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Synthesis of Benzoazepine Derivatives via Azide Rearrangement and Evaluation of Their Antianxiety Activities

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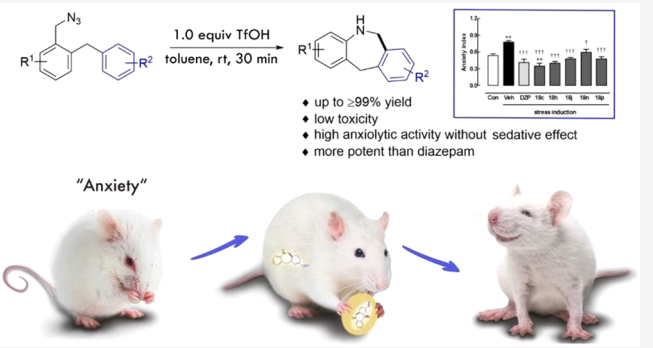
ABSTRACT: A new synthetic method for the construction of benzoazepine analogues has been developed employing *ortho*-arylmethylbenzyl azide derivatives as precursors using an azide rearrangement reaction. In this work, 14 benzoazepine compounds were successfully synthesized in moderate to excellent yields. All synthetic benzoazepines were evaluated for their cytotoxicity against normal human kidney cell line (HEK cell). The results showed that compound **18c** had the lowest cytotoxicity (IC_{50} = 65.68 μM) among tested compounds, which was comparable with the antianxiety drug diazepam (IC_{50} = 87.90 μM). Based on the cytotoxicity results, five benzoazepine analogues (compounds **18c**, **18h**, **18j**, **18n**, and **18p**) were selected to determine the antianxiety effect on stressed rats using elevated plus maze (EPM) and open field test (OFT) methods. Interestingly, compound **18c** showed better anxiolytic activity than diazepam without a sedative effect by showing superior hyperlocomotor activity. Therefore, this discovery could pave the way for drug development to treat patients with anxiety disorder.



KEYWORDS: *Benzoazepine, Azide, Antianxiety, Diazepam*

The impact of rapid growth of technology and social media has changed human behavior in both positive and negative manners.[1,2] The negative effects can lead to mental health problems such as major depressive disorder and dysthymic disorder.[3] In addition, genetics is another factor of this symptom. Anxiety disorder is a type of psychiatric disorder involving an excessive behavioral response to stress.[4] This symptom can affect job duties, career path, relationship among friends, and family. Nowadays, the prevalence of this disorder has increased every year and could contribute to depression and suicide.[5] Due to these negative effects, patients need behavioral therapy and/or medical treatment. However, patients have different responsiveness to the drug treatment. Therefore, a variety of drugs is needed for the treatment of these symptoms.[6] The benzoazepine core structure is a well-known pharmacophore that is present in several mood disorder drugs,[7,8] especially in the class of mood-stabilizing drugs such as imipramine, diazepam, and lorazepam.[9,10] In addition, this core structure shows various biological activities associated with several commercial drugs. For example, this core structure is present in commercial anticancer drugs such as olanzapine, rucaparib, clozapine, anthramycin, and tomaymycin.[11−14] Furthermore, the benzoazepine motif plays key roles in other biological activities including antibacterial,[15] anticonvulsant,[16,17] anti-HIV-1,[18,19] and antihypertension properties[20] (Figure 1).

As a result of its prominent biological activities, the benzoazepine core structure has a high potential to be used in drug design to develop novel pharmaceutical agents. According to our research interest in azide chemistry,[21−27] we envision that benzoazepine could be prepared using azide as the nitrogen source. In addition, azide was employed in a broad range of reactions for the synthesis of *N*-containing compounds, including *N*-methylarylamines, indoloquinolines, quinolines, tetrahydroquinolines, and phenanthridines.[21−27] Our research group has been interested in azide rearrangement chemistry to develop new methods for the synthesis of biologically active compounds. Benzyl azide derivatives were employed as a common precursor for the generation of an iminium ion intermediate, which could react with a variety of nucleophiles either in an intramolecular or intermolecular reaction to provide the corresponding *N*-containing com-

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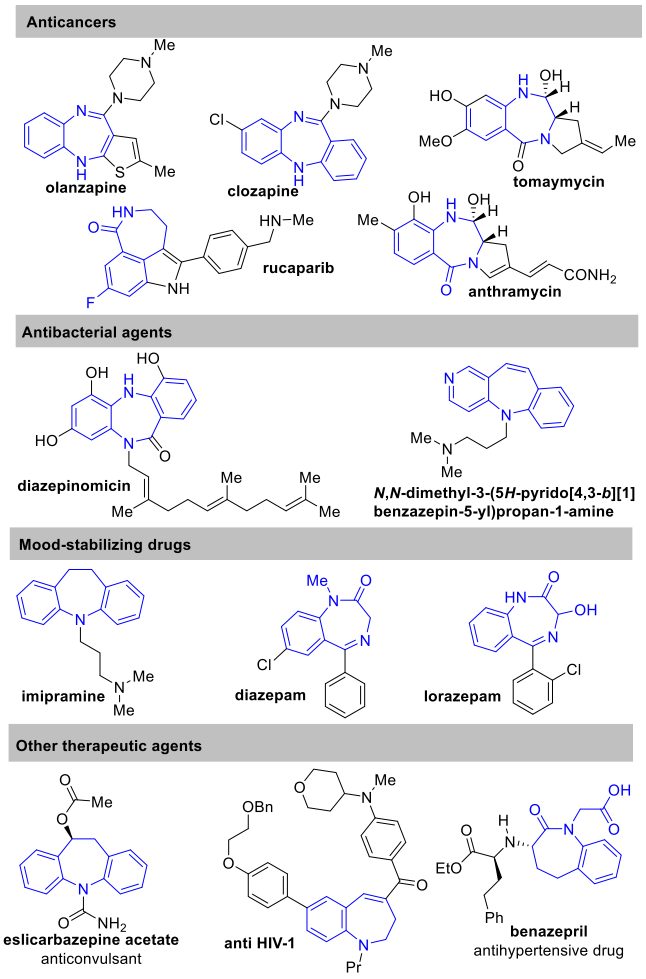


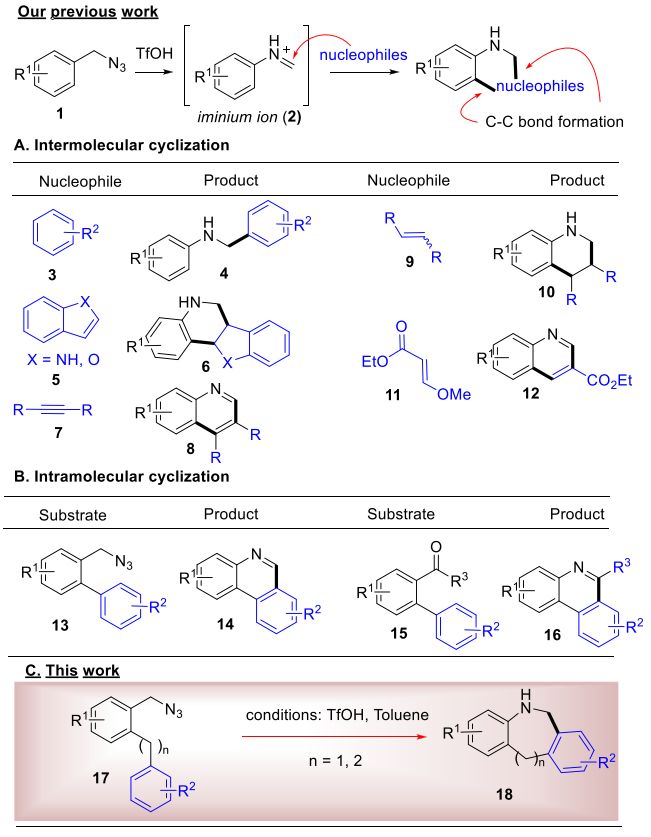
Figure 1. Representatives of commercial benzoazepine drugs.

pounds as shown in Scheme 1. In this work, we aimed to utilize our research background in azide rearrangement chemistry in the design and synthesis of benzoazepine derivatives for the development of an antianxiety agent (Scheme 1).

In this study, *ortho*-arylmethylbenzyl azide derivatives (**17**) were prepared as substrates for the synthesis of benzoazepines. The results are reported in Scheme 2. 2-Formylphenyl boronic acid **19a** was used as the starting material, which was coupled with 3-methoxybenzyl bromide **20c-Br** (X = Br) via Suzuki cross-coupling[28,29] to obtain biarylmethane aldehyde product **21c** in 36% yield along with the formation of a benzylethyl ether side product. Therefore, toluene was used instead of EtOH to avoid the formation of this side product and provide only compound **21c** in 48% yield due to the low solubility of the boronic acid in toluene. Later on, we found that the best partner to couple with 2-formylboronic acid **19a** via a Suzuki cross-coupling reaction was 3-methoxybenzyl chloride **20c-Cl** (X = Cl), which could give the corresponding biarylmethane derivatives **21c** in 76% yield. Therefore, commercially available benzyl chloride derivatives were employed as the partner for a Suzuki cross-coupling reaction. However, in some cases, benzyl bromide derivatives were used for the synthesis of compounds **21**, because they were commercially available. The results are demonstrated in Scheme 2.

Then, substrate **21** was reduced using $NaBH_4$ in MeOH to provide the corresponding alcohol product. After purification, this alcohol was converted to a benzyl bromide derivative using

Scheme 1. Our Proposed Synthetic Method



PBr_3 in DCM. Upon completion, DCM was removed to give crude product, which was then taken up in DMSO before NaN_3 was added. The reactions could provide the desired products **17** in moderate to good yields in three steps. However, *ortho*-arylmethylbenzyl aldehydes **21d** and **21m** could not be purified, and they were therefore subjected to the reduction conditions to provide alcohol products **28d** and **28m** in good yields. Furthermore, alcohol products from the reduction of compounds **21g**, **21j**−**21l**, **21p**, and **21s** could not be purified. Therefore, they were directly used in the bromination and azidation steps to give the corresponding azides **17** in moderate to good yields. In the case of substrate **17f**, it was prepared using the same protocol as shown in Scheme 2. However, both aldehyde and alcohol products could not be obtained in pure forms; thus, the bromination and azidation reactions were carried out in successive order to give azide **17f** in 27% overall yield (Scheme 3). In addition, a different method was used for the preparation of compound **17e**. Compound **22e**, prepared from a Friedel−Crafts reaction between phthalic anhydride and 1,2,4-trimethoxybenzene followed by esterification, was employed as the starting material. Compound **22e** was subjected to reduction conditions to give compound **23e**, which was converted to the corresponding product **17e** by reduction, bromination, and azidation as shown in Scheme 3.

Substrate **17t**, which would cyclize to give an eight-membered ring product, was prepared by the coupling reaction between 2-bromobenzaldehyde (**24**) and 3-methoxystyrene (**25**) via a Heck reaction to give product **26** in 84% yield. Then, this compound was hydrogenated using Pd/C and hydrogen gas to obtain compound **27** in 95% yield. Finally, the aldehyde moiety was converted to azide using the same

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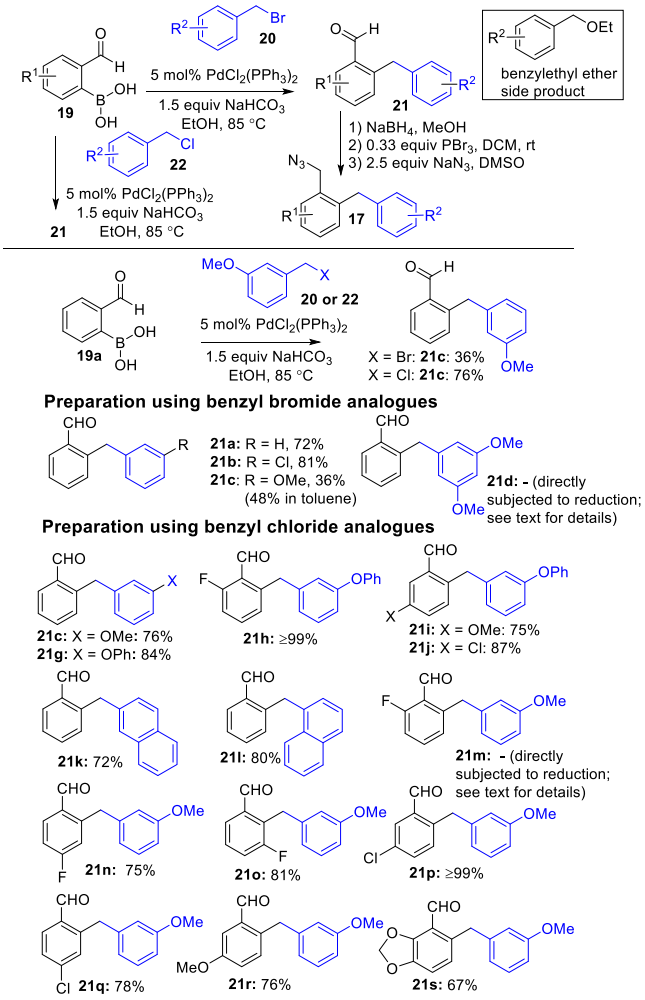
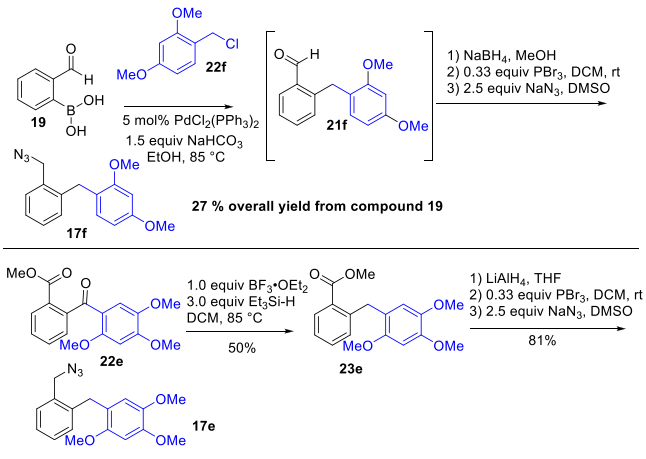
Scheme 2. Preparation of Compound 21



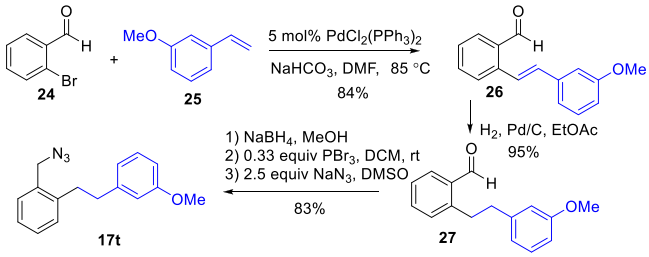
Scheme 3. Preparation of Compounds 17e and 17f



reaction sequence as discussed above to give compound **17t** in 83% yield (Scheme 4).[30]

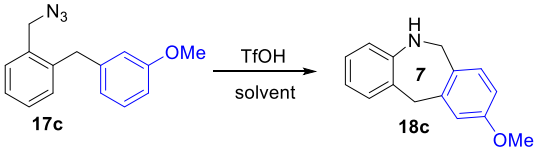
According to the previous works,[23] TfOH was the best acid to induce the generation of an iminium ion via azide rearrangement. The reaction conditions were easily optimizable using this acid. As we know, only TfOH in DCM or toluene could be used for an azide rearrangement; therefore, only the solvent and concentration of reactions were

Scheme 4. Preparation of Compound 17t



investigated as shown in Table 1. The results showed that dry DCM provided the desired product in lower yield (89%)

Table 1. Reaction Conditions



entry	TfOH (equiv)	solvent	yield (%)[a] **18c**
1	1.0	DCM (0.10 M)	89
2	1.0	PhMe (0.10 M)	93
3	1.0	PhMe (0.05 M)	84

[a]Isolated yield.

than dry toluene (93%), while the reaction at higher concentration (0.10 M) (entry 2) provided the desired product in a better yield than the lower concentration (0.05 M). The results were demonstrated in Table 1.

After we have the optimal conditions in hand, several substrates were subjected to this rearrangement. Substrate **17a** containing a benzyl substituent could not provide the desired product possibly due to the poor nucleophilicity of the phenyl ring. A similar result was obtained with the substrate containing 3-chlorobenzyl group **17b**; the reaction could not give the corresponding product as well. Therefore, we attempted the reaction with a substrate bearing the 3-methoxybenzyl group **17c**. The transformation smoothly took place to give benzoazepine product **18c** in 93% yield, while substrate **17d** could also provide the desired product **18d** in excellent yield (93%). These results showed that an intramolecular reaction and the resonance effect of halogen atom of the phenyl and 3-chlorophenyl groups were not sufficient to drive electrophilic aromatic substitution reactions to occur under these optimal conditions. The substrate containing a 3-methoxyphenyl group, however, efficiently proceeded under these conditions. These results implied that the minimum requirement for this electrophilic aromatic substitution to proceed, the substrates need to contain one methoxy group, which served as an electron-donating group of aromatic nucleophiles. It is important to note that the resonance effect of the methoxy group at the 3-position (entry 3) also gave the desired product in a comparable yield (93%). Unfortunately, the substrate containing a 2,4,5-trimethoxybenzyl group (**17e**) failed to give the desired product; only compound **27e** was obtained in 40% yield. This result showed that the inductive effect of the 2,4-dimethoxy group imposed a stronger effect than the resonance effect of the 5-methoxy group (entry 5). These results demonstrated that the resonance effect of the 3-substituent group was very

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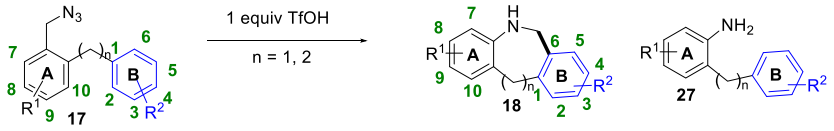
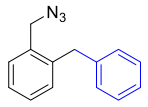
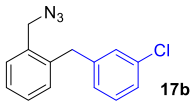
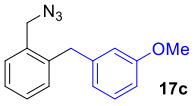
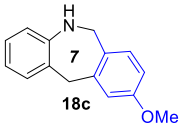
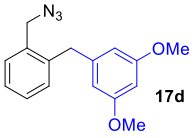
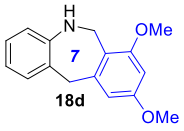
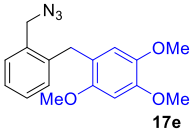
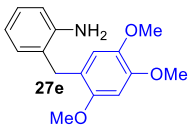
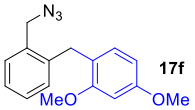
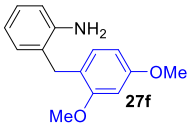
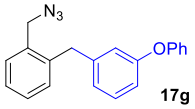
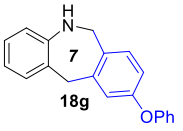
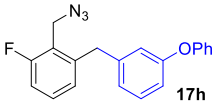
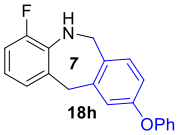
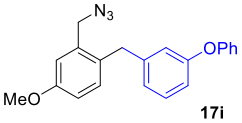
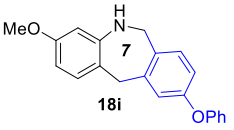
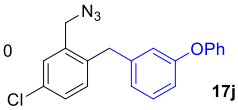
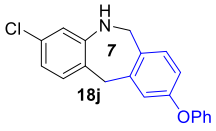
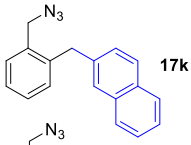
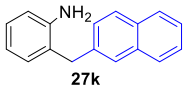
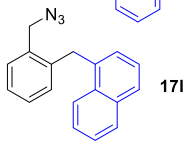
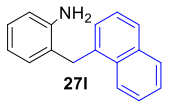
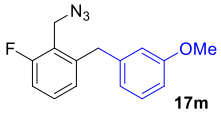
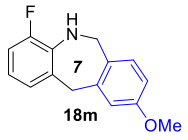
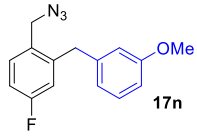
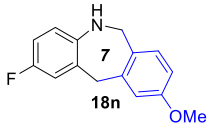
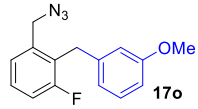
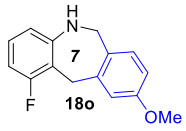
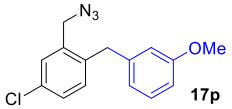
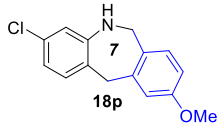
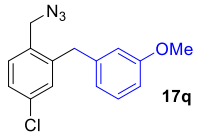
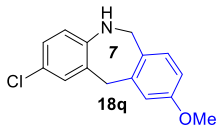
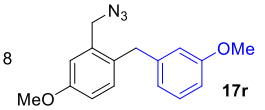
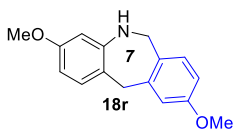
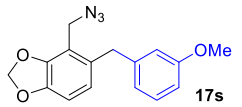
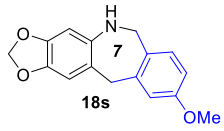
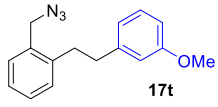
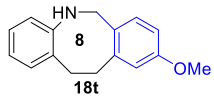
Table 2. Substrate Scope[a]



Entry	Substrate	Product	Yield
1	 17a	-	0%
2	 17b	-	0%
3	 17c	 18c	93%
4	 17d	 18d	93%
5	 17e	 27e	40%
6	 17f	 27f	15%
7	 17g	 18g	73%
8	 17h	 18h	98%
9	 17i	 18i	54%
10	 17j	 18j	97%
11	 17k	 27k	28%
12	 17l	 27l	45%
13	 17m	 18m	95%
14	 17n	 18n	99%
15	 17o	 18o	94%
16	 17p	 18p	75%
17	 17q	 18q	92%
18	 17r	 18r	75%
19	 17s	 18s	12%
20	 17t	 18t	53%

[a]Isolated yield.

crucial in this conversion. Next, the reaction of a substrate containing 3-phenoxybenzyl yielded the desired product **18g** in a slightly lower yield (73%), possibly due to the lower nucleophilicity of the 3-phenoxybenzyl group. To evaluate the electronic effect of R^1, several substrates were prepared and subjected to the optimal conditions. Substrate **17h** containing a fluorine atom at position 7 on aryl ring A was converted to the desired product **18h** in 98% yield, while substrate **17i**

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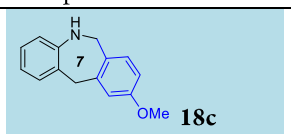
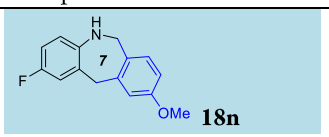
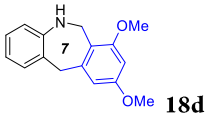
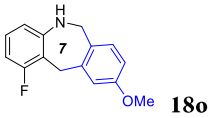
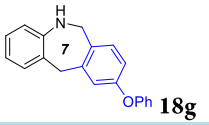
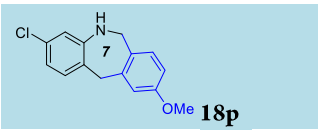
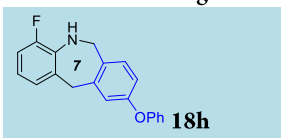
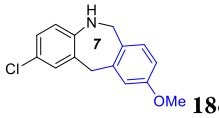
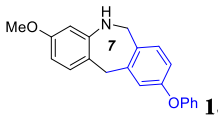
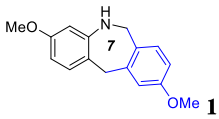
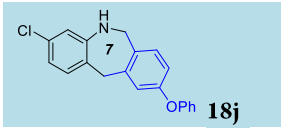
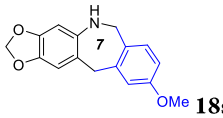
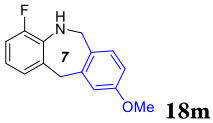
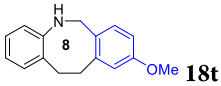
Table 3. In Vitro Cytotoxic Activity of Benzoazepine Analogues 18[a] against Normal Human Kidney Cell Line (HEK)

Entry	Compound	HEK (IC_{50} μM)	Entry	Compound	HEK (IC_{50} μM)
1	 **18c**	65.68	8	 **18n**	0.16
2	 **18d**	6.44	9	 **18o**	0.77
3	 **18g**	4.43	10	 **18p**	12.64
4	 **18h**	13.73	11	 **18q**	2.29
5	 **18i**	2.12	12	 **18r**	1.09
6	 **18j**	14.16	13	 **18s**	14.46
7	 **18m**	6.19	14	 **18t**	14.11
15	Diazepam (IC_{50} μM)	87.90			

[a]Benzoazepine analogues were tested as free bases.

containing a methoxy group at position 8 on aryl ring A underwent the reaction to provide the benzoazepine product **18i** in a much lower yield (54%). This was to be expected as electron-rich benzyl azide derivatives provided unstable iminium ion intermediates leading to decomposition and giving products in low yields. On the contrary, the substrate containing a chlorine atom at the same position could furnish the desired product **18j** in excellent yield (97%).

Next, substrates bearing a more highly nucleophilic group than the benzyl group, such as α- and β-naphthylmethyl groups (**17k** and **17l**), were examined. However, the reactions were unsuccessful in both cases. Substrates containing a variety of R^1 on a 1-(azidomethyl)-2-(3-methoxybenzyl)benzene core structure were next studied (entries 13−19). Compound **17m** (R^1 = 7-F) smoothly converted to the desired product in 95% yield. Moreover, compounds containing a fluorine atom at other positions, compounds **17n** (R^1 = 9-F) and **17o** (R^1 = 10-F), were also examined. The reactions of these substrates provided the corresponding products in 99 and 94% yields, respectively. Furthermore, substrates with chlorine substituents were prepared and subjected to the optimal conditions. Thus, the reactions of compounds **17p** (R^1 = 8-Cl) and **17q** (R^1 = 9-Cl) gave the corresponding products **18p** and **18q** in 75 and 92% yields, respectively. These results showed that substrates containing a halogen atom at various positions were well-tolerated under these conditions; their presence did not significantly affect the yields of products. In the case of substrate **17r**, bearing 5-methoxybenzyl azide, the reaction provided the desired product in 75% yield, which was better than the result of compound **17i** possibly due to the OMe group being more electron-donating than the OPh group, making the aryl ring become more nucleophilic in adding to an iminium ion intermediate. However, these conditions were not compatible with the dioxymethylene protecting group in substrate **17s**, leading to decomposition of the substrate to give the corresponding product **18s** in 12% yield. Moreover, this method was also applied to synthesize eight-membered product **18t** (entry 20), which produced a 53% yield of the eight-membered product.

In vitro cytotoxicity evaluation: Upon obtaining these synthetic benzoazepine derivatives, all of them were subjected to evaluation of their cytotoxicity. The results are shown in Table 3. A total of 14 benzoazepine analogues in their free base forms were subjected to in vitro cytotoxicity evaluation. The cytotoxic assay was performed against a normal human kidney cell line (HEK) using the MTT method.[31] The cytotoxicity of diazepam was employed to compare with our tested compounds. It is important to note that several tested compounds have low toxicity, especially compound **18c**, which had the lowest toxicity (IC_{50} = 65.68 μM) among the series and had slightly higher cytotoxicity than diazepam (IC_{50} = 87.90 μM), which is currently used as an antianxiety drug.

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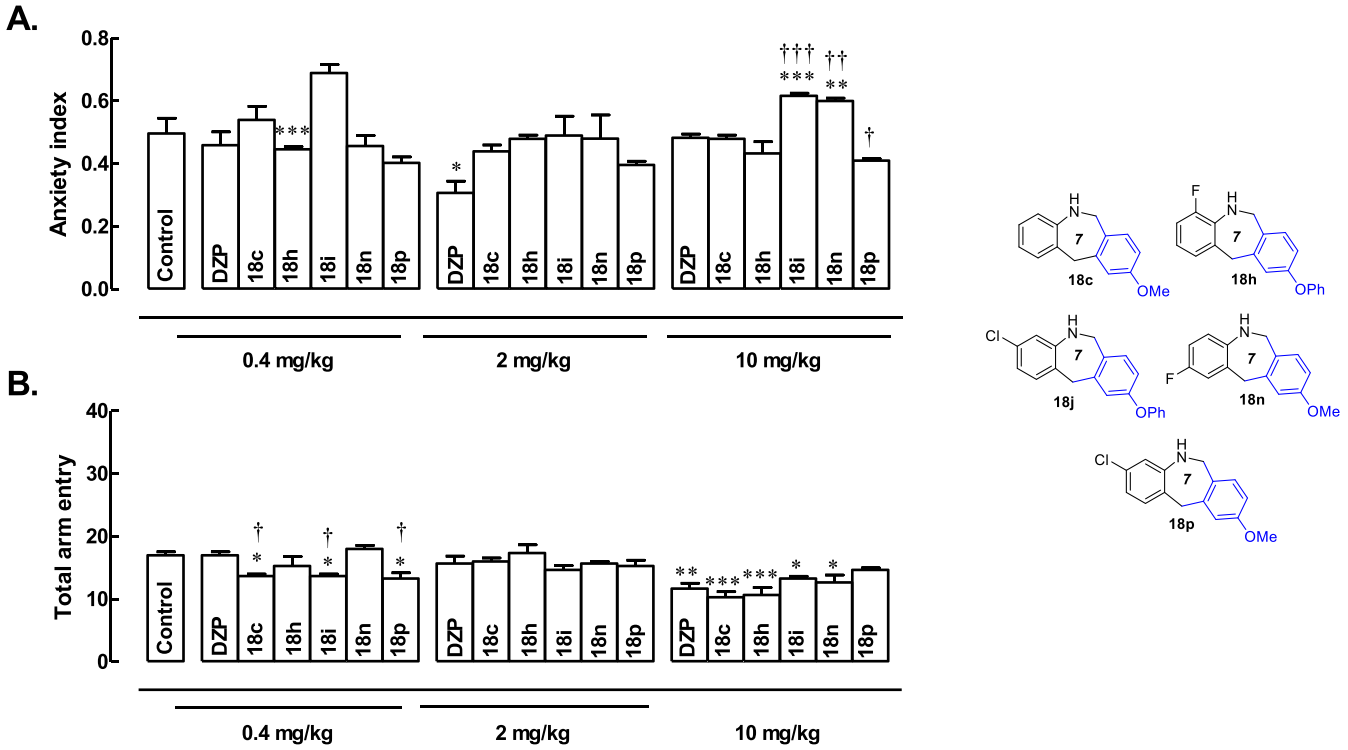

Figure 2. Effect of benzoazepine analogues on anxiety- and locomotion-related behaviors in rats as determined by EPM (three rats per group). (A) The anxiety index reflected the exploratory behaviors (the greater anxiety index indicated higher anxiety). (B) The total open arm entry in normal rats with acute 0.4, 2, or 10 mg/kg diazepam (**DZP**) or benzoazepine analogues (**18c**, **18h**, **18j**, **18n**, and **18p**). *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ compared with the control nonstress vehicle (control)-treated group. †$p < 0.05$, ††$p < 0.01$, and †††$p < 0.001$ compared with the stress **DZP**-treated group.

However, the toxicity increased when dimethoxy groups were placed in aryl ring B of compound **18d** having an IC_{50} value of 6.44 μM. Then, a substrate containing an OPh group at position 3 was examined (entry 3, compound **18g**) with the toxicity enhanced to give an IC_{50} value of 4.43 μM. The results from entries 1−3 suggested that compounds with an anisole moiety exhibited less toxicity than dimethylresorcinol and diaryl ether moieties. Interestingly, the addition of a fluorine substituent at position 7 of aryl ring A in compound **18h** could decrease the toxicity of the compound with an IC_{50} value of 13.73 μM when compared with compound **18g**. However, the toxicity of compound **18i** was increased when a methoxy substituent was installed at carbon position 8 on aryl ring A, giving an IC_{50} value of 2.12 μM. Therefore, we decided to prepare the benzoazepine derivatives containing an anisole moiety on aryl ring B while also varying the substituent groups on aromatic ring A. Unexpected results were obtained when the substrate contained a fluorine atom at position 7: compound **18m** (IC_{50} = 6.19 μM) exhibited significantly higher toxicity than compound **18h** with an IC_{50} value of 13.73 μM. In addition, we found that when a fluorine atom was located at positions 9 (compound **18n**) and 10 (compound **18o**), it provided even higher toxicity with IC_{50} values of 0.16 and 0.77 μM, respectively. Changing from a fluorine substituent to a chlorine atom, compound **18p**, containing a chlorine atom at position 8, showed lower toxicity with an IC_{50} value of 12.64 μM, while compound **18q**, bearing a chlorine atom at position 9, showed higher toxicity with an IC_{50} value of 2.29 μM.

In the case of compound **18r** containing a methoxy group at position 8, it also provided an unimpressive result with an IC_{50} value of 1.09 μM, whereas compound **18s** containing a methylenedioxy moiety could decrease the toxicity of the benzoazepine analogue with an IC_{50} value of 14.46 μM. However, the synthesis of this compound gave the desired product in low yield. In further evaluation, the substrate with a larger cyclic amine, tetrahydrodibenzoazocine **18t**, was prepared, which also provided the product in a moderate yield. This compound showed an IC_{50} value of 14.11 μM. For the biological activity evaluation, the four lowest toxicity benzoazepine derivatives were selected. However, compounds **18s** and **18t** were excluded from the list, because the synthesis of these compounds provided only low to moderate yields of products that were not suitable for further study in drug development. Therefore, compounds **18c**, **18h**, **18j**, and **18p** were selected to test for their antianxiety property. In addition, we also selected compound **18n** having the highest toxicity in this study to compare the results with the lowest toxicity compounds.

Effect of benzoazepine analogues on anxiety- and locomotion-related behaviors in rats: In this study, benzoazepines **18c**, **18h**, **18j**, **18n**, and **18p** were examined for their anxiolytic property in rats. The stress was induced by restraint stress induction. This method was accomplished to induce the anxiety-like behaviors in rats in numerous studies. Initially, stress was induced in rats by being immobilized in a plastic restrainer for 2 h/day for 14 days. The stress was evaluated using two methods including elevated plus maze (EPM) and open field test (OFT).[32,33] As compared to the control group, the 2-week stress induction showed anxiety-like behavior as demonstrated by both EPM and OFT. To establish a suitable dose of substances for antianxiety evaluation in rats, diazepam

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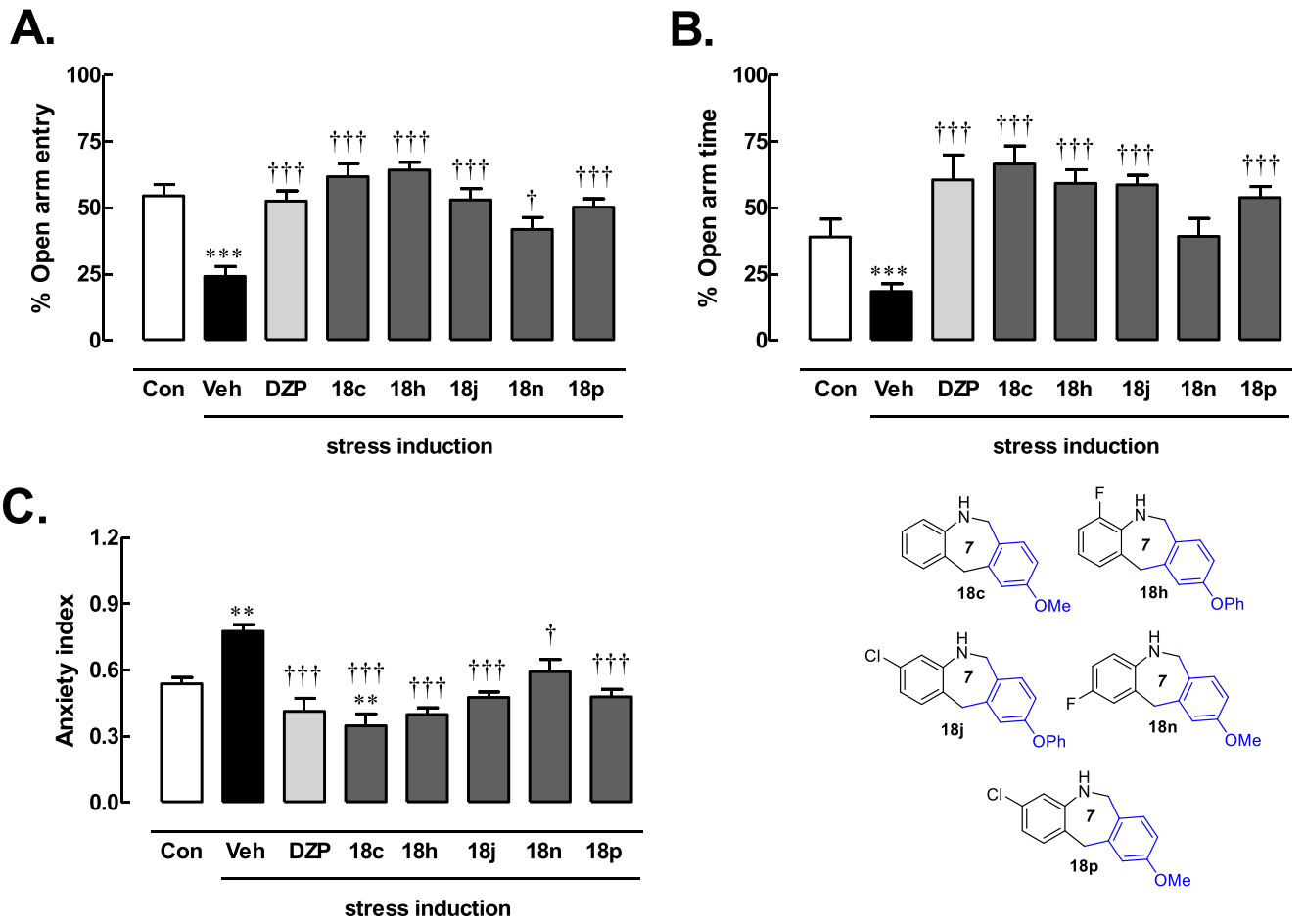

Figure 3. Effect of benzoazepine analogues on anxiety-like behaviors in stressed rats as determined by EPM (six rats per group). (A) The percent open arm entry, (B) the percent open arm time, and (C) the anxiety index in stressed rats with acute 2 mg/kg diazepam (**DZP**) or benzoazepine analogues (**18c**, **18h**, **18j**, **18n**, and **18p**). $^{**}p < 0.01$ and $^{***}p < 0.001$ compared with the control nonstress vehicle (**Con**)-treated group. $\dagger p < 0.05$ and $\dagger\dagger\dagger p < 0.001$ compared with the stress vehicle (**Veh**)-treated group.

(**DZP**) and benzoazepine analogues (**18c**, **18h**, **18j**, **18n**, and **18p**) were employed for the screening for the optimal dosage using EPM. The results were illustrated in Figure 2. For the EPM test, 2 mg/kg **DZP**-treated rats showed a significant decrease in anxiety index (Figure 2A), while 0.4 and 10 mg/kg **DZP**-treated rats did not change in the total open arm entry. Similarly, the treatment of 2 mg/kg of benzoazepine analogues (**18c**, **18h**, **18j**, **18n**, and **18p**) had not affected locomotor activity as compared to the vehicle control group (Figure 2B). Therefore, diazepam (2 mg/kg) and benzoazepine analogues (2 mg/kg) were proposed to be a suitable anxiolytic dose for the study of anxiolytic-like effects of benzoazepine analogues in the animal model.

The elevated plus maze (EPM) test: The effect of benzoazepine analogues (**18c**, **18h**, **18j**, **18n**, and **18p**) on anxiety-like behaviors in nonstressed control rats and stressed rats was determined using EPM. This protocol correlated the fear of novelty and open spaces with stress. The behavior of rats with a high percentage of the frequency of open-space entries and high percentage of time spent in the open arms was used to indicate a decrease of stress level in rats. To determine the therapeutic effects of benzoazepine analogues on the anxiety-like behaviors, stressed rats were administered with 2 mg/kg of test compounds for 30 min prior to the behavioral tests. In this study, all experiments were conducted employing six rats per group. The results in Figure 3A showed that the percentage of the open arm entry of nonstressed rats (**Con**) was significantly higher than the stressed rats (**Veh**). These results showed that the restraint stress induction was successful to induce anxiety-like behaviors in rats (**Veh**). Next, the stressed rats were treated with diazepam (**DZP**), an antianxiety drug, which resulted in a slightly lower percent open arm entry compared with **Con**. These results showed the effectiveness of diazepam, which corresponded with the results from other reported works. Acute amphetamine withdrawal rats were found to show increased anxiety-like behavior in the EPM.[34] Diazepam administration demonstrated anxiolytic efficacy in hemiparkinsonian rats.[35] In this study, five synthetic benzoazepine analogues (**18c**, **18h**, **18j**, **18n**, and **18p**) were evaluated for an antianxiety property. Interestingly, compound **18c** containing a methoxy group at position 3 of aromatic ring B provided a higher percent open arm entry than the **DZP** standard. In addition, this compound showed very low cytotoxicity against normal human kidney cell line (HEK cells) with a comparable IC_{50} to diazepam. Then, compound **18h** containing a fluorine atom at position 7 of aromatic ring A and a phenoxy group at position 3 of aromatic ring B was employed and showed the best activity among the tested compounds and the **DZP** standard. Compound **18j** containing a phenoxy group at the same position with **18h** and chlorine atom at position 8 of aromatic ring A showed slightly lower activity than compound **18h** but provided a comparable

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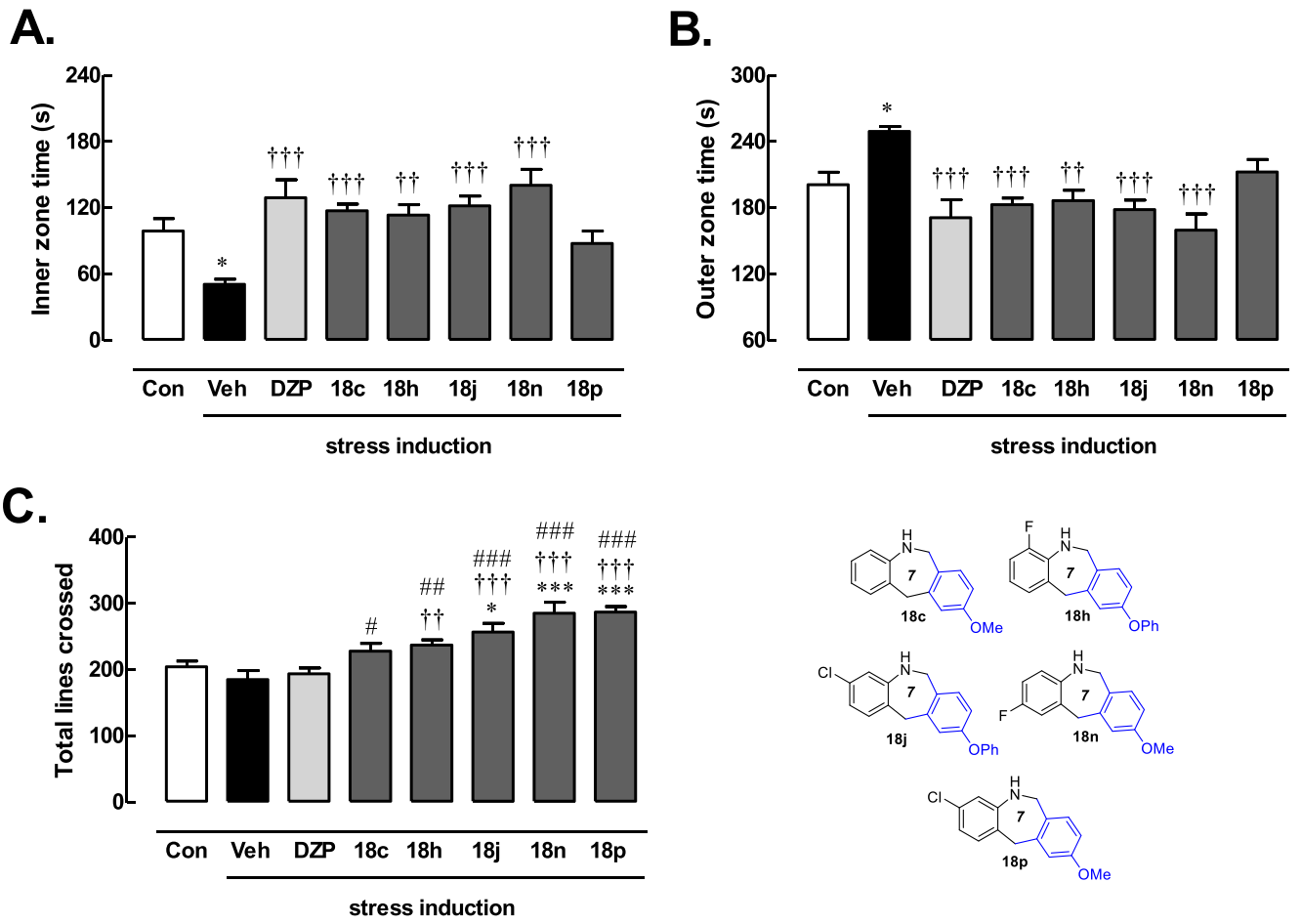

Figure 4. Effect of benzoazepine analogue treatments on anxiety-like behaviors and locomotor activity in stressed rats as determined by OFT (six rats per group). (A) The inner zone time, (B) the outer zone time, (C) the number of total lines crossed in stressed rats with acute 2 mg/kg diazepam (**DZP**) or benzoazepine analogues (**18c**, **18h**, **18j**, **18n**, and **18p**). *$p < 0.05$ and ***$p < 0.001$ compared with the control nonstress vehicle (**Veh**)-treated group. ††$p < 0.01$ and †††$p < 0.001$ compared with the stress vehicle (**Veh**)-treated group. #$p < 0.05$, ##$p < 0.01$, and ###$p < 0.001$ compared with the stress **DZP**-treated group.

activity to **DZP**. In the case of compound **18n** containing a fluorine atom and methoxy group at positions 9 of ring A and 3 of ring B, respectively, displayed the lowest percent open arm entry among tested compounds. Meanwhile, compound **18p** containing a chlorine atom and methoxy group at positions 8 of ring A and 3 of ring B, respectively, provided a comparable activity with the diazepam standard. This study showed that all tested benzoazepines had a significant effect in reducing the anxiety level of stressed rats, especially compounds **18c** and **18h**, which showed higher efficacy than the diazepine drug. In addition, time spent in open arms was also examined as shown in Figure 3B. The results showed that only compound **18c** showed the highest amount of time spent in the open arms by the stressed rats when compared with **DZP** and other synthetic benzoazepines (**18h**, **18j**, **18n**, and **18p**). In addition, compounds **18h**, **18j**, and **18p** showed a comparable amount of time spent in open arms with **DZP**, whereas compound **18n** had the lowest ability to reduce anxiety in the stressed rats. Figure 3C indicated the increased anxiety level of rats. These results clearly showed an anxiolytic property of benzoazepine analogues in stressed rats. It is important to note that compound **18c** gave the best antianxiety property compared with diazepam and other benzoazepine analogues.

The open field test (OFT) test: The effect of benzoazepine analogue treatments on anxiety-like behaviors and locomotor activity in stressed rats was determined by an OFT test (six rats per group). According to the OFT results, restraint stress induction was successful to induce anxiety-like behaviors in rats as shown in Figure 4. For the OFT test, a decrease in the inner zone time indicated a high anxiety level of rats while changing the number of total lines crossed and rearing correlated to locomotor activity and a tendency to want to explore the spaces of rats, respectively. In our study, the results showed that the stressed rats had a lower amount of time spent in the inner zone (Figure 4A) and spent more time in the outer zone (Figure 4B) without any alteration in the number of total lines crossed (Figure 4C). Next, the stressed rats were treated with the **DZP** standard when measuring a stress response; we found that rats spent more time both in the inner and outer zones (Figure 4A,B) and also showed a lower number of rearing than vehicle-treated stressed rats (**Veh**) (Figure 4C). In addition, the treatment of stressed rats with **DZP** could alter the number of line crossings in the OFT test (Figure 4C). Compared to vehicle-treated stressed rats (**Veh**, Figure 4A), rats treated with benzoazepine analogues, **18c**, **18h**, **18j**, **18n**, and **18p**, displayed a significant increase in the time spent in the inner zone of OFT (Figure 4A). It should be noted that compound **18n** demonstrated a higher increase in the amount of time spent in the inner zone by the stressed rats compared to the **DZP**-treated ones. Moreover, all tested compounds,

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including **DZP**, showed hyperlocomotor activity in stressed rats as illustrated in Figure 4C. Therefore, these results suggested that benzoazepine analogues possessed anxiolytic effects and could enhance the behavior response in stressed rats without the sedative effect.

In conclusion, we have developed a new method for the synthesis of benzoazepine analogues using azide rearrangement chemistry. In this work, 14 derivatives were successfully prepared in moderate to excellent yields. All benzoazepine analogues were evaluated for their cytotoxicity against normal human kidney cell line (HEK cell). The results showed that several compounds had low cytotoxicity. A total of five derivatives were employed to evaluate the antianxiety property including compounds **18c**, **18h**, **18j**, **18n**, and **18p**. In this work, we discovered that compound **18c** had higher potency to reduce the anxiety of the stressed rats and also had very low cytotoxicity comparable with diazepam. Therefore, compound **18c** could be used as a lead compound for further drug development to treat patients with anxiety disorder.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.1c00275.

Copies of ^{1}H and ^{13}C NMR spectra for compounds **21a−21c**, **21g−21l**, **21n−21s**, **23e**, and **26**. Copies of ^{1}H and ^{13}C NMR spectra for compounds **28a−28d**, **28h**, **28i**, **28m−28o**, **28q**, and **28r**. Copies of ^{1}H and ^{13}C NMR spectra for compounds **17a−17t**. Copies of ^{1}H and ^{13}C NMR spectra for compounds **18a−18d**, **18g−18j**, **18m−18t**, **27e**, **27f**, **27k**, and **27l**. Chromatographic purity analysis of compounds **18c**, **18h**, **18j**, **18n**, and **18p** using HPLC (PDF)

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Notes

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