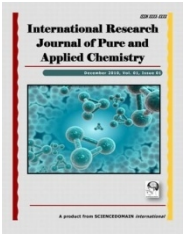

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Comparative Study of Benzyl and Phenyl Compounds Chemical Structure Effects on the Inhibition of Methane Production by Digested Pig Manure Methanogens

K. Kayembe[1], L. Basosila[1], P. T. Mpiana[1*], P. C. Sikulisimwa[1] and K. Mbuyu[1]

[1]Department of Chemistry, Faculty of Science, University of Kinshasa, P.O. Box 190 KINSHASA, D.R. Congo.

Authors' contributions

This work was carried out in collaboration between all authors. Author KK designed the study and performed the experimental work, author LB performed the statistical analysis, author PCS wrote the protocol; author PTM wrote the first draft of the manuscript, managed the analyses of the study, author KM made the literature searches. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: The present paper aims to study the effect of aromatic structure on the inhibition of biogas production and more specifically the comparative study of benzyl and phenyl compounds chemical structure effects on the inhibition of methane production by digested pig manure methanogens. The objective of this study was also to examine the structure-toxicity relationships of aromatic compounds to acetoclastic methanogens.
Study Design: Anaerobic digestion of pig manure, anaerobic toxicity essay, comparison of benzyl and phenyl compounds structure effects on the inhibition of methane gas biosynthesis.
Place and Duration of Study: Department of Chemistry, University of Kinshasa (DR Congo), between September 2011 and May 2012.
Methodology: The toxicity to acetoclastic methanogenic bacteria was performed with the

**Corresponding author: Email: ptmpiana@yahoo.fr;*

standard method of serum bottles; digested pig manure was utilized as inoculums and acetate as substrate. The methane gas volume produced was measured by serum bottles liquid displacement systems (Mariotte flask system).
Results: The obtained results indicate exist between the chemical structure of benzyl and phenyl compounds and their inhibitory effects on biosynthesis of methane by methanogenic bacteria. According to these results, α-chlorotoluene and bromobenzene with 0.61 and 2.90 mg/l IC_{50} values, respectively, are the most toxic compounds, while phenol and benzyl alcohol with IC_{50} values of 1248.90 and 2391.37 are less toxic.
Comparing the phenyl and benzyl compounds with the same substituents (Cl, Br, H, OH), their behavior was diverse. The benzyl chloride is more toxic than phenyl chloride and benzyl bromide is less toxic than phenyl bromide. Otherwise, benzyl alcohol is less toxic than phenol. A significant correlation between the toxicity of phenyl compounds and their hydrophobicity (R^2 = 0.911). But any correlation was not found for benzyl compounds.
Conclusion: The obtained results indicate that relationships exist between the chemical structure of benzyl and phenyl compounds and their inhibitory effects on the production of methane by methanogenic archaea. The behavior of phenyl and benzyl are different in inhibition of methane production by the methanogens because their chemical structures are not identic.

Keywords: Benzyl; phenyl; methane production; anaerobic digestion; toxicity; methanogens; digested pig manure; aromatic structure.

1. INTRODUCTION

In organic chemistry, benzyl is the substituent or molecular fragment possessing the structure $C_6H_5CH_2$-. Benzyl features a benzene ring attached to a CH_2 group. The term benzyl refers most commonly to benzyl compounds, such as benzyl chloride or benzyl benzoate . Sometimes, benzyl and phenyl are confused, but their formulas and behavior are very different. The term benzylic refers to the position on a carbon skeleton next to a phenyl or other aromatic ring [1].

The abbreviation "Bn" is frequently used to denote benzyl groups in nomenclature and structural depictions of chemical compounds. For example, benzyl alcohol can be represented as BnOH. The Fig. 1 shows benzyl and phenyl groups formulas.

Fig. 1. The structure of the benzyl group (A) and phenyl group (B) attached to an "R" group

Besides, in organic chemistry, the phenyl group or phenyl ring is a cyclic group of atoms with the formula C_6H_5. Phenyl groups are closely related to benzene. Phenyl groups have six carbon atoms bonded together in a hexagonal planar ring, five of which are bonded to individual hydrogen atoms, with the remaining carbon bonded to a substituent. Phenyl groups are pervasive in organic chemistry. Although often depicted with alternating double

and single bonds, phenyl groups are chemically aromatic and show nearly equal bond lengths between carbon atoms in the ring [1,2]

Phenyl groups are often represented by the symbol Ph or, archaically, Φ. Benzene is sometimes denoted as PhH. Phenyl groups are generally attached to other atoms or groups. Many or even most phenyl compounds are not described with the term "phenyl". For example the chloro derivative C_6H_5Cl is normally called chlorobenzene, although it could be called phenyl chloride. Phenyl compounds are derived from benzene (C_6H_6), at least conceptually and often in terms of their production. The phenyl group is hydrophobic and tends to resist oxidation and reduction.

Phenyl groups (like all aromatic compounds) have enhanced stability in comparison to equivalent bonding in aliphatic (non-aromatic) groups. This increased stability is due to the unique properties of aromatic molecular orbitals [2].

Phenyl groups are found in many organic compounds, both natural and synthetic. Most common among natural products is the amino acid phenylalanine, which contains a phenyl group. A major product of the petrochemical industry is "BTX" consisting of benzene, toluene, and xylene - all of which are building blocks for phenyl compounds. One of the simplest phenyl-containing compounds is phenol, C_6H_5OH. It is often said the resonance stability of phenol makes it a stronger acid than that of aliphatic alcohols such as ethanol (pK_a = 10 vs. 16–18). As described above, benzyl and phenyl compounds are two forms of aromatic compounds and are, usually, present in the environment.

Aromatic compounds are inhibitors of methane biosynthesis in anaerobic treatment of solid wastes and industrial effluents. Anaerobic treatment of solid wastes and industrial effluents may be limited by the methanogenic bacteria inhibition exerted by these types of compounds, the production of biogas is not possible and the organic matter contained in the effluent is not reduced. These effluents poured in the nature can be the basis of the pollution
Benzyl and phenyl compounds as the aromatic compounds can be biodegraded in anaerobic digestion process but they can also be toxic to methanogenic bacteria [3,4,6].

At our knowledge, few works are published on the methanogenic inhibition of aromatic compounds. Most of these works were performed with the granular sludge as inoculums from an industrial anaerobic reactor called "Upflow Anaerobic Sludge Bed" (UASB reactor). Generally, the digesters are heated to about 30ºC and more, but in this work digested pig manure from a laboratory scale digester was used as inoculum and the experiment was conducted at room temperature of an African tropical country (27±1ºC). Unlike granular sludge, digested pig manure is a natural inoculum and has never been in contact with aromatic compounds.

Indeed, although the anaerobic biodegradability of aromatic compounds has been extensively studied, less attention has been given to the correlation of aromatic compounds structure and their toxic effects on the community of anaerobic bacteria. The knowledge of the effect of aromatic structure on the inhibition of biogas biosynthesis is essential in predicting the impact of these xenobiotics on anaerobic waste and wastewater treatments, thereby preventing potentially costly upsets of treatment plant operations. A better understanding of the structure-toxicity relationships will make feasible the application of anaerobic technologies to waste and wastewater containing aromatic compounds [5].
The present paper aims to study the effect of aromatic structure on the inhibition of biogas biosynthesis and more specifically the comparative study of benzyl and phenyl compounds

chemical structure effects on the inhibition of methane production by digested pig manure methanogens. The influence of the nature, the number and the position of substituents has to be investigated. The objective of this study was also to examine the structure-toxicity relationships of aromatic compounds to acetoclastic methanogens.

2. MATERIALS AND METHODS

2.1 Biomass

Pig manure from DAIPN farm of N'sele /KINSHASA (DR CONGO) was digested in laboratory scale digester during about five months. The digested pig manure (sludge) was utilized as inoculums in our anaerobic toxicity tests. The digested pig manure was not previously acclimated to any aromatic compounds. Characteristic of inoculums: total suspended solids (TSS) concentration 91.10 g/l, volatile suspended (VSS) concentration 56.59 g/l, specific acetoclastic methanogenic activity 163.40 -210.81 mg COD-CH_4/g VSS .d (27±1ºC).

2.2 Stock Solutions

2.2.1 Stock substrate solution

The stock solution of the substrate is composed of acetic acid neutralized to pH = 7 with NaOH solution. It is at the concentration of 100 g COD-CH_3COOH /l (chemical oxygen demand per liter).

2.2.2 Stock solution 1

Macro-nutrients: NH_4Cl (170g/l); KH_2PO_4 (37 g/l); $CaCl_2$. $2H_2O$ (10 g/l); $MgSO_4.4H_2O$ (37 g/l).

2.2.3 Stock solution 2

Trace elements: $FeCl_3.4H_2O$ (2000mg/l); $CoCl_2.6H_2O$ (2000 mg/l); $MnCl_2.4H_2O$ (500 mg/l); $CuCl_2$ (50 mg/l); H_3BO_3 (50 mg/l); $(NH_4)6Mo7O_2.4H_2O$ (90 mg/l); Na_2SeO_3. 5 H_2O (100 mg/l); $NiCl_2$. 6 H_2O (50 mg/l mg/l); EDTA (1000 mg/l) ; HCl 36% (1 mg/l) ; yeast extract(200 mg/l) resazurin (500 mg/l).

2.2.4 Stock solution 3

Sulfide Na_2S (100 g/l) [6,13,14].

2.3 Aromatic Compounds

The used benzyl and phenyl compounds included: α-chlorotoluene (benzyl chloride), α-bromotoluene (benzyl bromide), benzyl alcohol, chlorobenzene (phenyl chloride), bromobenzene (phenyl bromide), and phenol. Otherwise benzene and toluene were utilized as reference aromatic compounds. All aromatic compounds were of high purity available, pure for analysis supplied by MERCK.

2.4 Anaerobic Toxicity Assay

Specific acetoclastic methanogenic activity measurements were performed with 1L glass serum bottles sealed with butyl rubber septa. Add to each serum bottle from the scale laboratory digester 1.5 g VSS of digested pig manure and add to this:

- Two ml stock solution 1;
- 1 ml stock solution 2;
- 2 drops stock solution 3;
- 40 ml stock substrate solution;

Fill the serum bottle to about 1000 ml with oxygen free tap water which is flushed with nitrogen gas for at least 15 minutes [12-14]. The flask were sealed with rubber septum cap and placed in a reciprocating shaker at 27±1ºC (room temperature).

The required quantity of inhibitory compound was added to each flask to provide the toxic concentration to be investigated. No toxicant was added to the controls. The toxicant concentrations had chosen as to cause an inhibition of the acetoclastic methanogenic activity ranging from 0-100 % [6,17].

The specific methanogenic activity was calculated from the slope of the cumulative methane production versus time curve and the quantity of VSS. The compound concentration that caused 50% inhibition of the methanogenic activity had referred to as “50% IC”. All specific methanogenic activity measurements were conducted in triplicate. To determine the degree of inhibition, the specific methanogenic activities of the control and samples containing inhibitory compounds were determined [6,7,13].

2.5 Methane Gas Measurement

The methane volume produced was measured by serum bottle liquid displacement systems (Mariotte flask system) as previously described [6,13,15]. The liquid in the displacement serum bottle should contain a concentrated solution of NaOH or KOH in order to rapidly convert CO_2 to carbonate and dissolve it into the NaOH solution [6].

3. RESULTS AND DISCUSSION

3.1 Inhibition of Specific Methanogenic Activity

All concentrations of aromatic compounds examined exerted an inhibitory effect on the specific acetoclastic methanogenic activity. Fig. 2 shows the decrease in specific methanogenic activity with the increasing of the concentration of chlorobenzene. The IC_{50} is calculated as the concentration of chlorobenzene corresponding to 50% of inhibition.

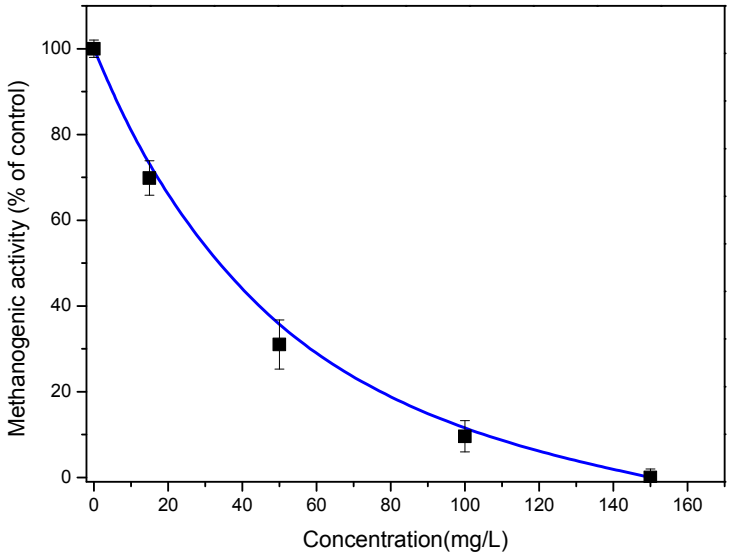

Fig. 2. Methanogenic activity of digested pig manure exposed to chlorobenzene versus chlorobenzene concentration

3.2 Methanogenic Toxicity of Benzyl and Phenyl Compounds

The inhibitory effect of benzyl and phenyl compounds: α-chlorotoluene (benzyl chloride), α-bromotoluene (benzyl bromide), benzyl alcohol, chlorobenzene (phenyl chloride), bromobenzene (phenyl bromide) , phenol and reference compounds (benzene, toluene) on the activity of acetoclastic methanogenic bacteria was studied at various levels, from concentrations that were nontoxic to those that were completely inhibitory concentration to acetoclastic methanogenic activity, as typified by the experiment with chlorobenzene in Fig. 1. Table 1 summarizes the 50% inhibiting concentrations (IC_{50}) of monomeric tannin compounds evaluated in this study, ranked in decreasing order of toxicity.

Table 1. The IC_{50} values observed in the study for the benzyl (Bn) and phenyl (Ph) compounds and reference aromatics

N°	Compounds	IC_{50} (mg/l)	logPoct
1	α-Chlorotoluene (BnCl)	0.6±0.1	2.30
2	Bromobenzene (PhBr)	2.9±0.2	2.99
3	α-Bromotoluene (BnBr)	8.7±0.2	2.92
4	Chlorobenzene (PhCl)	30.1±2.0	2.84
5	Toluene (BnH)	146.6±6.3	2.73
6	Benzene (PhH)	208.8±6.3	2.13
7	Phenol (PhOH)	1248.9±29.3	1.47
8	Benzyl alcohol (BnOH)	2391.4 ±28.7	1.10

The methanogenic inhibition as exhibited by the benzyl and phenyl compounds is illustrated in Fig. 3.

The obtained results indicate that relationships exist between the benzyl and phenyl compounds chemical structure and their inhibitory effects on biosynthesis of methane by methanogens. According to the Fig. 3, α-chlorotoluene and bromobenzene with 0.61 and 2.90 mg/l IC_{50} values, respectively, are the most toxic, while phenol and benzyl alcohol with IC_{50} values of 1248.90 and 2391.37 are less toxic.

With some exception, the results obtained in this work are compared quite well with those reported in our previous works [18,19] and by Sierra and Lettinga, [17] for monosubstituted aromatic compounds, as far as acetate was the substrate used in the bioassay. The addition of a functional group containing an oxygen or sulfur heteroatom to benzene, our reference compound, decreased the benzyl and phenyl compounds toxicity as in the case of OH substitution. However, the substitution of benzene H by Cl, Br, and CH_3 was associated with an increase in benzyl and phenyl compounds toxicity. But the results obtained with digested pig manure are in complete agreement with the above theory, which is not the case of the granular sludge.

This sufficiently demonstrates that the grafting of hydrophobic or hydrophilic substituent on the benzene, make the obtained compound more or less toxic [6,10,11]. In fact, it is known that a substitution on the aromatic ring that enhances the hydrophobicity render the molecule more toxic and that enhances the hydrophobicity of aromatic ring causes the molecule to be less toxic. According to Hansch and Leo works [9]:

- Hydrocarbon or halogenated substituents on the benzene ring are lipophilic in the case of CH_3, F, Cl, Br, I, NO_2 ...
- The substituents containing electronegative atoms such as O and N are generally hydrophilic (OH, SH, NH_2, CHO, COOH, $CONH_2$, OCH_3, $OCOCH_3$).

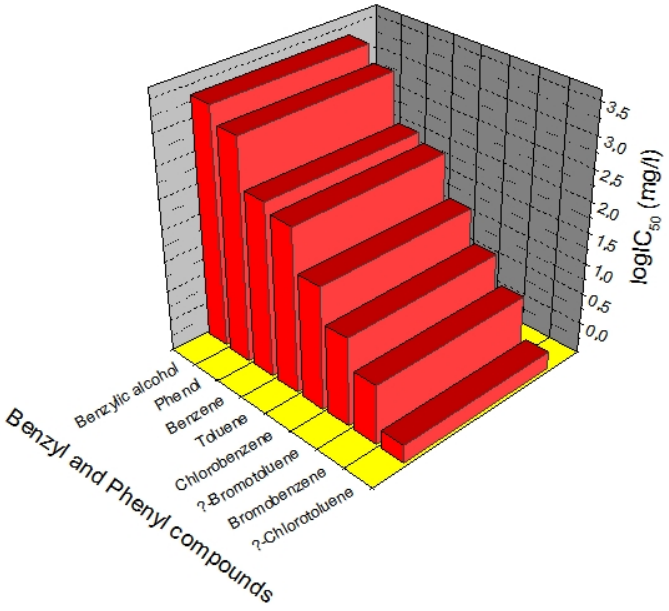

Fig. 3. Variation of the methanogenic toxicity as exhibited by benzyl and phenyl compounds

The diffusion of a molecule across a membrane depends on the permeability of this membrane. However, the membrane permeability is a function of the partition coefficient logPoct (hydrophobicity). So the more hydrophobic a molecule is, the higher is its membrane permeability and the greater is its toxicity [5,16].

Thus, the higher value of logPoct of an aromatic compound indicates that the compound readily penetrates the bacterial membrane and becomes toxic. It follows: alteration of the membrane structure, leakage of substances, the disruption of metabolism, cell degeneration and ultimately his death [5].

3.3 Comparison of Benzyl and Phenyl Methanogenic Toxicity on Methanogenic Bacteria

The methanogenic toxicity of benzyl and phenyl compounds with the same substituents is compared in the Fig. 4. About our references compound, the benzene is a phenyl compound with H as the substituent.

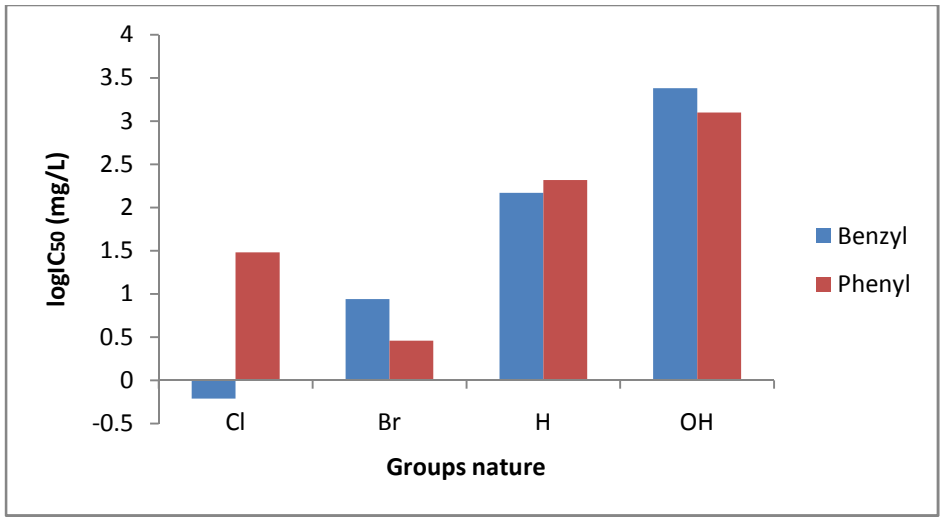

Fig. 4. Methanogenic toxicity of benzyl compounds versus phenyl one

According to the Fig. 4, the toluene is more toxic than benzene. Comparing the phenyl and benzyl compounds with the same substituents, the benzyl chloride is more toxic than phenyl chloride while benzyl bromide is less toxic than phenyl bromide. Otherwise, benzyl alcohol is less toxic than phenol. This implies that the behavior of phenyl and benzyl are different in inhibition of methane production by the methanogens because their chemical structures are not identic.

These results are comparable to those obtained by Sierra and Lettinga [17] for benzyl alcohol and phenol; toluene and benzene. But the others couples of benzyl and phenyl compounds were not studied by these authors. The other data on methanogenic toxicity of aromatic compounds has been investigated in our previous papers [18,19].

3.4 Correlation of the Methanogenic Toxicity with Aromatic Compounds Hydrophobicity

Correlations between toxicity and partition coefficient within series of organic contaminants structurally related have been reported by a number of research groups using fish, ciliate or microorganisms as tests organisms. Therefore, when comparing compounds that possess different types of substitutions, a perfect correlation with logPoct of the compound cannot be expected. A higher correlation could potentially be obtained by comparing compounds in homologues series [5,8,17].

To determine if the lipophilic character of benzyl and phenyl compounds tested could be correlated with their methanogenic toxicity, the logarithm of the IC_{50} values of these aromatics were plotted against the logarithm of the octanol-water partition coefficient (logPoct) of the aromatic compounds. Fig. 5 shows the correlation line between the methanogenic toxicity and partition coefficient logPoct for phenyl compounds (chlorobenzene, bromobenzene, benzene and phenol). No correlation was found for benzyl compounds (benzyl chloride, benzyl bromide, benzyl alcohol and toluene) methanogenic toxicity (IC50) and partition coefficient (logPoct) correlation.

It can be notice that there is a significant correlation between the toxicity of phenyl compounds and their hydrophobicity (R^2 = 0.911). This causes that the more hydrophobic molecule is, the more readily it crosses the cell membrane and becomes highly toxic and inversely [6,16].

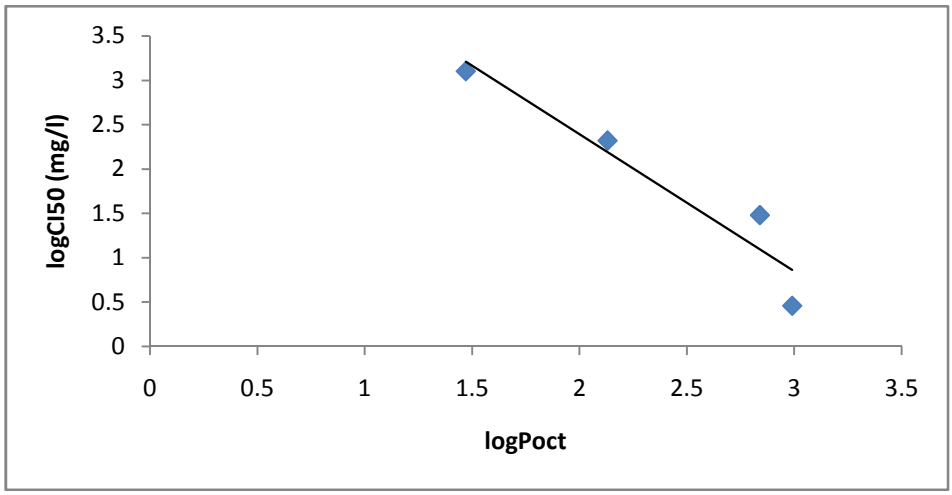

Fig. 5. Effect of hydrophobicity on phenyl methanogenic toxicity: methanogenic toxicity (IC_{50}) and partition coefficient (logPoct) correlation (R^2 = 0.911)

4. CONCLUSION

The obtained results indicate that relationships exist between the benzyl and phenyl compounds chemical structure and their inhibitory effects on biosynthesis of methane by methanogens. According to these results, α-chlorotoluene and bromobenzene with 0.61 and

2.90 mg/l IC_{50} values, respectively, are the most toxic compounds, while phenol and benzyl alcohol with IC_{50} values of 1248.90 and 2391.37 are less toxic.

Comparing the phenyl and benzyl compounds with the same substituents (Cl, Br,H,OH), the benzyl chloride is more toxic than phenyl chloride while benzyl bromide is less toxic than phenyl bromide. Otherwise, benzyl alcohol is less toxic than phenol. This implies that the behavior of phenyl and benzyl are different in inhibition of methane production by the methanogens because their chemical structures are not identic. A significant correlation was found between the toxicity of phenyl compounds and their hydrophobicity (R^2 = 0.911). But any correlation was not found for benzyl compounds.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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