Bioorganic & Medicinal Chemistry Letters 20 (2010) 1705–1711

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Arylpiperazine-containing pyrrole 3-carboxamide derivatives targeting serotonin 5-HT$_{2A}$, 5-HT$_{2C}$, and the serotonin transporter as a potential antidepressant

Suk Youn Kang [a], Eun-Jung Park [a], Woo-Kyu Park [b], Hyun Jung Kim [a], Daeyoung Jeong [b], Myung Eun Jung [a], Kwang-Seop Song [a], Suk Ho Lee [a], Hee Jeong Seo [a], Min Ju Kim [a], MinWoo Lee [a], Ho-Kyun Han [a], Eun-Jung Son [a], Ae Nim Pae [c], Jeongmin Kim [a], Jinhwa Lee [a,*]

[a] Central Research Laboratories, Green Cross Corporation, 303 Bojeong-Dong, Giheung-Gu, Yongin 446-770, Republic of Korea
[b] Pharmacology Research Center, Korea Research Institute of Chemical Technology (KRICT), 100 Jang-Dong, Yuseong-Gu, Daejeon 305-343, Republic of Korea
[c] Biochemical Research Center, Korea Institute of Science and Technology, 39-1 Hawolgok-Dong, Seongbuk-Gu, Seoul 136-791, Republic of Korea

ARTICLE INFO

Article history:
Received 8 December 2009
Revised 7 January 2010
Accepted 8 January 2010
Available online 21 January 2010

Keywords:
Depression
Antidepressant
Serotonin
Piperazine
Pyrrole

ABSTRACT

Arylpiperzine-containing pyrrole 3-carboxamide derivatives were synthesized and evaluated as novel antidepressant compounds. The various analogues were efficiently prepared and bio-assayed for binding to 5-HT$_{2A}$, 5-HT$_{2C}$ receptor, and 5-HT transporter. Based on their in vitro and in vivo activities as well as selectivity over other neurotransmitter receptors and PK profiles, **33** and **34** were identified as lead compounds. Consequently, this pyrrole series of compounds appears to be promising enough to warrant further investigation.

Depression, especially major depression is among the serious psychiatric disorders.[1,2] Symptoms of depression include sadness, loss of interest or pleasure in activities that were once enjoyed, change in appetite or weight, difficulty sleeping or oversleeping, physical slowing or agitation, energy loss, feeling of worthlessness or inappropriate quilt, difficulty thinking or concentrating, and recurrent thoughts of death or suicide. And depression is estimated to affect 7–8% of the world population.[3] Since the synaptic actions of monoamine neurotransmitters such as norepinephrine (NE) and serotonin (SER, 5-HT) were known as important drug target of psychiatric diseases, an increasing number of treatment options have become more available over the past two decades for individuals with major depression disorder.[4,5] Tricyclic antidepressants (TCAs) and nonselective monoamine oxidase inhibitors (MAOIs) were developed as first generation of antidepressant. Although they are highly effective in treating depression, they also have side effects, for example dry mouth, blurred vision, urinary retention, postural hypotension, and insomnia.[6,7] The first class of psychotropic drugs to be rationally designed, selective serotonin reuptake inhibitors (SSRIs) have been the most widely prescribed antidepressants since 1980s.[8] Although SSRIs such as fluoxetine, sertraline, paroxetine, and citalopram have achieved great success in treating depression, they also have some troublesome effects including sedation, anxiety, headache, tremor, and sexual dysfunction (especially anorgasmia) and generally effective only less than two-third patients. Additionally, delayed onset of action (2–6 weeks) is less than desirable in the treatment of depression.[9]

In recent years, the novel antidepressant model was studied to prepare compounds with dual or multiple activities.[10] Because numerous side effects are associated with non-selective binding at post-synaptic 5-HT receptors, it has been proposed that addition of a 5-HT receptor antagonist component could increase synaptic 5-HT levels, eventually achieve rapid onset time.[11–13] With those approaches, various compounds have been proposed and developed as potential antidepressants, with dual activity at SERT while binding antagonistically to the 5-HT$_{2A}$ receptor. For example, Eli Lilly's LY367265, **1** exhibits excellent binding affinities (K_i = 2.3 nM for SERT; K_i = 0.81 nM for 5-HT$_{2A}$).[14] Yamanouchi has also discovered YM-35992, **2** as an antidepressant with moderate affinities for SERT/5-HT$_{2A}$ (K_i = 21 nM and 86 nM, respectively).[15] Bristol–Myers Squibb's Nefazodone, **3** has been described as having a similar mode of action with an improved side effect profile.[16] This compound had advantages over other antidepressants including reduced possibility to disturbed sleep or sexual dysfunction, and ability to treat some patients who did not respond to other antidepressant drugs.

* Corresponding author. Tel.: +82 31 260 9892; fax: +82 31 260 9020.
E-mail address: jinhwalee@greencross.com (J. Lee).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.01.093

However, Bristol–Myers Squibb discontinued the sale on 2004 with adverse hepatic events including liver failure.[17] More recently, aripiprazole, **4**, which was approved for the treatment of atypical antipsychotics, was also used together with other medications to treat major depressive disorder in adults. Unlike other FDA-approved atypical antipsychotics targeting D_2 receptor, aripiprazole appears a 5-HT_{1A} partial agonist, 5-HT_{2A} antagonist, and 5-HT_{2C} partial agonist. In addition, it has moderate affinity for the serotonin transporter. However, it has numerous side effects including headache, nausea, constipation, anxiety, restlessness, insomnia, nervousness, and so on. In this regard, there are still urgent medical needs on 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 pyrrole 3-carboxamide derivatives targeting serotonin 5-HT_{2A}, 5-HT_{2C}, and the serotonin transporter as a potential antidepressant (see Fig. 1).

From the literature, common structure of antidepressants having dual activities appeared to exhibit combination of two distinct structural motifs. Aryl piperazine elements and heterocyclic motifs were connected to each other using a linker frequently to serve as SERT and 5-HT receptor inhibitors. With this in mind, the general structure of our target compounds is shown in Figure 2. We envisioned that novel target compounds **A** can be readily prepared by typical amide coupling with acid and amine at the final stage. Pyrrole 3-carboxylic acid **B** and arylpiperazinyl alkyl amine **C** can be used as acid and amine partners, respectively.

Preparation of pyrrole derivatives, particularly consisting of 5-phenyl and 3-carboxylic acid, started from alkylation of ethyl acetoacetate (**5**) with 2-bromo-1-phenylethanone (**6**) using sodium hydride to produce alkylated acetoacetate **7** (Scheme 1).[18] Cyclization of **7** to ethyl 2-methyl-5-phenyl-1H-pyrrole-3-carboxylate (**8**) was accomplished by heating ammonium acetate in acetic acid at 80 °C. Various alkyl groups were substituted at NH of pyr-

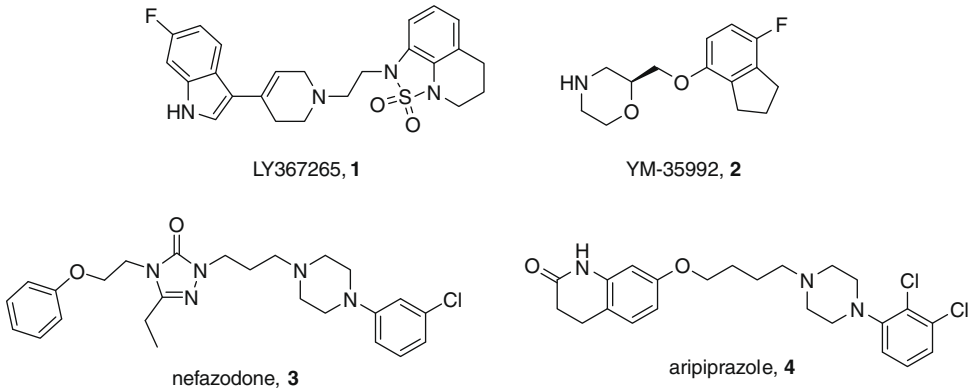


Figure 1. Chemical structures of representative antidepressant compounds.

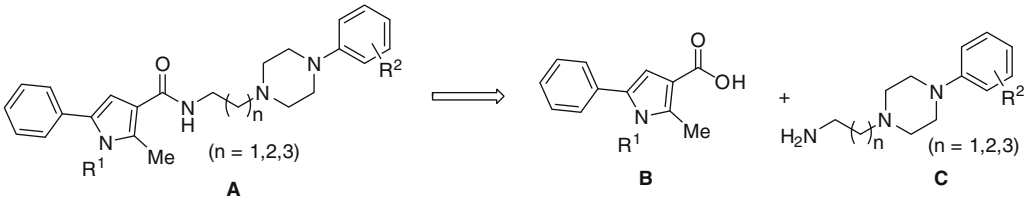
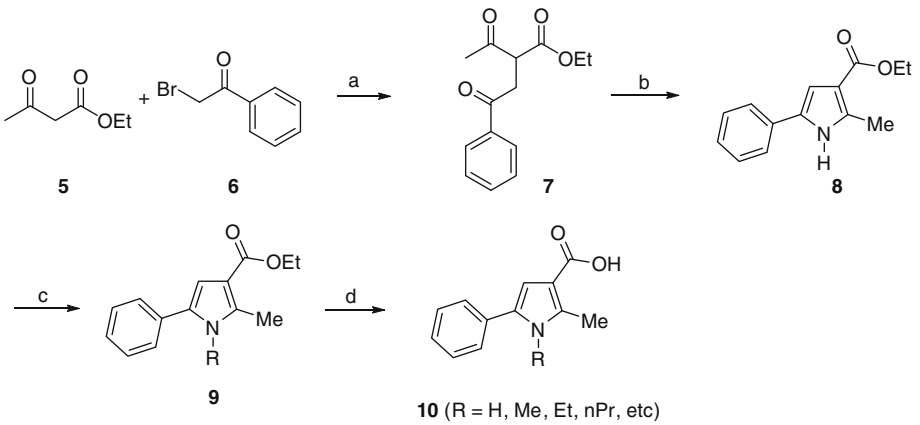


Figure 2. Preparation of target compounds using peptide bond formation.



Scheme 1. Reagents and conditions: (a) NaH, THF; (b) NH_4OAc, AcOH, 80 °C; (c) NaH, RI, DMF; and (d) NaOH, EtOH, reflux.

role under the conditions of sodium hydride and the corresponding alkyl iodides in DMF. Hydrolysis of pyrrole ester **9** using sodium hydroxide in refluxed ethanol afforded the corresponding acids **10**.

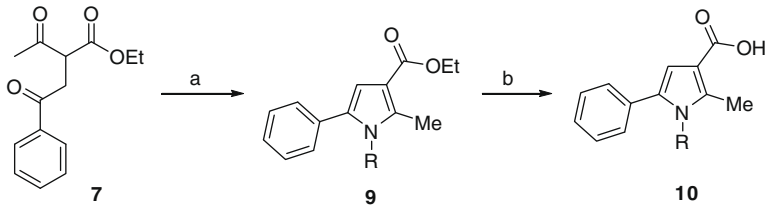
Meanwhile, direct cyclization of acetoacetate **7** to N-substituted pyrrole compounds was also accomplished using various amines instead of ammonium acetate to produce the corresponding pyrrole compounds **9** as shown in Scheme 2.

As shown in Scheme 3, preparation of aminoalkyl-arylpiperazine **14** started from the corresponding bromoalkyl-phthalimide **11** and arylpiperazine **12**.[19] Treatment of bromoalkyl-phthalimide **11** with arylpiperazine hydrochloride **12** in the presence of potassium carbonate in DMF at room temperature afforded N-protected amine **13**. Compound **13** was then treated with hydrazine in ethanol at 80 °C to give amine **14**. For the sake of convenience of handling on scale, liquid amine **14** was transformed to hydrochloride salt form **14a** with 4 N HCl in dioxane.

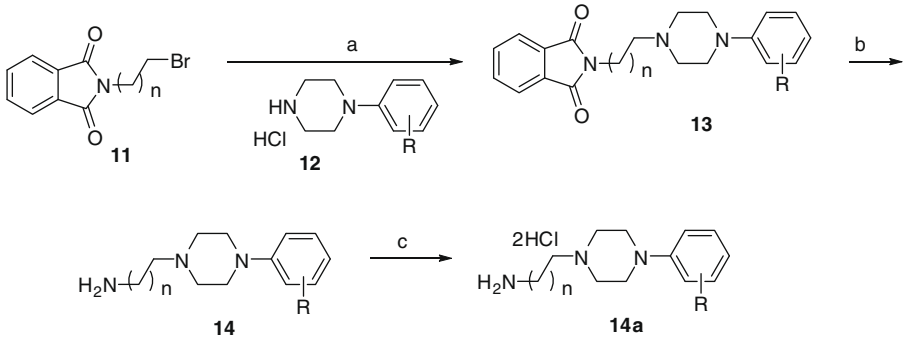
Finally, coupling reaction of acid **10a** and aminoalkyl-arylpiperazine **14a** was conducted as shown in Scheme 4. With 2-methyl-5-phenyl-1H-pyrrole-3-carboxylic acid (**10a**) and 3-(4-(3-chlorophenyl)piperazin-1-yl)propan-1-amine dihydrochloride (**14a**) in hand, typical amide coupling was conducted under conditions involving EDCI, HOBT, and NMM in methylene chloride to produce amide **15**. As reaction was completed, purification was performed using preparative reverse-phase HPLC with acetonitrile and water containing 0.2% TFA or not. The neutral form of product **15** was converted to HCl salt form **15a** 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, was evaluated by displacement binding using [^{3}H]Ketanserin, [^{3}H]Mesulergine, and [^{3}H]Imipramine, respectively, as radioligands.[20,23]

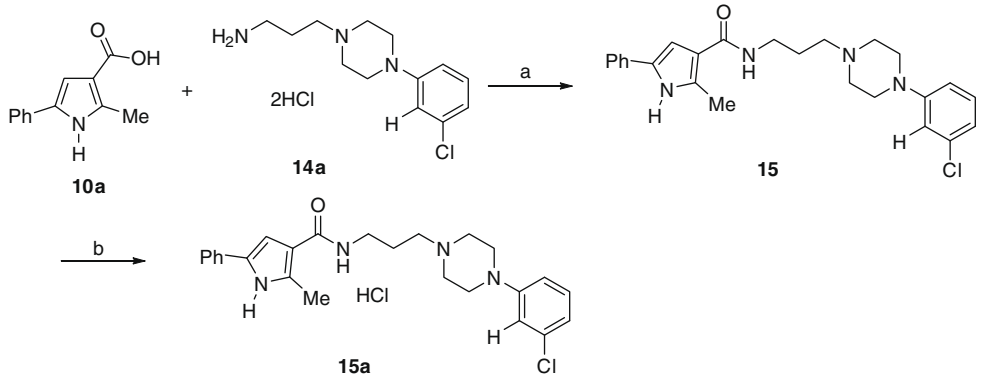
The initial work was focused on exploration of the central pyrrole moiety and substitution of arylpiperazine group (Table 1). N-Alkylated pyrrole derivatives and 3-chlorophenyl, 2,3-dimethylphenyl, and 2,3-dichlorophenyl piperazine were connected with propyl-carboxamide as a linker. The binding affinities of prepared compounds against the 5-HT_{2A}, 5-HT_{2C}, and SERT are shown in



Scheme 2. Reagents and conditions: (a) RNH_2, *p*-TsOH, EtOH, 80 °C and (b) NaOH, EtOH, reflux.

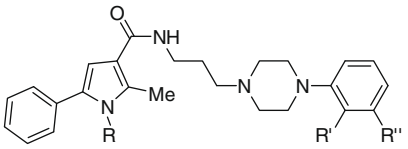


Scheme 3. Reagents and conditions: (a) **12**, K_2CO_3, DMF, rt; (b) $NH_2NH_2{\cdot}H_2O$, EtOH, rt; and (c) 4 N HCl in dioxane.



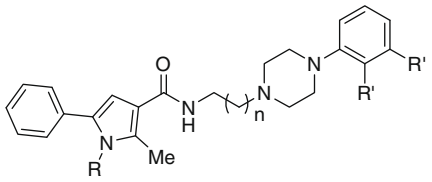
Scheme 4. Reagents and conditions: (a) EDCI, HOBT, NMM, CH_2Cl_2 or DMF and (b) HCl, MeOH, 0 °C.

Table 1
Competition binding assays at 5-HT_{2A} and 5-HT_{2C} receptor and the serotonin transporter to arylpipierazinyl pyrrole 3-carboxamide derivatives (IC_{50} values, unit:nM)



R	R′, R″											
	H, Cl				Me, Me				Cl, Cl			
	No.	5-HT_{2A}	5-HT_{2C}	SERT	No.	5-HT_{2A}	5-HT_{2C}	SERT	No.	5-HT_{2A}	5-HT_{2C}	SERT
H	**15**	245	280	92	**24**	300	615	77	**33**	300	599	137
Me	**16**	92	160	11.1	**25**	57	133	11.8	**34**	46.2	12	61.7
Et	**17**	92	192	25.8	**26**	75	139	35.9	**35**	376	66	182
*n*Pr	**18**	191	273	93	**27**	129	225	76	**36**	324	628	420
*i*Bu	**19**	333	1602	110	**28**	897	1298	350	**37**	406	342	381
N-Piperidinyl	**20**	441	448	110	**29**	133	417	92	**38**	67.1	795	298
4-Fluorophenyl	**21**	22.9	443	80.2	**30**	6.9	162	53.6	**39**	4.1	466	266
Benzyl	**22**	179	1436	235	**31**	224	297	14.3	**40**	354	220	125
Cyclohexylmethyl	**23**	1067	611	213	**32**	1233	638	352	**41**	4383	1034	412

Table 2
Binding affinities for 5-HT_{2A}, 5-HT_{2C} receptor, and serotonin transporter of arylpipierazinyl pyrrole 3-carboxamide derivatives (IC_{50} values, unit:nM)



R′ = Cl	*n* = 1				*n* = 2				*n* = 3			
R	No.	5-HT_{2A}	5-HT_{2C}	SERT	No.	5-HT_{2A}	5-HT_{2C}	SERT	No.	5-HT_{2A}	5-HT_{2C}	SERT
H	**42**	40	37	87	**33**	300	599	137				
Me	**43**	175	156	57	**34**	46.2	12	61.7	**45**	28	32	93
*n*Pr	**44**	408	310	32	**36**	324	628	420	**46**	104	20.9	76

R′ = Me	*n* = 2				*n* = 3			
R	No.	5-HT_{2A}	5-HT_{2C}	SERT	No.	5-HT_{2A}	5-HT_{2C}	SERT
H	**24**	300	615	77	**47**	10	63	21
Me	**25**	57	13	11.8	**48**	61	61	149
*n*Pr	**27**	129	225	76	**49**	792	149	76

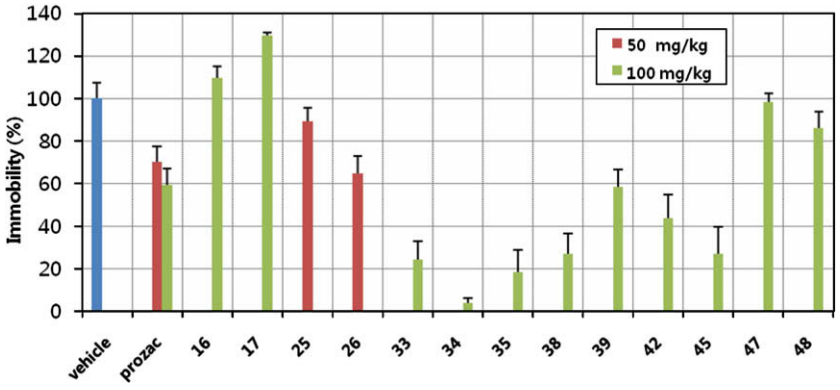


Figure 3. Effects of drugs on immobility in forced swimming test on mice. Drugs (100 and 50 mg/kg) were injected orally (po) 60 min before the testing, and total duration of immobility was recorded during the last 5 min of the 6-min testing period. Values are means ± S.E.M.

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. Thus, it is more reasonable to compare not only binding affinities against specific receptors, but also overall binding tendency of our compounds against the 5-HT_{2A}, 5-HT_{2C}, and SERT. Initially, most compounds tested displayed IC_{50} <1 μM, implying that this series of arylpiperazinyl pyrrole 3-carboxamide might hold prom-

ises as a potential antidepressant. Although 1H-pyrrole-3-carboxamides (**15**, **24**, and **33**) have moderate in vitro activities, substitution of NH in pyrrole with small alkyl such as methyl (**16**, **25**, and **34**), ethyl (**17**, **26**, and **35**), and *n*-propyl (**18**, **27**, and **36**) increased binding affinities against 5-HT_{2A}, 5-HT_{2C}, and SERT. But the binding affinities were reduced when bulkier alkyl groups (*i*-butyl **19**, **28**, **37**; cyclohexylmethyl **23**, **32**, **41**; benzyl **22**, **31**, **40**) were installed at the NH of pyrrole. Similar phenomenon was observed if a heteroatom was included as in *N*-piperidinyl **20**, **29**, **38**. Particularly, 4-fluorophenyl group responded selectively to 5-HT_{2A} receptor (**21**, **30**, and **39**). Also, it showed little difference in binding affinity between 3-chlorophenylpiperazinyl compounds and 2,3-dimethylphenyl piperzinyl compounds. Therefore, it is safe to say that such SAR could be observed to only short alkylated (*N*-Me, *N*-Et, and *N*-*n*-propyl) pyrrole compounds.

In order to improve binding affinity against 5-HT_{2A} and 5-HT_{2C} receptors and the serotonin transporter, varying linker size around key compounds was undertaken. Initially, dichlorophenylpiperazinyl derivatives were synthesized and screened. In the case of unsubstituted pyrrole **33**, overall binding affinity was increased when the length of linker shorten from C3 to C2 (**33** vs **42**). On the contrary, methylated pyrrole displayed increased binding affinity when linker was elongated (**34**, **43**). In case of substitution of NH in pyrrole with *n*-propyl, C2- and C3-linker compounds made no clear difference, but increased binding affinity as linker was extended from C3 to C4 (**36**, **44**, and **46**). In the case of dimethylphenylpiperazinyl group, binding affinity enhancement was observed for unsubstituted pyrroles (**24** vs **47**). However, overall binding affinity was decreased when NH in pyrrole was substituted with alkyl group. (**25** vs **48** and **27** vs **49**) (see Table 2).

To evaluate antidepressant activity of the interesting compounds, immobility in forced swimming test (FST) on mice was measured.[21,24] Prozac (fluoxetine) was used as a reference compound for comparison. The results are shown in Figure 3. At 100 mg/kg, 3-chlorophenylpiperazine analogues **16**, **17** show virtually no appreciable immobility effect. Compared with fluoxetine, dimethylphenylpiperazine compounds **25**, **26** display similar or a little off activity at 50 mg/kg, and *N*-ethyl pyrrole **26** shows more effective than *N*-methyl congener **25**. Improved results are consistently obtained with dichlorophenylpiperazine moieties. Substitution of NH in pyrrole with small alkyl groups such as methyl **34** or ethyl **35** shows extremely potent in vivo efficacy on animal model (less than 25% at 100 mg/kg). *N*-Methylpyrrole **34** is sixfold more active than NH pyrrole **33**, and fivefold more active than *N*-ethylpyrrole compound **35**. Its in vivo structure–activity relationship appears to go well with in vitro assay results. *N*-Piperidinylpyrrole **38** displays a similar level of efficacy to NH pyrrole counterpart **33**.

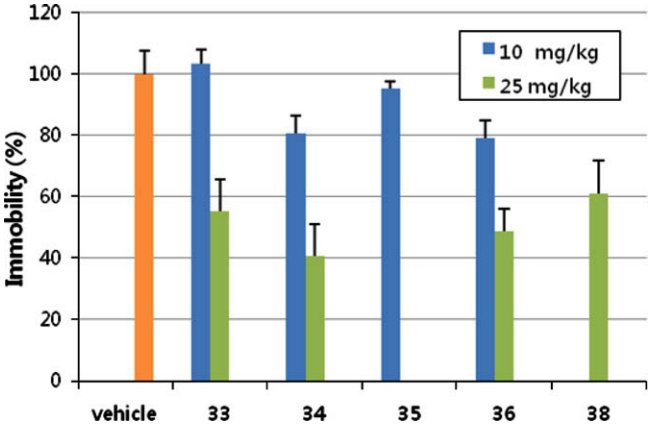


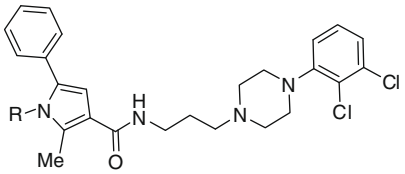
Figure 4. Effects of drugs on immobility in forced swimming test on mice. Drugs (25 and 10 mg/kg) were injected orally (po) 60 min before the testing, and total duration of immobility was recorded during the last 5 min of the 6-min testing period. Values are means ± S.E.M.

Although 4-fluorophenylpyrrole derivative **39** shows good inhibitory activity against 5-HT_{2A} receptor (IC_{50} = 4.1 nM), it displays only modest efficacy (58.3 ± 8.4%). From the viewpoint of linker size, propyl (C3-linker) shows better efficacy than C2 (**42** vs **33**) and C4 (**34** vs **45**). In the case of dimethylphenylpiperazine analogues, C4-linker appears to be more efficacious than C3-linker compounds (**16**, **17** vs **47**, **48**). However, all these compounds display relatively low immobility effects.

Dose-dependent immobility test was also conducted. At 25 mg/kg, compounds **33**, **34**, and **36** still showed good efficacy (immobility <50%). Compound **34** proved to be most potent in vivo efficacy (immobility = 40.7 ± 10.4%). When oral dose reduced from 25 mg/kg to 10 mg/kg, all the compounds tested showed insufficient antidepressant activity. Either **34** or **36** demonstrated merely modest in vivo efficacy (immobility ~80%)[25] (see Fig. 4).

Interesting compounds were further evaluated with the receptor selectivity studies. The results were shown in Table 3. It was demonstrated that binding affinities of these compounds against 5-HT_{2A}, 5-HT_{2C} and SERT were stronger than binding affinities against any other neurotransmitter receptors except 5-HT_{1A}. It is well-known that inhibition of 5-HT_{1A} receptor affects depressive disorder. Inhibition of dopamine receptor was only marginal. Thus, **34** not only shows favorable binding affinities against 5-HT_{2A}, 5-HT_{2C} receptors, and the serotonin transporter, but also provides good selectivity profiles over other neurotransmitter receptors.

Table 3
Profiles of interesting compounds via competition binding assay at 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_6, 5-HT_7, SERT, D_2, D_3 and D_4. (*D*; Dopamine) (IC_{50} values, unit:nM)



Compound	R	Unit: nM Binding affinity								
		5-HT_{1A}	5-HT_{2A}	5-HT_{2c}	5-HT_6	5-HT_7	SERT	D_2	D_3	D_4
33	H	117	300	599	1412	456	133	1071	334	1845
34	Me	54	46	12	713	333	62	2542	915	984
36	*n*Pr	94	324	628	1904	408	420	3736	1072	645
38	*N*-Piperidinyl	–	67.1	795	–	493	298	–	–	–
39	4-Fluorophenyl	–	6.9	599	–	524	54	–	–	–

Table 4
Pharmacokinetic parameters and brain/plasma ratio of compounds **33** and **34** after single oral (5 mg/kg) and i.v. (1 mg/kg) administration to rat

Compound	**33**[a]				**34**[a]			
PK parameters	i.v. (1 mg/kg $n = 2$)	Oral (5 mg/kg, $n = 3$)	Plasma	Brain	i.v. (1 mg/kg $n = 3$)	Oral (5 mg/kg, $n = 3$)	Plasma	Brain
C_{max} (μg/mL)	0.690	0.245	0.19	0.09	0.454	0.118	0.07	0.03
C_{last} (μg/mL)	0.004	0.001	0.10	0.06	0.008	0.001	0.04	0.02
T_{max} (h)	—	1.167	2.00	2.00	—	0.750	1.00	2.00
$t_{1/2}$ (h)	1.756	2.521	1.97	3.42	2.822	3.137	3.71	4.28
AUC_{irrf} (min μg/mL)	20.945	3.868			18.703	23.150		
AUC_{irlf}/Dose	20.945	12.783			18.703	4.630		
AUC_{all} (min μg/mL)			32.25	14.84			14.38	4.96
BA (%)	61.03				24.76			
Brain/plasma (%)	46.01				34.47			

[a] HCl salt compounds were used.

With promising in vitro and in vivo results in hand, pharmacokinetic properties of selected compounds **33**, **34** were evaluated, and these results are summarized in Table 4.[22] After oral administration of a 5 mg/kg dose of **33** to rats, a C_{max} of 0.245 μg/mL was obtained at 1.167 h. The elimination half-life for **33** following oral administration was 2.521 h in rats. Compound **33** showed relatively good oral bioavailability ($F = 61.03\%$) in rats. Meanwhile, after oral administration of a 5 mg/kg dose of **34** to rats, a C_{max} of 0.118 μg/mL was obtained at 0.750 h. The elimination half-life for **34** following oral administration was 3.137 h in rats. Compound **34** showed moderate oral bioavailability ($F = 24.76\%$) in rats. Compound **33** has slightly superior brain/plasma ratio (46.01%) to that of compound **34** (34.47%). For many companies, B/P (%) >30 is used as a minimum guideline for CNS discovery projects.[26] From these results, both compounds **33** and **34** showed good PK profile and B/P ratio. Compound **34** was further evaluated for effects of CYP inhibition. Compound **34** showed no appreciable inhibition against CYP1A2, CYP2D6, and CYP3A4, showing $IC_{50} > 20$ μM, while **34** is a moderate inhibitor against CYP2C9 ($IC_{50} = 9.7$ μM).[27]

In summary, we investigated a series of arylpiperazine containing pyrrole 3-carboxamide derivatives for the treatment of depressive disorders. As an approach to overcome side effects of known antidepressants and reach unmet needs in the field of antidepressants, novel pyrrole-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 NH in pyrrole, variation of the linker size by different number of carbons, and modification of substituents on aryl group. Based on the outcomes of in vitro SAR studies and forced swimming test, **34** was identified as a lead compound for this antidepressant program. Its receptor selectivity and PK properties were promising enough to warrant further studies around this pyrrole scaffold.

Acknowledgments

This Letter is dedicated to the memory of Mr. Young-Sup Huh, Chairman, CEO, Green Cross Corporation (GCC). We appreciate Dr. Chong-Hwan Chang for his leadership and dedication during his tenure of office as Head of GCC R&D. Also we are grateful to Dr. Eun Chul Huh for his leadership and supports during his tenure as Head of GCC R&D Planning and Coordination. This work was in part supported by Korea Ministry of Knowledge Economy.

References and notes

1. Greenberg, D. E.; Kessler, R. C.; Birnbaum, H. G.; Leong, S. A.; Lowe, S. W.; Berglund, P. A.; Corey-Lisle, P. K. *J. Clin. Psychiatry* **2003**, *64*, 1465.
2. *The Global Burden of Disease; A Comprehensive Assessment of Mortality and Disability from Disease, Injuries and Risk Factors in 1990 and Projected to 2020*; Murray, C. J. L., Lopez, A. D., Eds.; Harvard University Press: Cambridge, MA, 1996; p 38.
3. WHO *The World Health Report 2001, Mental Health: New Understanding, New Hope*; Fact Sheet No. 265.
4. *5-Hydroxyltryptamine Mechanisms in Primary Headaches*; Olesen, J., Saxena, P., Eds., 1st ed.; Raven Press: New York, 1992.
5. Rang, H.; Dale, M.; Ritter; Pharmacol, J., 5th ed.; Churchhill Livingstone: Edinburgh, 2003.
6. Blackwell, B. *Drugs* **1981**, *29*, 201.
7. Evrard, D. A.; Harrison, B. L. *Annu. Rep. Med. Chem.* **1999**, *34*, 1.
8. Blier, P.; Bergeron, R. *J. Clin. Psychiatry* **1998**, *59*, 16.
9. Katz, M. M.; Tekell, J. L.; Bowden, C. L.; Brannan, S.; Houston, J. P.; Berman, N.; Frazer, A. *Neuropsychophamacology* **2004**, *29*, 566. and references cited therein.
10. (a) Morphy, R.; Kay, C.; Rankovic, Z. *Drug Discovery Today* **2004**, *9*, 641. and reference cited therein; (b) Pacher, P.; Kecskemeti, V. *Curr. Med. Chem.* **2004**, *11*, 925.
11. Ballesteros, J.; Callado, L. F. *J. Affect. Disord.* **2004**, *79*, 137. and reference cited therein.
12. Romeo, L.; Arigas, F. *J. Neurochem.* **1997**, *68*, 2593.
13. Berman, R. M.; Anand, A.; Cappiello, A.; Miller, H. L.; Xu, X. S.; Oren, D. A.; Charney, D. S. *Biol. Psychiatry* **1999**, *45*, 1170.
14. Pullar, I. A.; Carney, S. L.; Colvin, E. M.; Lucaites, V. L.; Nelson, D. L.; Wedley, S. *Eur. J. Pharm.* **2000**, *407*, 39.
15. Hatanaka, K.; Nomura, T.; Hidaka, K.; Takeuchi, H.; Yatsugi, S.; Fujii, M.; Yamaguchi, T. *Neuropharmacology* **1997**, *35*, 1621.
16. (a) Rush, A. J.; Armitage, R.; Gillin, J. C.; Yonkers, K. A.; Winokur, A.; Moldofsky, H.; Vogel, G. W.; Kaplita, S. B.; Fleming, J. B.; Montplaisir, J.; Erman, M. K.; Albala, B. J.; McQuade, R. D. *Biol. Psychiatry* **1998**, *44*, 3; (b) Avila, A.; Vardona, X.; Martin-Baranera, M.; Maho, P.; Sastre, F.; Bello, J. *J. Clin. Psychopharmacol.* **2003**, *23*, 509.
17. (a) Greene, D.; Barbhaiya, R. H. *Clin. Pharmacokinet.* **1997**, *33*, 260; (b) Kent, J. M. *Lancet* **2000**, *355*, 911.
18. Vanotti, E; D'alessio, R.; Tibolla, M.; Varasi, M.; Montagnoli, A.; Santocanale, C.; Martina, K.; Menichincheri, M. WO2005/013986, **2005**.
19. Robarge, M. J.; Husbans, S. M.; Kieltyka, A.; Brodbeck, R.; Thurkauf, An.; Newman, A. H. *J. Med. Chem.* **2001**, *44*, 3175.
20. Park, W.-K.; Jeong, D.; Cho, H.; Lee, S. J.; Cha, M. Y.; Pae, A. N.; Choi, K. I.; Koh, H. Y.; Kong, J. Y. *Pharmacol., Biochem. Behav.* **2005**, *82*, 361.
21. Porsolt, R. D.; Bertin, A.; Jalfre, M. *Eur. J. Pharmacol.* **1978**, *51*, 291.
22. Spectrum Data of representative compounds; **33** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (br s, 1H), 7.53 (m, 1H), 7.39 (d, $J = 7.6$ Hz, 2H), 7.21–7.13 (m, 2H), 6.98 (t, $J = 8.0$ Hz, 1H), 6.81 (d, $J = 8.0$ Hz, 1H), 6.62 (d, $J = 2.4$ Hz, 1H), 3.57–3.53 (m, 2H), 3.13 (br s, 4H), 2.71 (br s, 5H), 2.63 (s, 3H), 1.84–1.79 (m, 2H). MH^+ 471. Compound **34** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (br s, 1H), 7.34–7.30 (m, 4H), 7.13 (dd, $J = 8.0$, 1.6 Hz, 1H), 7.00 (t, $J = 8.4$ Hz, 1H), 6.66 (dd, $J = 8.0$, 1.2 Hz, 1H), 6.36 (s, 1H), 3.53 (dd, $J = 11.6$, 5.6 Hz, 2H), 3.49 (s, 3H), 3.06 (br s, 4H), 2.68 (m, 5H), 2.65 (s, 3H), 2.61 (t, $J = 5.6$ Hz, 2H), 1.83–1.77 (m, 2H). MH^+ 485.
23. 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) was 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.

24. The forced swimming test was performed according to the methods described by Porsolt et al. (1978).[21] 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. Twenty-four hours later, the mouse was replaced 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 (10, 25, 50, and 100 mg/kg) and fluoxetine (50 and 100 mg/kg) were suspended in 3%-Tween 80 solution, and administered (i.p.) 30 min before the testing.
25. Considering that **36** shows only moderate binding affinities against 5-HT_{2A}, 5-HT_{2C} and SERT, **34** is regarded as a lead compound for our antidepressant program.
26. Kerns, E. H.; Di, D. *Drug-like Properties: Concepts, Structure Design and Methods: from ADME to Toxicity Optimization*; Academic Press: Burlington, MA, 2008.
27. For comparison, fluoxetine showed relatively strong inhibition against CYP2C9, CYP2D6, and CYP3A4, demonstrating IC_{50} = 18.4 μM, IC_{50} = 0.74 μM, IC_{50} = 5.8 μM, respectively in in-house assay, whereas it turned out to be a weak inhibitor against CYP1A2 (IC_{50} >20 μM) in this experiment.