The activity of Benzocaine benzene ring bioisosteres

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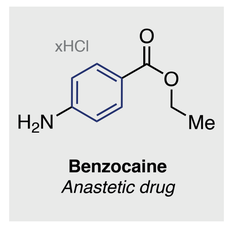
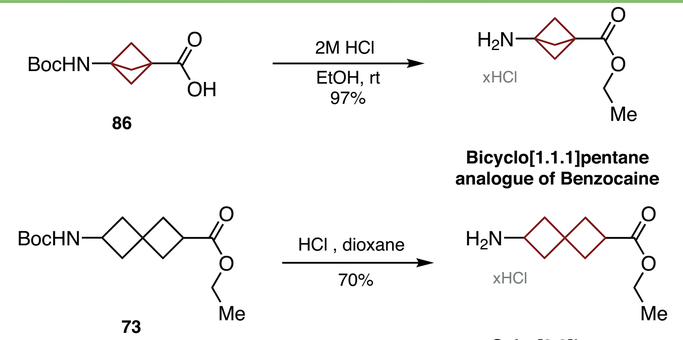
Introduction and Aim

The benzene ring is the most popular ring in natural products, bioactive compounds, and drugs. During the past decade, it was shown that bicyclo[1.1.1]pentane, cubane, bicyclo[2.2.2]octane and 2-oxabicyclo[2.2.2]octane etc., scaffolds could mimic the central benzene ring in bioactive compounds and might be useful to improve their physicochemical properties.

In this work, we incorporated bicyclo[1.1.1]pentane and spiro[3.3]heptane scaffolds into a structure of an FDA-approved local anaesthetic drug Benzocaine instead of the para-substituted benzene ring and tested how these structural modifications influence the biological activity.



Benzocaine
Anastetic drug

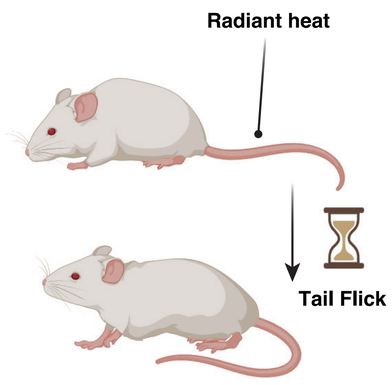


Scheme 1. Synthesis of benzocaine analogues from N-Boc amino acids 86 and 73; acidic N-Boc cleavage and the simultaneous esterification of the carboxyl group gave the desired analogues as hydrochloride salts.

Methods

Kinetic solubility (2% final DMSO) and LogD7.4 of all three compounds were assessed by the shake-flask method using LC-MS/MS detection. Metabolic stability was tested in human liver microsomes (HLM).

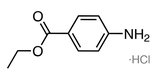
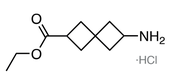
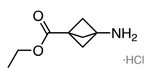
The anaesthetic activity of Benzocaine and its analogues was tested in mice using the “tail flick test” in which the tail is exposed to localized infrared irradiation and animal response-to-heat latency is registered (Scheme 2). Study design, animal selection, handling and treatment were performed in accordance with Bienta Animal Care and Use Guidelines, and European Union Directive 2010/63/EU. CD-1 female mice (n=5-6 per group) were single-dosed subcutaneously with Benzocaine (50 uL, 130 mg/ml), analogues (50 uL, 130 mg/ml) or vehicle 2 cm distally from the tail base. The site of tail exposure to infrared radiation was 2-3 cm below the base of the tail. The level of analgesia for each mouse was measured 5, 10, 15, 30, 60, 120, and 180 min after compound/vehicle injection. The cut-off time for the heat source was set at 12 sec to avoid tissue damage. Tail flick latency was registered by an analgesia meter (Columbus Instruments, USA).



Scheme 2. Tail-flick assay

Results

Table 1. ADME properties of Benzocaine and analogs

Compound	Structure	Solubility*, PBS pH 7.4, μM	LogD, pH 7.4	CLint, ul/min/mg, HLM	$t_{1/2}$, min, HLM	k_{el}, min^{-1}, HLM
Benzocaine hydrochloride		385±1.4	1.8±0.02	83#	20.1	0.034
Spiro[3.3]heptane analogue		332±2.5	-0.4±0.06	29#	56.7	0.012
Bicyclo[1.1.1] pentane analogue		319±3.5	0.1±0.05	140#	12.0	0.058

*Kinetic solubility, 2% final DMSO
#“No cofactor” control data indicates some instability of the compound in the test system without cofactors
HLM – human liver microsomes

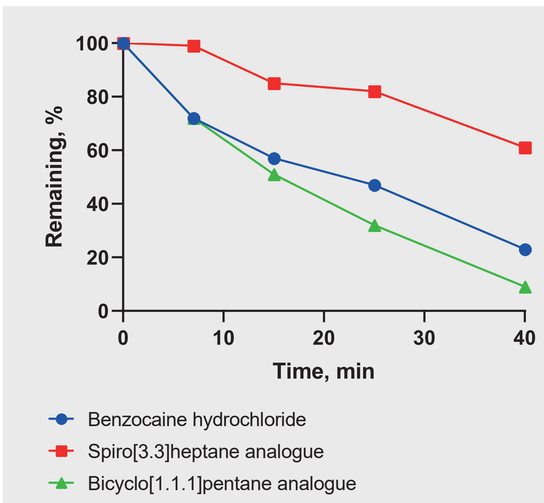


Figure 1. Stability data for benzocaine and analogs in human liver microsomes.
“No cofactor” control data indicates some instability of the compound in the test system without cofactors

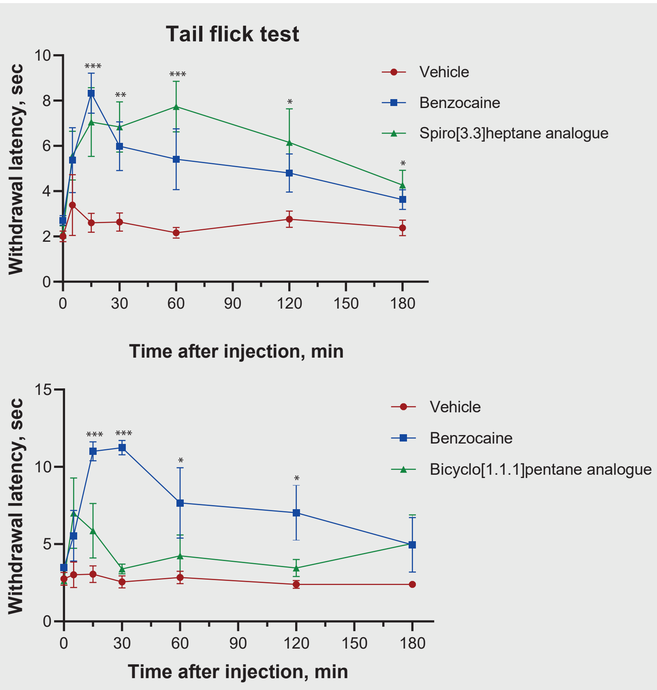


Figure 2. Time course of the antinociceptive effect of Benzocaine and its analogues in tail flick test on inbred mice. The data were presented as mean ± SEM. * - indicates P < 0.05; ** - indicates P < 0.01, and *** - indicates P < 0.001 compared with the vehicle-treated group.

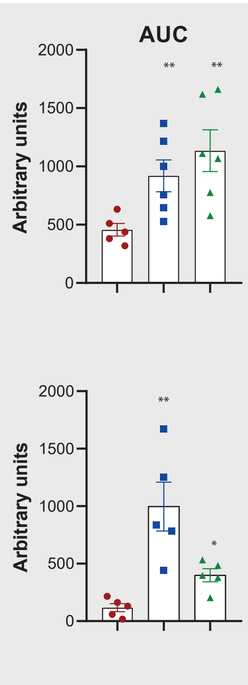


Figure 3. The area under the curve (AUC) of withdrawal latency of Benzocaine and its analogues in the tail flick test. The data were presented as mean ± SEM. * - indicates P < 0.05; ** - P < 0.01 compared with the vehicle-treated group.

Conclusions

- Spiro[3.3]heptane analogue demonstrated significantly lower $LogD_{7.4}$ and higher stability compared to benzocaine. The antinociceptive activity of this analogue was significantly higher compared to that of the vehicle. Moreover, its activity was very similar to that of Benzocaine throughout the whole observation period. In addition, this analogue showed a significant increase in coverage of analgesia time (AUC) compared to that of the vehicle.
- Bicyclo[1.1.1]pentane analogue of Benzocaine was found to be less active compared to the original drug Benzocaine - no significant difference in response time to tail flick was present throughout the observation period. On the other hand, this analogue demonstrated a clear analgesic activity – a significant increase in coverage of analgesia by time compared to that of the vehicle.
- These data show that spiro[3.3]heptane and bicyclo[1.1.1]pentane scaffolds mimic para-substituted benzene rings and might be used for making analogues of benzene-containing molecules.

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References

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M. A. M. Subbaiah, N. A. Meanwell. Bioisosteres of the Phenyl Ring: Recent Strategic Applications in Lead Optimization and Drug Design. *J. Med. Chem.* **2021**, *64*, 14046–14128