Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. 77 (6) 717–723 (2012) UDC 547.583.1+547.21+5-42.913:615.28–188
JSCS–4302 *Original scientific paper*

Synthesis and antimicrobial activity of novel tetrabromo-bis(substituted benzyl)cycloalkanones

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(Received 8 April 2011, revised 6 January 2012)

Abstract: A series of novel tetrabromo-bis(substituted benzyl)cycloalkanones have been synthesized through a rapid, simple, and efficient methodology in an excellent isolated yield and characterized *via* IR, NMR (^{1}H-, ^{13}C-NMR, DEPT 135, DEPT 90) and mass spectrometry. All compounds were assayed for their *in vitro* antimicrobial activities against eight bacteria and five fungi. They showed stronger antibacterial than antifungal activities. Compounds **4c**, **4d** and **4i**, containing a methoxy or chloro substituent on the *para* or *meta* position of the phenyl ring, showed comparable minimum inhibitory concentration (*MIC*) values to those of the standard antibiotics streptomycin and tetracycline. Among all the tested compounds, **4i** exhibited good to moderate antifungal activity against all the tested fungal strains used.

Keywords: antibacterial; antifungal; tetrabromo-bis-(substituted benzyl)cycloalkanones.

INTRODUCTION

In the past few decades, the incidence of serious antimicrobial infection has increased dramatically[1] due to the problem of multi-drug resistant microorganisms that has reached alarming levels around the world. For the treatment of microbial infections, the synthesis of new anti-infectious compounds has become a critical need. The organohalogen compounds have received much attention due to their important use in the field of medicinal and agro-chemistry.[2–4] A survey of the literature also revealed that different kinds of bis(substituted benzylidene)cycloalkanones, including organohalogen compounds, have been used for the treatment of the central nervous system, cardiovascular and obesity diseases,

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doi: 10.2298/JSC110408005M

and also showed antibacterial,[5] antifungal,[5] anti-angiogenic,[6,7] quinine reductase inducer,[8] cytotoxic,[9,10] and cholesterol-lowering activity.[11]

In 1980, Hoeve and Wynberg[12] prepared tetrabromo-di-*m*-anisalcyclopentanone from 2,5-dibenzylidenecycloalkanones using bromine in chloroform in a 47 % yield. Later, in 1988, Desiraju and Kishan[13] used the same starting material and obtained dibromo derivatives in which the bromine addition occurred on the methylene carbon of 2,5-dibenzylidenecycloalkanones. In the present study, we have synthesized and performed antimicrobial evaluation of a series of tetrabromo-bis(substituted benzyl)cycloalkanone analogues (**4a**–**j**). The structures of the new compounds were established by IR, NMR (^{1}H-, ^{13}C-, DEPT 135 and DEPT 90) and mass spectrometry.

EXPERIMENTAL

Chemistry

General. Melting points were recorded on a Fisher–Jones melting point apparatus and are uncorrected. IR spectra were obtained on an FTIR-300E (Jasco Corporation, Tokyo, Japan) in KBr disc. ^{1}H-NMR (250 MHz) and ^{13}C-NMR (62.5 MHz) spectra were recorded on a Bruker 250 spectrometer (Bruker, USA) in $CDCl_3$ with TMS as an internal standard. ESI--MS were measured on an LCQ advantage-trap mass spectrometer (Thermo Finnigan, San Jose, CA, USA).

*Synthesis of bis(substituted benzyl)cycloalkanones **3a**–**j***

The bis(substituted-benzyl)cycloalkanones were obtained by the cross-aldol condensation of substituted aldehydes with cycloalkanones in the presence of sodium acetate–acetic acid, Scheme 1.[14]

*Synthesis of tetrabromo-bis(substituted benzyl)cycloalkanones **4a**–**j***

The tetrabromo-bis(substituted benzyl)cycloalkanones (**4a**–**j**) were prepared by adding a Br_2 solution (2 mmol) in dioxane (10 mL) dropwise over 10 min to a stirred solution of the respective bis(substituted benzyl)cycloalkanone (**3a**–**j**, 1 mmol) in dioxane (20 mL). The stirring was continued for 3–4 h. The completion of the reaction was monitored by thin layer chromatography (TLC). The solvent was evaporated and the dry residue subjected to column chromatography (*n*-hexane/dichloromethane, 1:1), which afforded the respective compound **4a**–**j** as a white solid.

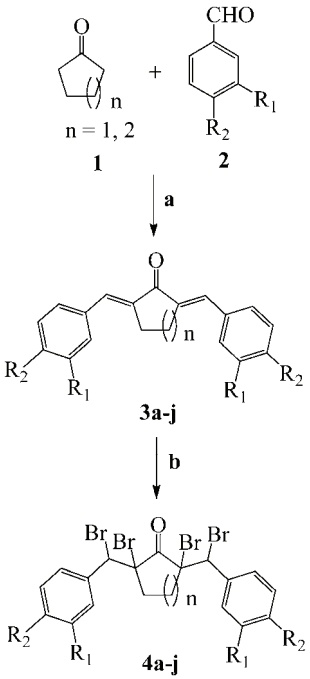
Data of synthesized compounds are given in Supplementary material.

Biology

Antibacterial screening. The *in vitro* antibacterial activity of the synthesized compounds **4a**–**j** were evaluated against eight bacterial strains, *i.e*., *Staphylococcus aureus* (KCTC 1916), *Bacillus subtilis* (ATCC 6633), *Listeria monocytogenes* (ATCC 19166), *Salmonella enteritidis* (KCCM 12021), *Pseudomonas aeruginosa* (KCTC 2004), *Enterobacter aerogenes* (KCTC 2190), *Salmonella typhimurium* (KCTC 2515) and *Escherichia coli* (ATCC 8739). All the bacterial strains were obtained from the Korea Food and Drug Administration (KFDA), Daegu, South Korea. Cultures of each bacterial strain were maintained on Luria broth (LB) agar medium at 4 °C.

The minimum inhibitory concentrations (*MIC*s) of compounds **4a**–**j** were tested by the two-fold serial dilution method.[15] The test compounds **4a**–**j** were incorporated into Luria–

–Broth medium to obtain a concentration of 1,000 μg ml^{-1} and serially diluted to a concentration in the range from 500 to 7.8 μg ml^{-1}. A standardized suspension (10 μl) of each of the tested organism (10^8 CFU ml^{-1}) was transferred to each tube and incubated at 37 °C for 24 h. Control tubes containing only bacterial suspensions were also incubated at 37 °C for 24 h. The lowest concentrations of the test samples that did not show any growth of the tested organism after macroscopic evaluation were determined as the *MICs*.



Compound	*n*	R_1	R_2
3a, 4a	1	H	H
3b, 4b	1	H	CH_3
3c, 4c	1	Cl	H
3d, 4d	1	H	OCH_3
3e, 4e	1	H	F
3f, 4f	2	H	H
3g, 4g	2	H	CH_3
3h, 4h	2	H	F
3i, 4i	2	H	OCH_3
3j, 4j	2	H	Br

Scheme 1. Reagents and conditions: a) AcONa, AcOH, 3–8 h, 120 °C; b) Br_2–dioxane, 3–4 h, room temperature.

Antifungal screening. The *in vitro* antifungal activities of the synthesized compounds **4a**–**j** were evaluated against various fungal species, *i.e.*, *Botrytis cinerea* (KACC 40573), *Rhizoctonia solani* (KACC 40111), *Fusarium oxysporum* (KACC 41083), *Sclerotonia sclerotiorum* (KACC 41065) and *Phytophthora capsici* (KACC 40157). The fungal cultures were obtained from the Korean Agricultural Culture Collection (KACC). Cultures of each fungal species were maintained on potato dextrose agar (PDA) slants and stored at 4 °C.

Potato dextrose agar (PDA) medium was used as the basal medium for the antifungal activity assay, which was performed by disc diffusion method.[16] Sterile Whatman No. 1 paper discs (6 mm diameter) were pierced in agar plates, equidistant and near the border, where 10 μl of compounds **4a**–**j** (200 μg $disc^{-1}$) were applied. A disc of fungal inoculum 6 mm in diameter was removed from the pre-grown cultures of all the tested fungal strains and placed upside down in the center of the Petri dishes. The plates were incubated at 28 °C for 5–7 days, the time in which the growth of the control would have reached the edges of the plates. Discs with $CHCl_3$ and nystatin were used as negative and positive controls, respectively. The inhibitory activity was measured (in mm) as the diameter of the observed inhibition zones.

The minimum inhibitory concentration (*MIC*) was determined by the two-fold dilution method,[15] against *B. cinerea*, *R. solani*, *F. oxysporum*, *S. sclerotiorum* and *P. capsici*. Samples were dissolved in $CHCl_3$ accordingly with their respective known weight. These solutions were serially diluted with $CHCl_3$ and were added to PDA to final concentrations of 500, 250 125 and 62.5, 31.2, 15.6 and 7.8 μg ml^{-1}, respectively. A 10 μl spore suspension of each test strain was inoculated in test tubes in PDA medium and incubated for 2–7 days at 28 °C. The control tubes containing PDA medium were inoculated only with fungal suspension. The minimum concentration at which no visible growth was observed was defined as the *MIC*, which was expressed in μg ml^{-1}.

RESULTS AND DISCUSSION

Chemistry

The synthetic route to the tetrabromo-bis(substituted benzyl)cycloalkanones (**4a**–**j**) are outlined in Scheme 1. The compounds **4a**–**j** were obtained from the intermediate compounds **3a**–**j**, respectively, in excellent yields (70–93 %). The structures of the newly synthesized compounds were established based on their IR, ^{1}H-NMR, ^{13}C-NMR, DEPT 135, DEPT 90 and mass spectral data. IR spectra of compounds **4a**–**j** showed an absorption peak in the 1660–1681 cm^{-1} region, indicating the presence of a carbonyl (>C=O) group. In the ^{1}H-NMR spectra, the characteristic benzylic protons of compounds **4a**–**j** appeared as a singlet at 5.50––6.03 ppm. The aliphatic protons of the cyclopentanone ring of compounds **4a**–**e** appeared as two AB-quartets at around 3.28–3.32 and 2.37–2.42 ppm equivalent to two protons, respectively. On the other hand, the six protons of the cyclohexanone ring of compounds **4f**–**j** were observed as triplets of a doublet at 2.99–3.07 ppm and two multiplets at around 2.14–2.46 and 1.90–2.03 ppm equivalent to two, three and one proton(s), respectively. The aromatic protons of the phenyl groups of compounds **4a**–**j** appeared in usual way. The methyl protons of compounds **4b** and **4g** were observed as a singlet at 2.35–2.36 ppm, whereas the methoxy protons of compounds **4d** and **4i** appeared as a singlet at 3.81–3.82 ppm. In the ^{13}C-NMR spectra of compounds **4a**–**j**, the characteristics carbonyl carbons and benzylic carbons appeared at 197.77–198.57 ppm and 51.40–55.30 ppm, respectively. In addition, the α-carbons were observed in the range of 63.95––67.98 ppm. In summary, all the newly synthesized compounds exhibited satisfactory spectral data consistent with the proposed structures. In addition, electron spray ionization mass spectra (ESI-MS) of **4a**–**j** showed a metastable ion peak as $[M(^{79}Br)+H]^+$ (100 %) in the positive mode along with other isotopic peaks.

Antibacterial activity

The *in vitro* antibacterial activities of the novel compounds **4a**–**j** were assayed against three Gram-positive bacteria *viz*. *S. aureus*, *B. subtilis* and *L. monocytogenes* and five Gram-negative bacteria, *viz*. *S. enteritidis*, *P. aeruginosa*, *E. aerogenes*, *S. typhimurium* and *E. coli*. The minimum inhibitory concentration

(*MIC* / μg ml^{-1}) was determined by the serial dilution method[15] for all the compounds and compared with the controls (streptomycin and tetracycline). The *MIC* values of the tested compounds are presented in Table I, from which it can be seen that all the tetrabromo-bis-benzylcycloalkanone analogues (**4a**–**j**) showed antibacterial activity against both the Gram-positive and Gram-negative bacteria. Among the tested compounds, compound **4i** exhibited the highest activity against both Gram-positive and Gram-negative strains, followed by compounds **4c** and **4d**. The *MIC* values of compounds **4i**, **4c** and **4d** are almost comparable to those of the standard drugs used in the present study against all bacterial strains.

TABLE I. Antibacterial activity (*MIC* / μg ml^{-1}) of compounds **4a**–**j**

Compound	*S. aureus*	*B. subtilis*	*L. monocytogenes*	*S. enteritidis*	*P. aeruginosa*	*E. aerogenes*	*S. typhimurium*	*E. coli*
4a	125	125	125	125	125	125	62.5	62.5
4b	62.5	62.5	31.2	62.5	125	62.5	62.5	125
4c	31.2	15.6	31.2	15.6	31.2	15.6	31.2	15.6
4d	31.2	15.6	32.2	15.6	31.2	15.6	31.2	31.2
4e	31.2	31.2	31.2	62.5	62.5	31.2	31.2	31.2
4f	62.5	125	62.5	125	62.5	62.5	125	125
4g	62.5	62.5	62.5	62.5	62.5	125	62.5	32.2
4h	31.2	62.5	62.5	62.5	62.5	62.5	31.2	31.2
4i	31.2	15.6	31.2	15.6	31.2	15.6	15.6	15.6
4j	62.5	62.5	31.2	31.2	62.5	31.2	31.2	31.2
Streptomycin	31.2	15.6	31.2	31.2	31.2	15.6	31.2	15.6
Tetracycline	15.6	15.6	31.2	15.6	31.2	15.6	15.6	15.6

Structure–activity relationships (SAR) may be explained briefly as follows: the ring size of cycloalkanones has no strong effect on the antibacterial activity, whereas the unsubstituted compounds (**4a** and **4f**) showed lower activity than the substituted compounds (**4b**–**d** and **4g**–**j**). Introduction of hydrophilic substituents (halogen, OMe) at the R_2 or R_1 position resulted in better activity than the hydrophobic substituent (CH_3). Among the compounds containing halogen substitution at the phenyl ring, the chlorine analogue (**4c**) showed better antibacterial activity than the fluorine or bromine analogues (**4e**, **4h** and **4j**).

Antifungal activity

The newly synthesized novel **4a**–**j** were evaluated for their *in vitro* antifungal activities against five fungal strains, *i.e.*, *B. cinerea*, *R. solani*, *F. oxysporum*, *S. sclerotiorum* and *P. capsici* by disc diffusion methods. The inhibition zones of the synthesized compounds were measured at a dose of 200 µg disc^{-1} and nystatin, a positive control, were evaluated at a dose of 30 µg disc^{-1}. As presented in Table II, among the all tetrabromo-bis-benzylcycloalkanone derivatives (**4a**–**j**) tested, 5 compounds inhibited the growth of some fungal strains. In detail, compound **4i**

showed good to moderate activity against all the tested fungal strains, while compounds **4d** and **4j** exhibited moderate activity against *R. solani* and *F. oxysporum*. Compounds **4f** and **4h** demonstrated moderate activity against one bacterial strain only. Compounds **4a**–**c**, **4e** and **4g** showed no activity against all the tested fungal strains. Therefore, the minimum inhibitory concentrations (*MIC*s) of compound only **4i** against *B. cinerea*, *R. solani*, *F. oxysporum*, *S. sclerotiorum*, and *P. capsici* were determined and found *MIC* values of 125, 31.2, 31.2, 125 and 250 μg ml^{-1}, respectively, were found (Table III).

TABLE II. Antifungal profile of compounds **4a**–**j** in terms of the inhibition zone in mm (data are means ± *SD* for at least three experiments. Concentrations of compounds **4a**–**j** and netilmycin were 200 and 30 μg $disc^{-1}$, respectively)

Compound	*B. cinerea*	*R. solani*	*F. oxysporum*	*S. sclerotiorum*	*P. capsici*
4d	–	08±1.1	10±1.1	–	–
4f	–	09±1.1	–	–	–
4h	–	–	07±1.1	–	–
4i	07±1.2	19±1.0	19±1.1	08±1.1	07±1.0
4j	–	11±1.1	11±1.2	–	–
Nystatin	23±1.1	25±1.2	25±1.1	27±1.1	33±1.0

TABLE III. Antifungal activity (*MIC* / μg ml^{-1}) of compound **4i**

Compound	*B. cinerea*	*R. solani*	*F. oxysporum*	*S. sclerotiorum*	*P. capsici*
4i	125	31.2	31.2	125	250
Nystatin	15.6	15.6	15.6	31.2	30.2

CONCLUSIONS

In this paper, an efficient synthesis of novel tetrabromo-bis(substituted benzyl)cycloalkanone derivatives and their antimicrobial activities against eight bacterial and five fungal strains were presented. All the tetrabromo-bis(substituted benzyl)cycloalkanone analogues showed potent antibacterial activity compared to their antifungal activity. Compounds **4i**, **4c** and **4d**, having a methoxy- or chloro-substituent on the *para* or *meta* position of the phenyl ring exhibited strong activity against all the tested bacterial strains. Hydrophilic substitution on the phenyl ring resulted in a better antibacterial activity than that of hydrophobic substitution. Among all the tested compounds, **4i** exhibited good antifungal activity against *R. solani* and *F. oxysporum*. The potential antimicrobial activity of compounds **4i**, **4c** and **4d** would be helpful in synthesis of a huge number of tetrabromo-bis(substituted benzyl)cycloalkanone analogues for extensive antimicrobial studies, which could be used to develop more suitable drug candidates.

SUPPLEMENTARY MATERIAL

Analytical and spectral data of synthesized compounds are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

ИЗВОД

СИНТЕЗА И АНТИМИКРОБНА АКТИВНОСТ НОВИХ ТЕТРАБРОМ-БИС(СУПСТИТУИСАНИХ БЕНЗИЛ)ЦИКЛОАЛКАНОНА

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Синтетисана је серија нових тетрабром-бис(супституисаних бензил)циклоалканона, брзим и ефикасним поступком. Производи су добијени у високом приносу и окарактерисани су спектроскопским методама (ИЦ, NMR (^{1}H-, ^{13}C-NMR, DEPT 135, DEPT 90)) и масеном спектрометријом. Испитана је *in vitro* антимикробна активност синтетисаних једињења према осам врста бактерија и 5 врста гљива. Тестирана једињења показују добру антибактеријску и антифунгалну активност. Једињења **4c**, **4d** and **4i** која садрже метокси- и хлор--супституенте у *пара* и *мета* положају на фенил групи имају вредности *MIC* које су блиске вредностима које дају стандардни антибиотици стрептомицин и тетрациклин. Од свих испитиваних једињења, дериват **4i** показује добру до умерену активност према све четири врсте гљива.

(Примљено 8. априла 2011, ревидирано 6. јануара 2012)

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