound (25 mg/kg) and sertraline (25 mg/kg) were suspended in 3%-Tween 80 solution, and administered (ip) 30 min before the testing.
19. Spectrum data of representative compound; **16d** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (br s, 1H), 7.62 (s, 1H), 7.09 (t, J = 7.6 Hz, 1H), 6.92–6.90 (m, 2H), 3.59 (q, J = 6.0 Hz, 2H), 2.93 (t, J = 4.4 Hz, 4H), 2.70–2.67 (m, 6H), 2.64 (s, 3H), 2.55 (s, 3H), 2.27 (s, 3H), 2.23 (s, 3H). MH^+ 400.
20. We have other supporting data involving in vivo efficacy tests in different animal model (e.g., tail suspension test) at the stage of lead selection. However, functional assay for selected compounds will be performed in the due course.