British Journal of Multidisciplinary and Advanced Studies,7(4),1-7,2026
Chemistry and Biochemistry
Print ISSN: 2517-276X
Online ISSN: 2517-2778
https://bjmas.org/index.php/bjmas/index
Publication of the European Centre for Research Training and Development -UK

Study of the Antibacterial Activities of Some Oximes and Their Corresponding Benzyl Oxime Ethers

Ramadan Ali Bawa and Maryam Ibrahim Aleer
Department of Chemistry, Faculty of Science, Misurata University, P. O. Box 2400, Misurata, Libya
Corresponding email: r.bawa@sci.misuratau.edu.ly

doi: https://doi.org/10.37745/bjmas.0581 Published August 08, 2026

Citation: Bawa R.A. and Aleer M.I. (2026) Study of the Antibacterial Activities of Some Oximes and Their Corresponding Benzyl Oxime Ethers, *British Journal of Multidisciplinary and Advanced Studies*,7(4),1-7

Abstract: *Five oximes and five of their corresponding benzyl oxime ethers **1 – 10** were synthesized. The antibacterial activities of these oxime derivatives were determined against the E. coli at the concentration of 30 ppm in DMSO. The obtained results were compared with the commercially available antibacterial drug, cefotaaxime. The benzyl oxime ether **6** was the only oxime derivative in this study that showed an excellent inhibition rate reaching 90%, whereas the commercially available antibacterial agent, the cefotaaxime, exhibited low inhibition effect of just 16%. The other oximes and the benzyl oxime ethers **1 – 5** and **7** – **10** exhibited low inhibitory levels of 17% – 34%.*

Keywords: oximes, oxime ethers, synthesized, antibacterial, inhibition, *E. coli*.

INTRODUCTION

Oximes and oxime ethers have been reported to have significant biological properties , such as, *anti*-inflammatory, antimicrobial, antioxidant and *anti*-cancer properties [1,2]. Escherichia coli (*E. coli*) is a varied group of bacteria that exist in in the human gastrointestinal tract and the environment, including both beneficial and pathogenic strains. Pathogenic *E. coli* strains could cause a variation of infections, including diarrheal diseases, urinary tract infections, bacteremia, sepsis, neonatal meningitis, and intra-abdominal infections [3,4]. These pathogenic strains are classified into enteric and extra-intestinal pathotypes, each with specific virulence factors and disease manifestations. Because of its flexibility, genetic variety, and spreading, *E. coli* is a major human pathogen worldwide, contributing ausing the increase in antibiotic resistance and healthcare costs [5]. Furthermore, *E.coli* is a common choice for laboratory research, biotechnology, and industrial applications because of its easy to handle. Obtainability of broad genome sequences and the ability to grow under a variation of conditions [6]. Efforts are ongoing to improve vaccines against pathogenic *E coli* to fight infections and antibiotic resistance efficiently [3]. The commonness of extraintestinal invasive *E. coli* infection (IEI) in the United States is 0.50 events per 1000 visits and 1.82 events per 1000 patients [7]. Moreover, *E. coli* is the top leading pathogen for bloodstream infections, responsible for 17% of cases [8]. Additionally, *E. coli* is a common cause of periprosthetic joint infection (PJI) in patients [7,9]. Cefotaxime belongs to the cephalosporin type of antibiotics which possess a quite wide range of

effects. It could be also used as an antibiotic before surgery, and to treat serious infections caused by sensitive microbial strains, such as lower respiratory tract infections, urinary tract infections, gynecological infections, skin infections, and central nervous system infections [10].

Experimental

Materials

Acetaldehyde, acetone, benzaldehyde, acetophenone, benzophenone, hydroxylamine hydrochloride, benzyl chloride, potassium hydroxide, potassium iodide, chloroform, DMSO, ethanol and Nutrient Agar. Were purchased from Riedel-de Haen Chemical, CARLO ERBBA, AVONCHEM and Sigma – Aldrich. These chemicals were used without further purification. Escherichia coli (*E. coli*) was obtained from the department of microbiology, Misurata university.

Instrumentation

The following analytical tools were used for the structure elucidation of the synthesized oximes and benzyl oxime ethers **1 – 10**. Melting points were measured on a Bio Cote apparatus. The pH was measured using JENWAY pH meter 3505. Infrared spectrum was recorded on Perkin Elmer FT-IR spectrometer Frontier. Nuclear magnetic resonance ^{1}HNMR was recorded on a JEOL 500 spectrometer Residual proton signal from the deuterated solvents were used as references [$CDCl_3$ (1H, 7.27 ppm)]. Coupling constants were measured in Hz. Mass spectrometer was recorded on a Shimadzu Qp – 2010 Plus spectrometer.

General procedure for the synthesis of oximes:

A literature procedure [11] was adapted towards the synthesis of the desired oxime. Solution of hydroxylamine hydrochloride (71.94 mmol in 10 cm^3 of distilled water) and a solution of potassium hydroxide (53.48 mmol in 5 cm^3 of distilled water) were placed in a round-bottomed flask and stirred at room temperature. Acetaldehyde (65.71 mmol) was then added while stirring and the reaction mixture was refluxed. At the start of the boiling, small amounts of ethanol (5 cm^3) were added from time to time to the reaction mixture through the condenser until the boiling solution became clear. The reaction was left under reflux for further an hour after which the reaction vessel was allowed to cool gradually to the room temperature. The pH of the reaction mixture was measured and found, as expected, to be acidic. A solution of 1N KOH was added to the reaction mixture until the solution became neutral. The reaction mixture was then refluxed for further 30 min, cooled to room temperature and the pH was measured and found to be still acidic. Addition of 1N KOH solution was required and the reaction mixture was refluxed for further 10 min, cooled to room temperature and the pH was measured and found to be neutral. The reaction mixture was transferred into a beaker containing ice-water (100 cm^3). The acetaldehyde oxime was extracted from chloroform, dried over anhydrous Na_2SO_4, filtered and evaporated to give the desired compound. **Compound 1:** brown oil (3.28 gm, 55.56 mmol, 85% yield); IR ν_{max} (cm^{-1}) 3261 (OH), 1715 (C=N). ^{1}HNMR (500 MHz, $CDCl_3$) **Major isomer** (formation ratio of **53%**): δ 7.09 (1H, s, O*H*), 6.80 (1H, q, *J* = 10 Hz, C*H*O), 1.31 (3H, d, *J* = 10 Hz, CH_3); **Minor isomer** (formation ratio of **47%**) δ 7.43 (1H, q, *J* = 10 Hz, C*H*O), 7.09 (1H, s, O*H*), 1.31 (3H, d, *J* = 10 Hz, CH_3). **Compound 2:** white semisolid (2.88 gm, 39.45 mmol, 60% yield);

IR ν_{max} (cm^{-1}) 3337 (OH), 1677 (C=N). ^{1}HNMR (500 MHz, $CDCl_3$) δ 8.62 (1H, br s, O*H*), 1.91 (6H, s, 2 × CH_3). **Compound 3:** oil (5.44 gm, 44,93 mmol, 68% yield); IR ν_{max} (cm^{-1}) 3337 (OH), 1677 (C=N). ^{1}HNMR (500 MHz, $CDCl_3$) δ 9.50 (1H, br s, O*H*), 8.30 (1H, s, C*H*O), 7.69 – 7.66 (2H, m, Ar-*CH*), 7.45 – 7.43 (3H, m, Ar-C*H*). **Compound 4:** white solid (7.37 gm, 54.59 mmol, 82% yield); IR ν_{max} (cm^{-1}) 3209 (OH), 1979, 1496 (C=N). ^{1}HNMR (500 MHz, $CDCl_3$) δ 8.86 (1H, br s, O*H*), 7.68 – 7.65 (2H, m, Ar-C*H*), 7.43 – 7.41 (3H, m, Ar-C*H*), 2.36 (3H, s, CH_3). **Compound 5:** a white powder (11.90 gm, 64.32 mmol, 98.70% yield); IR ν_{max} (cm^{-1}) 3298 (OH), 1649 (C=N). ^{1}HNMR (500 MHz, $CDCl_3$), δ 7.84 – 7.82 (3H, m, Ar-C*H*), 7.63 – 7.47 (7H, m, Ar-C*H*), 6.27 (1H, br s. O*H*).

General procedure for the synthesis of oxime ethers:

An adapted literature procedure [12] was followed to synthesize this oxime ether. The acetaldehyde oxime (5.0 mmol) was placed into a round-bottomed flask (50 cm^3) and dissolved in DMSO (5 cm^3). Benzyl chloride (10.0 mmol), KI and KOH (10.0 mmol) were then added. The reaction mixture was intensively stirred for 4 h at the room temperature (monitored by TLC). Water (30 cm^3) and chloroform (30 cm^3) were added to the reaction mixture after which the organic layer was extracted, washed with water (4 × 25 cm^3), dried over anhydrous Na_2SO_4 and filtered. The solvent was evaporated to obtain the desired the oxime ether. **Compound 6:** dark oil (0.445 gm, 3.053 mmol, 37%); IR ν_{max} (cm^{-1}) 1602 (C=N), 1026 (C-O). ^{1}HNMR (500 MHz, $CDCl_3$), δ 8.15 (1H, q, *J* = 15 Hz, C*H*O), 7.49 – 7.39 (10H, m, Ar-C*H*), 4.61 (2H, s, PhCH_2), 1.93 (3H, d, *J* = 10 Hz, CH_3). Mass spec (EI) m/z ($C_9H_{11}NO$, calculated MWt 149.08) Found: 149 (0.08), 134 (0.1), 91 (100). **Compound 7:** oil (1.22 gm, 7.53 mmol, 95% yield); IR ν_{max} (cm^{-1}) 1602 (C=N), 1016 (C-O). ^{1}HNMR (500 MHz, $CDCl_3$), δ 6.71 – 6.68 (H, m, Ar-C*H*), 6.56 – 6.54, (H, m, Ar-C*H*), 4.34 (2H, s, PhCH_2), 3.68 (3H, s, 2 × CH_3). Mass spec (EI) m/z ($C_{10}H_{13}NO$, calculated MWt 163.10) Found: 163 (0.02), 91 (100), 72 (0.24). **Compound 8:** dark oil (1.83 gm, 8.65 mmol, 83% yield); IR ν_{max} (cm^{-1}) 1603 (C-O), 1025 (C=N). **^{1}**HNMR (500 MHz, $CDCl_3$), δ 8.16 **(**1H, s, C*H*O**)**, 7.42 – 7.38 (10H, m, 2 × Ar-C*H*), 4.62 (2H, s, PhCH_2). Mass spec (EI) m/z ($C_{10}H_{13}NO$, calculated MWt 211.10) Found: 211 (0.07), 196 (0.11), 120 (0.53), 91 (100). **Compound 9:** brown oil (1.78 gm, 7.93 mmol, 88%yield); IR ν_{max} (cm^{-1}) 1602 (C=N), 1024 (C-O). ^{1}HNMR (500 MHz, $CDCl_3$) δ 7.79 – 7.76 (H, m, Ar-C*H*), 7.50 – 7.40, (H, m, Ar-C*H*), 4.67 (2H, s, PhCH_2), 2.38 (3H, s, CH_3). Mass spec (EI) m/z ($C_{14}H_{13}NO$, calculated MWt 225.12) Found: 225 (0.08), 211 (3.0), 134 (0.26), 91 (100). **Compound 10:** yellow oil (1.34 gm, 4.67 mmol, 73% yield); IR ν_{max} (cm^{-1}) 1658 (C=N), 1027 (C-O).**^{1}**HNMR (500 MHz, $CDCl_3$), δ 7.87 – 7.79 (14H, m, Ar-C*H*), 7.62 – 7.34 (H, m, Ar-C*H*), 4.62 (2H, s, PhCH_2). Mass spec (EI) m/z ($C_{20}H_{17}NO$, calculated MWt 287.13) Found: 287 (4.05), 210 (0.04), 196 (0.37), 91 (100).

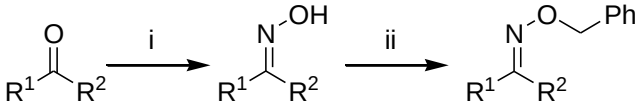
A procedure for the determination of the inhibitory levels of the synthesized oxime derivatives 1 – 10: [13,14]

The agar dilution technique is used to qualitatively determine the in vitro behavior of an antimicrobial agent against the test fungi or bacteria. In this method, graded concentrations of antibiotics are incorporated into agar plates and inoculated in spots with the microorganisms under study. If the microorganism under study is notably affected by the incorporated antimicrobial agent, no microbial growth is expected in agar plates with higher amounts of this antimicrobial drug. Microbial growth is observed as the antibiotic concentration in the agar plate diminishes. Inhibition of growth at the minimum concentration of the antibiotic is considered the end point [8,9]. Here, the antibacterial

potentials of the oximes and oxime ethers were realized against *E. coli* at a constant concentration of 30 ppm, using the commercially available antibacterial agent Cefotaxime as a reference. This antibacterial agent showed rather low inhibitory level against the selected bacteria, *E. coli*, in which their capacity to inhibit the growth of the bacteria was found to be 16%.

RESULTS AND DISCUSSION

An adapted synthetic procedure [8] was used towards the synthesis of the targeted oximes **1 – 5**. The carbonyl compounds were reacted with the hydroxylamine hydrochloride under basic conditions and reflux for 3 h. The targeted oximes **1 – 5** were obtained in good to excellent yields. Subsequently, the resulting oximes were converted to their corresponding benzyl oxime ethers **6 – 10** in good yields by following an adapted synthetic procedure [9] **(Scheme1)**. The structures of all synthesized oxime derivatives were confirmed by using a number of spectroscopic techniques, such as IR, NMR and mass spec.



R^1 may be Me or Ph
R^2 may be H, Me or Ph

Reagents & reaction conditions: (i) $NH_2OH.HCl$, KOH, Na_2SO_4, 0 - 5 °C, then reflux for 3 h; (ii) BnCl, KOH,KI, DMSO, $CHCl_3$, stirring in the temperture room, 4 h

Scheme 1: Synthesis of the targeted oxime derivatives

The inhibition level of the acetaldehyde oxime ether **6** was found to be the highest amongst all the synthesized oximes and their corresponding benzyl oxime ethers being tested against the *E. coli* including the commercially available antibacterial the Cefotaxime, which was used as a reference, exhibited low to moderate inhibitory levels against the *E. Coli* **(Table 1)**. The acetaldehyde oxime ether exhibited an excellent antibacterial level reaching 90%, whereas the inhibitory level of the benzophenone oxime ether was found to be 17%, which is almost as same as the inhibition level that was recorded for the commercially available antibacterial agent, the Cefotaxime 16%. The other eight oxime derivatives showed, in general, low to moderate inhibition potentials against the *E. Coli*. The benzophenone oxime, acetophenone oxime ether, benzaldehyde oxime ether and acetone oxime ether showed nearly two-fold antibacterial potentials of that was recorded for the reference, the Cefotaxime **(Fig. 1)**.

Table 1 The inhibition level of the oxime and oxime ethers at a concentration of 30 ppm against E. coli, comparison of commercially available antibacterials against cefotaxime references

Compound No	Compound name	Inhibition %
Reference	Cefotaxime	16
1	Acetaldehyde oxime	27
2	Acetone oxime	28
3	Benzaldehyde oxime	26
4	Acetophenone oxime	26
5	Benzophenone oxime	31
6	Acetaldehyde oxime ether	90
7	Acetone oxime ether	34
8	Benzaldehyde oxime ether	33
9	Acetophenone oxime ether	32
10	Benzophenone oxime ether	17

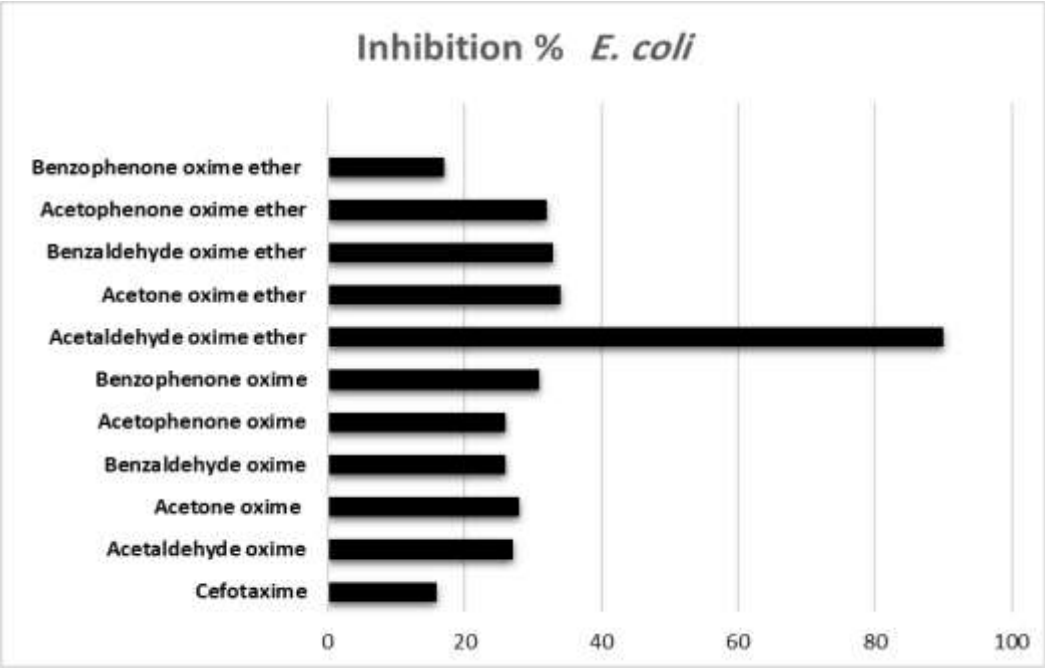


Figure 1: The levels of inhibition that were recorded for all oximes and their corresponding benzyl oxime ethers in comparison with the antibacterial agent, Cefotaxime as a reference

CONCLUSION

The desired oxime derivatives **1 – 10** were synthesized in moderate to excellent yields. The inhibition capacities of the oxime derivatives **1 – 10** were investigated against the *E. coli*, which they exhibited promising results in comparison with the commercially available antibacterial agent (Cefotaxime; its inhibitory level of 16%). Only one of the ten synthesized oxime derivatives **6** showed an excellent

level of inhibition against the *E. coli* reaching 90% of inhibition. The other nine oxime compounds **1 – 5** and **7 – 10** exhibited low inhibitory potentials ranging from 17% to 34%, although, their inhibitory potentials were generally found to be almost two-fold higher than the inhibitory level of the reference, the Cefotaxime.

Acknowledgements:
Authors would like to thank the department of chemistry and the bioresearch center at Misurata University for providing all possible facilities to finish this work. Special thanks go to prof. Huda S. Elgobbi and her research group for their help with the antifungal testing.

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