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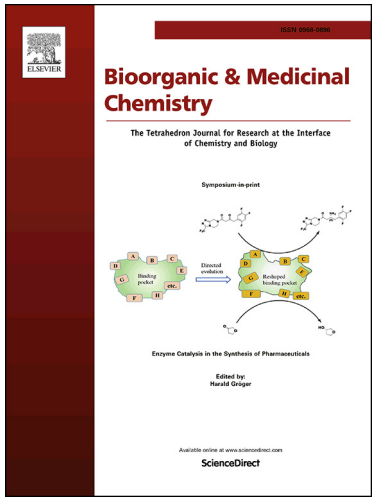

Novel Benzodiazepines Derivatives as analgesic Modulating for Transient receptor potential vanilloid 1

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PII: S0968-0896(18)31210-0
DOI: https://doi.org/10.1016/j.bmc.2018.08.002
Reference: BMC 14489

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 3 July 2018
Revised Date: 28 July 2018
Accepted Date: 1 August 2018

Please cite this article as: Liu, Y., Liao, C., Zhou, J., Liu, C., Li, Q., Jiang, Y., Qian, H., Novel Benzodiazepines Derivatives as analgesic Modulating for Transient receptor potential vanilloid 1, *Bioorganic & Medicinal Chemistry* (2018), doi: https://doi.org/10.1016/j.bmc.2018.08.002

Novel Benzodiazepines Derivatives as analgesic Modulating for Transient receptor potential vanilloid 1

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Abstract

A new series of derivatives of 3-(7-chloro-5-(2-fluorophenyl)-2-oxo-2,3-dihydro-1*H*-benzo[e][1,4]diazepin-3-yl)propanoic acid were designed and synthesized as analgesic modulating for Transient receptor potential vanilloid 1. They were investigated for TRPV1 antagonistic activity in vitro, analgesic activity and sedative activity in vivo and aqueous solubility. Preliminary studies identified 3-(7-chloro-5-(2-fluorophenyl)-2-oxo-2,3-dihydro-1*H*-benzo[e][1,4]diazepin-3-yl)-N,N-dimethylpropanamide(Compound **11**), as a potent analgesic modulating for TRPV1 with potent activity and good aqueous solubility.

Keywords: benzodiazepine, transient receptor potential vanilloid 1, analgesic, sedation

1. Introduction

Pain is an unpleasant feeling and also a global health challenge, which has strong clinical demand. In the past two decades, advancements have come in the form of quality improvement initiatives, the introduction of novel analgesics, the advent of innovative techniques and improved understanding of pain signaling[1]. Unfortunately, most analgesic drugs on the market today is saddled by some side-effects[2]. Transient receptor potential vanilloid type 1 (TRPV1) is a non-selective cation channel and found on unmyelinated C fibers in the periphery, for which many have been developed and entered clinical trials for the treatment of pain [3, 4]. TRPV1 antagonists are usually made up of three parts, including: aryl interaction head, H-bond interaction linker and lipophilic sidechain tail [5]. For example, the TRPV1 antagonist BCTC (**1**), containing the three parts described above, possesses the potent analgesic activity.

Moreover, benzodiazepines drugs, which act on γ-aminobutyric acid type A ($GABA_A$), have a strong sedative anesthesia effect and are widely used in postoperative analgesia[6] (**Fig.1**), Diazepam (**2**) is usually used to be as sedative-hypnotic medicine. And Midazolam (**3**) was optimized from Diazepam, which possesses potent sedative and anesthetic activity[7]. Furthermore, optimization of adding the side chain of methyl propionate make Remimazolam (**4**) become safer and more effective, for its metabolite having very little activity, so that Remimazolam exerts and loses efficacy quickly[8]. However, a variety of side effects including dependence, unwanted sedation and amnesia, complicating their long-term use[9-12]. Thus, there is a great need for novel, potent analgesic drugs with improved safety and tolerability.

In this context, in order to keep analgesia potency and reduce the side effects, we regard aromatic area of benzodiazepine derivatives as aryl interaction head, and γ-aminobutyric as linker, and connect them to different lipophilic sidechain by using amide bond, resulting in novel benzodiazepine derivatives as analgesic modulated for Transient receptor potential vanilloid 1.

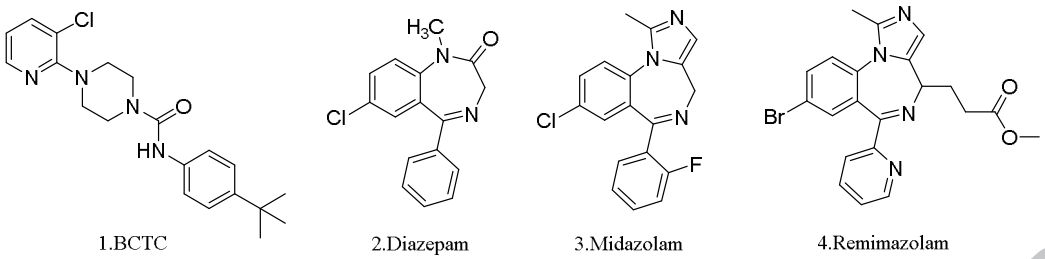


Figure 1. Structure of BCTC and benzodiazepine sedative drugs

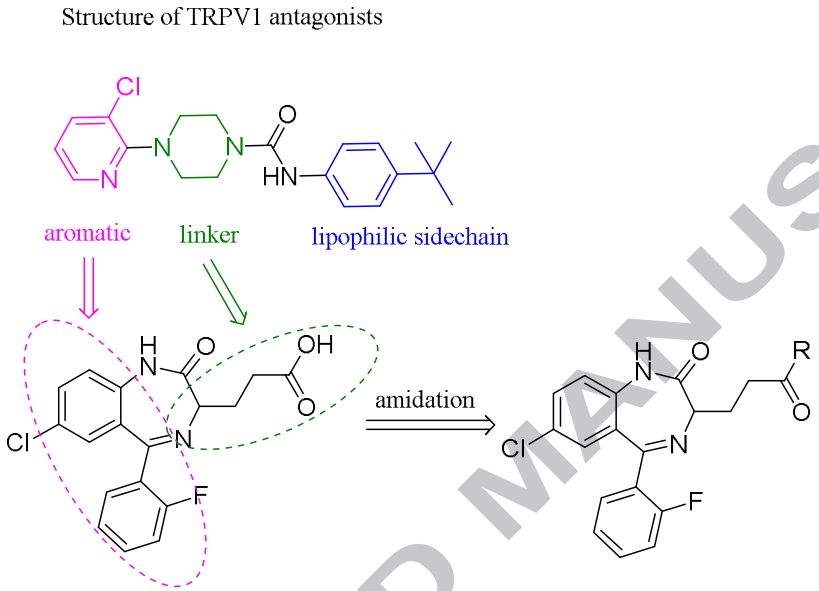


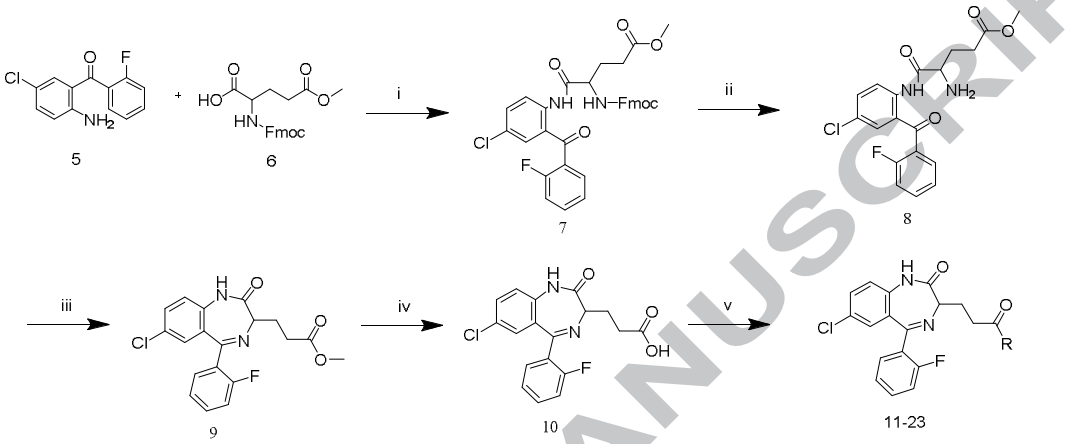
Figure 2. Modified benzodiazepines structure by amidation according to the common structure of TRPV1 antagonists

2. Result and Discussion

2.1 Chemistry

The synthetic route employed to prepare benzodiazepine derivatives **11-23** is shown in **Scheme 1**. Commercially available 2-Amino-5-chloro-2'-fluorobenzophenone reacted with Fmoc-L-Glu(OMe) under the catalysis of dicyclohexylcarbodiimide (DCC) in dichloromethane at room temperature for 4 h to produce intermediate **7**. The Fmoc protecting group of intermediate **7** then was removed by dissolving the intermediate in the mixed solvents of trimethylamine-dichloromethane, and the seven-membered ring was synthesized by dissolving in the mixed solvents of acetic acid-ethanol, producing methyl

4-amino-5-((4-chloro-2-(2-fluorobenzoyl)phenyl)amino)-5-oxopentanoate. Hydrolyzing methyl ester in THF-MeOH-H_2O with LiOH produced intermediate **10**. Compound **10** reacted with differently substituted primary amines or amino heteroaromatic compounds to produce corresponding targets **11-23** in moderate yields. Total yield range is 40 to 65%.



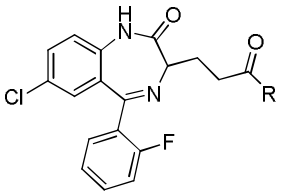
Scheme 1. Reagents and conditions: (i) DCC, DCM; (ii) 50% morpholine/DCM; (iii) 10% acetic acid/EtOH, 85°C; (iv) LiOH, THF/MeOH/H_2O=1:1:1; (v) HOBt, DIC, DCM→rt.

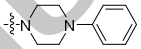
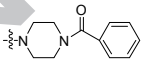
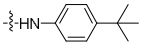
2.2 Biological studies

2.2.1 Transient receptor potential vanilloid type 1 antagonistic activity assays in vitro

In order to test the antagonistic activity of the compounds, we used TRPV1 over-expressed cells HEK-293 for vitro activity test (**Table 1**). Compound **11**, **19**, **20** and **23** has relatively good antagonistic activity against capsaicin-activated TRPV1. But the inhibition rate is lower than that of positive control BCTC, and the remaining compounds have weaker antagonistic activity. The more carbon atoms of the aliphatic chain connected at the terminal nitrogen atom, the weaker its antagonistic activity is; compound with six-membered nitrogen-containing heterocycle is more potent than one with five-membered nitrogen-containing heterocycle, and the inhibition rate significantly increases when the benzyl group is connected to the terminal nitrogen. However, as the steric hindrance between the aromatic ring and the three-carbon linker increases, the inhibition rate decreases.

Table 1. Structures and inhibitory activity against TRPV1 of target compounds **10-23**



Comp	R	hTRPV1(CAP) IC_{50}[a] (μM)	Comp	R	hTRPV1(CAP) IC_{50}[a] (μM)
BCTC		0.016	17	$-N(CH2)_5$	ND
10	OH	ND	18	morpholinyl	ND
11	$-NMe_2$	0.046	19	Thiomorpholiyl	0.033
12	$-NEt_2$	0.297	20	benzyl	0.025
13	$-NPr_2$	0.352	21		0.458
14	$-NiPr_2$	0.684	22		0.461
15	$-NnBu_2$	0.621	23		0.020
16	$-N(CH_2)_4$	ND			

[a] Human TRPV1 receptor activated by capsaicin. Unless otherwise stated, all values are the mean (SEM of at least three separate experiments). ND, not determined.

2.2.2 Analgesic activity in vivo

The TRPV1 antagonist BCTC with better analgesic activity was used as a positive control. In the capsaicin test, results showed that all the compounds can reduce the time of the mice licking paw to varying degrees, so all the compound are able to reduce the pain induced by capsaicin. Among them, compound **12**, **13**, **14**, **15** and **22** have best analgesic activities. And their analgesic activity was better than that of positive control BCTC; In abdominal constriction test, compound **11**, **12**, **17**, **19** and **23** can significantly reduce the number of abdominal constriction. Among them **11**, **12**, **17** and **23** have better analgesic activity than positive control BCTC; In the tail-flick test, compared with the blank group, except **12**, **13**, **17** and **21**, the remaining compounds can increase the mice MPE %, Compounds **11**, **15**, **16**, **18,** **19** and **20** have a significant effect of reducing the pain induced by the hot. **16**, **1**8 and **20** have better analgesic activity than BCTC.

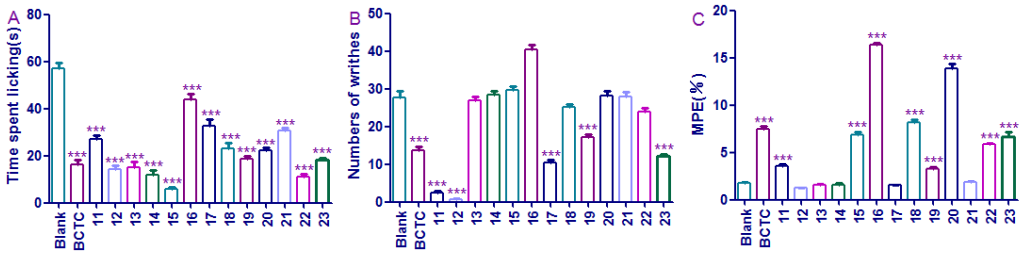


Figure 3. Antinociceptive activities of compound **11~23**. (A) The results of capsaicin test; (B) The results of abdominal constriction test; (C)The results of tail-flick test. Each bar represents the mean ± SEM (n = 6). Statistical analysis was evaluated using a one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. *p <0.05; **p <0.01; ***p <0.001 compared with the vehicle group.

2.2.3 Sedative activities screened in vivo

Table 2. Sedative activities of target compounds 11-23 in mice

Comp	Pharmacodynamics
11	5 min after the administration, the mice stopped running. They walked slowly and stayed motionless within a short time after 10 min, and after 20 min, they recover to normal gradually.
12	5 min after the administration, the mice stopped running and shook. After 8 min, they stayed still, but minor activities such as finishing hair began to appear, then they moved once every 2 min; After 15 min, they began to recover to normal.
13	After administration, the mice appeared some phenomenon such as dizziness and unstable behavior gradually, after about 19 min they recovered to normal and they did not enter a state of complete sedation.
14	After administration, the mice appeared some phenomenon such as dizziness and unstable behavior gradually, they did not enter a state of complete sedation.
15	After administration, the mice appeared some phenomenon such as dizziness and unstable behavior gradually, they did not enter a state of complete sedation.
16	6 min after administration, the mice stayed still, then they crawled once every four min. About after 12 min they recovered to normal.
17	Within 2 min after administration, the mice began to stay still, they completely recovered to normal after about 13 min
18	Within 5 min after administration, the mice stayed still, they crawled once every 4.5 min, after about 12 min they recovered to normal.
19	Within 5 min after administration, the mice began to stay still. The mice appeared some minor movements and then stayed still again, after about 10 min they began to run and jump and recovered to normal basically.
20	No significant effect.
21	No significant effect.

22	No significant effect.
23	5 min after the administration, the mice stopped running. They walked slowly and stayed motionless within a short time after 8 min, and after 18 min, they recovered to normal gradually.
triazolam	Within 10 s after administration, it entered a state of sedation with weakness and disability. After 8 min, there was an activity phenomenon. Minor activities such as finishing hairs began to appear, but they could not move around. After 44 min, they basically recovered and appeared normal behaviors such as running and jumping.

2.2. 4 Aqueous solubility test

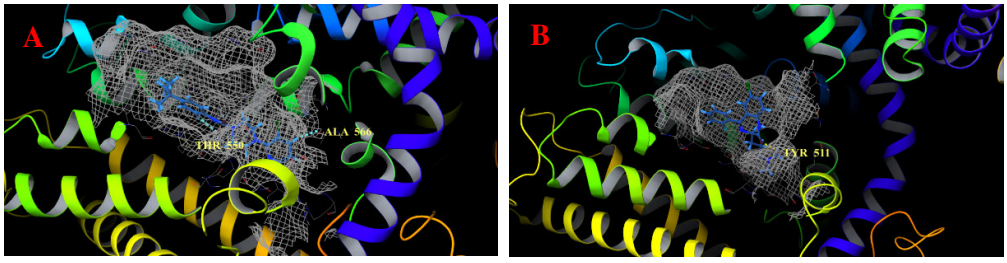
Data in the **Table 3** displays that expect for compound **16**, **19** and **22**, aqueous solubility of other compounds has improved to varying degrees compared to BCTC.

Table 3 Aqueous solubility of compound **11-23**

Comp	Solubility(mg/mL)	Comp	Solubility(mg/mL)
11	1.78	17	0.66
12	1.16	18	0.63
13	2.93	19	0.20
14	2.74	20	1.70
15	6.35	21	1.89
16	0.20	22	0.30
BCTC	0.60	23	1.64

2.3 Docking results

Docking experiments have been conducted to study structure-activity relationships of target compounds (Figure 4). Results show that both BCTC and Compound **11** occupied the cavity of the TRPV1 ion channel. BCTC molecular has aromatic H-bond interactions with the Key residue ALA 566 and THR 550. Compound **11** with designed novel structures has H-bond interaction with residue TYR 511, elucidating that designed structure change is of significance. Comparing compound **15** with **11**, compound with longer carbon chain may show better affinity. Actually compound with heterocycle attached to terminal nitrogen (Compound **18**) may bind relatively weakly with the receptor.



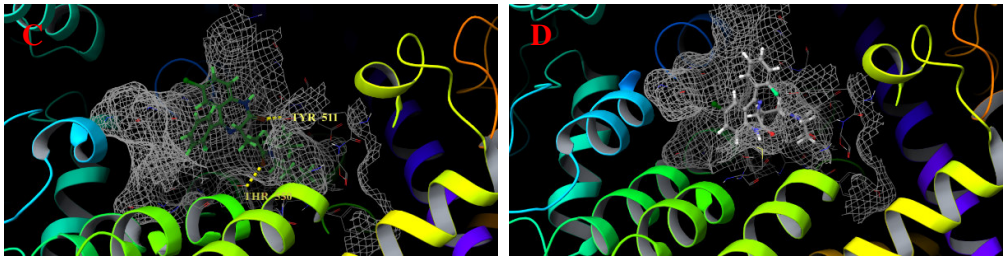


Figure 4 (A) Structure docking results with TRPV1 for BCTC;(B) Structure docking results with TRPV1 for **11**;(C) Structure docking results with TRPV1 for **15**;(D) Structure docking results with TRPV1 for **18**

3. Conclusions

Designed and synthesized benzodiazepines derivatives as novel TRPV1 antagonists were studied on TRPV1 antagonistic activity in vitro, analgesic activity in vivo and sedative activity in vivo. Their antagonistic activity against TRPV1 is basically consistent with their analgesic activity in vivo. The longer the carbon chain on the terminal nitrogen is, the stronger the analgesic effect of the compounds is in capsaicin-induced Tail-flick test (**15** > **14** > **13** > **11**). When the nitrogen-containing heterocycle and aromatic ring are attached to the terminal nitrogen, the analgesic activity in this model would be reduced to some extent. However, such law was not found in abdominal constriction test and capsaicin test. Compound **11** and **19** have significant activity in all three analgesia models in vivo. When a saturated aliphatic chain and a five-membered or six-membered nitrogen-containing heterocycle is connected to the terminal nitrogen, the compounds have a certain degree of sedation. But compounds with unsaturated aromatic group connected to the terminal nitrogen have little sedative activity. It is speculated that the steric hindrance of the terminal aromatic ring hinders the combination of the compound and the benzodiazepine receptor and then the sedative effect disappears. In addition, compounds **11-15** with saturated aliphatic chains linked to the terminal nitrogen atom have good aqueous solubility. Based on the results above, compound **11** was found possessing strong analgesic, certain sedative activity and good aqueous solubility, providing the potential development prospects.

4. Experimental

4.1 General

All reagents were purchased from Shanghai Chemical Reagent Company. Column chromatography (CC): silica gel 60 (100-200 mesh). Thin-layer chromatography (TLC): silica gel 60 F254 plates (250 mm; Qingdao Ocean Chemical Company, Qingdao, China). M.p.: capillary tube; uncorrected. IR spectra: Shimadzu FTIR-8400S spectrophotometer; in cm^{-1}. ^1H NMR spectra: Bruker ACF-300Q apparatus at 300 MHz, in DMSO or $CDCl_3$ unless otherwise indicated; δ in ppm rel. to Me_4Si, *J* in Hz. ^{13}C NMR spectra: Bruker ACF-300Q apparatus at 75 MHz; δ in ppm rel. to Me_4Si, *J* in Hz. Mass spectrometry (MS): Waters UPLC/MS/MS: ACQUITY UPLC/TQD, in m/z; Elemental analyses: CHN-O-Rapid instrument; %Purity of the target compounds (> 97%) were determined by HPLC analysis (UV detector, wavelength: 254 nm).

4.1.1 Synthesis of 3-(7-chloro-5-(2-fluorophenyl)-2-oxo-2,3-dihydro-1H-benzo [e][1,4]diazepin-3-yl)propanoic acid (9)

4.1.1.1 The preparation of 7

(2-amino-5-chlorophenyl)(2-fluorophenyl)methanone(4.0 mmol)and Fmoc-L-Glu(OMe) (4.8 mmol) were dissolved into 10 mL dichloromethane, adding 1,3-dicyclohexylcarbodiimide (4.8mmol) into stirred solution. The reaction mixture was stirred at room temperature for 2 h. The mixture was filtered through celite and washed with ethyl acetate. The combined organic layers were washed with water and brine, then dried over anhydrous Na_2SO_4 and concentrated. The residue was recrystallized by the mixture of petroleum/ethyl acetate (4:1). Compound **7** was obtained and the yield was 56.87%. ^1H NMR (300 MHz, DMSO-d_6) δppm: 9.66 (s, 1H, NH), 8.02-7.58 (m, 9H, Ar-H), 7.41-7.29 (m, 6H, Ar-H), 4.76 (d, *J* = 6.7 Hz, 2H, CH_2), 4.57- 4.49 (m, 2H, CH), 3.78 (s, 3H, CH_3), 2.45-2.27 (m, 4H, CH_2).

4.1.1.2 The preparation of 9

Compound **7** was dissolved into the mixed solution of morpholine and dichloromethane (1:1). The reaction mixture was stirred at room temperature for 4 h. The reaction mixture was monitored by TLC and concentrated in vacuo. Then the concentrated product was dissolved into ethanol with 10% acetic acid. The reaction mixture was stirred at 85°C for 2 h. The reaction mixture was monitored by TLC and concentrated in vacuo. The residue was

recrystallized by the mixture of petroleum/ethyl acetate (1:1). Compound **9** was obtained and the yield was 60.32%. ^{1}H NMR (300 MHz, DMSO-d_6) δppm: 9.80 (s, 1H, NH), 8.14-7.71 (m, 4H, Ar-H), 7.59-7.33 (m, 3H, Ar-H), 4.12 (t, J = 8.0 Hz, 1H, CH), 3.68 (s, 3H, CH_3), 2.53-2.30 (m, 4H, CH_2).

4.1.1.3 The preparation of 10

Compound **9** (0.5 mmol, 1 eq) was dissolved into the mixed solution of THF/MeOH/H_2O (2:1:1). Adding LiOH (1.5 mmol, 3 eq). The reaction mixture was stirred at room temperature for 2 h. The mixture was added to ice water. The pH was adjusted by the solution of 2 N HCl to 4-5. The mixture was cooled overnight and filtered. Compound **10** was obtained and the yield was 93.58%. ^{1}H NMR (300 MHz, DMSO-d_6) δppm: 10.80 (s, 1H, OH), 9.83 (s, 1H, NH), 8.16-7.88 (m, 4H, Ar-H), 7.71-7.43 (m, 3H, Ar-H), 4.16 (t, J = 8.1 Hz, 1H, CH), 2.58-2.34 (m, 4H, CH_2).

4.1.2 Preparation of derivatives 11-23

Compound **10** (0.8 mmol, 1 eq) was dissolved into dichloromethane (5 mL) , then HOBt (1.20 mmol, 1.5 eq) , DIC (1.20 mmol, 1.5 eq) and nitrogen substituent (0.8 mmol, 1 eq) were added into the solution in turn, the mixture was stirred at room temperature for 2 h and monitored by TLC. The reaction mixture was washed with saturated brine(15 mL) and extracted with ethyl acetate (15 mL x 2). The organic layers were combined and dried by anhydrous sodium sulfate. The organic layer was concentrated in vacuo. The residue was purified by flash column -chromatography on silica gel using ethyl petroleum/ethyl (2:1) as eluent to obtain **11-23**.

4.1.2.1 3-(7-chloro-5-(2-fluorophenyl)-2-oxo-2,3-dihydro-1H-benzo[e][1,4]diazepin-3-yl)-N,N-dimethylpropanamide (11)

Yield 40.59%, white solid, m.p.104-106°C; ^{1}H NMR (300 MHz, DMSO-d_6) δppm: 10.69 (s, 1H, NH), 7.91 – 7.49 (m, 3H, Ar-H), 7.49 – 7.18 (m, 3H, Ar-H), 7.13 (s, 1H, Ar-H), 5.53 (d, J = 5.9 Hz, 1H, CH), 3.48 (d, J = 84.0 Hz, 2H, CH_2), 2.98 (s, 3H, CH_3), 2.80 (s, 3H, CH_3), 2.25 (d, J = 5.4 Hz, 2H, CH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δppm: 172.33, 170.37, 164.00, 137.65, 132.06, 129.51, 128.73, 127.32, 125.09, 123.61, 116.62, 62.97, 37.13, 33.80, 27.12,

24.90; ESI-MS m/z: 386.21 ([M+H]$^-$). Anal. Calcd for $C_{20}H_{19}ClFN_3O_2$: C, 61.94; H, 4.94; N, 10.83. Found: C, 61.95; H, 4.96; N,10.84.

4.1.2.2 3-(7-chloro-5-(2-fluorophenyl)-2-oxo-2,3-dihydro-1H-benzo[e][1,4]diazepin-3-yl) -N,N-diethylpropanamide (12)

Yield 65.26%, white solid, m.p.107-108°C; ^{1}H NMR (300 MHz, DMSO-d_6) δppm: 10.70 (s, 1H, NH), 7.70-7.48 (m, 3H, Ar-H), 7.48-7.19 (m, 3H, Ar-H), 7.13 (s, 1H, Ar-H), 5.54 (s, 1H, CH), 3.47 (m, 4H, 2CH_2), 2.26 (s, 2H, CH_2), 1.83-1.57 (m, 2H, CH_2), 1.11 (s, 3H, CH_3), 0.98 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δppm: 171.41, 170.38, 137.69, 132.89, 132.06, 128.71, 127.32, 123.60, 116.35, 92.24, 62.96, 33.82, 28.88, 27.49, 24.90, 14.72, 13.52; ESI-MS m/z: 414.24 ([M+H]$^-$). Anal. Calcd for $C_{22}H_{23}ClFN_3O_2$: C, 63.54; H, 5.57; N, 10.10. Found: C, 63.55; H, 5.56; N,10.11.

4.1.2.3 3-(7-chloro-5-(2-fluorophenyl)-2-oxo-2,3-dihydro-1H-benzo[e][1,4]diazepin-3-yl) -N,N-dipropylpropanamide (13)

Yield 45.16%, white solid, m.p.108-110°C; ^{1}H NMR (300 MHz, $CDCl_3$-d_6) δppm: 9.90 (s, 1H, NH), 7.60-7.33 (m, 3H, Ar-H), 7.30-6.96 (m, 4H, Ar-H), 4.64 (d, J = 5.6 Hz, 1H, CH), 3.27 (s, 4H, CH_2), 2.91-2.36 (m, 4H, CH_2), 1.79-1.37 (m, 4H, CH_2), 0.88 (m, 6H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δppm: 172.13, 170.40, 164.20, 157.53, 137.46, 133.03, 132.09, 129.49, 128.59, 127.46, 127.12, 125.10, 123.61, 62.70, 49.24, 40.04, 28.80, 23.65, 22.28, 20.92, 11.53, 11.37; ESI-MS m/z: 422.31 ([M+H]$^-$). Anal. Calcd for $C_{24}H_{27}ClFN_3O_2$: C, 64.93; H, 6.13; N, 9.47. Found: C, 64.94; H, 6.16; N,9.48.

4.1.2.4 3-(7-chloro-5-(2-fluorophenyl)-2-oxo-2,3-dihydro-1H-benzo[e][1,4]diazepin-3 -yl)-N,N-diisopropylpropanamide (14)

Yield 50.06% , white solid, m.p.109-112°C; ^{1}H NMR (300 MHz, DMSO-d_6) δppm: 9.80 (m, 1H, NH), 7.74 (m, 2H, Ar-H), 7.48-7.30 (m, 4H, Ar-H), 7.10 – 6.80 (m, 1H, Ar-H), 4.15 (s, 1H, CH), 3.92-3.36 (m, 2H, CH_2), 2.61 (d, J = 6.6 Hz, 2H, CH_2), 2.55-2.29 (m, 2H, CH), 1.57 -1.12 (m, 12H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δppm: 172.50, 171.22, 136.17, 135.16,

132.07, 131.62, 129.20, 126.55, 125.84, 124.32, 122.83, 122.52, 117.51, 116.18, 110.94, 62.54, 45.89, 42.42, 31.34, 29.70, 29.36, 23.30, 20.92, 20.55; ESI-MS m/z: 442.27 ($[M+H]^-$). Anal. Calcd for $C_{24}H_{27}ClFN_3O_2$: C, 64.93; H, 6.13; N, 9.47. Found: C, 64.93; H, 6.15; N,9.48.

4.1.2.5 N,N-dibutyl-3-(7-chloro-5-(2-fluorophenyl)-2-oxo-2,3-dihydro-1H-benzo[e][1,4] diazepin-3-yl)propanamide (15)

Yield 45.76% , white solid, m.p.112-113°C; ^{1}H NMR (300 MHz, DMSO-d_6) δppm: δ 9.12 (s, 1H, NH), 7.45 (m, 3H, Ar-H), 7.13 (m, 4H, Ar-H), 6.34 (s, 1H, CH), 4.34 (s, 4H, CH_2), 3.29 (s, 4H, CH_2), 2.68-2.45 (m, 4H, CH_2), 1.36-1.23 (m, 10H, CH_2, CH_3).^{13}C NMR (75 MHz, $CDCl_3$-d_6) δppm:172.21, 157.16, 151.80, 131.59, 129.53, 122.65, 109.96, 59.22, 52.38, 42.07, 35.22, 29.01, 28.92, 25.68, 24.84, 23.49, 20.17, 13.88; ESI-MS m/z: 470.34 ($[M+H]^-$). Anal. Calcd for $C_{26}H_{31}ClFN_3O_2$: C, 66.16; H, 6.62; N, 8.90. Found: C, 66.17; H, 6.62; N,8.91.

4.1.2.6 7-chloro-5-(2-fluorophenyl)-3-(3-oxo-3-(pyrrolidin-1-yl)propyl)-1,3-dihydro-2H -benzo[e][1,4]diazepin-2-one (16)

Yield 56.26% , white solid, m.p.107-109°C; ^{1}H NMR (300 MHz, DMSO-d_6) δppm: 10.69 (s, 1H, NH), 7.66-7.48 (m, 3H, Ar-H), 7.47-7.18 (m, 3H, Ar-H), 7.13 (s, 1H, Ar-H), 4.01 (s, 1H, CH), 3.51 (m, 4H, $2CH_2$), 2.46 (d, J = 7.9 Hz, 2H, CH_2), 2.26 (d, J = 6.3 Hz, 2H, CH_2), 1.86-1.70 (m, 4H, $2CH_2$); ^{13}C NMR (75 MHz, DMSO-d_6) δppm: 174.59, 170.64, 166.79, 157.05, 138.64, 138.07, 132.34, 132.09, 132.02, 130.23, 129.21, 128.50, 127.88, 127.20, 123.55, 62.83, 55.35, 50.51, 30.41, 26.10, 25.88, 24.42; ESI-MS m/z: 428.20 ($[M+H]^-$). Anal. Calcd for $C_{22}H_{21}ClFN_3O_2$:C, 63.85; H, 5.11; N, 10.15. Found: C, 63.85; H, 5.12; N,10.16.

4.1.2.7 7-chloro-5-(2-fluorophenyl)-3-(3-oxo-3-(piperidin-1-yl)propyl)-1,3-dihydro-2H -benzo[e][1,4]diazepin-2-one (17)

Yield 65.20%, white solid, m.p.101-103°C ; ^{1}H NMR (300 MHz, DMSO-d_6) δppm: 10.69 (s, 1H, NH), 7.85-7.47 (m, 3H, Ar-H), 7.31 (dd, J = 23.8, 8.1 Hz, 3H, Ar-H), 7.13 (s, 1H, Ar-H), 5.53 (d, J = 7.1 Hz, 1H, CH), 3.42 (s, 4H, $2CH_2$), 3.19 (d, J = 4.3 Hz, 2H, CH_2), 2.24 (d, J = 6.9 Hz, 2H, CH_2), 1.67-1.44 (m, 6H, $3CH_2$); ^{13}C NMR (75 MHz, DMSO-d_6) δppm: 170.71,

170.58, 170.40, 164.04, 161.88, 158.57, 137.59, 132.96, 132.10, 129.50, 128.71, 127.33, 125.16, 123.61, 116.64, 116.36, 62.89, 46.34, 45.67, 30.38, 29.09, 26.08, 25.79, 24.41; ESI-MS m/z: 426.25 ([M+H]-). Anal. Calcd for $C_{23}H_{23}ClFN_3O_2$: C, 64.56; H, 5.42; N, 9.82. Found: C, 64.57; H, 5.44; N,9.82.

4.1.2.8 7-chloro-5-(2-fluorophenyl)-3-(3-morpholino-3-oxopropyl)-1,3-dihydro-2H-benzo[e][1,4]diazepin-2-one (18)

Yield 55.74%, white solid, m.p.101-102°C; ^{1}H NMR (300 MHz, DMSO-d_6) δppm: 10.69 (s, 1H, NH), 7.59 (s, 4H, Ar-H), 7.29 (dt, J = 21.0, 9.7 Hz, 3H, Ar-H), 7.11 (s, 1H, Ar-H), 5.55 (d, J = 7.1 Hz, 1H, CH), 3.60 (s, 8H, 4CH_2), 2.52 (s, 2H, CH_2), 2.25 (d, J = 6.7 Hz, 2H, CH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δppm: 171.36, 170.38, 164.13, 137.48, 132.14, 129.53, 128.69, 127.40, 125.17, 123.63, 66.55, 62.85, 45.85, 41.90, 28.90, 27.09; ESI-MS m/z: 428.20 ([M+H]-). Anal. Calcd for $C_{22}H_{21}ClFN_3O_3$: C, 61.47; H, 4.92; N, 9.78. Found: C, 61.48; H, 4.94; N,9.80.

4.1.2.9 7-chloro-5-(2-fluorophenyl)-3-(3-oxo-3-thiomorpholinopropyl)-1,3-dihydro-2H -benzo[e][1,4]diazepin-2-one (19)

Yield 60.15%, white solid, m.p.103-105°C; ^{1}H NMR (300 MHz, DMSO-d_6) δppm: 10.70 (s, 1H, NH), 8.30-7.48 (m, 4H, Ar-H), 7.30 (dt, J = 21.0, 9.8 Hz, 3H, Ar-H), 7.13 (s, 1H, Ar-H), 5.53 (d, J = 7.0 Hz, 1H, CH), 3.72 (s, 4H, 2CH_2), 3.54 (m, 4H, 2CH_2), 2.59 (s, 2H, CH_2), 2.26 (d, J = 7.0 Hz, 2H, CH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δppm: 171.31, 170.35, 157.18, 137.59, 132.90, 132.15, 129.52, 128.70, 127.35, 125.12, 123.63, 116.49, 66.58, 62.93, 48.02, 33.79, 28.94, 25.79, 24.90; ESI-MS m/z: 444.18 ([M+H]-). Anal. Calcd for $C_{22}H_{21}ClFN_3O_2S$: C, 59.26; H, 4.75; N, 9.42. Found: C, 59.28; H, 4.76; N,9.42.

4.1.2.10 3-(7-chloro-5-(2-fluorophenyl)-2-oxo-2,3-dihydro-1H-benzo[e][1,4]diazepin-3-yl) -N-phenylpropanamide (20)

Yield 49.85%, white solid, m.p.105-107°C; ^{1}H NMR (300 MHz, DMSO-d_6) δppm: 9.80 (s, 1H, NH), 7.78 (s, 1H, Ar-H), 7.34 (dt, J = 17.4, 16.4 Hz, 4H, Ar-H), 7.12-6.60 (m, 5H, Ar-H), 6.59-6.08 (m, 3H, Ar-H), 4.48 (d, J = 7.5 Hz, 1H, CH), 2.17 (d, J = 14.4 Hz, 2H, CH_2), 1.97

(d, J = 14.0 Hz, 2H, CH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δppm: 173.42, 169.12, 161.60, 158.34, 144.76, 137.81, 132.15, 131.51, 131.08, 129.99, 128.56, 128.29, 125.69, 124.23, 120.20, 119.47, 116.63, 116.33, 78.17, 30.91, 24.91; ESI-MS m/z: 434.24 ([M+H]-). Anal. Calcd for $C_{24}H_{19}ClFN_3O_2$: C, 66.13; H, 4.39; N, 9.64. Found: C, 66.12; H, 4.39; N,9.63.

4.1.2.11 7-chloro-5-(2-fluorophenyl)-3-(3-oxo-3-(4-phenylpiperazin-1-yl)propyl)-1,3 -dihydro-2H-benzo[e][1,4]diazepin-2-one (21)

Yield 56.65%, white solid, m.p.110-112°C;^1H NMR (300 MHz, DMSO-d_6) δppm: 10.71 (s, 1H, NH), 7.58 (d, J = 7.6 Hz, 3H, Ar-H), 7.49-7.07 (m, 7H, Ar-H), 6.88 (m, 4H, Ar-H), 4.03 (s, 1H, CH), 3.62 (s, 4H, piperazine), 3.14 (s, 4H, piperazine), 2.60 (s, 2H, CH_2), 2.29 (d, J = 6.3 Hz, 2H, CH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δppm: 171.04, 170.39, 170.06, 164.07, 157.05, 151.29, 137.60, 133.00, 132.20, 129.45, 128.74, 127.34, 125.16, 123.63, 119.78, 116.32, 62.90, 55.38, 49.20, 48.83, 45.18, 29.07, 27.25; ESI-MS m/z: 503.32 ([M+H]-). Anal. Calcd for $C_{28}H_{26}ClFN_4O_2$: C, 66.60; H, 5.19; N, 11.09. Found: C, 66.61; H, 5.19; N,11.10.

4.1.2.12 3-(3-(4-benzoylpiperazin-1-yl)-3-oxopropyl)-7-chloro-5-(2-fluorophenyl)-1,3 -dihydro-2H-benzo[e][1,4]diazepin-2-one (22)

Yield 40.36%, white solid, m.p.112-113°C;^1H NMR (300 MHz, DMSO-d_6) δppm: 10.69 (s, 1H, NH), 7.57 (d, J = 8.6 Hz, 4H, Ar-H), 7.43 (d, J = 11.3 Hz, 6H, Ar-H), 7.27 (d, J = 7.9 Hz, 3H, Ar-H), 7.11 (s, 1H, Ar-H), 4.03 (d, J = 5.9 Hz, 1H, CH), 3.61 (s, 2H, CH_2), 3.53 (d, J = 35.9 Hz, piperazine), 2.26 (d, J = 6.4 Hz, 2H, CH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δppm: 171.51, 170.34, 169.87, 164.13, 158.56, 137.53, 136.11, 132.95, 132.10, 130.10, 129.54, 128.79, 127.39, 125.15, 123.64, 116.31, 62.93, 29.16, 27.14; ESI-MS m/z: 531.35 ([M+H]-). Anal. Calcd for $C_{29}H_{26}ClFN_4O_3$: C, 65.35; H, 4.92; N, 10.51; O, 9.01. Found: C, 65.36; H, 4.93; N,10.52.

4.1.2.13 N-(4-(tert-butyl)phenyl)-4-(7-chloro-5-(2-fluorophenyl)-2-oxo-2,3-dihydro -1H-benzo[e][1,4]diazepin-3-yl)butanamide (23)

Yield 40.22%, white solid, m.p.108-111°C; ^1H NMR (300 MHz, DMSO-d_6) δppm: 10.45 (s, 1H, NH), 7.67 (d, J = 8.6 Hz, 4H, Ar-H), 7.43 (d, J = 7.9 Hz, 3H, Ar-H), 7.27-7.39 (m, 6H,

Ar-H), 4.14 (d, J = 5.9 Hz, 1H, CH), 2.37 (m, 2H, CH_2), 2.10 (t, J = 6.4 Hz, 2H, CH_2), 1.33(s, 9 H,3CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δppm: 179.77, 172.05, 168.64, 163.25, 146.85, 136.36, 135.54, 132.58, 131.24, 130.02, 129.50, 128.54, 128.16, 127.88, 127.87, 125.26, 124.48, 121.23, 121.14, 115.58, 64.88, 37.88, 37.62, 34.18, 31.34, 20.03. ESI-MS m/z: 491.18 ([M+H]$^-$). Anal. Calcd for $C_{28}H_{27}ClFN_3O_2$: C, 68.36; H, 5.53; N, 8.54; O, 9.01. Found: C, 68.36; H, 5.52; N, 8.52.

4.2 Pharmacology

4.2.1 Transient receptor potential vanilloid type 1 antagonistic activity assays in vitro

The TRPV1 aequorin cells (PerkinElmer, USA) were collected from culture plates with Ca^{2+} and Mg^{2+}-free phosphatebuffered saline supplemented with 5 mM ethylenediaminetetra-acetic acid; pelleted for 2 min at 1000g; resuspended in Dulbecco's minimum essential medium-F12 medium with 15 mM HEPES (pH 7.0) and 0.1% BSA (assay buffer) at a density of 3×10^5 cells/mL; incubated for 4 h in the dark in the presence of 5 mM coelenterazine h (Promega, USA). After loading, cells were diluted with assay buffer to a concentration of 5×10^6 cells/mL. Twenty microliters of cells were injected over 20 mL of the sample solution plated on 384-well plates, respectively, unless otherwise indicated. The digitonin, ATP (Sigma–Aldrich, USA) assay buffer was added in the blank control wells for reference, and final concentrations of digitonin and ATP were 100 mM and 50 mM. The sample solution and the cells were incubated for 2.5 min before adding agonist capsaicin (Tocris, England), then immediately detected. The light emission was recorded during variable times using EnVision 2014 Multilabel Reader (PerkinElmer, USA)[13].

4.2.2 Analgesic activity in vivo

Capsaicin test: The capsaicin test was assessed as previously described. License number Following an acclimation period of 30 min, 20 μL of solution of capsaicin (0.8 μg/mL) was injected subcutaneously into the dorsal aspect of the right hind paw[14]. The mouse was then placed in an individual cage. Mice were observed for a continuous period of 5 min. The amount of time spent licking the injected paw was measured and expressed as the cumulative licking time during the 5 min observation period.

Abdominal constriction test: Abdominal constriction test was performed as described

previously. Mice were placed in individual glass cylinders for a 30 min acclimatization period[15]. The writhes were induced by injection with 0.6% acetic acid (0.1 mL/10 g/mouse i.p.), and immediately placed inside transparent glass cylinders. The number of muscular contractions was counted for 15 min after acetic acid injection.

Tail-flick test: Tail-flick test was performed as described previously. In the experiment evaluating the time course of antinociception, we elected to use the warm water tail withdrawal test in order to minimize damage to the tail because of the repeated testing[15]. In brief, the distal one-third of the tail was immersed into 52.0°C water and latency to withdrawal the tail was recorded before and after drug treatment. The antinociception response was presented as percent maximal possible effect (%MPE) as defined by percent maximal possible effect=100%×(drug response time - basal response time)/(cut-off time - basal response time). To avoid tissue damage, the cut-off time was suitable for 12 s.

License number for test animals: NO.201811021.All animal experimental protocols were approved by the ethical committee at China Pharmaceutical University and conducted according to the Laboratory Animal Management Regulations in China and adhered to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication NO. 85-23, revised 2011).

4.2.3 Sedative activities screened in vivo

Healthy male BALB/c mice weighting 16-20 g and were purchased from laboratory animal center of Nanjing Qinglongshan. Mice were kept at 24 ± 1°C and 55 ± 10% humidity, with 12 h of light (artificial illumination; 08:00-20:00) for 1 week. The test compound was administered intravenously to animals through tail veins, while those animals were placed in plastic restrainers[16]. When onset of loss of righting reflex was recorded, the animals were immediately removed from the restrainers. The loss of ability to right itself from supine positions was defined as LRR. Once an animal was able to right itself for the first time, it was placed on its back once again. If they recovered itself consecutively two more times back to normal, the animals actions were labeled as recovered from LRR. Each compound, including positive controls, was prepared in a 0.9% wt/vol saline solution. A dose of 9.0 mg/kg b.w. was administered by rapid bolus via the lateral tail vein to six mice.

4.2.4 Aqueous solubility test

HPLC chromatographic conditions: Column: 4.6×150mm, Inertsil ODS-SP; Mobile phase: CH_3CN/H_2O; Detection wavelength: 254 nm; Flow rate: 1 mL/min; Detector: Photo-Diode Array; Column temperature: 40°C; Injection volume: 10 μL ; Run time：20 min.

Establishment of a standard curve: Weigh compounds accurately, dissolve the compound in the 100 mL volumetric flask and add distilled water to the scale. Respectively take 0.5 mL, 1 mL, 1.5 mL, 2 mL and 2.5 mL from solution prepared above into 10 mL centrifuge tubes and add distilled water to the scale. Each solution was measured in parallel for three times by HPLC. Calculate linear regression equation.

Preparation of compounds' saturated solution: Weigh excess compounds and dissolve in distilled water, shake on the shaker for 72 h in 37°C. Centrifuge then take the supernatant. Analyze the supernatant by HPLC and then calculate the solubility according to linear regression equation of standard curve.

4.3 Docking experiments

Receptor protein (PDB ID:5IS0) was prepared and optimized using relevant module by Schrodinger software. Receptor Grid Generation Module was used to generate docking area. Structure of ligands were drawn by ChemOffice2014 software and prepared using LigPrep module. In the end, molecular docking between ligands and receptor was conducted with extra precision.

Acknowledgments

This study was supported by Scientific and Technological Research Program of Chongqing and Municipal Education Commission (No. KJ1702012) and National Natural Science Foundation of China (No.81673299).

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Novel Benzodiazepines Derivatives as analgesic Modulating for Transient receptor potential vanilloid 1

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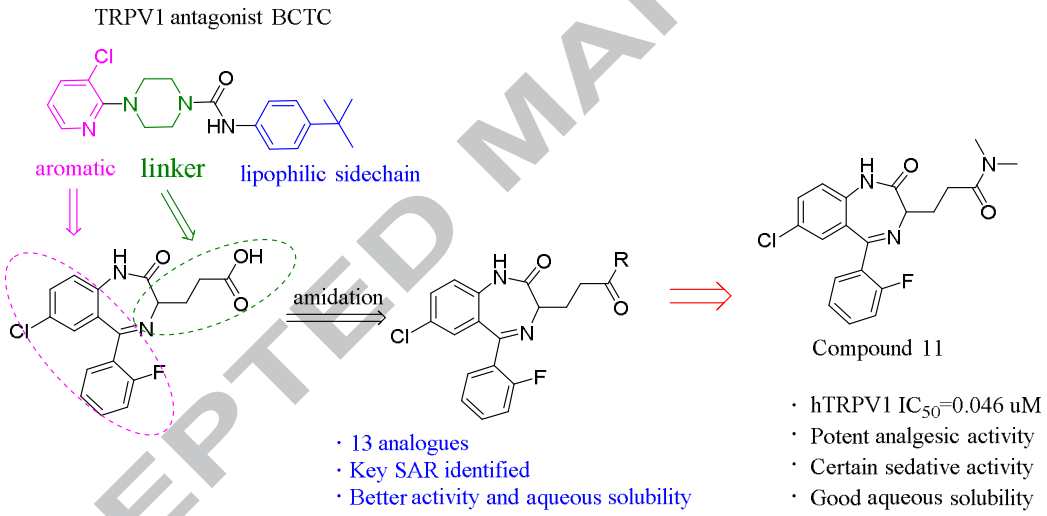

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