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SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF NOVEL SUBSTITUTED BENZO[*d*] ISOXAZOL-3-OL DERIVATIVES

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Abstract
A novel 5/6/7-substitited benzo[*d*]isoxazol-3-ol derivatives **4a-j** obtained by the reaction of substituted salicylic acid **1** with thionyl chloride to get **2**, which on treatment with hydroxyl amine hydrochloride to yield **3**. The subsequent reaction with carbonyldiimidazole to results title compounds **4a-k** with good yields and the structures of these compounds were confirmed by IR, ^{1}H NMR & Mass spectral analysis. The newly synthesized compounds were evaluated for antimicrobial activity against variety of bacterial and fungal strains some of these compounds have shown significant antibacterial and antifungal activities.

Keywords: benzoisoxazole, thionyl chloride, hydroxyl amine hydrochloride, carbonyldiimidazole, antimicrobial activity.

Introduction

The growing interest in heterocyclic compounds is basically because of their raised biological activity and also they make possible development of novel materials with unique properties. One very interesting and promising class of heterocycle is the series of 3-substituated-1,2-benzisoxazole [1]. This type of heterocycle has significant pharmacological and biological activity such as anticonvulsant [2], antipsychotic [3], anticancer [4], presented affinity for serotonergic and dopaminergic receptors [5]. In particular, 3-(*N*-benzylpiperidinylethyl)-1,2-benzisoxazoles inhibit acetyl cholinesterase, making them suitable candidates for the palliative treatment of Alzheimer's disease [6]. Due to great importance, many synthetic strategies have been employed for the synthesis of 1,2-benzisoxazoles such as Ac2O/K2CO3 [7], Ac2O/pyridine [4], SOCl2/pyridine [8]. However, these methods usually carried out in two steps where first step involves the conversion of hydroxyl group of oxime to good leaving group and in second step cyclization occurs under basic condition. Very few methods described for the synthesis of 1,2-benzisoxazole in one step using trichloroacetyl isocyanate [9]. In recent years, application of ionic liquids in organic synthesis have attracted considerable attention due to their special properties such as good solvating capability, wide liquid range, negligible vapor pressure, easy recycling, high thermal stability and rate enhancers [10-12]. Nowadays, much attention has been focused on organic reactions catalyzed by ionic liquids [13-15]. Particularly, imidazolium ionic

liquids have been successfully used in many organic transformations includes Diels–Alder [16], Wittig [17], Hantzsch condensation [18]. The basic ionic liquid [bmim]OH used in various organic transformation such as Michael reaction [19], Knoevenagel condensation [20]. Thus, the development of a new method for the synthesis of substituated-1,2-benzisoxazole derivatives would be highly desirable

Antimicrobial activity

The minimum inhibition concentration (MIC) was determined using the streak plate and cup plate method by measuring the zone of inhibition according to a standard procedure [21]. All the synthesized compounds were screened in vitro for their antimicrobial activity against a variety of bacterial strains such as *Staphylococcus aureus, Salmonella paratyphi, Escherichia coli, Shigella flexneri, Pseudomonas auregenosa, Bacillus subtilis,* and fungi such as *Cerevesae vitae, Candida albicans, Aspergillus niger* (**Table-1**). The MIC of the compounds was defined, as the lowest concentration at which there was 80% inhibition of growth compared with the growth for a drug free control [22]. Standard inhibition of zone size for Ciprofloxacin, Cloxacillin and for Gentamycin21 is (++++) at ≤ 50 µgm/mL against all microbes.

Table 1: Antimicrobial activities of the compounds **4a – j.**

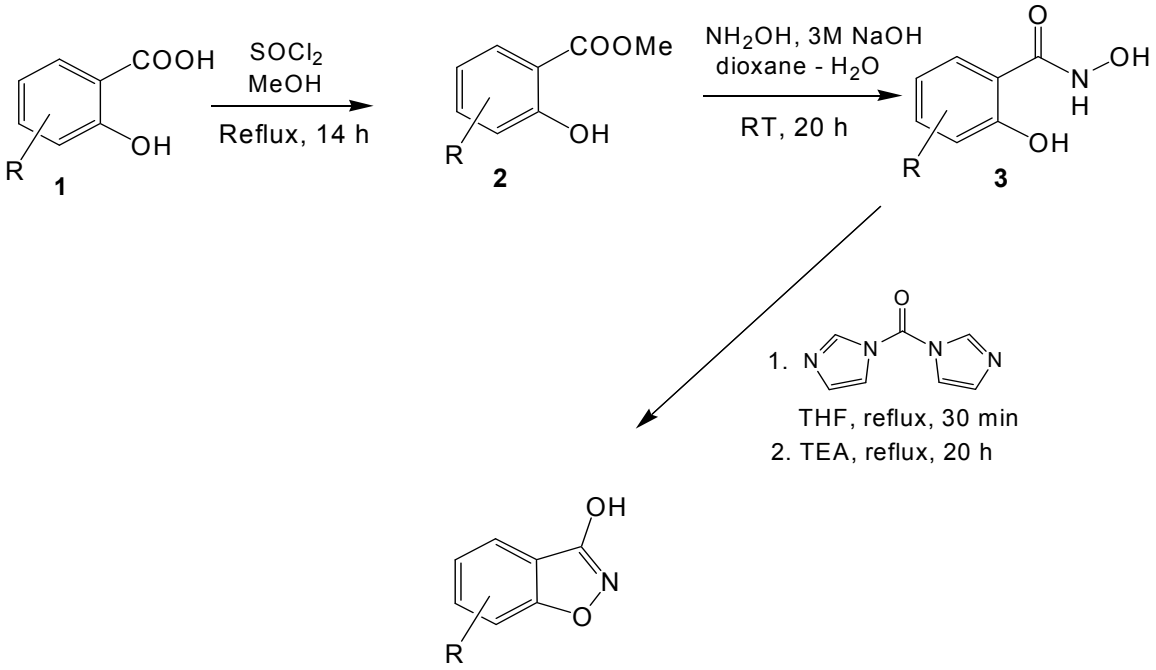
Compound	**Antibacterial activity**						**Antifungal activity**		
	S.a	*E. c*	*S. a*	*S. f*	*P. a*	*B. s*	*A. n*	*C. a*	*C. v*
4a	++	++	++	++	+++	++	++	++	++
4b	++	++	++	++	++	+++	---	---	---
4c	+++	++++	+++	++	+	---	++	+	+
4d	+++	+++	++	+	++	+	++	++	---
4e	++++	++	+	+	+	++	++	++	---
4f	+++	++	++	++	++	++	++	++	++
4g	+++	++	+++	++	+++	++	++	++	++
4h	++	++	+	++	++	++	++	++	++
4i	++++	++	++	++	+++	++	++	++	++
4j	++	++	++	++	+++	++	++	++	++

50 µgm/mL = ++++, 100 µgm/mL = +++, 150 µgm/mL = ++, 200 µgm/mL = +, Not active upto 200 µgm/mL = – Ciprofloxacin, Cloxacillin & Gentamycin21 is (++++) at ≤ 50 µgm/mL *S. a = Staphylococcus aureus, S. p = Salmonella paratyphi, E. c = Escherichia coli, S. f = Shigella flexneri, P. a = Pseudomonas auregenosa, B. s = Bacillus subtilis, C. v = Cerevesae vitae, C. a = Candida albicans, A. n = Aspergillus niger.*

Results and Discussions

A novel 5/6/7-substitited benzo[*d*]isoxazol-3-ol derivatives **4a-k** obtained by the reaction of substituted salicylic acid **1** with thionyl chloride to get **2**, which on treatment with hydroxyl amine hydrochloride to yield **3**. The subsequent reaction with carbonyldiimidazole to results title compounds **4a-j** with good yields and the structures of these compounds were confirmed by IR, ^{1}H NMR & Mass spectral analysis. **(Scheme I)**.

All synthesized compounds were characterized on the basis of FTIR, 1H NMR and Mass spectroscopic analysis. The IR spectral data of all compounds showed the characteristic peaks of C=N stretching at 1590–1584 cm^{-1}, aromatic tertiary C–N stretching at 1354–1340 cm^{-1}, and C–N stretching at 1153–1143 cm^{-1} showing the formation of the benzoisoxazole ring. The peaks at 3415 and 1523 cm^{-1} indicating the presence of -OH and -NO_2 groups respectively were also observed in the corresponding synthesized compounds. Figure **1a**, **1b** and **1c** shows the IR, ^1H NMR and Mass spectrum of the title compound **4a** respectively.



4a, R = 6-CH_3, **4b**, R = 6-OCH_3, **4c**, R = 6-Cl, **4d**, R = 6-Br, **4e**, R = 6-F, **4f**, R = 6-NO_2
4g, R = 5-F, **4h**, R = 5-NO_2, **4i**, R = 5-Br, **4j**, R = 5,7-NO_2

Scheme 1: Synthetic route for title compounds **4a-j.**

Experimental Section

Melting points were determined using Gallenkamp apparatus and uncorrected. IR spectra were recorded on a FT-IR 1605 Perkin-Elmer machine; ^1H NMR in CDCl3 on a Varian FT-80A spectrometer with TMS as an internal standard and mass spectra on a VG-Micromass 7070H mass spectrometer. TLC was run on silica gel G coated plates with iodine vapour as visualizing agent.

General procedure for the synthesis of 4a-j

Thionyl chloride (0.845 mol) was added drop-wise to the solution of compound **1** (0.1297 mol) in methanol (200 mL) at 0 ^oC and heated to reflux for 14 h. After completion of the reaction, the solvent was evaporated. The residue was dissolved in EtOAc (50 mL), washed with sat. $NaHCO_3$, dried over anhydrous Na_2SO_4, filtered and evaporated to yield **2.** The compound **2** (0.022 mmol) in dioxane (10 mL) was added to the well-stirred solution of NaOH (3 mol),

hydroxylamine hydrochloride (0.00552 mmol) in water and stirring was continued for 20 h at RT. After the reaction, the reaction mixture was acidified to pH~5, extracted with EtOAc, dried over anhydrous Na_2SO_4, filtered and evaporated to give the product **3** then the corbonyldiimidazole (0.233) was added to the solution of compound **3** (0.77 mol) in THF (190 mL) and refluxed for 30 min and then TEA (11.8 ml) was added and reflux continued for further 20 h. After the reaction, the solvent was evaporated and the residue was dissolved in EtOAc, washed with 1M HCl, dried over anhydrous Na_2SO_4, filtered and evaporated. All the title compounds **4a-j** were purified by silica gel (100-200 mesh) column chromatography using 0-20% EtOAc in pet ether containing 2% AcOH as a eluent.

Compound 4a. Off-white solid, yield 52% m.p. 153-154 °C, IR (KBr, cm^{-1}): 866, 932, 948, 978, 1035, 1108, 1164, 1256, 1319, 1412, 1465, 1525, 1551, 1621, 1662, 2559, 2706, 2754, 2920, 3444; ^1H NMR (400 MHz $CDCl_3$): δ 2.52 (1H, s, CH3), 7.14 (1H, d, Ar-H), 7.36 (1H, s, Ar-H), 7.62 (1H, d, Ar-H), 12.28 (1H, s, OH) ppm; MS: *m/z* 148 (M -1), 149 (M+1). Anal. Calcd for $C_8H_7NO_2$: C, 64.38; H, 4.75; N, 9.39. Found: C, 64.58; H, 4.30; N, 9.62 %.

Compound 4b. Yellow solid, yield 56% m.p. 187-189 °C, IR (KBr, cm^{-1}): 862, 930, 944, 977, 1054, 1114, 1168, 1250, 1317, 1417, 1463, 1524, 1551, 1620, 1665, 2354, 2458, 2558, 2716, 2753, 2927, 3448 (OH) ; ^1H NMR (400 MHz $CDCl_3$): δ 3.76 (1H, s, OCH3), 7.08 (1H, d, Ar-H), 7.25 (1H, s, Ar-H), 7.64 (1H, d, Ar-H), 12.14 (1H, s, OH) ppm; MS: *m/z* 164 (M -1), 166 (M+1). Anal. Calcd for $C_8H_7NO_3$: C, 58.14; H, 4.28; N, 8.46. Found: C, 58.10; H, 4.30; N, 8.57 %.

Compound 4c. White solid, yield 54% m.p. 71-73 °C, IR (KBr, cm^{-1}): 785, 858, 925, 949, 973, 1061, 1118, 1174, 1252, 1310, 1418, 1467, 1525, 1556, 1623, 1665, 2554, 2717, 2752, 2928, 3442; ^1H NMR (400 MHz $CDCl_3$): δ 7.24 (1H, d, Ar-H), 7.46 (1H, s, Ar-H), 7.82 (1H, d, Ar-H), 12.32 (1H, s, OH) ppm; MS: *m/z* 169 (M^+), 171 (M+2). Anal. Calcd for $C_7H_4ClNO_2$: C, 49.58; H, 2.39; Cl, 20.95; N, 8.25. Found: C, 48.65; H, 2.30; Cl, 20.91; N, 8.42 %.

Compound 4d. yellow solid, yield 63% m.p. 161-162 °C, IR (KBr, cm^{-1}): 763, 854, 931, 959, 981, 1065, 1123, 1175, 1258, 1318, 1414, 1460, 1529, 1558, 1621, 1669, 2550, 2719, 2757, 2935, 3454; ^1H NMR (400 MHz $CDCl_3$): δ 7.28 (1H, d, Ar-H), 7.60 (1H, s, Ar-H), 7.86 (1H, d, Ar-H), 12.22 (1H, s, OH) ppm; MS: *m/z* 212 (M^+), 214 (M+2), 216 (M+4). Anal. Calcd for $C_7H_4BrNO_2$: C, 39.25; H, 1.88; Br, 37.34; N, 6.56. Found: C, 39.58; H, 2.03; Br, 37.19; N, 6.56 %.

Compound 4e. white powder, yield 63% m.p. 189-194 °C, IR (KBr, cm^{-1}): 743, 869, 939, 947, 973, 1034, 1114, 1160, 1258, 1320, 1416, 1466, 1528, 1550, 1627, 1669, 2554, 2714, 2757, 2986, 3456; ^1H NMR (400 MHz $CDCl_3$): δ 7.28 (1H, d, Ar-H), 7.64 (1H, s, Ar-H), 7.86 (1H, d, Ar-H), 12.21 (1H, s, OH) ppm; MS: *m/z* 152 (M -1), 154 (M+1). Anal. Calcd for $C_7H_4FNO_2$: C, 54.99; H, 2.63; F, 12.48; N, 9.18. Found: C, 54.98; H, 2.74; F, 12.56; N, 9.18 %.

Compound 4f. Oil, yield 58%, IR (KBr, cm^{-1}): 785, 858, 925, 949, 973, 1061, 1118, 1174, 1252, 1310, 1418, 1467, 1525, 1556, 1623, 1665, 2554, 2717, 2752, 2928, 3442; ^1H NMR (400 MHz $CDCl_3$): δ 7.24 (1H, d, Ar-H), 7.46 (1H, s, Ar-H), 7.92 (1H, d, Ar-H), 12.32 (1H, s, OH) ppm; MS: *m/z* 179 (M-1), 181 (M+1). Anal. Calcd for $C_7H_4N_2O_4$: C, 46.68; H, 2.28; N, 15.53. Found: C, 46.65; H, 2.24; N, 15.46 %.

Compound 4g. white solid, yield 63% m.p. 214-216 °C, IR (KBr, cm^{-1}): 745, 871, 934, 942, 974, 1038, 1117, 1164, 1255, 1321, 1417, 1461, 1522, 1554, 1621, 1660, 2558, 2716, 2750, 2984, 3452; ^1H NMR (400 MHz $CDCl_3$): δ 7.14 (1H, d, Ar-H), 7.36 (1H, s, Ar-H), 7.74 (1H, d, Ar-H), 12.00 (1H, s, OH) ppm; MS: *m/z* 152 (M -1), 154 (M+1). Anal. Calcd for $C_7H_4FNO_2$: C, 54.99; H, 2.63; F, 12.48; N, 9.18. Found: C, 54.99; H, 2.70; F, 12.54; N, 9.24 %.

Compound 4h. solid, yield 68%, m.p. 128-131 °C, IR (KBr, cm^{-1}): 781, 854, 920, 944, 972, 1064, 1121, 1175, 1250, 1315, 1413, 1464, 1528, 1550, 1623, 1664, 2557, 2719, 2754, 2921, 3443; ^1H NMR (400 MHz $CDCl_3$): δ 7.28 (1H, d, Ar-H), 7.48 (1H, s, Ar-H), 7.75 (1H, d, Ar-H), 12.24 (1H, s, OH) ppm; MS: *m/z* 179 (M-1), 181 (M+1). Anal. Calcd for $C_7H_4N_2O_4$: C, 46.68; H, 2.28; N, 15.53. Found: C, 46.66; H, 2.28; N, 15.47 %.

Compound 4i. white solid, yield 64% m.p. 158-162 °C, IR (KBr, cm^{-1}): 766, 855, 938, 953, 982, 1063, 1124, 1179, 1250, 1317, 1412, 1464, 1527, 1550, 1625, 1665, 2551, 2725, 2754, 2938, 3450; ^1H NMR (400 MHz $CDCl_3$): δ 7.18 (1H, d, Ar-H), 7.64 (1H, s, Ar-H), 7.96 (1H, d, Ar-H), 12.04 (1H, s, OH) ppm; MS: *m/z* 212 (M^+), 214 (M+2), 216 (M+4). Anal. Calcd for $C_7H_4BrNO_2$: C, 39.25; H, 1.88; Br, 37.34; N, 6.56. Found: C, 39.49; H, 2.15; Br, 37.26; N, 6.46 %.

Compound 4j. solid, yield 68%, m.p. 264-268 °C, IR (KBr, cm^{-1}): 789, 857, 928, 949, 976, 1068, 1127, 1179, 1257, 1318, 1414, 1466, 1528, 1559, 1627, 1666, 2559, 2723, 2764, 2928, 3448; ^1H NMR (400 MHz $CDCl_3$): δ 7.68 (1H, s, Ar-H), 7.98 (1H, s, Ar-H), 12.34 (1H, s, OH) ppm; MS: *m/z* 224 (M-1), 226 (M+1). Anal. Calcd for $C_7H_3N_3O_6$: C, 37.36; H, 1.28; N, 18.68. Found: C, 36.99; H, 1.34; N, 18.63 %.

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