SYNTHESIS, CHARACTERIZATION, THEORETICAL CALCULATIONS AND ANTIMICROBIAL STUDIES OF SUBSTITUTED 3-AMINOCYCLOHEXANESPIRO-5-HYDANTOINS

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Abstract

This article presents the synthesis of 6- and 8- substituted 3-aminocyclohexanespiro-5-hydantoins. The initial substituted cyclohexanespiro-5-hydantoins were prepared by means of the *Bucherer-Lieb* method. The products obtained were treated with hydrazine hydrate. As a result of this interaction the corresponding substituted 3-aminocyclohexanespiro-5-hydantoins were synthesized. The compounds obtained were characterized by physicochemical parameters, ^{1}H, ^{13}C NMR and IR spectroscopy. The structures of the target products were optimized using Density Functional Theory (DFT) methods at B3LYP/6-31G(d,p) level. The theoretical IR and NMR spectra of the compounds were calculated at B3LYP/6-31G(d,p) and B3LYP/6-31+G(2d,p) levels, respectively and compared with the experimental data. The antimicrobial efficiency of the substituted 3-aminocyclohexanespiro-5-hydantoins was examined using the agar well diffusion method.

The antimicrobial tests were carried out against the following microorganisms: Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*, Gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella abony*, the yeasts *Candida albicans* and *Saccharomyces cerevisiae*, the molds *Penicillium chrysogenum* and *Aspergillus niger*, plant pathogenic fungi *Fusarium oxysporum* and *Pythium ultimum* and the plant pathogenic bacterium *Pseudomonas syringae*.

Key words: substituted 3-aminocyclohexanespiro-5-hydantoins, DFT, antimicrobial activity.

INTRODUCTION

Substituted cycloalkanespiro-5-hydantoins have been prepared and tested for toxicity, for gross effects on behavior, and for anticonvulsant and analgesic activity in mice. It has been found that certain cyclohexanespiro-5-hydantoins showed analgesic and antiinflammatory activity (Oldfield and Cashin, 1965). Various cycloalkanespiro-5-hydantoins have been presented as herbicides (Schröder et al., 1990). Furthermore, the strong insecticidal action of cyclohexanespiro-5-hydantoin against alfalfa weevil, *Hypera postica* (Gyll.) (Coleoptera: Curculionidae) has been established (Atanasova et al., 2014).

It is well known that the interaction of spirohydantoins with hydrazine hydrate leads to formation of the corresponding 3-amino derivatives (Wildonger and Winstead, 1967; Naydenova et al., 2002; Marinova et al., 2014; Marinov et al., 2014). Different 3-aminospirohydantoins were synthesized and their biological properties were reported in the previous works of ours. The anticonvulsive effect of a series of 3-aminocycloalkanespiro-5-hydantoins with 5-, 6-, 7-, 8- and 12- membered rings was studied. The tested compounds showed no such type of activity. Moreover, it was found that some of the products induced seizures (Naydenova et al., 2002). Recently, the investigation of the antimicrobial effect of 3-amino-9'-fluorenespiro-5-hydantoin showed that this compound exhibited pronounced antibacterial activity against the bacterium *Escherichia coli* (Marinova et al., 2014).

The aim of the current research is to present the synthesis and structural elucidation of some 6- and 8- substituted 3-aminocyclohexanespiro-5-hydantoins and to evaluate their antimicrobial activity.

MATERIALS AND METHODS

General

All chemicals used were purchased from Merck and Sigma-Aldrich. The melting points were determined with a digital melting point apparatus SMP 10. The elemental analysis data were obtained with an automatic analyzer Carlo Erba 1106. The purity of the compounds was checked by thin layer chromatography on Kieselgel 60 F_{254}, 0.2 mm Merck plates, eluent system (vol. ra-

tio): benzene : ethanol = 5 : 1. The IR spectra were registered in KBr pellets on a Bruker FT-IR VERTEX 70 Spectrometer from 4000 cm^{-1} to 400 cm^{-1} at resolution 2 cm^{-1} with 25 scans. The NMR spectra were taken on a Bruker DRX-250 spectrometer, operating at 250.13 and 62.90 MHz for ^{1}H and ^{13}C, respectively, using the standard Bruker software. Chemical shifts were referenced to tetramethylsilane (TMS). Measurements in DMSO-d_6 solutions were carried out at ambient temperature. The substituted cyclohexanespiro-5-hydantoins (**2a-2d**, Scheme 1) were synthesized *via* the Bucherer-Lieb method (Bucherer and Lieb, 1934). The substituted 3-aminocyclohexanespiro-5-hydantoins (**3a-3d**, Scheme 1) were obtained in accordance with Marinov et al. (Marinov et al., 2014).

Computational details

The geometries and normal mode vibrational frequencies of the compounds synthesized **3a-3d** were computed at the DFT level using Firefly QC package (Granovsky), which is partially based on the GAMESS (US) (Schmidt et al., 1993; Gordon and Schmidt, 2005) source code. Geometry optimization of these structures was carried out by the hybrid B3LYP functional which combines the three-parameter exchange functional of Becke (Becke, 1993) with the LYP correlation one (Lee et al., 1988) using 6-31G(d) basis set. Vibration frequency calculations were performed numerically to obtain vibrational zero point and thermal energies and to validate that the found structures corresponded to the energy minima. The calculations were carried out without symmetry constraints by the gradient procedure. A gradient convergence threshold of 1 x 10^{-4} hartree $Bohr^{-1}$ was used.

The proton and carbon chemical shieldings were calculated with the B3LYP functional and 6-31+G(2d,p) basis set using the gauge-including atomic orbitals (GIAO) approach (Ditchfield, 1974; Wolinski et al., 1990) and B3LYP/6-31G(d) optimized geometry. Solvent effect was accounted by using the self-consisted reaction field method with the conductor polarizable continuum model (C-PCM) formalism (Cossi et al., 2003). The including of the solvent as dielectric in GIAO NMR calculations was used to estimate the effect of the medium (DMSO) on the chemical shifts of **3a-3d** compounds. In order to compare with the experimental data, the calculated absolute shieldings were transformed to chemical shifts using the reference compound tetramethylsilane (TMS): $\delta = \delta_{calc}(TMS) - \delta_{calc}$. Both $\delta_{calc}(TMS)$ and δ_{calc} were evaluated with the same method and basis set. The NMR calculations were carried out using GAUSSIAN 09 program package (Frisch et al., 2009).

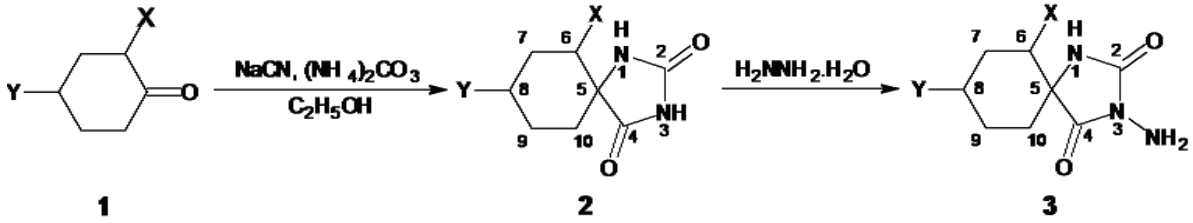
Antimicrobial assay

The microbial cultures were purchased from National Bank of Industrial Microorganisms and Cell Cultures (NBIMCC), Sofia. The plant pathogenic microorganisms were isolated from plant tissue materials. The antimicrobial effect of the synthesized compounds ***3a-3d*** against Gram-positive bacteria *Staphylococcus aureus* ATCC 6538 and *Bacillus subtilis* ATCC 6633, Gram-negative bacteria *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella abony* NTCC 6017, the yeasts *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* ATCC 9763, the molds *Penicillium chrysogenum*, and *Aspergillus niger*, as well as towards plant pathogenic fungi *Fusarium oxysporum* and *Pythium ultimum* and a plant pathogenic bacterium *Pseudomonas syringae* was studied. The technique used for this investigation was the agar well diffusion method (Perez et al., 1990). The agar media used were as follows: Tryptic soy agar (Biolife) for the tests with bacteria, Sabouraud-dextrose agar (Biolife) for yeasts and molds, Potato dextrose agar (PDA) (Merck) for plant pathogenic fungi and Tryptic soy agar medium (TSA) for a plant pathogenic bacterium. The bacteria were grown at 37 °C. The yeasts, molds and plant pathogenic microorganisms were cultured at 28 °C. The wells (Ø = 7 mm) were filled with solutions (50 µl) of the compounds synthesized. The concentrations of compounds ***3a-3d*** in pure dimethyl sulfoxide (DMSO) were as follows: 1, 0.1, 0.01 and 0.001 %. The pure DMSO (50 µl) was used as a control.

RESULTS AND DISCUSSION

The synthesis of the target compounds was performed in accordance with Scheme 1. The substituted 3-aminocyclohexane-5-hydantoins (**2a-2d**) were synthesized by the Bucherer-Lieb method (Bucherer and Lieb, 1934). The reaction was carried out by an interaction between the corresponding substituted cyclohexanone (**1a-1d**), sodium cyanide, ammonium carbonate and ethanol. The compounds obtained (**2a-2d**) were treated with concentrated hydrazine hydrate in accordance with Marinov et al. (Marinov et al., 2014). As a result of this interaction the corresponding substituted 3-aminocyclohexanespiro-5-hydantoins (**3a-3d**) were synthesized. The products obtained were characterized by physicochemical parameters, elemental analysis and spectral data. The results obtained from these analyses are listed in Tables 1-4 respectively.

To obtain information for the geometric parameters of the compounds presented in Fig. 1, DFT calculations in the gas phase were performed. Full geometry optimization of the structures was carried out using B3LYP functional and 6-31G(d,p) basis set. Since two of the structures (**3a** and **3b**) are positional isomers, the free Gibbs energies was calculated. It was found that the isomer **3b** is most stable than **3a** one by 1.02 kcal mol^{-1}. The different position of the -CH_3 group (6-methyl- to 8-methyl-) in compounds **3a** and **3b** does not lead to any changes in bond lengths of the five-membered ring. The alteration occurs only in cyclohexane ring - the C5-C6 and C5-C10 bonds became shorter in **3b** while the C8-C7 and C8-C9 ones are elongated. The bond lengths in cyclohexane moiety as well as in imidazolidine ring are not changed by elongation of the hydrocarbon chain from methyl to propyl group.



a) X = Me-, Y = H-; **b)** X = H-, Y = Me-; **c)** X = H-, Y = Et-; **d)** X = H-, Y = Pr-

Scheme 1. *Synthesis of substituted 3-aminocyclohexanespiro-5-hydantoins*

Table 1. Physicochemical parameters of compounds **2a-2d** and **3a-3d**

Compound	X	Y	Systematic name	Yield, %	M. p., °C	R_f
2a	Me-	H-	6-methyl-1,3-diazaspiro[4.5]decane-2,4-dione	55	217-218	0.56
2b	H-	Me-	8-methyl-1,3-diazaspiro[4.5]decane-2,4-dione	88	276-277	0.53
2c	H-	Et-	8-ethyl-1,3-diazaspiro[4.5]decane-2,4-dione	91	265-266	0.62
2d	H-	Pr-	8-propyl-1,3-diazaspiro[4.5]decane-2,4-dione	95	301-302	0.59
3a	Me-	H-	3-amino-6-methyl-1,3-diazaspiro[4.5]decane-2,4-dion	80	215-216	0.47
3b	H-	Me-	3-amino-8-methyl-1,3-diazaspiro[4.5]decane-2,4-dione	95	211-212	0.44
3c	H-	Et-	3-amino-8-ethyl-1,3-diazaspiro[4.5]decane-2,4-dione	92	190-191	0.56
3d	H-	Pr-	3-amino-8-propyl-1,3-diazaspiro[4.5]decane-2,4-dione	97	205-206	0.52

Table 2. Elemental analysis data of compounds **2a-2d** and **3a-3d**

Compound	Molecular formula	Elemental analysis, %					
		Calculated			Found		
		C	H	N	C	H	N
2a	$C_9H_{14}N_2O_2$ 182.22	59.32	7.74	15.37	59.18	7.61	15.17
2b	$C_9H_{14}N_2O_2$ 182.22	59.32	7.74	15.37	59.07	7.58	15.20
2c	$C_{10}H_{16}N_2O_2$ 196.25	61.20	8.22	14.27	61.01	8.00	14.11
2d	$C_{11}H_{18}N_2O_2$ 210.27	62.83	8.63	13.32	62.68	8.45	13.18
3a	$C_9H_{15}N_3O_2$ 197.23	54.81	7.67	21.30	54.63	7.51	21.09
3b	$C_9H_{15}N_3O_2$ 197.23	54.81	7.67	21.30	54.72	7.57	21.15
3c	$C_{10}H_{17}N_3O_2$ 211.26	56.85	8.11	19.89	56.66	8.02	19.79
3d	$C_{11}H_{19}N_3O_2$ 225.29	58.64	8.50	18.65	58.53	8.37	18.55

Owing to the great similarity of the structures, DFT calculations of the carbon and hydrogen NMR chemical shifts were also performed and it has been found to be a reliable method for structural determination. We present our GIAO NMR results considering DMSO solvation employing the polar-continuum model (Table 4). The predicted values of the chemical shifts for compounds **3a-3d** are unsatisfactory if the NMR cal-

Table 3. Experimental IR, ^{1}H and ^{13}C NMR data of compounds **2a-2d**

Compound	IR (KBr) ν_{max}/cm^{-1}	^{1}H NMR (DMSO-d_6) δ/ppm	^{13}C NMR (DMSO-d_6) δ/ppm *
2a	3273, 3066, 2935, 1767, 1725	0.67 (s, CH_3), 1.21-2.52 (m, 8H), 8.25 (s, 1H), 10.60 (s, 1H)	15.9 (CH_3), 21.1, 25.5, 29.6 (CH_2), 36.2 (CH), 66.4 (C_5), 157.5 (C=O), 178.8 (C=O)
2b	3254, 3068, 2925, 1773, 1734	0.91 (s, CH_3), 1.19-2.52 (m, 8H), 8.18 (s, 1H), 10.33 (s, 1H)	15.7 (CH_3), 25.1, 29.5 (CH_2), 37.1 (CH), 65.8 (C_5), 158.2 (C=O), 178.1 (C=O)
2c	3188, 3048, 2929, 1777, 1734	0.95 (s, CH_3), 1.26-2.52 (m, 10H), 8.28 (s, 1H), 10.51 (s, 1H)	15.1 (CH_3), 27.8, 34.1 (CH_2), 32,1 (CH_2, aliph.), 35.9 (CH), 64.7 (C_5), 157.0 (C=O), 178.8 (C=O)
2d	3194, 3070, 2936, 1776, 1734	0.86 (s, CH_3), 1.15-2.52 (m, 12H), 8.45 (s, 1H), 10.57 (s, 1H)	14.7 (CH_3), 27.7, 33.6 (CH_2), 24.5, 39.1 (CH_2, aliph.), 35.8 (CH), 62.8 (C_5), 156.9 (C=O), 179.2 (C=O)

** These assignments are confirmed by the DEPT-135 spectral data.*

Table 4. Selected experimental and B3LYP/6-31G(d,p) calculated (in bold) IR frequencies and B3LYP/6-31+G(2d,p)//B3LYP/6-31G(d,p) GIAO NMR chemical shifts in DMSO of compounds **3a-3d.** The frequencies are *scaled by 0.945*

Compound	IR (KBr) ν_{max}/cm^{-1}	^{1}H NMR (DMSO-d_6) δ/ppm	^{13}C NMR (DMSO-d_6) δ/ppm *
3a	3503, 3421, 3273, 2932, 1778, 1726, 1610 **3429, 3341, 3264, 2929, 1773, 1732, 1610**	0.62; **0.77** (s, CH_3), 1.56-2.52; **1.43-2.37** (m, 8H), 4.74; **3.6** (s, 2H), 8.25; **4.7** (s, 1H)	14.8; **18.1** (CH_3), 20.6, 25.1, 29.5, 33.6; **21.9, 25.4, 30.0, 36.0** (CH_2), 35.7; **38.9** (CH), 67.6; **67.5** (C_5), 157.1; **157.6** (C=O), 177.5; **176.9** (C=O)
3b	3500, 3432, 3269, 2930, 1775, 1729, 1612 **3429, 3344, 3267, 2928, 1774, 1733, 1610**	0.88; **0.97** (s, CH_3), 1.51-2.52; **1.21-2.21** (m, 8H), 4.71; **3.8** (s, 2H), 8.18; **5.01** (s, 1H)	15.2; **23.8** (CH_3), 24.7, 29.0; **29.6, 34.3** (CH_2), 34.3; **31.6** (CH), 66.1; **64.6** (C_5), 157.9; **157.2** (C=O), 177.9; **176.8** (C=O)
3c	3492, 3416, 3243, 2932, 1776, 1731, 1613 **3430, 3342, 3264, 2931, 1774, 1733, 1609**	0.86; **0.90** (s, CH_3), 1.48-2.52; **1.27-2.21** (m, 10H), 4.75; **3.56** (s, 2H), 8.28; **5.02** (s, 1H)	14.6; **14.9** (CH_3), 27.6, 34.0; **27.3, 34.2** (CH_2), 29.9; **33.1** (CH_2, aliph.), 35.2; **38.3** (CH), 65.6; **64.6** (C_5), 156.8; **157.2** (C=O), 178.7; **176.7** (C=O)
3d	3495, 3421, 3240, 2930, 1775, 1728, 1610 **3427, 3341, 3263 2935, 1773, 1732, 1610**	0.83; **0.99** (s, CH_3), 1.50-2.52; **1.21-2.21** (m, 12H), 4.77; **3.59** (s, 2H), 8.45; **5.00** (s, 1H)	14.4; **17.1** (CH_3), 28.2, 34.5; **29.7, 34.2** (CH_2), 24.1, 38.4; **30.3, 40.8** (CH_2, aliph.), 35,7; **37.8** (CH), 64.9; **64.2** (C_5), 158.7; **157.1** (C=O), 178.6; **176.8** (C=O)

** These assignments are confirmed by the DEPT-135 spectral data*

culations are performed in gas phase. More adequate results are obtained when NMR spectra is calculate in solution taking into account DMSO as solvent.

Because of the sensitivity of ^{13}C NMR chemical shifts to the presence of polarization and diffuse functions in the basis set the 6-31+G(2d,p) basis set was employed (Blicharska and Kupka, 2002; d'Antuono et al., 2005). Our theoretical results are in agreement with the ^{1}H and ^{13}C NMR measurements in DMSO-d_6 solution of compounds **3a**-**3d**. The change of the substituent position from 6-methyl- to 8-methyl- influences NMR spectra of compounds **3b** and **3a**. In the ^{13}C NMR spectra of **3a** and **3b** the most significant chemical shift changes are observed for the three carbon nuclei – in CH_3, CH_2 and CH groups (Table 4). Because of the elongation of the hydrocarbon chain in **3b-3d** from methyl to propyl group only unsignificant chemical shift alterations in all spectra were detected (in carbons from aliphatic $–CH_2$ group). Similar changes are observed in ^{1}H NMR spectra of four compounds. The alterations are related to the position of the methyl group in cyclohexane ring. The most significant chemical shift changes are observed for the protons of $–CH_3$ group and cyclohexane moiety.

The infrared spectra of 3-amino-6-methyl-1,3-diazaspiro[4.5]decane-2,4-dione (**3a**), 3-amino-8-methyl-1,3-diazaspiro[4.5]decane-2,4-dione (**3b**), 3-amino-8-ethyl-1,3-diazaspiro[4.5]decane-2,4-dione (**3c**) and 3-amino-8-propyl-1,3-diazaspiro[4.5]decane-2,4-dione (**3d**) were computed at B3LYP/6-31G(d,p) level. Available experimental data for the vibrational frequencies of the four compounds in KBr are presented for comparison. All results are listed in Table 4. Our assignments for the DFT calculated frequencies are based upon the analysis of the corresponding vibrational eigenvectors. Some modes such as N-H, C=O, N-N stretching and NH_2 deformation were found to be characteristic.

To verify the structures, we compared our DFT calculated wavenumbers for the compounds synthesized (Fig. 1) with the experimental data in KBr (Table 4). There is good agreement between the calculated and experimentally observed frequencies for **3a-3d**. The typical strong carbonyl bands at 1775-1778 cm^{-1} is calculated to be at 1773-1774 cm^{-1}. Good correspondence is found for the characteristic ν(N-H), ν(NH_2) and in-plane deformation δ(NH_2) as well as other low-frequency bands. The calculated amino in-plane deformation δ(NH2) is found to be 1609-1610 cm^{-1} while the experimental one is 1610-1613 cm^{-1}. Generally, the changes in the type or position of the substituents do not affect to characteristic modes in IR spectra of compounds **3a-3d**.

The antimicrobial activity of the products **3a-3d** was studied against Gram-positive bacteria *Staphylococcus aureus* ATCC 6538 and *Bacillus subtilis* ATCC 6633, Gram-negative bacteria *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella abony* NTCC 6017, the yeasts *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* ATCC 9763, the molds *Penicillium chrysogenum*, and *Aspergillus niger*, as well as towards plant pathogenic fungi *Fusarium oxysporum* and *Pythium ultimum* and a plant pathogenic bacterium *Pseudomonas syringae*. It was found that compounds **3a-3d** showed no activity against the tested microorganisms.

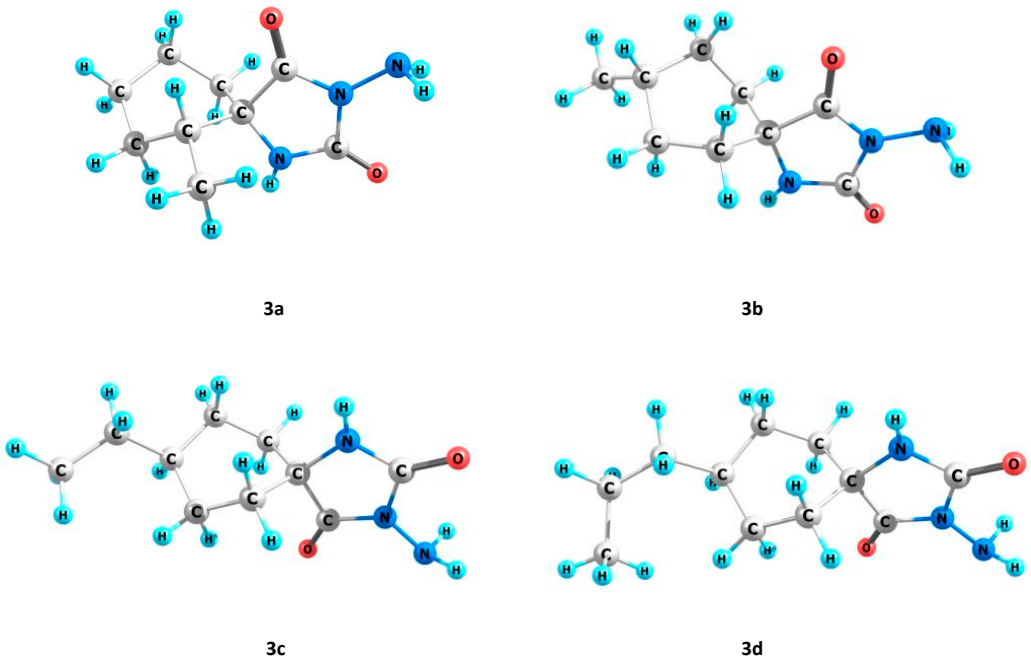


Fig. 1. *B3LYP/6-31G(d,p) optimized structures of compounds* ***3a-3d***

CONCLUSIONS

1. The synthesis and structural characterization of 6- and 8-substituted 3-aminocyclohexanespiro-5-hydantoins (**3a-3d**) were presented. DFTB3LYP/6-31G(d,p) calcu-lations were performed to obtain information about the geometric parameters, NMR and IR spectral behavior of the compounds synthesized.

2. It was found that elongation of the hydrocarbon chain of the substituent in cyclohexane ring of **3b-3d** did not affect on the bond lengths as well as on the characteristic modes in IR spectra.

3. The most significant chemical shift changes in ^{1}H and ^{13}C NMR scpectra are observed when the position of substituent (-CH_3 group) is changed.

4. The conducted tests for the presence of biological activity revealed that compounds **3a-3d** could not serve as antimicrobial agents.

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