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Modification of benzoxazole derivative by bromine-spectroscopic, antibacterial and reactivity study using experimental and theoretical procedures

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ABSTRACT

N-[2-(2-bromophenyl)-1,3-benzoxazol-5-yl]-2-phenylacetamide (NBBPA) was synthesized in this study as an original compound in order to evaluate its antibacterial activity against representative Gram-negative and Gram-positive bacteria, with their drug-resistant clinical isolate. Microbiological results showed that this compound had moderate antibacterial activity. Study also encompassed detailed FT-IR, FT-Raman and NMR experimental and theoretical spectroscopic characterization and assignation of the ring breathing modes of the mono-, ortho- and tri-substituted phenyl rings is in agreement with the literature data. DFT calculations were also used to identify specific reactivity properties of NBBPA molecule based on the molecular orbital, charge distribution and electron density analysis, which indicated the reactive importance of carbonyl and NH_2 groups, together with bromine atom. DFT calculations were also used for investigation of sensitivity of the NBBPA molecules towards the autoxidation mechanism, while molecular dynamics (MD) simulations were used to investigate the influence of water. The molecular docking results suggest that the compound might exhibit inhibitory activity against GyrB complex.

1. Introduction

Benzoxazole ring is one of the most common heterocycles in medicinal chemistry. Previous reports revealed that substituted benzoxazoles possess diverse chemotherapeutic activities including antimicrobial [1–5], antiviral [6], topoisomerase I and II inhibitors [7] and antitumor activities [8,9]. Bacterial resistance to antibacterial agents or antibiotics is of grave concern in the medical community, as many species of bacteria have evolved resistance to certain antibiotics and synthetic agents. Therefore, there could be a rapidly growing global crisis in the clinical management of life-threatening infectious diseases caused by multidrug-resistant strains of the Gram-positive pathogens like Streptococcus, Enterococcus, and Staphylococcus, and Gram-negative pathogens like Escherichia, Salmonella, and certain Pseudomonas strains. Especially the emergence of multidrug-resistant strains of Gram-positive bacterial pathogens such as methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermis* and vancomycin-resistant Enterococcus is an alarming problem of ever increasing significance [10–12]. To meet this crisis successfully, many researchers across the globe are working to unearth new compounds

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which can selectively attack novel targets in microorganisms. Hence, the development of novel, potent, and unique antibacterial agents is the preeminent way to overcome bacterial resistance and develop effective therapies [13]. In previous studies, we synthesized some compounds which bearing a hydrogen, chlorine, methyl, nitro, an amine, ester, and amide substitution at the 5th position on the benzoxazole ring and examined for their *in vitro* antimicrobial activity against some Gram-positive, Gram-negative bacteria and *Candida albicans* [1–3,14,15]. On the basis of these considerations, we synthesized *N*-[2-(2-bromophenyl)-1,3-benzoxazol-5-yl]-2-phenylacetamide (NBBPA) as antimicrobial agent reported in this work, choosing a bromine atom at the 2nd position of phenyl of second carbon of benzoxazole ring. The strategy employed was to examine the effect of the bromine against some Gram-positive, Gram-negative bacteria and their drug-resistant isolates.

One of the main characteristics of biologically active molecules is their overall stability, especially in aquatic mediums. These organic molecules are synthesized to be highly stable, which hardens their removal from the water and soil [16]. Since pharmaceutical care products are frequently used and improperly dumped into the environment their active components accumulate in water, which is very harmful because it has been shown that aforementioned molecules exhibit toxic effects towards aquatic organisms [16,17]. The fact that biologically active molecules that serve as active components of pharmaceutical products have been detected in all types of water is particularly upsetting, since conventional methods for the removal of these molecules are ineffective [18,19]. Removal of these molecules based on forced degradation by advanced oxidation processes could be fine alternative [17,18,20,21] and it also might serve as a basis for the studies of their toxic effects [22–24]. Rationalization of studies based on forced degradation can be done by application of DFT calculations and MD simulations [25–27]. Principles of molecular modeling enable prediction of reactive properties of investigated molecules, further influencing the improvement and development of procedures for the purification of water. Bearing in mind the usefulness of theoretical analysis, in this work we have investigated reactivity in order to gain an insight into the possible degradation properties of NBBPA molecule.

2. Experimental section

2.1. Synthesis of N-[2-(2-bromophenyl)-1,3-benzoxazol-5-yl]-2-phenylacetamide

The firstly synthesis of *N*-[2-(2-bromophenyl)-1,3-benzoxazol-5-yl]-2-phenylacetamide was obtained in two step procedures as given below (Scheme 1):

First step: 5-Amino-2-(2-bromophenyl) benzoxazole was synthesized by heating 0.01 mol 2,4-diaminophenol.2HCl with 0.01 mol 2-bromobenzoic acid in 12.5 g polyphosphoric acid (PPA) and stirring at 200 °C for 4 h. At the end of the reaction period, the residue was poured into ice water mixture and neutralized with excess of 10 M NaOH solution extracted with benzene and then this solution was dried over anhydrous sodium sulphate and evaporated under diminished pressure. The residue was boiled with 200 mg charcoal in ethanol and filtered. After the evaporation of solvent *in vacuo*, the crude product was obtained and recrystallized from ethanol.

Second step: Phenylacetic acid (0.5 mmol) and thionyl chloride (1.5 ml) were refluxed in benzene (5 ml) at 80 °C for 3 h. Excess thionyl chloride was removed *in vacuo*. The residue was dissolved in ether (10 ml) and this solution was added during 1 h to a stirred, ice-cold mixture of 5-amino-2-(2-bromophenyl)benzoxazole (0.5 mmol), sodium bicarbonate (0.5 mmol), diethyl ether (10 ml) and water (10 ml). The mixture was kept stirred overnight at room temperature and filtered. The precipitate was washed with water, 2 N HCl and water and finally with ether to give *N*-[2-(2-bromophenyl)-1,3-benzoxazol-5-yl]-2-phenylacetamide. The product was recrystallized from ethanol-water as needles, which was dried *in vacuo*. The chemical, physical and spectral data of the compound are reported below; $C_{21}H_{15}BrN_2O_2.1{,}75H_2O$, yield: 61,42%, mp: 145–147 °C. MS (70 eV) *m/z*: 429 (M^++H+23(Na)), 431 (M^++H+2 + 23(Na)). Elemental Analysis: Calculated: C: 57.48, H: 4.25, N:6.38; Found: C: 57.19, H: 3.92, N: 6.32.

The chemicals were purchased from the commercial venders and were used without purification. The reactions were monitored and the purity of the products was checked by thin layer chromatography TLC. Kieselgel HF 254 chromatoplates (0.3 mm) was used for TLC and the solvent system was ethylacetate:n-hexane (2:1). The melting point was taken on a Buchi SMP 20 capillary apparatus and is uncorrected. ^{1}H NMR spectra was obtained with a Varian 400 MHz spectrometer in dimethylsulfoxide-d6 (DMSO-d_6) and tetramethylsilane (TMS) was used as an internal standard. Mass analyses was carried out with a Waters Micromass ZQ by using ESI^+ method. Elemental analysis was performed on LECO 932 CHNS (Leco 932, St. Joseph, MI, USA) instrument and was within 0.4% of the theoretical values. All chemicals and solvents were purchased from Aldrich Chemical Co. or Fischer Scientific.

The FT-IR spectrum (Fig. 1) was recorded using KBr pellets on a DR/Jasco FT-IR 6300 spectrometer. The FT-Raman spectrum (Fig. 2) was obtained on a Bruker RFS 100/s, Germany. For excitation of the spectrum the emission of Nd:YAG laser was used, excitation wavelength 1064 nm, maximal power 150 mW, measurement on solid sample. The spectral resolution after apodization was 2 cm^{-1}.

2.2. Microbiology

Microorganisms *Pseudomonasaeruginosa* isolate (gentamicin-resistant), *Escherichiacoli* isolate, which has an extended spectrum beta lactamase enzyme (ESBL), *Staphylococcus aureus* isolate (meticilline-resistant (MRSA)), *P. aeruginosa* ATCC 27853 (American Type Culture Collection), *E. coli* ATCC 25922, *S. aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *E. faecalis* isolate (vancomycin-resistant enterococci). Methods Standard strains of *P. aeruginosa* ATCC 25853, *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *E. faecalis* ATCC 29212 and clinical isolates of these microorganisms resistant to various antimicrobial agents were included in the study. Resistance was determined by Kirby Bauer Disk Diffusion method according to the guidelines of Clinical and Laboratory Standards Institute (CLSI) [28] in the clinical isolates. Standard powders of ampicillin trihydrate, gentamycin sulphate, ofloxacin were obtained from the manufacturers. Stock solutions were dissolved in dimethylsulphoxide (ofloxacin), pH 8 phosphate buffer saline (PBS) (ampicillin trihydrate) and distilled water (gentamicin sulphate). Newly synthesized compound was dissolved in 80% DMSO-20% EtOH. Bacterial isolates were subcultured in Mueller Hinton Agar (MHA) plates and incubated over night at 37 °C for 24–48 h. The microorganisms were passaged at least twice to ensure purity and viability. The solution of the newly synthesized compound and standard drugs were prepared at 400, 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.562, 0.78, 0.39, 0.19, 0.095, 0.047, 0.024 μg/ml concentrations, in the wells of microplates by diluting in Mueller Hinton Broth (MHB). Bacterial susceptibility testing was performed according to the guidelines of CLSI M100-S16 [29].

The bacterial suspensions used for inoculation were prepared at 10^5 cfu/ml by diluting fresh cultures at MacFarland 0.5 density (10^7 cfu/ml). Suspensions of the bacteria at 10^5 cfu/ml concentration were inoculated to the twofold diluted solution of the