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Synthesis and Antibacterial Activity of Novel N-Alkylbenzoxazol-2-ones Derivatives

Yadi Reddy Bonuga*[1,2], A. Ravinder Nath[1] and B. Ram[2]

1. University College of Technology, Osmania University, Hyderabad, Telangana State, **INDIA**
2. Green Trends Laboratories, Plot No.43, Kushai guda, Telangana State, Hyderabad, 500062, **INDIA**

Email: yadireddybonuga@gmail.com

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ABSTRACT

*The synthesis of N-Alkylbenzoxazol-2-ones derivatives 4a-j was prepared in three steps from commercially available 2-amino-phenol **1**. The structures of these compounds were established on the basis of the spectroscopic techniques like ^{1}H NMR, mass and IR data. Compounds **4a-j** were screened in-vitro at a concentration of 100 µg/mL for antibacterial activity against two Gram-positive (Staphylococcus aureus and Staphylococcus pyogenes) and two Gram-negative strains (Escherichia coli and Pseudomonas aeruginosa) with reference to the standard drug ciprofloxacin (100 µg/disc). Compounds **4f** and **4g** exhibited excellent activity and the compounds **4a**, **4b**, **4c**, **4i** and **4j** showed god antibacterial activity while the compounds **4d** and **4e** showed moderate antibacterial activity.*

Keywords: Synthesis, 2-amino-phenol, N-alkylbenzoxazol-2-ones, Antibacterial activity.

INTRODUCTION

Benzoxazol-2(3H)-one heterocycles is well thought-out 'advantaged scaffolds' in the design of pharmacological probes. These heterocycles have, in fact, high adaptability in chemical modifications, allowing changes to the characteristics of side-chains on a rigid platform; moreover, they have received significant consideration from medicinal chemists, due to their ability to mimic a phenol or a catechol moiety in a metabolically stable template[1] This class of compounds has led to the discovery of a number of derivatives exhibiting various pharmacological activities *viz.*, anti-inflammatory and analgesic [2-5], antitubercular, antibacterial and antimicrobial [6-8], and normolipemic [9,10], effects.

Alkylation of benzoxazol-2(3H)-ones and benzothiazol-2(3H)-ones gave intermediates for the synthesis of compounds used in pharmacotherapy for their anticocaine activity, as these substituted heterocycles interact with Sigma-1 receptors [11], Courtois et al. described the syntheses of new fosmidomycin analogues containing the benzoxazolone ring, which present a potential interest in the treatment of malaria [12].

Since resistance of pathogenic microorganisms towards available antibiotics is rapidly becoming a worldwide problem, the design of new compounds to deal with these resistant strains has become a major

area of research today. Therefore, in the present paper we have N-Alkylbenzoxazol-2-ones 4a-j and evaluated for antibacterial activity.

MATERIALS AND METHODS

Chemical and solvents used were purchased either from Fluka or Merck. All the reagents were of analytical grade. Thin-layer chromatography (TLC) was performed on E.Merck AL silica gel 60 F254 plates and visualized under UV light. The IR spectra were recorded on a Perkin Elmer FT-IR spectrometer. The ^{1}H NMR spectra were recorded in $CDCl_3$ on a Varian EM-360 spectrometer (400MHz). The ^{13}C NMR spectra recorded in $CDCl_3$ on a Varian EM-360 spectrometer operating at 100MHz. All the chemical shifts were reported in δ (ppm) using TMS as an internal standard. The mass spectra were recorded on Agilent ion trap MS. All the reactions were carried out under argon atmosphere.

Preparation of benzo[d]oxazol-2(3H)-one (2): To a stirred solution of 2-aminophenol (3 g, 0.0275 mol) in 30 mL of anhydrous THF was added 6.7 g of CDI (0.0412 mol). The solution was refluxed for 4 hours. After completion of the reaction, the THF was removed under reduced pressure, and the dry residue was partitioned between 2N hydrochloric acid and chloroform. The organic layer was dried over anhydrous sodium sulfate, and was evaporated under reduced pressure to obtain compound **2**. White solid; Yield: 90%; IR (KBr): ν_{max} 3220, 3018, 1803, 1764, 1621, 1479, 1397, 1306, 1252, 1146, 1098, 1008, 919, 893,760, 739, 574 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 9.57 (brs, 1H); 7.26-7.11 (m, 4H); ESI MS: m/z (rel.abund. %): 248.0 (M+1).

Preparation of 3-(2-bromoethyl)benzo[d]oxazol-2(3H)-one (3): A mixture of compound **2** (3.2 g, 0.0237 mol) and potassium carbonate (8.17 g, 0.0592 mol) was dissolved in 50 mL of acetonitrile and the solution was refluxed for 10 min. 1,2-dibromoethane (5.08 mL, 0.0592 mol) and a catalytic amount of KI were added, and the reaction mixture was stirred for an additional 12 h at reflux temperature. The inorganic salts were filtered off, and the solvent was evaporated under reduced pressure. Distilled water (50 mL) was added, and the residue was extracted thrice with chloroform (3 x 100 mL). The organic phase was separated and dried over anhydrous Na_2SO_4. The filtered solution was concentrated under reduced pressure, and the remaining residue was purified by column chromatography (10% EtOAc-hexane as eluent) to afford compound **3**. White solid. Yield: 55%, m.p: 97-98 °C; ^{1}H NMR (DMSO-d_6): δ 3.06 (brs, 2H); 4.32 (brs, 2H); 7.20-7.38 (m, 4H).

General experimental procedure for the synthesis of compounds 4a-4f: A mixture of compound **3** (242 mg, 1 mmol), secondary amines **a-f** (430 mg, 5 mmol), anhydrous K_2CO_3 (690 mg, 5 mmol), and a catalytic amount of KI was dissolved in 30 mL of CH_3CN, and the solution was refluxed for 3 h. The completion of the reaction was monitored by TLC. The inorganic salts were filtered off and the solvent was evaporated under reduce pressure. Distilled water (50 mL) was added, and the residue was extracted with $CHCl_3$ (3 X 100 mL). The organic phase was separated and dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to obtain a crude compound which was further purified by column chromatography (80 % EtOAc-hexane as eluent) to obtain compounds **4a-f**.

General experimental procedure for the synthesis of compounds 4g-i: A solution of compound **3** (150 mg, 0.607 mmol), in dry DCM was taken, and cooled to 0-5 °C in an ice bath. Triethyl amine (3 mmol) was added to the above reaction mixture and was stirred for 10 minutes followed by the addition of secondary amines **g-j** (115 mg, 0.607 mmol). The reaction mixture was stirred at room temperature for 5 h. After completion of the reaction, the solvent was evaporated under reduced pressure and the obtained residue was diluted in water and extracted with ethylacetate. The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated to get crude product, which was purified by column chromatography over silica gel using ethylacetate-hexane (80%) as an eluent to obtain the compounds **4g-i**.

3-(2-(piperidin-1-yl)ethyl)benzo[d]oxazol-2(3H)-one (4a): Pale yellow viscous; Yield:80%; IR (KBr): ν_{max} 2929, 2715, 1779, 1613, 1486, 1364, 1237, 1102, 1011, 967, 751, 681 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.34 (broad spins 1H), 1.66 (broad spins, 2H), 1.89 (broad spins, 2H), 2.14 (s, 1H), 2.22 – 2.64 (m, 4H), 2.89 (broad spins, 2H), 3.06 (broad spins, 1H); 4.32 (s, 2H); 7.20-7.38 (m, 4H); ESI MS: m/z (relative abundance. %): 248.0 (M+1).

3-(2-morpholinoethyl)benzo[d]oxazol-2(3H)-one (4b): Pale yellow viscous; Yield: 78%; IR (KBr): ν_{max} 2955, 2854, 2812, 1777, 1614, 1487, 1455, 1393, 1360, 1320, 1299, 1272, 1239, 1146, 1117, 1089, 1058, 1035, 1010, 960, 918, 870, 755, 676 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.56 (s, 4H), 2.74 (t, J = 3.3 Hz, 2H), 3.67 (s, 4H), 3.97 (t, J = 3.6 Hz, 2H), 7.01-7.28 (m, 4H); ESI MS: m/z (relative abundance. %): 248.0 (M+1).

3-(2-(piperazin-1-yl)ethyl)benzo[d]oxazol-2(3H)-one (4c): Yellow viscous liquid; Yield: 82%; IR (KBr): ν_{max} 2942, 2818, 1767, 1613, 1485, 1445, 1392, 1359, 1320, 1255, 1134, 1088, 1056, 1009, 951, 872, 798, 753, 732, 687, 581 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.54 (broad spins, 4H), 2.69-2.77 (m, 4H), 3.01(s, 2H), 3.92 (s, 2H), 5.30 (broad spins 1H); 6.97-.28 (m, 4H); ESI MS: m/z (relative abundance. %): 248.0 (M+1).

3-(2-(dimethylamino)ethyl)benzo[d]oxazol-2(3H)-one (4d): Brown semi solid; Yield: 76%; IR(KBr): ν_{max} 2942, 2818, 1767, 1613, 1485, 1445, 1392, 1359, 1320, 1255, 1134, 1088, 1056, 1009, 951, 872, 798, 753, 732, 687, 581 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.33 (s, 6H), 2.70 (t, J = 3.3 Hz, 2H), 3.98 (t, J = 3.6 Hz, 2H), 7.01-7.28 (m, 4H); ESI MS: m/z (relative abundance %): 207.0 (M+1).

3-(2-(diisopropylamino)ethyl)benzo[d]oxazol-2(3H)-one (4e): Yellow semi solid; Yield: 74%; IR (KBr): ν_{max} 2923, 2852, 1773, 1649, 1604, 1487, 1399, 1335, 1311, 1255, 1160, 1134, 1046, 966, 895, 758, 673, 574 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.11-1.32 (m, 12H), 1.65-1.70 (m, 2H), 5.03 (dd, J = 0.6, 4.8 Hz, 2H), 5.55 (dd, J = 0.9, 8.1 Hz, 2H), 7.08-7.22 (m, 4H); ESI MS: m/z (relative abundance %): 263.0 (M+1).

3-(2-(1H-imidazol-1-yl)ethyl)benzo[d]oxazol-2(3H)-one (4f): Yellow semi solid; Yield: 82%; IR (KBr): ν_{max} 2924, 2852, 1773, 1649, 1604, 1487, 1398, 1335, 1311, 1255, 1160, 1134, 1046, 966, 895, 759, 673 cm^{-1}; ^1H NMR (DMSO-d_6): δ 5.10 (d, J = 4.8 Hz, 2H), 5.62 (d, J = 4.8 Hz, 2H), 6.87-6.91 (m, 2H), 7.17-7.30 (m, 5H); ESI MS: m/z (relative abundance %): 230.1 (M+1).

3-(2-(4-tosylpiperazin-1-yl)ethyl)benzo[d]oxazol-2(3H)-one (4g): Reddish brown liquid; Yield: 82%; IR (KBr): ν_{max} 2924, 1769, 1712, 1614, 1597, 1486, 1454, 1393, 1349, 1329, 1305, 1164, 1121, 1092, 1065, 1092, 1033, 1009, 727, 679, 651, 594 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.44 (s, 3H), 2.55 (broad spins 2H), 2.90 (brs, 2H), 3.52 (broad spins 2H), 3.80 (broad spins 2H); 6.98-7.20 (m, 4H); 7.60 (d, J = 3.9 Hz, 2H), 7.72 (d, J = 4.2 Hz, 2H); ESI MS: m/z (relative abundance %): 402.0 (M+1).

3-(2-(4-benzylpiperazin-1-yl)ethyl)benzo[d]oxazol-2(3H)-one (4h): Reddish brown viscous liquid; Yield: 82%; IR (KBr): ν_{max} 3414, 2955, 2850, 1769, 1613, 1485, 1453, 1391, 1361, 1247, 1211, 1156, 1100, 1033, 1010, 958, 912, 755, 704 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.26 (broad spins 4H), 3.62 (broad spins 4H), 5.20 (s, 2H), 3.50 (broad spins 2H), 3.80 (broad spins 2H), 6.98-7.18 (m, 4H), 7.34-7.56 (m, 5H); ESI MS: m/z (relative abundance %): 338.0 (M+1).

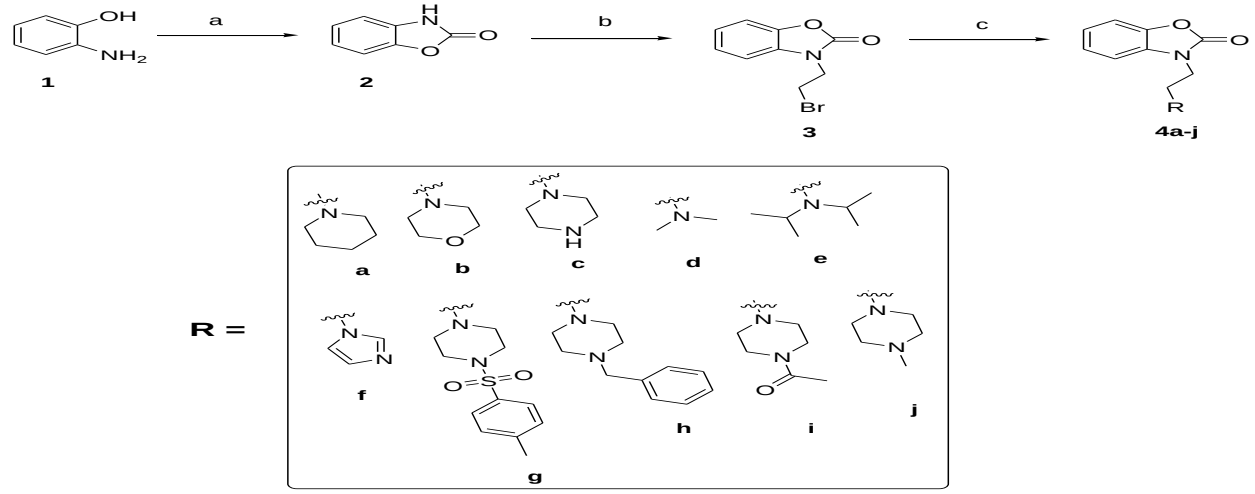
3-(2-(4-acetylpiperazin-1-yl)ethyl)benzo[d]oxazol-2(3H)-one (4i): Brownish yellow viscous liquid; Yield: 78%; IR (KBr): ν_{max} 2933, 2806, 1757, 1638, 1489, 1439, 1356, 1282, 1245, 1136, 1097, 999, 898, 872, 805, 752, 735, 681 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.08 (s, 3H), 2.50 (t, J = 3.0 Hz, 2H), 2.55 (t, J = 3.0 Hz, 2H), 2.71-2.78 (m, 2H), 3.43 (t, J = 3.0 Hz, 2H), 3.58 (t, J = 3.0 Hz, 2H), 3.97 (t, J = 3.9 Hz, 2H), 7.01 (d, J = 4.8 Hz, 1H), 7.11-7.23 (m, 3H); ESI MS: m/z (relative abundance %): 290.0 (M+1).

3-(2-(4-methylpiperazin-1-yl)ethyl)benzo[d]oxazol-2(3H)-one (4j): Yellow viscous liquid; Yield: 78%; IR (KBr): ν_{max} 2925, 2852, 1769, 1650, 1614, 1485, 1455, 1393, 1358, 1283, 1257, 1164, 1138, 1092, 1052, 1009, 962, 872, 789, 773, 736, 688 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.50 (t, J = 3.0 Hz, 2H), 2.55 (t, J = 3.0 Hz, 2H), 2.71-2.78 (m, 2H), 3.30 (s, 3H), 3.43 (t, J = 3.0 Hz, 2H), 3.58 (t, J = 3.0 Hz, 2H), 3.97 (t, J = 3.9 Hz, 2H), 7.01 (d, J = 4.8 Hz, 1H), 7.11-7.23 (m, 3H); ESI MS: m/z (relative abundance %): 262.0 (M+1).

Antimicrobial Bioassay: The antimicrobial activity was determined using disc diffusion method [13, 14] by measuring zone of inhibition in mm. All the compounds, **4a-j** were screened *in vitro* at a concentration of 100 µg/mL for antibacterial activity against two Gram-positive (*Staphylococcus aureus and Staphylococcus pyogenes*) and two Gram-negative strains (*Escherichia coli and Pseudomonas aeruginosa*). Standard antibacterial drug ciprofloxacin (100 µg $disc^{-1}$) was also tested under similar conditions against these organisms. Each experiment was done in triplicate and the average reading was taken.

RESULTS AND DISCUSSION

The synthesis of N-Alkylbenzoxazol-2-ones derivatives **4a-j** is illustrated in **scheme-1**. Carbonylation of 2-aminophenol in presence of CDI in THF at reflux for 4 h resulted in the formation of benzo[d]oxazol-2(3H)-one **2** in 90% yield. Alkylation of compound **2** with 1,2 bromoethane in presence of potassium carbonate and potassium iodide in acetonitrile at reflux for 12 h resulted in 3-(2-bromo ethyl) benzo[d]oxazol-2(3H)-one **3** in 55% yield. Reaction of compound **3** with secondary amines a-j in presence of potassium carbonate (4a-4f) and triethylamine (4g-4j) in dichloromethane at room temperature for 5 h resulted in N-Alkylbenzoxazol-2-ones derivatives **4a-j.** The structural confirmation of N-Alkylbenzoxazol-2-ones derivatives **4a-j** was established by ^1H NMR, IR and mass spectroscopic methods. The mass spectra of compounds showed (M+1) peaks, is in agreement with their molecular formula. The IR spectral data of the compounds **4a-j** gave the evidence for the expected functional groups. As an representative example the ^1H NMR of 3-(2-morpholinoethyl)benzo[d]oxazol-2(3H)-one **4b**, is described here, the protons resonating at 2.74 ppm and 3.97 ppm as triplets is assigned for the methylene protons while the protons resonating at 2.56 ppm and 3.67 ppm as singlets is assigned to the morpholine protons. The aromatic protons resonated in the expected region i.e at 7.01-7.28 ppm as a multiplet with two proton integration.



Scheme 1: N-Alkylbenzoxazol-2-ones derivatives **4a-j**

Experimental conditions: a) CDI, THF, reflux, 4h; b) K_2CO_3, KI, 1,2,-dibromo ethane, acetonitrile, reflux, 12h; c) (i) **4a-4f**: secondary amine, K_2CO_3, KI, acetonitrile, reflux, 3h; (ii) **4g-j**: secondary amine, triethylamine, DCM, r.t., 5h

Antibacterial Activity: The results of antibacterial activity are expressed in terms of zone of inhibition and presented in **Table 1**. In case of Gram negative bacteria (*Escherichia coli and Pseudomonas aeruginosa)*: The antibacterial activity was classified as excellent activity (≥28 mm), good activity (22-27 mm) and moderately active (19-21 mm), compounds 4f and 4g exhibited excellent activity and the compounds 4a, 4b, 4c, 4i and 4j showed good antibacterial activity while the compounds 4d and 4e showed moderate antibacterial activity. Similar trends of antibacterial activity were observed in case of Gram-negative bacterial strains *viz., Staphylococcus aureus* and *Staphylococcus pyogenes*.

Table 1: Antibacterial Activity of N-Alkylbenzoxazol-2-ones derivatives **4a-j**

Compound No.	*Gram negative bacteria*		*Gram positive bacteria*	
	E. coli	*P. aeruginosa*	*S.aureus*	*S.pyogenes*
	Zone of inhibition (mm)			
4a	26	24	19	20
4b	27	23	19	21
4c	26	24	18	17
4d	20	19	16	13
4e	21	20	14	15
4f	31	28	23	25
4g	29	27	22	24
4h	--	--	--	--
4i	25	22	18	19
4j	24	23	18	18
Standard drug	28	26	21	22

*Ciprofloxacin: (Conc. 100 µg mL^{-1}); --: no activity;

APPLICATIONS

Some of the synthesized N-alkylbenzoxazol-2-ones derivatives was found to exhibit antibacterial activity and may emerge as a potential active pharmacophore. A further investigation into various biological activities is the future scope of the work.

CONCLUSIONS

Synthesis of some N-alkylbenzoxazol-2-ones **4a-j** is described utilizing commercially available 2-amonio-phenol as starting material in three steps. These compounds were screened against a panel of four bacterial strains viz., *Staphylococcus aureus and Staphylococcus pyogenes, Escherichia coli and Pseudomonas aeruginosa*. Compounds **4f** and **4g** exhibited excellent activity and the remaining compounds in the series showed good to moderate antibacterial activity.

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AUTHORS' ADDRESSES

1. **Dr. A. Ravindranath**
University College of Technology, Osmania University,
Hyderabad, India
E-mail: dranisetti@yahoo.co.in, Ph: 9849144243
2. **B.Yadi Reddy**
Director, Green Trends Laboratories,
Plot No.43, Kushai guda, Hyderabad, 500062, India
E-mail: yadireddybonuga@gmail.com, Ph: 9392533357
3. **Dr. Ram Bhavani**
Director, Green Trends Laboratories
Plot No.43, Kushai guda, Hyderabad, 500062, India
E-mail Id: ram_19_73@yahoo.co.in, Ph: 8179802942