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PII: S0960-894X(15)00511-9
DOI: http://dx.doi.org/10.1016/j.bmcl.2015.05.049
Reference: BMCL 22739

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 15 January 2015
Revised Date: 29 April 2015
Accepted Date: 15 May 2015

Please cite this article as: Suzuki, S., Okano, T., Horiuchi, R., Hareyama, N., Amikura, K., Yamamoto, N., Yoshizawa, Y., Yagi, M., Serizawa, K., Hayashi, R., Discovery of potent α_{1L}-adrenoceptor agonists: Design and synthesis of bicyclic derivatives, *Bioorganic & Medicinal Chemistry Letters* (2015), doi: http://dx.doi.org/10.1016/j.bmcl.2015.05.049

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Abstract

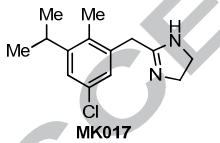
We aimed to create a novel and potent α_{1L}-adrenoceptor agonist because such agonists are possible drug candidates for stress urinary incontinence. We used ligand-based drug design and evaluated the α_{1L}-adrenoceptor agonist activity of the designed compounds. Among them, tetrahydroquinoline derivative **50** showed the most potent activity (ratio of noradrenaline half maximal effective concentration, 0.0028) and effectively induced contraction of rat bladder neck.

Keywords: Drug discovery, Adrenoceptor agonist, Stress urinary incontinence.

Abbreviations: CHO, Chinese hamster ovary; EC_{50}, half maximal effective concentration; DCE, 1,2-dichloroethane; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; NA, noradrenaline; NBS, *N*-bromosuccinimide; PMB, 4-methoxybenzyl ether; SUI, stress urinary incontinence; TosMIC, toluenesulfonylmethyl isocyanide; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TMS, trimethylsilyl.

α_1-Adrenoceptors are members of the 7TM superfamily of G protein-coupled receptors and are classified as α_{1H}-adrenoceptors and α_{1L}-adrenoceptors. α_{1H}-Adrenoceptors have high affinity for prazosin, which is a specific α_1-adrenoceptor antagonist[1], and they are classified into three subtypes (α_{1A}, α_{1B}, and α_{1D}). In contrast, α_{1L}-adrenoceptors, which result from expression of the α_{1a}-adrenoceptor gene[2], have low affinity for prazosin. Putative α_{1L}-adrenoceptors had been discussed for several years and recent research results have confirmed the existence of α_{1L}-adrenoceptor subtypes. Muramatsu and co-workers have reported an α_{1L}-adrenoceptor-expressing cell line[3]. The α_{1L}-adrenoceptor mediate contraction of the rabbit prostate [4], the mouse prostate[5, 6], the rat urethra[7], the human bladder[7] and prostate[8], and other mammalian urogenital structures. Accordingly, we hypothesized that strong α_{1L}-adrenoceptor agonists may be useful drugs for stress urinary incontinence (SUI). SUI is a common health problem caused by injury to the tissue of the bladder neck or pelvic floor muscle by childbirth or obesity. SUI significantly reduces quality of life.

Although the development of α_{1L}-adrenoceptor agonists would be useful for SUI drugs, little research on these agonists has been conducted. MK017 is one of the few known α_{1L}-adrenoceptor agonists (Fig. 1) [9]. Against this background, we commenced research aimed at finding stronger α_{1L}-adrenoceptor agonists than existing agonists for SUI drugs.



MK017

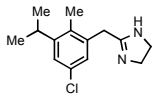
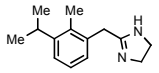
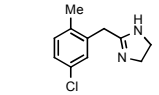
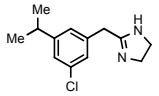
Figure 1. The structure of MK017. This compound is a known α_{1L}-adrenoceptor agonist.

To begin, we took MK017 as a starting point for ligand-based drug design because there is little ligand information available. We evaluated substituent effects for MK017 and discovered the 3-isopropyl substituent is essential for α_{1L}-adrenoceptor agonist activity (Table 1). Agonist activity is indicated as the ratio of noradrenaline (NA) half maximal effective concentration (EC_{50}) in Chinese hamster ovary (CHO) cells stably expressing human α_{1L}-adrenoceptor[3, 10, 11]. We subsequently

hypothesized that conformational restricted compounds in which the 2′ carbon was connected to the 1′, 3′, or 3″ carbon via a C1 or C2 unit, while retaining the isopropyl unit, would achieve high α_{1L}-adrenoceptor agonist activity. Accordingly, we designed the indane and tetraline frameworks shown in Fig. 2.

Table 1 In vitro α_{1L}-adrenoceptor agonist activity of MK017 derivatives.

Table 1
In vitro α_{1L}-adorenoceptor agonist activity of MK017 derivatives

	Compound	Ratio of NA EC_{50}
MK017		0.043
1		ND (36%)**
2		ND (0%)**
3		0.52

* NA EC_{50} = 1019 ± 247 nM
** ND : Not Determined
E_{max} responce to NA is taken as 100%

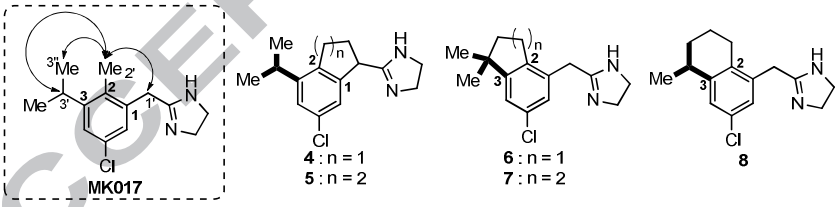
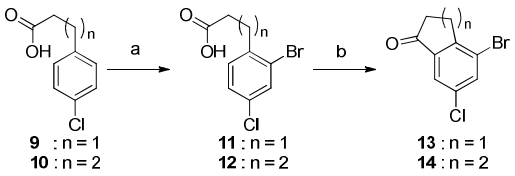


Figure 2. Designed compounds. Various indane and tetraline derivatives were designed to serve as α_{1L}-adrenoceptor agonists.

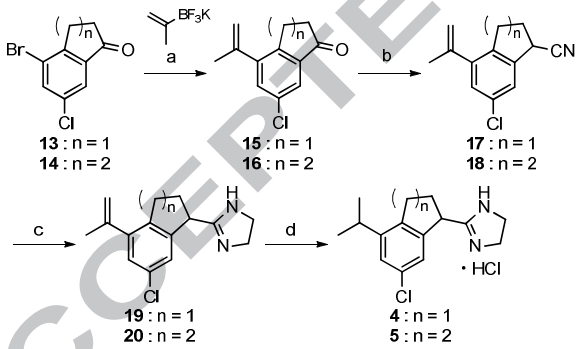
The key intermediates in our synthetic plan were bromoindanone **13** and bromotetralone **14**. These were chosen because the bromide can be converted to isopropenyl or methylene nitrile by Suzuki-Miyaura cross-coupling using isopropenyl borane reagent or isoxazole borane reagent followed by hydroxylation, respectively. Furthermore, the carbonyl oxygen can be converted to a

geminal dimethyl group or nitrile group by treatment with dimethylzinc or toluenesulfonylmethyl isocyanide (TosMIC), respectively. To prepare the key intermediates, we started from the corresponding carboxylic acids (**9**, **10**). Bromination and subsequent cyclization resulted in formation of indanone and tetralone (Scheme 1).



Scheme 1

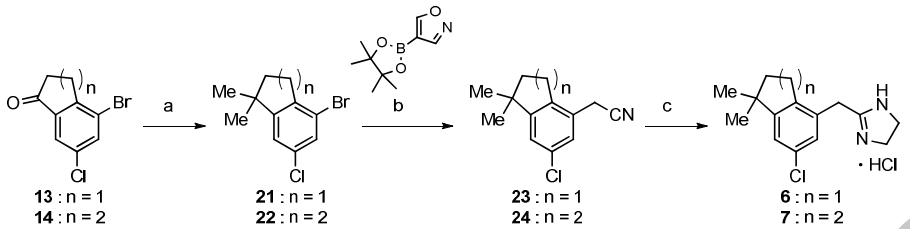
Compounds **4** and **5** were synthesized according to Scheme 2. The isopropenyl substituent was installed by Suzuki-Miyaura cross-coupling. The carbonyl oxygen was converted to a nitrile with TosMIC. Cyclization with ethylenediamine at the nitrile afforded imidazoline products **19** and **20**. Finally, reduction of the olefin could be accomplished under hydrogen (1 atm) using PtO_2, leading to the desired compounds.



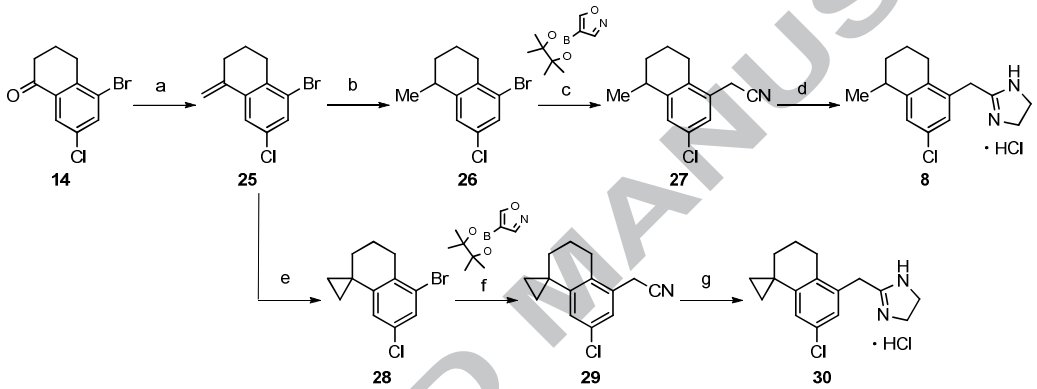
Scheme 2

Compounds **6** and **7** were synthesized according to Scheme 3. Dimethylation with dimethylzinc afforded **21** and **22**. Suzuki-Miyaura cross-coupling with isoxazole borane reagent, and subsequent hydroxylation gave the methylene nitrile derivatives **23** and **24**[12]. Because isoxazole intermediate remained in this step, yield was low. Finally, cyclization with ethylenediamine afforded the desired compounds. Additionally, monomethyl- and spirocyclopropyl-substituted derivatives (**8** and **30**) were prepared from **14**. The monomethyl substituent was installed by Wittig reaction and reduction.

The cyclopropyl substituent was installed by Wittig reaction and Simmons-Smith reaction (Scheme 4).



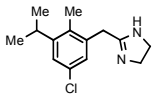
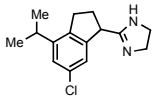
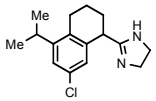
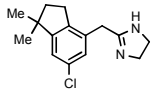
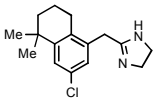
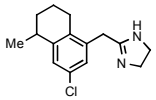
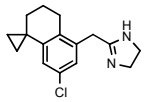
Scheme 3



Scheme 4

The results of α_{1L}-adrenoceptor agonist activity are shown in Table 2. Whereas indane derivatives tended to have lower agonist activity, tetraline derivatives tended to have higher agonist activity (**4** vs. **5**, **6** vs. **7**). Furthermore, a substituent at the 3-position had a marked effect on agonist activity (**7**, **30** vs. **8**). At this position, we presume that hydrophobic interaction and steric bulk played important roles in α_{1L}-adrenoceptor agonist activity.

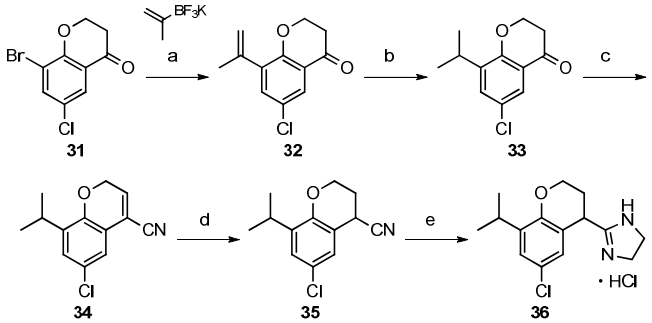
Table 2 In vitro α_{1L}-adrenoceptor agonist activity of indane and tetraline derivatives.

	Compound	Ratio of NA EC_{50}
MK017		0.043
4		0.08
5		0.024
6		0.12
7		0.028
8		0.063
30		0.025

* NA EC_{50} = 1019 ± 247 nM

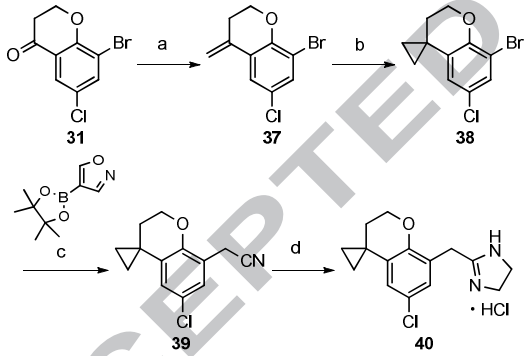
Aiming to further improve the α_{1L}-adrenoceptor agonist activity, we introduced a heteroatom at the 2-position of the benzene ring in tetralines **5** and **30**, while retaining the same structure conformation. We expected that the effect of hydrogen bonding and strong π-π stacking interaction with the α_{1L}-adrenoceptor protein could be altered by changing the electron density of the benzene ring.

Chromane derivatives were synthesized by the same method as in the benzene derivative syntheses. The isopropenyl substituent was installed by Suzuki-Miyaura cross-coupling from bromide **31**. Olefin reduction afforded the isopropyl substituent. The carbonyl oxygen was converted to a nitrile by treatment with trimethylsilyl cyanide (TMS-CN) and olefin reduction. Finally, cyclization with ethylenediamine at the nitrile afforded imidazoline **36** (Scheme 5).



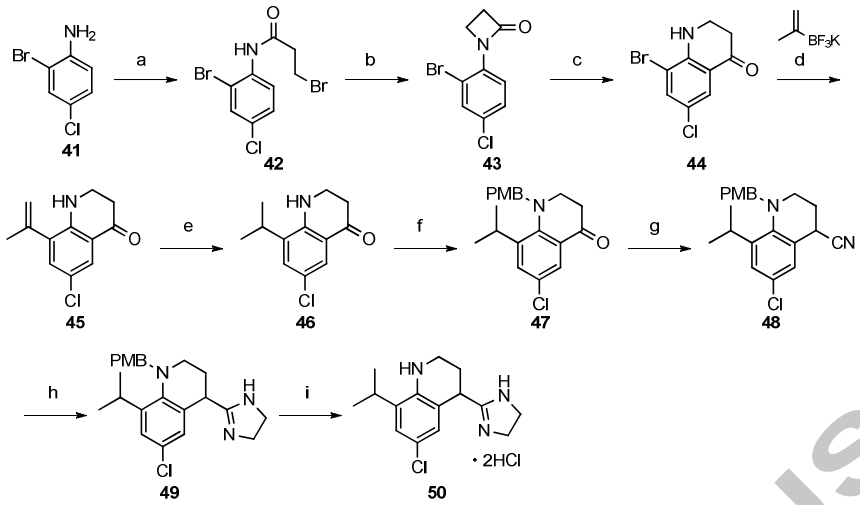
Scheme 5

Derivatives with a cyclopropyl group were synthesized by the method used for **30**. An exo olefin was installed by Wittig reaction and converted to a cyclopropyl group by Simmons-Smith reaction using trifluoroacetic acid (TFA), diethylzinc, and diiodomethane. Then, Suzuki-Miyaura cross-coupling with isoxazole borane reagent at the bromide followed by hydroxylation gave the methylene nitrile derivative **39**. Finally, cyclization with ethylenediamine at nitrile afforded target product **40** (Scheme 6).



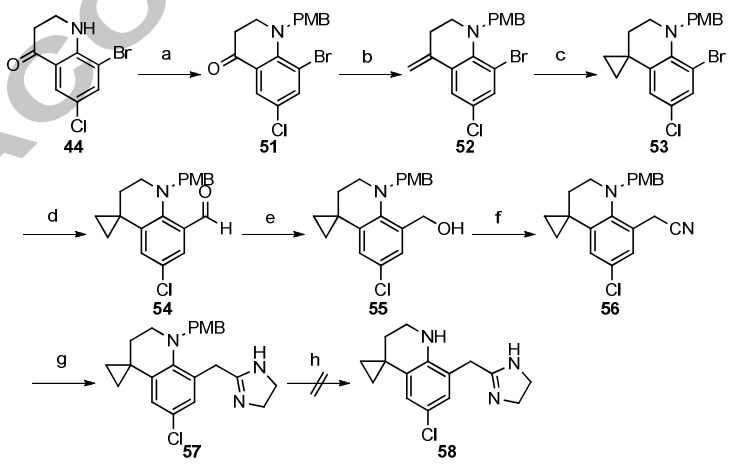
Scheme 6

To prepare tetrahydroquinoline derivatives, first we constructed the tetrahydroquinolinone framework by a general method from corresponding aniline **41**[13]. After that, the isopropenyl substituent was installed by Suzuki-Miyaura cross-coupling from the bromide. Olefin reduction afforded the isopropyl substituent. The carbonyl oxygen was converted to a nitrile with TosMIC after protecting the aniline nitrogen with a 4-methoxybenzyl ether (PMB) group. Cyclization with ethylenediamine at the nitrile afforded imidazoline **49** and deprotection with acid afforded target product **50** (Scheme 7).

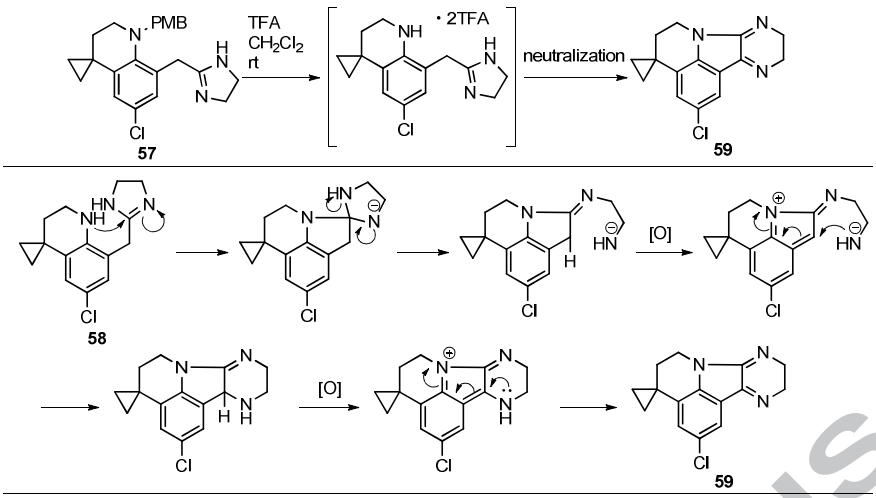


Scheme 7

We attempted to synthesize tetrahydroquinoline derivative **58** in the same way (Scheme 8). Unfortunately, we could not obtain the target product. In the final step, deprotection with TFA afforded a pentacyclic heterocompound (**59**). Its structure was determined by X-ray analysis (Fig. 3)[14]. By mass spectroscopy, we detected **58** but not **59** under acidic conditions, so we speculate that **59** was formed by the synthetic mechanism shown Scheme 9. After the reaction mixture was neutralized, the tetrahydroquinoline nitrogen attacked the C2 position of the imidazoline. An unstable intermediate was oxidized in air and then intramolecular attack by the nitrogen anion gave the pentacyclic framework. Finally, compound was oxidized again, giving **59**.



Scheme 8



Scheme 9

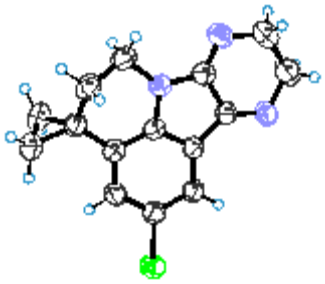
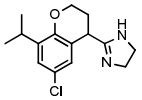
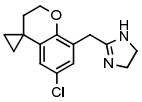
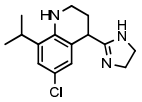


Figure 3. ORTEP drawing of compound **59**. This product was unexpectedly formed in the attempted synthesis of **58**. Thermal ellipsoids show 50% probabilities (white, carbon; green, chlorine; purple, nitrogen; blue, hydrogen).

Chromane compound **40** which was cyclized at the 2 and 3 positions in the benzene ring had lower α_{1L}-adrenoceptor agonist activity compared with **30**. In contrast, both heterocyclic compounds cyclized at the 1 and 2 positions of the benzene ring (**36**, **50**) had higher α_{1L}-adrenoceptor agonist activity compared with **5**. Tetrahydroquinoline **50** showed the most potent α_{1L}-adrenoceptor agonist activity with a ratio of NA EC_{50} of 0.0028 (Table 3). We presumed that the nitrogen and oxygen atoms effectively adjust the electron density of the benzene ring, which effectively interacts with the α_{1L}-adrenoceptor. The effect of the aniline NH is not yet fully understood, but it might serve as a proton donor.

Table 3 In vitro α_{1L}-adrenoceptor agonist activity of chromane and tetrahydroquinoline

derivatives.

	Compound	Ratio of NA EC_{50}
36		0.014
40		0.088
50		0.0028

* NA EC_{50} = 1029 ± 247nM

Because **50** showed potent α_{1L}-adrenoceptor agonist activity in CHO cells stably expressing human α_{1L}-adrenoceptor, we next evaluated the pharmacological effect of **50** in isolated rat bladder neck[15].Contraction of the bladder neck was induced dose-dependently by **50**, which showed stronger potency than NA (Fig. 4).

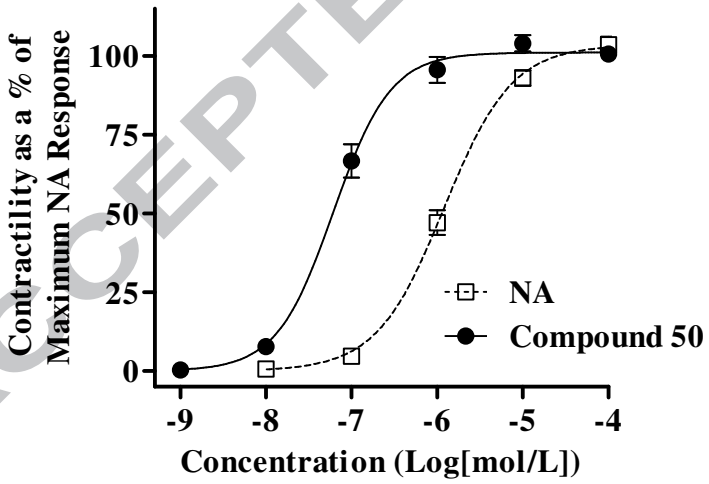


Figure 4. Log concentration-response curve in isolated rat bladder neck. Compound **50** induced contraction of isolated rat bladder neck dose-dependently and showed stronger potency than NA.

We successfully designed and discovered novel potent α_{1L}-adrenoceptor agonists, including

tetraline, chromane, and tetrahydroquinoline derivatives. Among the compounds synthesized in this study, **50** exhibited the highest agonist activity (ratio of NA EC_{50}, 0.0028) and induced contraction of isolated rat bladder neck. Further optimization of the tetrahydroquinoline derivatives is in progress and will be reported in the future.

The authors thank Dr. Ikunobu Muramatsu (Division of Pharmacology, Department of Biochemistry and Bioinformative Sciences, School of Medicine) for supporting our research.

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10. CHO cells stably expressing human α_{1L}-adrenoceptor are defined as the cells (α_{1L}-dominant) expressing human α_{1A}-*AR* and human *CRELD1α*.
11. The α1L-adrenoceptor agonist activity was evaluated by measuring the ability to induce a fluorescence-based calcium mobilization signal in a fluorometric imaging plate reader assay

using the cells (α_{1L}-dominant) expressing human *α_{1A}-AR* and human *CRELD1α.* The cells were plated onto 96-well plates and grown overnight at 37°C in a CO_2 incubator. The cells were incubated with calcium indicator dye (FLIPR® Calcium 5 Assay kit, Molecular Devices) for 60 min at 37°C. Calcium influx was measured using the fluorometric imaging plate reader (FLIPR$^{TETA®,}$ Molecular Devices).

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14. X ray experimental details : space group ; Pccn(#56), Z value ; 8, D_{calc} ; 1.390 g/cm^3, Residuals [R1 (I>2.00s(I)) ; 0.0616, R (All reflections) ; 0.0663, wR2 (All reflections) ; 0.2043]. CCDC No. ; 1059398.
15. Male Wistar rats were anesthetized with sodium pentobarbital (50 mg/kg) and killed. The bladder neck were isolated and cleaned in a modified Krebs-Henseleit solution aerated with 95% O_2 and 5% CO_2. Strips of bladder neck were placed in organ baths containing a modified Krebs-Henseleit solution (37°C). NA and other test agonists were applied cumulatively and the isometric tension changes were recorded through a transducer. This study was performed according to the Guidelines for Animal Experiments, University of Fukui.

List of captions

Figure 1. The structure of MK017. This compound is a known α_{1L}-adrenoceptor agonist.

Figure 2. Designed compounds. Various indane and tetraline derivatives were designed to serve as α_{1L}-adrenoceptor agonists.

Figure 3. ORTEP drawing of compound **59**. This product was unexpectedly formed in the attempted synthesis of **58**. Thermal ellipsoids show 50% probabilities (white, carbon; green, chlorine; purple, nitrogen; blue, hydrogen).

Figure 4. Log concentration-response curve in isolated rat bladder neck. Compound **50** induced contraction of isolated rat bladder neck dose-dependently and showed stronger potency than NA.

Table 1. In vitro α_{1L}-adrenoceptor agonist activity of MK017 derivatives.

Table 2. In vitro α_{1L}-adrenoceptor agonist activity of indane and tetraline derivatives.

Table 3. In vitro α_{1L}-adrenoceptor agonist activity of chromane and tetrahydroquinoline derivatives.

Scheme 1. Reagents and conditions: (a) NBS, $AuCl_3$, DCE, 80°C, 96–99%; (b) 1) $SOCl_2$, CH_2Cl_2, 90°C, 2) $AlCl_3$, CH_2Cl_2, reflux, 6–17% over 2 steps.

Scheme 2. Reagents and conditions: (a) $Pd(OAc)_2$, Cs_2CO_3, PPh_3, THF, H_2O, 70°C, 79–89%; (b) TosMIC, KOtBu, THF, -78°C, then MeOH, 60°C, 31-56%; (c) P_2S_5, ethylenediamine, 100°C, 28–76%; (d) 1) PtO_2, H_2, MeOH, rt, 2) HCl-MeOH, MeOH, rt, 50-65%.

Scheme 3. Reagents and conditions: (a) Dimethylzinc, $TiCl_4$, CH_3Cl_2, -78–0°C, 83–88%; (b) $PdCl_2$(dppf), KF, DMSO, H_2O, 3–11%; (c) 1) P_2S_5, ethylenediamine, 100°C, 2) HCl-MeOH, MeOH, rt, 87%-quant.

Scheme 4. Reagents and conditions: (a) $MePPh_3Br$, tBuOK, THF, rt, 97%; (b) PtO_2, H_2, MeOH, rt, 88%; (c) $PdCl_2$(dppf), KF, DMSO, H_2O, 130°C, 3%; (d) 1) P_2S_5, ethylenediamine, 100°C, 2) HCl-MeOH, MeOH, rt, quant.; (e) CH_2ICl, Et_2Zn, CH_2Cl_2, rt, 80%; (f) $PdCl_2$(dppf), KF, DMSO, H_2O, 130°C, 26%; (g) 1) P_2S_5, ethylenediamine, 100°C, 2) HCl-MeOH, rt, 96%.

Scheme 5. Reagents and conditions: (a) $Pd(OAc)_2$, PPh_3, Cs_2CO_3, THF, H_2O, 70°C, 91%; (b) Wilkinson's reagent, H_2, CH_2Cl_2, rt, 96%; (c) 1) TMS-CN, ZnI_2, CH_2Cl_2, reflux, 2) Amberlyst 15, toluene, reflux, 39%; (d) $NaBH_4$, EtOH, rt, 97%; (e) 1) P_2S_5, ethylenediamine, 100°C, 2) HCl-MeOH, MeOH, rt, 94%

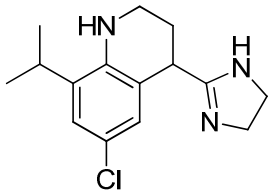
Scheme 6. Reagents and conditions: (a) $MePPh_3Br$, KOtBu, THF, rt, 15%; (b) TFA, Et_2Zn, CH_2I_2, DCE, 64%; (c) $PdCl_2$(dppf), KF, DMSO, H_2O, 16%; (d) 1) P_2S_5, ethylenediamine, 100°C, 2) HCl-MeOH, MeOH, rt, 49%

Scheme 7. Reagents and conditions: (a) 3-Bromopropanoyl chloride, K_2CO_3, CH_2Cl_2, rt; (b) NaOtBu, DMF, rt, 2 steps 72%; (c) TfOH, DCE, rt, 34%; (d) $Pd(OAc)_2$, PPh_3, Cs_2CO_3, 70°C, 85%; (e) Wilkinson's reagent, H_2, CH_2Cl_2, rt, 99%; (f) PMB-Cl, K_2CO_3, NaI, DMF, 50°C, 47%; (g) TosMIC, KOtBu, THF, -78°C, then MeOH, 60°C, 60%; (h) P_2S_5, ethylenediamine, 100°C, 91%; (i)

1) TFA, CH_2Cl_2, rt, 2) HCl-MeOH, MeOH, rt, quant.

Scheme 8. Reagents and conditions: (a) PMB-Cl, NaI, K_2CO_e, DMF, 50°C, 56%; (b) $MePPh_3Br$, KOtBu, THF, rt, 80%; (c) Et_2Zn, CH_2I_2, TFA, DCE, 0°C, 71%; (d) nBuLi, THF, -78°C, then DMF, 0°C; (e) $NaBH_4$, MeOH, THF, rt, 2 steps 76%; (f) 1) $SOCl_2$, CH_2Cl_2, rt, 16h, 2) NaCN, DMSO, rt, 49%; (g) P_2S_5, ethylenediamine, 100°C, 76%; (h) TFA, CH_2CL_2, rt.

Scheme 9. Putative synthetic mechanism of by-product **59**.



50

Ratio of NA EC_{50} = 0.0028

(NA EC_{50} = 1019 ± 247 nM)