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# Discovery of novel chiral diazepines as bombesin receptor subtype-3 (BRS-3) agonists with low brain penetration

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ABSTRACT

The discovery and optimization of a novel series of BRS-3 agonists are described. We explored a potent BRS-3 agonist with low brain penetration to avoid an adverse effect derived from central nervous system exposure. Through the derivatization process, chiral diazepines **9f** and **9g** were identified as possessing low brain penetration as well as potent in vitro activity against human and mouse BRS-3s.



Bombesin receptor subtype-3 (BRS-3) is known as an orphan G-protein coupled receptor and belongs to the bombesin receptor family. BRS-3 receptor is expressed not only in brain, but also in other peripheral organs such as intestine, liver, lung, testes and pancreas.[1] Since BRS-3 deficient mice develop obesity and impaired glycemic regulation,[2] discovering ligands that modulate BRS-3 signaling is attractive for treatment of diabetes and obesity.

Recently, Guan et al. have reported a potent and brain penetrant BRS-3 agonist as a clinical candidate (MK-5046),[3] which showed anti-obesity effects in rats and dogs. However, MK-5046 also caused adverse effects, such as an increase of body temperature, heart rate and blood pressure in animals.[4] Moreover, it raised at least blood pressure in humans in the clinical trial.[5] We speculated that these adverse effects were due to the activation of sympathetic nervous system by stimulating BRS-3 in the central nervous system (CNS), and therefore low exposure of compound to the CNS is expected to avoid them. On the other hand, since BRS-3 is also expressed in the peripheral tissues, there is the potential for some anti-obesity effects by stimulating peripheral BRS-3. In this paper, we describe the discovery and optimization of a series of BRS-3 agonists with low brain penetration.

We first designed and synthesized a novel benzodiazepine BRS-3 agonist derived from hit compounds **1a** and **1b** which were identified by a high throughput screening campaign of our corporate library, and a benzodiapzepine sulfonamide **2** which was previously reported as a potent BRS-3 agonist MK-7725 (Fig. 1).[6,7] Inspired by a structural similarity between our lead compounds **1** and the sulfonamide **2**, we surmised the sulfonyl group of **2** could be replaced to another linker and the benzene ring labeled with C in Fig. 1 could be removed from the rigid benzodiazepine structure (Table 1).

For an efficient derivatization, we initiated the SAR study by adopting 4-*tert*-butylphenyl group by reference to the previously reported compound **3a** as an (*S*)-atropisomer by Liu et al.[8] Although replacement of sulfonyl group with arylacyl group **3b** resulted in no activity, arylacetyl group **3c** showed a good human and mouse in vitro activities. The replacement *tert*-butyl group with phenoxy group **3d** resulted in almost no in vitro activities. However, the synthesis of *meta*-phenoxy compound **3e** by reference to a partial structure of **1b** led to dramatically improve its activities. Encouraged by the result, we continued further modifications of compound **3e** by reducing the phenyl ring to improve its solubility. Consequently, the introduction of 2-(3-phenoxyphenyl)acetyl group as the side chain of **3e** turned out to enhance the in vitro activity against both species (**3f**; human $EC_{50}$ = 8.5 nM, mouse $EC_{50}$ = 12.2 nM). On the other hand, conversion to the urea group **3g** provided a sharp loss of potency unlike hit compound **1b**.

Derivatization of the right terminal phenyl ring of **3f** was next focused on, as described in Table 2 because this part was crucial

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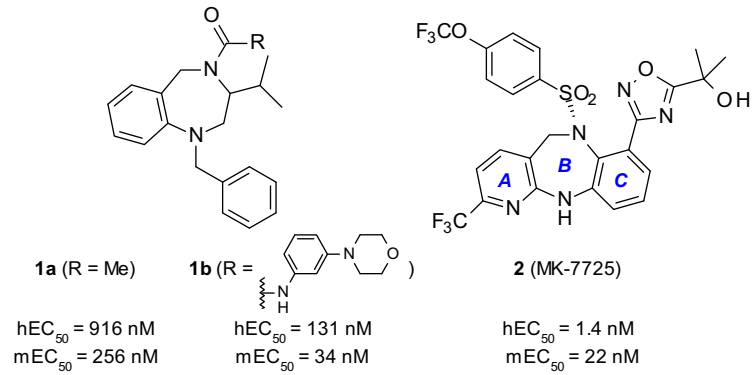
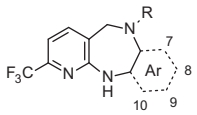
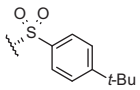
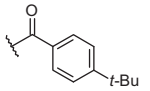
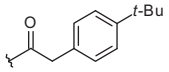
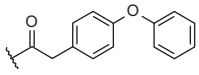
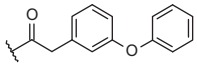
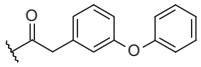
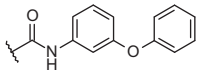



**Figure 1.** Our HTS hit compounds **1a** and **1b** and brain penetrant benzodiazepine sulfonamide **2** (MK-7725) with BRS-3 $EC_{50}$ values.

**Table 1**
$EC_{50}$ values of BRS-3 agonists **3a**–**3f**



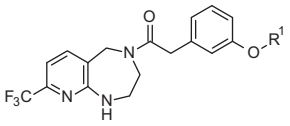
| Compound | R | Ar | Human BRS-3 $EC_{50}$[a] (nM) | Mouse BRS-3 $EC_{50}$[a] (nM) |
|---|---|---|---|---|
| **3a** ((*S*)-isomer) |  | 7,8-Dimethylphenyl | 97[b] | 33[b] |
| **3b** |  | Phenyl | >10000 | >10000 |
| **3c** |  | Phenyl | 48 | 227 |
| **3d** |  | Phenyl | 368 | >10000 |
| **3e** |  | Phenyl | 25 | 46 |
| **3f** |  | None | 8.5 | 12.2 |
| **3g** |  | None | 4378 | 1378 |

[a] Data are averages of at least 3 times repeated measurement.
[b] Data are cited from Ref. 8a.

for in vitro activity, and could be efficiently converted by amidation of the diazepine moiety with various carboxylic acids. Also **3c** with 4-*tert*-butylphenyl group was relatively potent, but we did not derivatize it further in the light of its rigidity, lipophilicity and synthetic variety. An increase of potency was observed by the installment of a fluoro atom on the right phenyl ring (**4a**–**4c**). Among them, the *para*-fluoro substituent **4c** showed the best efficacy, and *para*-tolyl substituent **4d** indicated further improved activity with an increase of log*D* value (>4.8). Although an installment of nitrogen atom on the phenyl ring reduced activities, **4f** showed the best among them. Meanwhile, the combination of *para*-methyl and 3-pyridyl group **4h** provided excellent activities with a modest lipophilicity (log*D* = 3.6). We also examined a replacement of the R1 part with aliphatic groups. Relative small alkyl derivatives **4i**–**4k** decreased the activities presumably because pharmacophore of the right terminal moiety required a certain size of lipophilic functional group. As we expected, an in vitro activity was retained in case that the number of carbon was more than four (**4l**–**4p**). These results suggested **4h** should be balanced in terms of activity and lipophilicity at this stage.

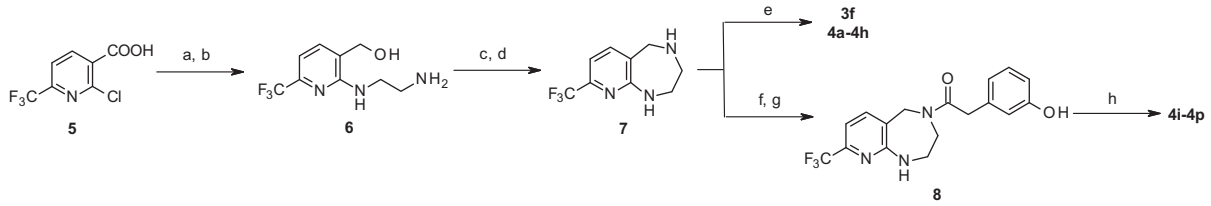
The preparation of diazepine derivatives listed in Tables 1 and 2 was shown in Scheme 1. The synthesis of **3b**–**3e** was performed via a tricyclic benzodiazepine in the usual manner.[8a] Subsequent amidation with the corresponding carboxylic acid provided the desired compounds **3b**–**3e**. New scaffold compounds (**3f**, **4a**–**4h**) were synthesized after synthetic examination for a formation of 7-membered diazepine ring. Initially, we tried to obtain the diazepine **7** by reduction of a corresponding lactam ring, but resulted in failure probably because of distortion of the ring. As a result, we succeeded to procure **7** from **5** by reduction of carboxyl group and additional substitution of ethylenediamine. Then, oxidation of **6** to aldehyde directly provided 7-membered imine in a moderate yield. A dimer imine compound was also isolated as a minor product in this step. Finally, hydrogenation by Pd/C catalyst in ethyl

**Table 2**
$EC_{50}$ values of BRS-3 agonists **3f** and **4a–4p**



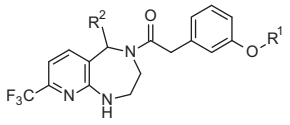
| Compound | R | Human BRS-3 $EC_{50}$[a] (nM) | Mouse BRS-3 $EC_{50}$[a] (nM) | Log*D*[b] |
|---|---|---|---|---|
| **3f** | Ph | 8.5 | 12.2 | 4.5 |
| **4a** | *o*-F-Ph | 6.9 | 3.5 | 4.5 |
| **4b** | *m*-F-Ph | 6.8 | 8.7 | 4.8 |
| **4c** | *p*-F-Ph | 3.6 | 2.7 | 4.6 |
| **4d** | *p*-tolyl | 1.3 | 1.0 | >4.8 |
| **4e** | 2-Py | 254 | 244 | 3.2 |
| **4f** | 3-Py | 156 | 106 | 3.2 |
| **4g** | 4-Py | 977 | 621 | 3.3 |
| **4h** |  | 9.5 | 2.8 | 3.6 |
| **4i** | Me | 2041 | 1781 | ND[c] |
| **4j** | Et | 2046 | 1208 | ND[c] |
| **4k** | *i*-Pr | 381 | 569 | ND[c] |
| **4l** | *n*-Bu | 59 | 52 | ND[c] |
| **4m** | *i*-Bu | 70 | 62 | 4.3 |
| **4n** |  | 199 | 121 | ND[c] |
| **4o** | *c*-Pentane | 41 | 100 | ND[c] |
| **4p** | *c*-Hexane | 8.1 | 8.7 | 4.4 |

[a] Data are averages of at least 3 times repeated measurement.
[b] The distribution coefficients (log*D*) were measured between 1-octanol and phosphate buffered saline (pH 7.4).
[c] Not determined.



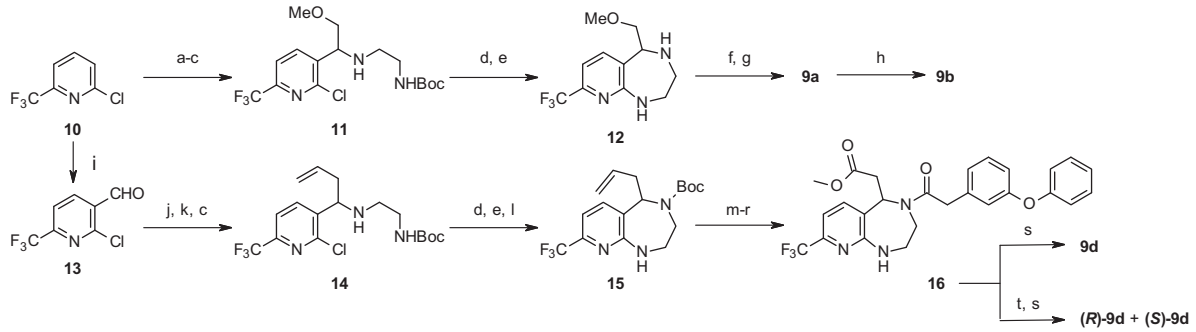

**Scheme 1.** Reagents and conditions: (a) $BH_3$-THF, THF, 91%; (b) ethylenediamine (neat), 125 °C, 79%; (c) $MnO_2$, acetone/$CH_2Cl_2$, 45%, (d) $H_2$, 10%Pd/C, AcOEt, 99%; (e) arylacetic acid, HATU, $(i\text{-Pr})_2$EtN, $CH_2Cl_2$, 72–95%; (f) [3-(benzyloxy)phenyl]acetic acid, $(i\text{-Pr})_2$EtN, HATU, $CH_2Cl_2$, 99%; (g) $H_2$, 10%Pd/C, EtOH, 99%; (h) alkylhalide, $K_2CO_3$, DMF, 31–83%. HATU: (Dimethylamino)-*N*,*N*-dimethyl(3*H*-[1,2,3]triazolo[4,5-*b*]pyridin-3-yloxy)methaniminium hexafluorophosphate.

**Table 3**
$EC_{50}$ values of BRS-3 agonists **3f** and **9a–9g**



| Compound | R1 | R2 | Human BRS-3 $EC_{50}$[a] (nM) | Mouse BRS-3 $EC_{50}$[a] (nM) | Log*D*[b] |
|---|---|---|---|---|---|
| **3f** | Ph | H | 8.5 | 12.2 | 4.5 |
| **9a** | Ph | $CH_2OH$ | 12 | 19 | 4.1 |
| **9b** | Ph | $CO_2H$ | 1562 | 2260 | ND[c] |
| **9c** | Ph | $CH_2CH_2OH$ | 11 | 11 | ND[c] |
| **9d** | Ph | $CH_2CO_2H$ | 13 | 15 | 1.2 |
| (*R*)-**9d** | Ph | (*R*)-$CH_2CO_2H$ | 1.6 | 3.4 | 1.2 |
| (*S*)-**9d** | Ph | (*S*)-$CH_2CO_2H$ | 2071 | 3124 | 1.2 |
| **9e** | *p*-F-Ph | (*R*)-$CH_2CO_2H$ | 1.7 | 2.7 | 1.2 |
| **9f** | *i*-Bu | (*R*)-$CH_2CO_2H$ | 17 | 62 | 0.9 |
| **9g** |  | (*R*)-$CH_2CO_2H$ | 1.7 | 3.5 | 0.3 |

[a] Data are averages of at least 3 times repeated measurement.
[b] The distribution coefficients (log*D*) were measured between 1-octanol and phosphate buffered saline (pH 7.4).
[c] Not determined.



**Scheme 2.** Reagents and conditions: (a) 2-methoxy-*N*-methoxy-*N*-methyl-acetamide, (*i*-Pr)$_2$NH, *n*-BuLi/hexane, THF, −78 °C, 30%; (b) *N*-boc-ethylenediamine, Ti(O*i*-Pr)$_4$, THF; (c) NaBH$_4$, EtOH, 0 °C, 2 steps, 62%; (d) 4 N HCl/AcOEt, rt; (e) (*i*-Pr)$_2$EtN, *N*-methylpyrrolidone, 150 °C; (f) arylacetic acid, (*i*-Pr)$_2$EtN, HATU, $CH_2Cl_2$, 3 steps, 35%; (g) $BBr_3$, $CH_2Cl_2$, −78~0 °C, 15%; (h) Jones reagent, $CH_2Cl_2$, 17%; (i) *n*-BuLi/hexane, (*i*-Pr)$_2$NH, DMF, THF, −78 °C, 95%; (j) *N*-boc-ethylenediamine, $Na_2SO_4$, $CH_2Cl_2$, rt, 82%; (k) allylmagnesium bromide/$Et_2O$, THF, 0 °C, 73%; (l) $Boc_2O$, $Et_3N$, $CH_2Cl_2$, 76%; (m) 2.5%$OsO_4$/*i*-PrOH, *N*-methylmorpholine oxide, acetone/$H_2O$, 0 °C to rt; (n) $NaIO_4$, THF/$H_2O$; (o) $NaClO_2$, $NaH_2PO_4$, isobutene, *t*-BuOH/$H_2O$; (p) iodomethane, $K_2CO_3$, acetone, 4 steps, 78%; (q) 4 N HCl/AcOEt, 92%; (r) arylacetic acid, (*i*-Pr)$_2$EtN, HATU, $CH_2Cl_2$, 72–99%; (s) aq. NaOH, THF/MeOH, 88–97%; (t) Chiral separation (CHIRALPAK, IC, EtOH/hexanes = 30/70). HATU: (Dimethylamino)-*N*,*N*-dimethyl(3*H*-[1,2,3]triazolo[4,5-*b*]pyridin-3-yloxy)methaniminium hexafluorophosphate.

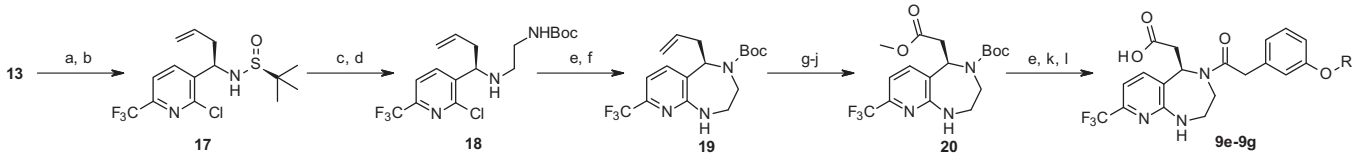
acetate and amidation of **7** with various carboxylic acids yielded **3f** and **4a**–**4h**. Aliphatic derivatives **4i**–**4p** was synthesized via $S_N2$ reaction of the phenol compound **8** and various alkylhalides.

We next turned our attention to introduction of a polar group to the diazepine structure to obtain a potent compound with lowered log*D* value aiming for low brain penetration. After a synthetic exploration and in vitro evaluation, we discovered an installment of aliphatic groups to R2 position depicted in Table 3 retained the potency. Therefore, we introduced various lengths of linkers with polar groups to the R2 position (**9a**–**9g**) and discovered all of derivatives except for **9b** showed excellent activities. A chiral separation was also examined by using chiral column chromatography and yielded each pure enantiomer (*R*)-**9d** and (*S*)-**9d**.[9] As a result, it was turned out that only (*R*)-**9d** retained the in vitro activities (compound (*R*)-**9d**; human $EC_{50}$ = 1.6 nM, mouse $EC_{50}$ = 3.4 -nM). The absolute configuration of (*R*)-**9d** was determined by the asymmetric synthesis mentioned later. Further SAR studies were pursued to improve potency and decrease of log*D* value by introduction of potent functional groups on the R1 position and a carboxy unit on the R2 position. This combination enabled us to obtain **9e**–**9g** with a good activity and low log*D* value, and was especially effective for **9g**, which indicated good activities in both species with the lowest log*D* value (compound **9g**; human $EC_{50}$ = 1.7 nM, mouse $EC_{50}$ = 3.5 nM, log*D* = 0.3).

Each compound listed in Table 3 was synthesized from a commercially available pyridine **10** as shown in Scheme 2. Similar to the synthetic route in Scheme 1, all derivatives were synthesized by amidation of the corresponding diazepines and carboxylic acids after cyclization of the substituted diamines. As for **9a** and **9b**, addition of lithiated **10** to the Weinreb amide, imine formation with *N*-boc protected diamine, and subsequent reduction yielded **11**. Then, deprotection of Boc group and cyclization under heating condition provided the diazepine **12**. Finally, amidation and deprotection of methyl ether were conducted to generate **9a**. Among several oxidative conditions, only Jones oxidation was successful to afford **9b**.[9] Next, as a first step to install an acetic acid group to the R1 position, aldehyde **13** was reacted with allyl Grignard reagent, and the resulting alcohol was oxidated to the ketone and condensed with the diamine followed by cyclization and Boc protection to afford **15**. Then, the allyl group of **15** was converted to methyl ester compound **16** after oxidative cleavage to aldehyde, Klaus oxidation reaction to carboxylic acid,[10] and methylation of the carboxy group. Compound **9d** was procured by deprotection of Boc group and amidation. After chiral separation of **16** by utilizing of chiral column chromatography, subsequent hydrolysis of methyl ester afforded each isomer (*R*)-**9d** and (*S*)-**9d**.[11]

A synthetic route of chiral diazepine derivatives **9e**–**9g** was shown in Scheme 3. First, Ellman's chiral sulfinamide ((*S*)-*t*-BuS(O)$NH_2$) was adopted to stereospecifically introduce an allyl group to the R1 position.[12] After imine formation with the aldehyde **13** and the Ellman's sulfinamide, an effective asymmetric addition of allyl group to the imine moiety was carried out under the condition using Indium catalyst in aqueous NaBr, which was reported by Sun et al.[13] A pure desired (*R*,*S*)-diastereomer **17** was easily isolated by column chromatography in a high yield.[14] Replacement of the sulfinamide with *N*-Boc ethylenediamine after several steps, deprotection of Boc group and cyclization yielded a key chiral intermediate **19**.[15] Finally, the desired compounds **9e**–**9g** were synthesized by use of **19** in the same manner illustrated in Scheme 2.[16,17]

Pharmacokinetic (PK) parameters in mice of compounds **3f**, **9f** and **9g** were evaluated (Table 4). Highly lipophilic compound **3f** was not enough exposed to blood due to low solubility and microsomal stability (MS) and it showed an undesired high brain penetration in mice. On the other hand, the solubility and unbound fractions (PB free) of diazepine derivatives **9f** and **9g** with a chiral carboxyl group were dramatically improved. As a result, blood exposure of **9f** and **9g** in mice was also improved. Moreover, the



**Scheme 3.** Reagents and conditions: (a) (*S*)-*t*-BuS(O)$NH_2$, Ti(OEt)$_4$, THF, 70 °C, 83%; (b) allylbromide, In, aq NaBr, 93%; (c) HCl/dioxane,MeOH, 82%; (d) *tert*-butyl *N*-(2-oxoethyl)carbamate, $NaBH(OAc)_3$, dichloroethane, quant.; (e) 4 N HCl/AcOEt, rt; (f) (*i*-Pr)$_2$EtN, *N*-methylpyrrolidone, 160 °C, then $(Boc)_2O$, 2 steps, 72%; (g) 2.5%$OsO_4$/*i*-PrOH, *N*-methylmorpholine oxide, acetone/$H_2O$, 0 °C to rt; (h) $NaIO_4$, THF/$H_2O$; (i) $NaClO_2$, $NaH_2PO_4$, isobutene, *t*-BuOH/$H_2O$; (j) $TMSCH_2N_2$, THF/MeOH, rt, 4 steps, 73%; (k) 1-arylacetic acid, (*i*-Pr)$_2$EtN, HATU, $CH_2Cl_2$, 74–95%; (l) aq NaOH, THF/MeOH, 90–96%.

**Table 4**
Physical properties and mouse PK parameters of **3f**, **9f** and **9g**

| Compound | In vitro $EC_{50}$ (nM) | Log $D$[b] | Solubility JP1/JP2 (μg/mL) | PB free[a] (%) | $C_{max}$[c] (μg/mL) | AUC[c] (hr∗μg mL) | $T_{1/2}$[c] (h) | MS[d] (%) | PSA[e] | Mouse $K_p$ brain[f] |
|---|---|---|---|---|---|---|---|---|---|---|
| **3f** | 12 | 4.5 | 11/5.1 | 0.67 | 0.060 | 0.041 | 0.82 | 0.70 | 53 | 0.72[g] |
| **9f** | 62 | 0.9 | 40/810 | 3.3 | 0.36 | 0.39 | 3.7 | 70 | 88 | 0.043[g] |
| **9g** | 3.5 | 0.3 | 960/950 | 4.6 | 0.090 | 0.20 | 2.6 | 94 | 98 | 0.002[g,h] |

[a] Unbound fractions (%) in mouse plasma. PB: Protein Binding.
[b] The distribution coefficients (log$D$) were measured between 1-octanol and phosphate buffered saline (pH 7.4).
[c] Average of 2 mice dosed at 10 mg/kg po. Each dose was administered by DMA/PG/(20%HPβCD/saline): 10/10/80 as a solvent.
[d] Remaining (%) of the tested compound after 0.5 h incubation with mouse liver microsome.
[e] Polar surfice area (Å$^2$).
[f] $K_p$ brain: brain to plasma concentration ratio.
[g] $K_p$ value was determined after a single administration of compound at 30 mg/kg po to mice. The measurement of each concentration was conducted at 2.5 h after administration.
[h] Measured by racemic compound of **9g**.

brain to plasma concentration ratios ($K_p$ brain) of them, especially **9g**, were fairly low in parallel with their log$D$ values as we expected. In addition, their high polar surface area (PSA) value also might contribute their low brain exposure because high PSA is supposed to lower blood–brain barrier penetration.[18] Thus, we found out two promising candidates **9f** and **9g** as BRS-3 agonists with high in vitro activity, blood exposure and low $K_p$ brain value.

In summary, we obtained chiral diazepine derivatives as novel BRS-3 agonists with low brain penetration. Removal of the benzene ring labeled with C of **2**, which was suggested from our hit compounds **1**, resulted in a first breakthrough to obtain an original scaffold. Introduction of a polar group to the diazepine structure provided the potent derivative **9d**. By the chiral separation of **9d**, we discovered only (*R*)-**9d** was highly active. Also an effective asymmetric synthesis of the desired (*R*)-enantiomers **9e**–**9g** was achieved. Finally, various polar compounds were obtained by combining each preferable functional group on the R1 and R2 position. This strategy enabled us to obtain chiral diazepines **9f** and **9g** with potent in vitro BRS-3 activity, good blood exposure and low brain penetration. These compounds are expected to avoid adverse effects derived from CNS, hence in vivo efficacy and safety evaluations of them are underway and to be reported in near future.

## Reference and notes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6. The 384-well IP-One HTRF® Assay (Cisbio, Bedford, MA) was performed as described by the manufacturer's protocol. CHO-K1 stably expressing human and mouse BRS-3 was plated at 200,000 cells per well in 50 μl HAM and incubated in the $CO_2$ incubator at 37 °C for 24 h. On the next day, the media were removed and 25 μl of compounds diluted in HAM containing 0.1% BSA and 20 mM LiCl were added to each well and serial diluted IP1 standards (Cisbio) were also added to corresponding wells at this step. After the cells were incubated for 1 h in the $CO_2$ incubator at 37 °C, all the media were removed again and d2-labeled IP1 and cryptate-labeled anti-IP1 monoclonal antibody diluted in lysis buffer were added sequentially. The assay plates were kept in dark at 4 °C overnight. Ratiometic measurements of fluorescence emission at 665 nm and 620 nm were obtained using a RUBYstar fluorometer (BMG Labtech, Ortenberg, Germany). IP1 levels in each well were calculated according to the standard curves on each plate. $EC_{50}$ values were obtained by fitting data to a nonlinear curve-fitting program (GraphPad Software, Inc., La Jolla CA).
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11. Each enantiomer (*R*)-**9d** and (*S*)-**9d** was obtained by hydrolysis after chiral HPLC separation of **15**: column, Chiral Pack IC (4.6 × 250 mm); eluent, ethanol/hexanes = 30/70. The ee value of the methyl esters of (*R*)-**9d** and (*S*)-**9d** were determined by chiral HPLC analysis: column, Chiral Pack IC (4.6 × 250 mm); flow rate 1.0 mL/min; temperature, 27 °C; $t_R$ of (*R*)-enantiomer = 6.56 min; $t_R$ of (*S*)-enantiomer = 7.47 min. The retention time of the methyl ester of (*R*)-enantiomer ((*R*)-**9d**) via chiral separation was well corresponded to the one sythesized by the stereospecific route in Scheme 3.
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14. An undesired (*S*,*S*)-diastereomer was also obtained in 2.0% yield.
15. The ee value of the key intermediate **19** was determined to 98.1% ee by chiral HPLC analysis: column, Chiral Pack IA (4.6 × 150 mm); eluent, ethanol/isopropylalcohols = 50/50; flow rate, 1.0 mL/min; temperature, 27 °C; $t_R$ of (*R*)-enantiomer = 3.56 min; $t_R$ of (*S*)-enantiomer = 4.11 min. The absolute configration of **19** was inferred by the reaction mechanism proposed by the Ellman's papers (see Ref. 12).
16. The synthesis of **9f** was performed as follows: Step 1. Synthesis of *N*-{(1*R*)-1-[2-chloro-6-(trifluoromethyl)pyridin-3-yl]but-3-en-1-yl}-2-methylpropane-2-sulfinamide (**17**): A mixture of 2-chloro-6-(trifluoromethyl) pyridine-3-carbaldehyde **13** (19.0 g, 90.8 mmol), $Ti(OEt)_4$ (41.4 g. 182 mmol), and (*S*)-(−)-*tert*-butylsulfinamide (11.2 g. 92.6 mmol) in THF (200 mL) was stirred at reflux for 5 h. The cooled reaction mixture was concentrated, and dissolved in ethylacetate (500 mL). The resulting suspension was filtered through a Celite pad, and the filtrate was concentrated and purified by column chromatography (hexane/EtOAc = 4:1) to provide the imine (28.9 g) as a light-yellow solid. A mixture of the imine (28.9 g, 92.5 mmol), allyl bromide (55.9 g, 0.46 mmol), and indium (42.5 g, 0.37 mmol) was vigorously stirred in sat. NaBr aq at room temperature overnight. After addition of sat. $NaHCO_3$ aq (500 mL), the reaction mixture was extracted with ethylacetate (250 mL × 3). The organic layers were filtered through a Celite pad, and dried ($Na_2SO_4$), concentrated and purified by column chromatography (hexane/EtOAc = 1:4 with 5% of $Et_3N$) to provide **17** as a white solid (26.0 g, 77%). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ 7.99 (1H, d, $J$ = 7.8 Hz), 7.67 (1H, d, $J$ = 7.8 Hz), 5.81–5.73 (1H, m), 5.27 (2H, dd, $J$ = 13.2, 17.6 Hz), 5.06–5.02 (1H, m), 3.84 (1H, d, $J$ = 2.4 Hz), 2.82–2.77 (1H, m), 2.53–2.47 (1H, m), 1.27 (9H, s). Step 2. Synthesis of *tert*-butyl[2-({(1*R*)-1-[2-chloro-6-(trifluoromethyl)pyridin-3-yl]but-3-en-1-yl}amino)ethyl carbamate (**18**): **17** (22.8 g, 64.3 mmol) was dissolved in 4 N HCl/MeOH (100 mL) and stirred at room temperature for 2 h. After the reaction mixture was concentrated, the residue was added to diethylether (300 mL). The resulting suspension was filtered, and dried in vacuo to provide the amine HCl salt (15.1 g, 82%) as a white solid. A mixture of the amine HCl salt (15.1 g, 52.8 mmol), *tert*-butyl *N*-(2-oxoethyl)carbamate (12.6 g, 79.3 mmol), and $NaBH(OAc)_3$ (25.2 g, 95.1 mmol) in $CH_2Cl_2$ (250 mL) was stirred at room temperature for 1 h, then warmed to room temperature for 4 h. After addition

of sat.$NaHCO_3$ aq (300 mL), the reaction mixture was extracted with $CH_2Cl_2$ (250 mL × 2). The organic layers were washed with brine, dried ($Na_2SO_4$), concentrated and purified by column chromatography (hexane/EtOAc = 2:5) to provide **18** as a white solid (21.6 g, quant.). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ 8.09 (1H, d, $J$ = 7.8 Hz), 7.60 (1H, d, $J$ = 8.2 Hz), 5.78–5.68 (1H, m), 5.14–5.07 (2H, m), 4.69 (1H, s), 4.15 (1H, dd, $J$ = 7.8, 3.9 Hz), 4.09 (1H, dd, $J$ = 6.6, 14.5 Hz), 3.15 (2H, br s), 2.61–2.56 (1H, m), 2.53–2.49 (1H, m), 2.44–2.38 (1H, m), 2.21–2.13 (1H, m), 1.18 (9H, d, $J$ = 2.0 Hz). Step 3. Synthesis of *tert*-butyl (5*R*)-5-(prop-2-en-1-yl)-8-(trifluoromethyl)-1,2,3,5-tetrahydro-4*H*-pyrido[2,3-*e*][1,4]diazepine-4-carboxylate (**19**): **18** (21.6 g, 54.8 mmol) was dissolved in 4 N HCl/ethylacetate (100 mL) and stirred at room temperature for 1 h. After the reaction mixture was concentrated, the residue was dissolved in $CH_2Cl_2$ (300 mL), washed with sat. $NaHCO_3$ aq and brine, dried ($Na_2SO_4$), concentrated and purified by column chromatography (MeOH/$CH_2Cl_2$ = 1:12) to provide the diamine as white solid (12.7 g). A mixture of the diamine (12.7 g. 43.3 mmol) and ($i$-Pr)$_2$EtN (23.7 mL, 130 mmol) in NMP (250 mL) was stirred at 160 °C for 8 h. Subsequently, $(Boc)_2O$ (14.2 g, 65.0 mmol) was added to the cooled mixture. After stirring at room temperature for 1 h, The mixture was diluted with $Et_2O$, washed with brine, dried ($Na_2SO_4$), concentrated and purified by column chromatography (EtOAc/hexane = 1:4) to provide **19** as brown oil (11.1 g, 72%). $^1H$ NMR (500 MHz, $CDCl_3$): a mixture of conformers $\delta$ 7.53–7.39 (1H, m), 7.06 (1H, d, $J$ = 7.8 Hz), 5.64–5.54 (1H, m), 5.31–4.96 (4H, m), 4.10–3.97 (1H, m), 3.35–3.27 (2H, m), 3.10 (1H, dt, $J$ = 24.9, 10.5 Hz), 2.80–2.68 (1H, m), 2.45 (1H, td, $J$ = 14.9, 7.2 Hz), 1.40 (9H, s). Step 4. Synthesis of *tert*-butyl (5*R*)-5-(2-methoxy-2-oxoethyl)-8-(trifluoromethyl)-1,2,3,5-tetrahydro-4*H*-pyrido[2,3-*e*][1,4]diazepine-4-carboxylate (**20**): A mixture of **19** (11.1 g, 31.1 mmol), 2.5%$OsO_4$ in *i*-PrOH (8.00 mL. 0.78 mmol), and *N*-methylmorpholine oxide (4.74 g. 40.5 mmol) in acetone/$H_2O$ (90 mL/30 mL) was stirred at 0 °C overnight. The mixture was diluted with AcOEt (300 mL), washed with brine, dried ($Na_2SO_4$), concentrated and purified by column chromatography (MeOH/$CH_2Cl_2$ = 1:8) to provide the diol as brown oil (12.9 g). A mixture of the diol (12.9 g, 31.2 mmol), sodium periodate (13.3 g. 62.3 mmol) in THF/$H_2O$ (150 mL/50 mL) was stirred at room temperature for 1.5 h. The mixture was diluted with AcOEt (300 mL), washed with brine, dried ($Na_2SO_4$), concentrated in vacuo to provide the aldehyde as colorless oil (11.9 g). A mixture of the aldehyde (11.9 g, 31.1 mmol), $NaH_2PO_4$ $2H_2O$ (36.4 g. 234 mmol), NaClO (7.04 g, 78 mmol), 2-methyl-2-butene (33.1 mL, 311 mmol) in *t*BuOH/$H_2O$ (150 mL/150 mL) was stirred at room temperature overnight. After concentration, the residue was diluted with AcOEt (300 mL × 2), washed with brine, dried ($Na_2SO_4$), concentrated in vacuo to provide the carboxylic acid as brown oil (16.1 g). A mixture of the carboxylic acid (15.2 g, 22.7 mmol), 2.0 M (Trimethylsilyl)diazomethane/hexane (18.0 mL, 36.0 mmol) in THF/MeOH (50 mL/50 mL) was stirred at 0 °C for 1 h. The mixture was concentrated and purified by column chromatography (EtOAc/hexane = 1:3) to provide **20** as colorless oil (8.40 g, 73%). $^1H$ NMR (500 MHz, $CDCl_3$): a mixture of conformers $\delta$ 7.76–7.61 (1H, m), 7.16 (1H, d, $J$ = 7.8 Hz), 5.70 (1H, d, $J$ = 90.8 Hz), 5.03 (1H, s), 4.14–4.04 (1H, m), 3.67 (3H, s), 3.51–3.44 (1H, m), 3.37 (1H, d, $J$ = 14.6 Hz), 3.21–3.06 (2H, m), 2.95 (1H, dd, $J$ = 15.6, 8.3 Hz), 1.36 (9H, s). Step 5. Synthesis of [(5*R*)-4-({3-[(6-methylpyridin-3-yl)oxy]phenyl}acetyl)-8-(trifluoromethyl)-2,3,4,5-tetrahydro-1*H*-pyrido[2,3-*e*][1,4]diazepin-5-yl]acetic acid (**9f**): **20** (8.82 g, 22.7 mmol) was dissolved in 4 N HCl/1,4-dioxane (50 mL) and stirred at room temperature for 1 h. ($i$-Pr)$_2$O (300 mL) was added to the mixture. The deposited hydrochloride salt was separated by filtration and dried in vacuo to provide the diazepine as white solid (7.1 g). A mixture of the diazepine (2.00 g, 4.38 mmol), 2-(3-isobutoxyphenyl)acetic acid (1.05 g, 5.04 mmol), ($i$-Pr)$_2$EtN (3.05 mL, 17.5 mmol), HATU (2.08 g, 5.48 mmol) in $CH_2Cl_2$ (20 mL) was stirred at room temperature overnight. The mixture was diluted in $CH_2Cl_2$ (100 mL), washed with sat. $NaHCO_3$ aq and brine, dried ($Na_2SO_4$), concentrated and purified by column chromatography (EtOAc/hexane = 1:1) to provide the acylated diazepine as colorless oil (2.36 g). A mixture of the acylated diazepine (2.26 g, 4.07 mmol), 2 N NaOH aq (40 mL) in THF/MeOH(20 mL/20 mL) was stirred at room temperature for 2 h. The mixture was acidified with 2 N HCl aq and extracted with EtOAc. The organic layer was washed with sat. $NaHCO_3$ aq and brine, dried ($Na_2SO_4$) and concentrated in vacuo. The obtained residue was triturated in $Et_2O$/hexane, filtered and dried in vacuo to provide **9f** as white solid (1.69 g, 89%) $^1H$ NMR (400 MHz, $CDCl_3$): a mixture of conformers $\delta$ 7.81 (0.5H, d, $J$ = 7.4 Hz), 7.21–7.13 (1.5H, m), 6.90 (1H, dd, $J$ = 26.6, 7.4 Hz), 6.79–6.71 (3H, m), 6.21 (0.5H, t, $J$ = 7.0 Hz), 5.46 (0.5H, t, $J$ = 7.8 Hz), 5.16–5.01 (1H, m), 3.99 (1H, dd, $J$ = 28.8, 15.5 Hz), 3.87-3.54 (5H, m), 3.47–3.22 (2H, m), 3.19–2.95 (2H, m), 2.88–2.75 (1H, m), 2.07–1.97 (1H, m), 1.01–0.97 (6H, m). MS (ESI) $m/z$: 466 $(M+H)^+$. HRMS (ESI) $m/z$: 464.1793 (calcd for $C_{23}H_{26}N_3O_4F_3$: 464.1797). $\alpha_D^{21.0}$ −40.2 ($c$ 1.00, $CHCl_3$).

17. The synthesis of **9g** was prepared in a similar manner described for **9f** (See Ref. 16, Step 5). [(5*R*)-4-{[3-(2-methylpropoxy)phenyl]acetyl}-8-(trifluoromethyl)-2,3,4,5-tetrahydro-1*H*-pyrido[2,3-*e*][1,4]diazepin-5-yl]acetic acid (**9g**): A white solid, $^1H$ NMR (400 MHz, $CD_3OD$): a mixture of conformers $\delta$ 8.14 (1H, dd, $J$ = 16.6, 2.4 Hz), 7.78 (0.5H, d, $J$ = 7.3 Hz), 7.35 (1H, t, $J$ = 5.6 Hz), 7.30–7.27 (2H, m), 7.16 (0.5H, d, $J$ = 7.3 Hz), 7.09–6.93 (3H, m), 6.85–6.72 (1H, m), 6.15 (0.5H, t, $J$ = 7.6 Hz), 5.56 (0.5H, t, $J$ = 7.3 Hz), 4.19–3.38 (5H, m), 3.26–2.81 (4H, m), 2.53 (3H, d, $J$ = 4.4 Hz). MS (ESI) $m/z$: 501 $(M+H)^+$. HRMS (ESI) $m/z$: 499.1597 (calcd for $C_{25}H_{23}N_4O_4F_3$: 499.1593). $\alpha_D^{21.0}$+32.9 ($c$ 1.00, $CHCl_3$).
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