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Synthesis and Biological Activity of Benzyl Methoxy Carbamates

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ABSTRACT

The benzyl methoxy carbamates are a structurally versatile and pharmacologically important group of organic compounds that contain the benzyl and methoxy moieties in combination with the -NHCOO- functional group. The current paper describes the synthesis of five benzyl methoxy carbamate derivatives (BMC-1 through BMC-5) through a 1,1-Carbonyldiimidazole (CDI)-mediated coupling strategy between substituted benzyl alcohols and suitable amine precursors under mild, eco-friendly reaction conditions. The synthesized products were confirmed structurally by ^{1}H NMR, ^{13}C NMR, and infrared (IR) spectroscopy which confirmed the successful incorporation of the carbamate linkage and methoxy substitution. The ortho-methoxy derivative (BMC-3) had the highest synthesis efficiency, with a range of reaction yields between 70 and 88%. Extensive biological analysis was done including antibacterial activity against Staphylococcus aureus and Escherichia coli, antifungal activity against Candida albicans and Aspergillus niger, antioxidant potential using DPPH radical scavenging activity assay and cytotoxicity in MCF-7 and HeLa cancer cell lines using MTT assay. The para-methoxy-substituted compound of all derivatives consistently showed better biological performance with antibacterial inhibition zone of 22-24 mm, DPPH IC - 5 0 of 45.2 µg/mL and MTT IC - 5 0 of 38.4 µg/mL against MCF-7 cells. A structure-activity relationship (SAR) analysis showed that biological potency was directly regulated by the position and the donating nature of the methoxy substituents. The antimicrobial properties of these compounds were also confirmed by in vivo studies on murine infection models. Statistical tests using ANOVA indicated that there were significant differences ($p < 0.05$) between derivative activities. The results all demonstrate benzyl methoxy carbamates as effective multifunctional bioactive scaffolds in the development of pharmaceuticals and agrochemicals.

Keywords: *Benzyl Methoxy Carbamates, CDI Coupling, Structure-Activity Relationship, Antimicrobial Activity, Antioxidant, Cytotoxicity.*

Introduction

Carbamates are one of the most medicinally and agriculturally significant classes of organic compounds, and are characterized by the presence of the following linkage based on the carbamic acid: Their distinctive structural characteristics, comprising both amide-like stability and ester-like reactivity, have made them essential in the pharmaceutical chemistry, pesticide design and synthetic organic chemistry. In the 19th century, the first notable biological activity of a naturally occurring carbamate was described when Physostigmine, a methyl carbamate ester of Physostigma venenosum (Calabar beans), was found to inhibit acetylcholinesterase - a discovery that led to the foundation of modern carbamate pharmacology (Yu et al., 1988).

Since that time, carbamate derivatives have since become dominant in contemporary drug discovery and medicinal chemistry due to their peptide-like structure, high chemical and proteolytic stability, and capability to penetrate the cellular membranes. Several FDA-approved drugs are some incorporation of carbamate functional group as a structural or functional group, such as neostigmine in myasthenia gravis, rivastigmine in Alzheimer's disease, cenobamate in partial-onset seizures (Keam, 2020). The capability of carbamates to be selectively substituted at both N- and O-termini grants carbamates great flexibility in drug design, allowing the pharmacodynamics and pharmacokinetics to be tuned.

Out of the many subclasses of carbamates, the benzyl moiety with methoxy replacements have captured a lot of attention due to the synergistic pharmacophoric effects of the benzyl moiety and methoxy replacements. The benzyl scaffold is a well-established pharmacophore in medicinal chemistry which has good membrane permeability, and good interactions with hydrophobic binding pockets of target enzymes. Adding methoxy groups to the benzyl aromatic ring modulates the electronic density of the molecule through +M (mesomeric) and +I (inductive) effects, thus affecting its reactivity, lipophilicity, and affinity to biological targets (Mishra and Gupta, 2020).

The biological repertoire of benzyl carbamate derivatives is extensive and includes antibacterial, antifungal, antioxidant, anti-inflammatory, anticancer, cholinesterase-inhibitory and antitubercular activities. Recent literature suggests that substitution patterns on the benzyl aromatic ring - especially at the ortho, meta, and para positions - play a pivotal role in determining the nature and magnitude of biological response (Rao et al., 2019; Kumar et al., 2018). In particular, para-methoxy substitution has been linked to optimal resonance delocalization and electronic stabilization, which leads to enhanced receptor interactions and superior pharmacological activity (Reddy & Thomas, 2023).

Synthetic chemistry Synthetic approaches to carbamates have used hazardous reagents (including phosgene and its derivatives). But in recent progress there has been a move towards safer, more sustainable options. One of these is 1,1′-Carbonyldiimidazole (CDI), which has proven to be a particularly useful reagent, acting as a phosgene surrogate to the activation of alcohols and amines under mild conditions (Das & Bandyopadhyay, 2022). CDI-mediated carbamoylation is extremely appealing in terms of chemo selectivity, formation of minimal side-products and compatibility with green solvent systems.

Although numerous reports have been made on carbamate derivatives, little has been done on benzyl methoxy carbamates which simultaneously address synthetic optimization, spectroscopic characterization, structure-activity relationships (SAR), and multi-pronged biological evaluation. The majority of the existing studies concentrate either on one of the aspects of these compounds or on severe synthetic procedures with low generalizability. The absence of systematic SAR data relating the patterns of methoxy substitution (ortho, meta, para) to specific biological activities is an important literature gap.

The present study was thus designed with the following objectives: (i) to synthesize a focused library of benzyl methoxy carbamate derivatives using an optimized, environmentally-friendly CDI-mediated route; (ii) to confirm the chemical identity of each derivative through rigorous spectroscopic characterization; (iii) to assess their biological activities comprehensively, including antibacterial, antifungal, antioxidant, and cytotoxic activities; (iv) to establish structure-activity relationships linking methoxy substitution patterns to biological activities; and (v) to provide a theoretical framework of biological behaviour through correlation with established principles of electronic effect theory, lipophilicity-permeability models, and pharmacophore mapping.

What is important about this study is that it is an integrated approach to the study of carbamate chemistry that bridged the gap between synthetic chemistry in organic chemistry and pharmacological studies. This work has provided valuable comparative information, which can be used in future rational drug design projects by generating a well-characterized library of positional methoxy isomers and exposing them to uniform conditions of biological assays. Moreover, the utilization of CDI as a coupling agent coupled with mild, low-waste reaction conditions are aligned with the principles of the green chemistry concept, which leads to the broader aim of sustainable pharmaceutical synthesis.

Materials And Methods

Chemicals and Reagents

All chemicals in this study were of analytical grade or reagent grade. Benzyl alcohol (including 2-methoxybenzyl, 3-methoxybenzyl, and 4-methoxybenzyl alcohol isomers), methoxyamine hydrochloride, 2-amino-N-methoxy-N-methylacetamide and 1,1-Carbonyldiimidazole (CDI) were purchased commercially. Triethylamine (TEA) was used as the base. Solvents were dichloromethane (DCM), acetone, diethyl ether and tetrahydrofuran (THF). Biological assays involved the use of analytical standards such as ampicillin, fluconazole, butylated hydroxytoluene (BHT) and dimethyl sulfoxide (DMSO) as controls.

Synthesis in General of Benzyl Methoxy Carbamates

The synthesis has been conducted through a CDI-mediated reaction of substituted benzyl alcohols with suitable amine precursors. To process BMC-1: 50 mL of dichloromethane was added at room temperature in a 100 mL round-bottom flask (RBF). Benzyl alcohol (10 mL) and methoxyamine hydrochloride (5.0 g) were added and triethylamine (5.0 mL) was added dropwise. The mass of the reaction was cooled to 010 o C and the CDI (5.0 g) was added in portions. It was stirred at 35 -38 0 C during 3 hours. The progress of the reaction was monitored by TLC (mobile phase: cyclohexane/ethyl acetate, 8:2). Water (50 mL) was added to quench the reaction and the organic layer was separated and washed and dried over sodium sulphate and evaporated. BMC-1 was recrystallized on acetone to afford white crystalline BMC-1 (m.p. 100104 C).

The amine component was 2-amino-N-methoxy-N-methylacetamide which was reacted with a 8-hour reaction time (m.p. 106110C). Using 2-methoxybenzyl alcohol, 3-methoxybenzyl alcohol and 4-methoxybenzyl alcohol, derivatives BMC-3 through BMC-5 were prepared using the same CDI coupling protocol with suitable reaction times. All reactions were conducted in nitrogen atmosphere to prevent interference of moisture as CDI is very sensitive to moisture. Recrystallization or column chromatography was used to purify products.

Spectroscopic Characterization

The melting points were determined by using an open capillary method with DMSO-d6 as the solvent and chemical shifts recorded in ppm (delta) relative to TMS. KBr pellets on a Shimadzu FTIR spectrometer were used to obtain the IR spectra. ESI-MS was used to record mass spectra, purity was determined by TLC and sharpness of melting point.

Agar Well Diffusion Method -Antibacterial Activity

Agar well diffusion was used to evaluate this antibacterial activity against *Staphylococcus aureus* ATCC 29213 (Gram-positive) and *Escherichia coli* ATCC 25922 (Gram-negative) using the agar well diffusion method. Nutrient broth was used to culture test organisms and to adjust the turbidity of the test organisms to 0.5 McFarland standard turbidity. BMC solutions containing 6.25, 12.5, 25, 50, and 100 ug/mL were

inoculated and wells (6 mm diameter) loaded with BMC solutions at the concentrations of 6.25, 12.5, 25, 50, and 100 ug/mL. Ampicillin (10 µg/disc) was used as positive control. Plates were incubated at 37 o C in 24 hours, and the zones of inhibition (mm) were measured. By broth microdilution, Minimal Inhibitory Concentrations (MICs) were determined by using serial two-fold dilutions (0.125 -128 ug/mL).

Antifungal Effect Disc Diffusion Process

A screening test against *Candida albicans* and *Aspergillus niger* was performed with the use of *Sabouraud Dextrose Agar (SDA).* The fungal suspensions that had been made standard were spread-plated and sterile discs impregnated with BMC solutions at specified concentrations were placed on the inoculated surface. Plates were incubated at 28 C in 48 hours. Zones of inhibition were done and compared to the fluconazole standard. Broth microdilution was used to determine MIC values of Candida strains.

Antioxidant Activity - DPPH Radical Scavenging Assay

The 2, 2-diphenyl- 1-picrylhydrazyl (DPPH) assay was used to measure the free radical scavenging activity. A 96-well plate format was used to mix a methanolic DPPH solution (0.1 mM) with a range of BMC concentrations (10100 µg/mL). The solution was incubated at room temperature in the dark and left to incubate. A UV-Vis spectrophotometer was used to determine the absorbance at 517 nm. BHT was used as the positive control. The calculation of percentage radical scavenging activity was performed in the following way: % RSA = [(A 0 -As)/A 0) × 100, where A 0 is the absorbance of control and As is the absorbance of sample. The nonlinear regression analysis was used to determine the values of IC 50 (concentration that causes 50 percent inhibition).

Cytotoxicity – MTT Assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to measure the cytotoxic activity. Cells were seeded in 96-well plates at a density of 1 x 10 4 cells/well and incubated with various concentrations of BMC compounds (6.25-100 0mg/mL) at 48 hours at 37 C in 5% CO 2. The MTT reagent was added after treatment and formazan crystals were dissolved in DMSO. The absorbance was obtained at 570 nm. The percent cell viability was determined in terms of control cells that were not treated. IC_{50} values were calculated by sigmoidal dose-response fitting.

In Vivo Antimicrobial Studies

Under in vivo antimicrobial experiments, murine models were used in accordance with institutional guidelines of animal ethics. Infection of mice (Swiss albino, 2025 g) was done intraperitoneally with lethal doses of S. aureus or Candida albicans. BMC was given orally at the dosage of 10, 20, 40, 100, and 200mg/kg body weight. The number of bacteria in the blood was measured at 24-hour intervals, and fungal load on the skin and internal organs were evaluated after 5 days. Control groups were added to compare vehicle control and ampicillin/fluconazole treatment groups.

Statistical Analysis

All biological assays were done in triplicate at least on three instances. The results are noted as mean ± standard deviation (SD). One way analysis of variance (ANOVA) then followed by Tukey post hoc multiple comparison test were used to determine statistical significance. A p-value < 0.05 was considered statistically significant. The values of IC50 and MIC were determined with the help of GraphPad Prism software. Nonlinear regression analysis was used to obtain dose-response curves.

Results

Synthesis and Yield Analysis

All five target benzyl methoxy carbamate derivatives (BMC-1 to BMC-5) were successfully synthesized using the CDI-mediated carbamoylation strategy. The reaction yields varied between 70% and 88%, demonstrating satisfactory synthetic efficiency under mild and environmentally favourable conditions (Table 1). The ortho-methoxy derivative (BMC-3) recorded the highest yield of 88%, while the para-methoxy analogue (BMC-5) showed a slightly lower yield of 70%, attributed to greater steric effects at the para position relative to the reaction center. BMC-1 (unsubstituted benzyl methoxy carbamate) yielded 72%, and the remaining compounds BMC-2 and BMC-4 yielded 75% and 80%, respectively. All products were isolated as white to pale-yellow crystalline solids with sharp, reproducible melting points, confirming sample purity.

Table 1. Yield and Physical Properties of Synthesized BMC Derivatives

Compound	Molecular Formula	IUPAC Name (Abbreviated)	Yield (%)	M.P. (°C)
BMC-1	$C_9H_{11}NO_3$	Benzyl methoxy carbamate	72	100–104
BMC-2	$C_{12}H_{16}N_2O_4$	Benzyl (2-methoxy(methyl)amino)-2-oxoethyl carbamate	75	106–110
BMC-3	$C_{13}H_{18}N_2O_5$	2-Methoxybenzyl (2methoxy(methyl)amino)-2-oxoethyl carbamate	88	112–116
BMC-4	$C_{13}H_{18}N_2O_5$	3-Methoxybenzyl (2-methoxy(methyl)amino)-2-oxoethyl carbamate	80	118–122
BMC-5	$C_{13}H_{18}N_2O_5$	4-Methoxybenzyl (2-methoxy(methyl)amino)-2-oxoethyl carbamate	70	124–128

Structural Characterization

All the compounds produced were confirmed structurally by a combination of spectroscopic methods. For BMC-1, ^{1}H NMR (400 MHz, DMSO-d_6) revealed aromatic protons as multiplets in the δ 7.23–7.32 region (5H), the benzyl –CH_2– as a doublet at δ 5.41 (2H), the N–H carbamate proton as a broad singlet at δ 6.13 (1H), and the methoxy –OCH_3 as a singlet at δ 3.87 (3H). ^{13}C NMR showed the carbonyl carbamate carbon at δ 155.42, aromatic carbons at δ 137.09, 128.32, and 128.17, the benzyl –CH_2– at δ 66.69, and the methoxy carbon at δ 62.11. NIR analysis (KBr, cm -1) was used to confirm N -H at 3085, aliphatic C -H at 2921, C=O carbamate at 1695, and C=C aromatic at 1401.

For BMC-2, ^{1}H NMR showed aromatic protons at δ 7.27 (multiplet, 5H), N–H at δ 6.96, benzyl –OCH_2– at δ 5.36, N–CH_2–CO singlet at δ 4.38, methoxy at δ 3.93, and N-methyl at δ 2.71. ^{13}C NMR confirmed the carbamate C=O at δ 157.43, amide C=O at δ 174.31, aromatic carbons between δ 128–137, and methoxy at δ 57.58. Characteristic changes in the aromatic region (7.21160 BMC-3 to BMC-5) and other aromatic carbon resonances at 7.21160 proved the presence of methoxy groups on the ring. BMC-5 mass spectrometry displayed M/Z of 225.10, which is in line with the calculated molecular weight. The entire spectral data was in complete conformity with the proposed structures.

Antibacterial Activity

All the products of the synthesis of the BMC derivatives were shown to possess measurable antibacterial activity against both Gram-positive (S. aureus) and Gram-negative (E. coli) bacteria strains (Table 2). The inhibition zones of BMC-1 (15mm), BMC-3 (24mm) and BMC-4 (20mm) against S. aureus, were

compared to that of ampicillin standard (14mm). Inhibition zones varied between 14 mm (BMC-1) and 22 mm (BMC-3), against E. coli. The values of MIC against S. aureus ranged between 0.25 and 2.1 µg/mL, against Bacillus spp. between 2.2 and 8.1 µg/mL, and against E. coli between 3.4 and 15.6 µg/mL, which means that it is more active against Gram-positive organisms. Inhibition depending on the concentration of the compound was clearly shown whereby the zone diameters were increasing in proportional relation to the concentration of the compound tested across the tested range of 6.25-100 1 g/mL.

Table 2. Antibacterial Activity of BMC Derivatives (Zone of Inhibition, mm)

Compound	S. aureus (mm)	E. coli (mm)
BMC-1	15	14
BMC-2	18	17
BMC-3 (ortho)	24	22
BMC-4 (meta)	20	19
BMC-5 (para)	19	18
Ampicillin (std.)	26	24

Antifungal Activity

The antifungal experiment on *Candida albicans* and *Aspergillus niger* demonstrated moderate to high inhibitory properties in all derivatives. The Candida MICs of BMC compounds were: 0.125 µg/mL (equivalent to 12.6 mm inhibition zone) to 2 µg/mL (3.4 mm). Once again the ortho-methoxy derivative (BMC-3) had the best antifungal activity and the zone of inhibition was comparable to the fluconazole standard. In A. niger, the same tendencies of substitution ortho > meta > para were observed. The BMC at 25, 50 and 100mg/kg doses, in the in vivo fungal model in mice infected with C. albicans, significantly reduced the number of fungal cells and prevented development of the disease. The highest therapeutic effect was observed when using 100 mg/kg and few adverse reactions were observed during the period of administration.

Antioxidant Activity (DPPH Assay)

The DPPH radical scavenging assay has demonstrated an obvious dose-dependent antioxidant activity to all of the BMC derivatives. The values of IC 50 ranged between 45.2 ug/mL (BMC-3, ortho-methoxy) to 67.8 ug/mL (BMC-1, unsubstituted) (Table 3). The antioxidant capacity of the ortho-methoxy derivative was the highest, followed by the BMC-4 (meta) and the BMC-5 (para). None of the derivatives had an antioxidant activity greater than 70 µg/mL IC 50, however, none were as strong as the standard BHT (IC 50 = 22.4 µg/mL). It was found that the proximity of the methoxy group to the reactive site led to the increased activity of ortho-methoxy compounds in radical scavenging.

Table 3. Antioxidant and Cytotoxic Activity of BMC Derivatives

Compound	DPPH IC_{50} (µg/mL)	MTT IC_{50} MCF-7 (µg/mL)	MTT IC_{50} HeLa (µg/mL)	SAR Trend
BMC-1	67.8	64.5	70.1	Baseline
BMC-2	55.3	52.8	58.9	Moderate
BMC-3 (ortho)	45.2	38.4	42.7	Highest
BMC-4 (meta)	50.7	46.2	49.3	High
BMC-5 (para)	58.1	55.7	59.5	Moderate
BHT / Standard	22.4	—	—	Reference

Cytotoxicity (MTT Assay)

The cytotoxic properties of BMC derivatives were compared to MCF-7 and HeLa cancer cell lines. Inhibition of cell proliferation was concentration dependent on all compounds. BMC-3 demonstrated the strongest cytotoxicity with IC_{50} values of 38.4 µg/mL (MCF-7) and 42.7 µg/mL (HeLa), while BMC-1 was the least active (IC_{50} = 64.5 µg/mL and 70.1 µg/mL, respectively). Notably, evaluation of selectivity index showed that the anti-proliferative activity was selective against cancer cell lines, and not against normal fibroblast cells, indicating the occurrence of selective anti-proliferative activity. ANOVA analysis showed that the differences between the cytotoxic activities of the derivatives were statistically significant ($p < 0.05$).

In Vivo Antimicrobial Activity

Murine models were used to determine the in vivo antimicrobial efficacy. Oral administration of BMC at 200mg/kg was shown to have a significant effect on reducing the bacterial load in blood of mice infected with *S. aureus*. BMC was also effective in reducing fungal burden in mice infected with *Candida albicans*, with the highest effect being observed with the 100 mg/kg dose. Both BMC-3 and BMC-5 were effective at all doses that were tested against both organisms. No major adverse effects were detected during the course of treatment and mice were well-tolerated to BMC at all doses administered. These findings were consistent with the in vitro findings and were supportive of the potential of these compounds to further preclinical development.

Discussion

The overall findings of this study demonstrate that benzyl methoxy carbamates represent a pharmacologically versatile due to the presence of methoxy substituents on the benzyl aromatic ring that are intricately regulated by the position and electronic nature of methoxy substituents on the benzyl aromatic ring. The invariable superiority of the ortho-methoxy form (BMC-3) in antibacterial, antifungal, antioxidant and cytotoxic reactions highlights the critical role of molecular geometry and electronic distribution in defining biological performance. The trend in the antibacterial and antifungal activity could be explained by the Lipophilicity permeability Relationship and the Electronic Effect Theory. The methoxy group, which acts as an electron-donating substituent due to ^{+}M and ^{+}I effects, increases the electron density of the aromatic ring, increasing nucleophilic character and facilitating hydrogen bonding with the active sites of microbial enzymes (Matošević and Bosak, 2020). The ortho-substituted compound will have the advantage of proximal interaction with the carbamate nitrogen, enhancing conformational directionality towards microbial targets. Greater lipophilicity further facilitates diffusion across the bacterial phospholipid bilayer, which has been described in the literature as mechanisms of action of carbamate-class antimicrobials (Pizova et al., 2017). The Hydrogen Atom Transfer (HAT) and Single Electron Transfer (SET) are the free radical scavenging mechanisms that are consistent with the antioxidant data. The electron-giving methoxy group in the donation of labile hydrogen atoms or electrons to the DPPH radicals, stabilizing these radicals by resonance. The ortho-methoxy position presumably allows the methoxy oxygen lone pairs to directly overlap the aromatic 2 system, reducing the HOMO-LUMO energy gap and enhancing radical scavenging activity, as demonstrated by Frontier Molecular Orbital Theory (Rajic et al., 2011). These findings are similar to those obtained by Mishra and Gupta (2020), who reported improved radical scavenging of methoxy-substituted carbamates as compared to unsubstituted counterparts. The selective cytotoxicity of BMC-3 and BMC-4 to MCF-7 and HeLa cancer cell lines as opposed to normal fibroblasts is in line with the Selective Cytotoxicity Hypothesis. The cancer cells have a modified membrane potential and increased metabolic rate that supports the differentiated accumulation of lipophilic compounds. The moderate polarity of the carbamate moiety (–NHCOO)

provides dual hydrogen-bonding capability, which may facilitate intracellular accumulation and interaction with biomolecular targets topoisomerases or a complex of mitochondrial electron transport chains, triggering apoptosis. These results are comparable to the anticancer activity reported by Bhole et al. (2018) and Reddy and Thomas (2023) of benzyl carbamate-based molecules.

Conclusion

This work has effectively presented the synthesis, structural characterization and overall biological assessment of five benzyl methoxy carbamate analogs (BMC-1 to BMC-5). The carbamamoylation strategy mediated by CDI was efficient and eco-friendly with high purity products being obtained in the range of 70-88%. The structural identity of all compounds was unambiguously proved by spectroscopic data (^{1}H NMR, ^{13}C NMR, IR, MS). Biological screening showed that all the derivatives had strong antibacterial, antifungal, antioxidant, and cytotoxic effects. The ortho-methoxy analog (BMC-3) was consistently the most potent in all assays, with inhibition zones of 24 mm in S. aureus, DPPH IC₫ - 0 of 45.2 µg/mL in DPPH and MTT IC - 0 of 38.4 µg/mL in MCF-7 cells. Analysis of structure-activity relationship identified a strong dependence of biological activity on the location of methoxy substitution, with the trend observed being ortho > meta > para most biological endpoints of interest. In vivo experiments also confirmed the efficiency of antimicrobial with no major adverse effects. Theoretical concepts with Electronic Effect Theory, Lipophilicity- Permeability Models, HAT/SET mechanisms and Pharmacophore Theory comprehensively explained the biological trends observed. The research paper fills the void in synthetic chemistry and pharmacological testing, and offers a validated chemical platform of rational design of next-generation carbamate-based therapeutics. Further research is needed on molecular docking studies, in vivo pharmacokinetic profiling, toxicity study in advanced animal models, and clinical translation study.

Future Scope

The encouraging findings realized in this research provide a number of opportunities to conduct new research. Computational studies based on validated crystal structures of enzymes (e.g., AChE, DNA gyrase, topoisomerase II) and molecular docking would be used to elucidate the specific binding modes of BMC derivatives, and support structure-based drug design. In vivo pharmacokinetic profiling, such as ADMET (absorption, distribution, metabolism, excretion, toxicity) studies in rodent models are necessary to assess bioavailability and to determine safe dose ranges before progressing to clinical trials. The compound library can be expanded to include multi-methoxy substituted analogs, halogenated analogs, and bio isosteric replacements of the benzyl moiety to enable comprehensive mapping of SAR in larger chemical space. Moreover, consideration of BMC derivatives as acetylcholinesterase inhibitors that might be used in the management of Alzheimer disease is justified, considering the already established pharmacological relevance of carbamates in the inhibition of cholinesterase. The development of formulations to deliver targeted drugs and testing of these compounds as agrochemical agents (fungicides, herbicides) are also key areas of future research. The synergistic activity with already known antibiotics and antifungal agents should be evaluated to identify potential combination therapy to the drug-resistant infections.

Limitations

The current research can be characterized by a number of limitations that have to be considered. Firstly, the compound library, despite its focus and chemical diversity in terms of positional isomers, was limited to five derivatives and may not be representative of the breadth of structure-activity space in benzyl methoxy carbamates. Second, all of the biological evaluations except the murine infection models were

performed in vitro, and in vitro activity does not necessarily predictably to in vivo efficacy due to factors such as protein binding, metabolic stability, and poor bioavailability. Third, X-ray crystallographic analysis had not been carried out to verify solid-state molecular geometries and computational studies (molecular docking, QSAR modeling) were not included in the current scope. Fourth, the pharmacokinetic characteristics of the synthesized compounds are not characterized, and therefore, it is not possible to assess the compounds synthesized on drug-likeness based on Lipinski Rule of Five parameters. Fifth, the cytotoxicity panel was only conducted on two cancer cell lines; the assessment on a broader panel of cancer cell lines including drug resistant cell lines would give more conclusive results. Lastly, the specific mechanism of action was not studied at the cellular and molecular level (receptor identification, pathway analysis, apoptosis markers) and it appears to be a topic of further research.

References

1. Adams R, Baron EA (1965) The ester grouping in compounds of biological interest. Adv Enzymol 25:365–426.
2. Bak A, Kozik V, Muzart J, et al. (2019) N-phenyl carbamate derivatives: synthesis and inhibition of acetylcholinesterase and butyrylcholinesterase. Molecules 24(3):471.
3. Bhole RP, Bhusari KP, Borkar VN, et al. (2018) Novel carbamate hybrid molecules targeting Hsp70 and Hsp90 in prostate cancer. Eur J Med Chem 157:1254–1265.
4. Bonini C, Chiummiento L, De Bonis M, et al. (2010) New saquinavir and nelfinavir analogues from enantiopure diols as HIV-protease inhibitors. Bioorg Med Chem 18:5451–5458.
5. Carter HE, Frank RL, Johnston HW (1943) Benzyl carbamate. Org Synth 23:30.
6. Cerminara I, D'Annibale A, Tiozzo Fasiolo E (2012) Heterocyclic peptidomimetics in medicinal chemistry. Mini-Rev Med Chem 12(2):174–182.
7. Chaturvedi AK (2012) Carbamate pesticides: toxicology, pharmacology, and chemical structure. J Anal Toxicol 36(1):1–12.
8. Chaturvedi AK, Ray PG (2007a) Organic carbamates and their role in drug design. Curr Org Chem 11(5):439–466.
9. Cheng C, Liu S, Liang J, et al. (2019) 3-Hydroxyphenyl carbamates as antitubercular agents: synthesis, in vitro and in vivo activity. Eur J Med Chem 163:375–384.
10. Chiummiento L, Funicello M, Lupattelli P, et al. (2009) Synthesis of HIV protease inhibitors from (S)-glycidol. Eur J Org Chem 2009:3302–3307.
11. Cho CY, Moran EJ, Cherry SR, et al. (1993) An unnatural biopolymer. Science 261(5126):1303–1305.
12. Das A, Bandyopadhyay S (2022) Green synthesis of benzyl carbamates using CDI under solvent-free conditions. Green Chem Lett Rev 15(2):245–256.
13. Dexheimer TS, Rosenthal AS, Luci DK, et al. (2014) Synthesis and SAR of ML323, a potent USP1/UAF1 deubiquitinase inhibitor. ACS Med Chem Lett 5(10):1099–1103.
14. Filip GZ, Schoenebeck F (2025) Synthesis and properties of fluorinated N-carbonyl compounds. J Am Chem Soc 147(5):3120–3132.
15. Gaunt MJ, Yu J, Spencer JB (1998) Catalytic hydrogenolysis of substituted benzyl groups: electronic effects on reactivity. J Org Chem 63(14):4172–4173.
16. George JE, Pound JM, Davey RB (2005) Chemical control of ticks on cattle: acaricide resistance management. Vet Parasitol 129(1–2):53–62.
17. Ghosh AK, Brindisi M (2015) Organic carbamates in drug design and medicinal chemistry. J Med Chem 58(7):2895–2940.

18. Ghosh AK, Brindisi M (2023) Carbamates as pharmacological tools. Bioorg Med Chem 85:117283.
19. Greene TW, Wuts PGM (1999) Protective Groups in Organic Synthesis, 3rd edition. Wiley, New York.
20. Guan AY, Liu CL, Sun XF, et al. (2011) Synthesis and fungicidal activity of strobilurin derivatives containing coumarin moiety. Bioorg Med Chem 19:6503–6509.
21. Gupta RC (2014) Carbamates in veterinary medicine and pesticide science. In: Biomarkers in Toxicology. Elsevier, pp 415–428.
22. Hugo AA, Perez JL, Sobarzo CM, et al. (2019) Synthesis and acaricidal activity of benzyl ethyl carbamates via computational and experimental approaches. Parasitol Res 118:2291–2305.
23. Jampilek J, Brychtova K (2012) Amicarbazone and its heterocyclic analogs as potential antimicrobial agents. Med Chem 8(4):523–545.
24. Kauerova T, Kollar P, Dobrovolna J, et al. (2016) Hydroxynaphthanilides with nitro substitution as potential anticancer agents. Molecules 21(7):876.
25. Kaushik NK, Kaushik N, Attri P, et al. (2013) Biomedical importance of indoles. Molecules 18(6):6620–6662.
26. Keam SJ (2020) Cenobamate: first approval for partial-onset seizures. Drugs 80(4):443–450.
27. Kim YW, Alluri SR, Bhatt J, et al. (2001) Bisnorcholane spermidine/spermine carbamates: antibacterial and hemolytic properties. Bioorg Med Chem Lett 11(14):1891–1893.
28. Kos J, Zadrazilova I, Nevin E, et al. (2015) Antimicrobial activity of salicylanilide N-alkylcarbamates. Chem Biol Drug Des 86(5):1189–1198.
29. Kumar D, Ritika (2020) Biological significance of indole derivatives: a review. Curr Org Chem 24(5):389–416.
30. Kumar S, Patel N, Rao K (2018) Synthesis and antifungal evaluation of substituted carbamate derivatives. Indian J Chem B 57B(9):1251–1259.
31. Lemke TL, Williams DA, Roche VF, et al. (2013) Foye's Principles of Medicinal Chemistry, 7th edition. Lippincott Williams & Wilkins, Philadelphia.
32. Matošević A, Bosak A (2020) Carbamate group as a structural motif in drug design: a review. Arh Hig Rada Toksikol 71(3):195–204.
33. Metcalf RL (1971) Structure-activity relationships for insecticidal carbamates. Bull WHO 44(1-3):43–78.
34. Meyer C, Schepmann D, Yanagisawa S, et al. (2008) Synthesis and binding affinity of sigma-1 receptor ligands. Eur J Med Chem 43(12):2537–2548.
35. Mishra P, Gupta SK (2020) Methoxy-substituted carbamates as antioxidant agents: synthesis and DPPH evaluation. J Heterocycl Chem 57(4):1782–1791.
36. Moraczewski AL, Banaszynski LA, From AM, et al. (1998) Rotational isomerism in N-arylcarbamates. J Org Chem 63(21):7258–7262.
37. Patel HR, Shah MK (2017) Carbamate derivatives in medicinal chemistry: a review of structural diversity and pharmacological scope. Pharma Chem 9(2):75–88.
38. Perez B, Gutierrez N, Rojas S, et al. (2010) Ivermectin resistance in cattle tick Rhipicephalus microplus in Mexico. Vet Parasitol 168(1):88–92.
39. Pizova H, Kauerova T, Opletalova V, et al. (2017) Proline-based carbamates as cholinesterase inhibitors: synthesis, SAR, and in silico analysis. Eur J Med Chem 133:75–88.
40. Pinho S, Lopes MS, Correia-de-Sa P, et al. (2013) Anticholinesterase natural compounds and synthetic derivatives in Alzheimer's disease therapy. Mini-Rev Med Chem 13(1):105–129.