SYNTHESIS OF SOME 5-NITRO-2-FURFURYLIDENE DERIVATIVES AND THEIR ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES

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Abstract: A number of new 5-nitro-2-furfurylidene derivatives **9a-k** were synthesized by the reaction of 2-methyl-4-(5-nitro-2-furfurylmethyliden)-Δ^2-oxazolin-5-one **6** or 2-phenyl-4-(2-furfurylmethyliden)-Δ^2-oxazolin-5-one **7** with appropriate 2-aminobenzothiazole. The compounds synthesized were identified by ^{1}H-NMR, IR, MS and micro analysis. All compounds studied in this work were screened for their *in vitro* antimicrobial and antifungal activities against the standard strains: *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, and the* yeast *Candida albicans*.

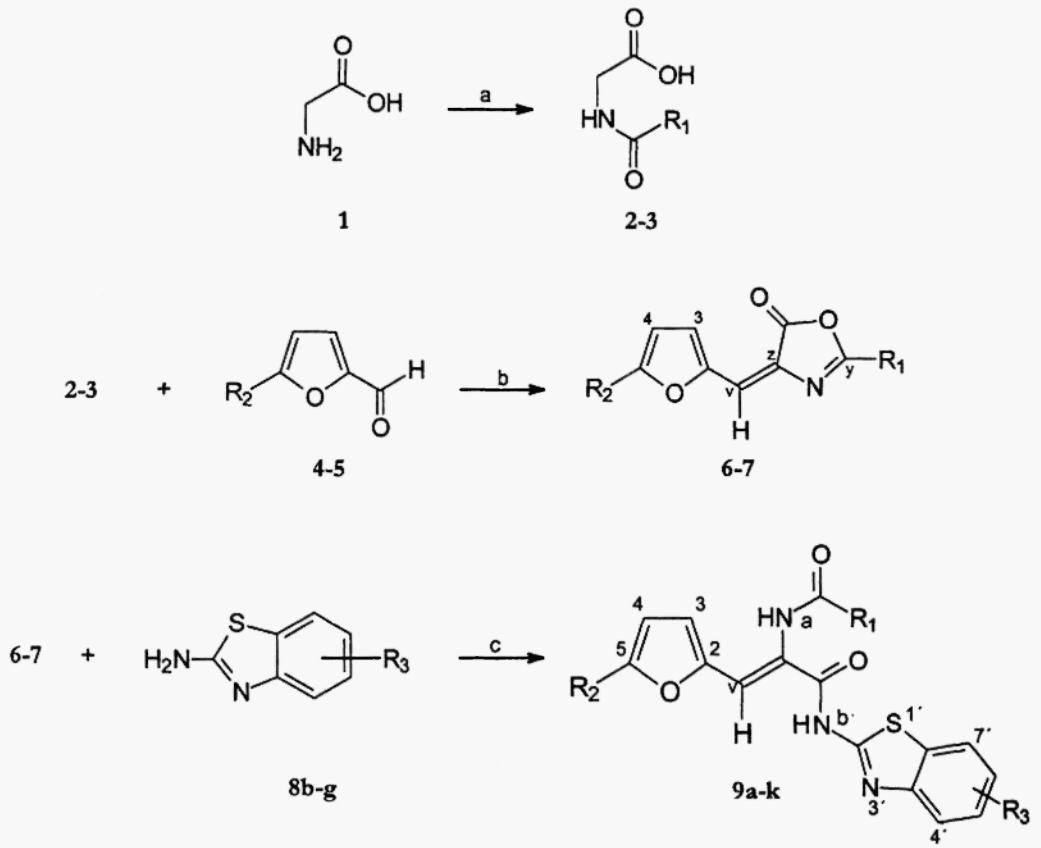
Introduction

Efficacy and safety play an important role in the selection of therapeutic agents (1). However, antibacterial therapy, amongst other proceedings, is highly susceptible to the progressive inefficiency of its pharmacological agents (2). This is due to misprescriptions, inadequate therapeutic dosages and the development of biochemical mechanisms of bacterial resistance most often as a result of treatment interruptions (2,3). Treating multiresistant infections has become increasingly difficult, posing a major challenge that demands continuous search for new antimicrobian agents. To this effect, nitrofurazone, furazolidone, and nitrofurantoin are synthetic antimicrobials utilized from the 50's (4). Also, it was reported that nifurtimox, was effective against *Trypanosoma americane* (Chagas) (5). More recently furazolidone and nitrofurantoin have been used against *Pneumocystis carinii pneumoniae* (PCP) (6), and the treatment of duodenal ulcer cause by *Helicobacter pylori* (7). Molecular modification methods, with views to identifying more potent antimicrobial analogues of 5-nitrofuran substituted at 2-position with a wide variety of substituents have been synthesized (8). The nitro group is essential for the activity, whereas the influence of the 2-substituent on the activity is not completely understood (9). Given these findings, we are introducing some benzothiazole derivatives into the 5-nitrofurane and nitrofurane molecules, and are finding that these compounds have shown considerable antibacterial and antifungal *in vitro* activities against the standard strains: *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, and the* yeast *Candida albicans*.

Results and discussion

The reactions involve nucleophilic acylic substitution between 2-methyl-4-(5-nitro-2-furfurylmethyliden)-Δ^2-oxazolin-5-one **6** or 2-phenyl-4-(2-furfurylmethyliden)-Δ^2-oxazolin-5-one **7** with appropiate 2-aminobenzothiazole **8a-g**, according to the procedure indicated at Scheme 1 and described in the experimental section. The azalactones **6** and **7** were synthesized from the reaction of 5-nitro2-furfuraldehyde 4 and 2-furfuraldehyde **5** respectively with acetylglycine and benzoylglycine **2-3** (10). 2-aminobenzothiazole derivatives were prepared by treating of aniline appropriate, and bromine similar to the literature procedure (11). The intermediates and final compounds **9a-k** were obtained in good yields. The melting points of all intermediates agreed with those reported in the literature. The analysis of ^{1}H NMR, IR, MS were consistent with the expected structure **8b-g** and **9a-k** Table 1, 2. The elemental analysis showed good agreement (±0.4%) with theoretical values. 2-methyl-4-(5-nitro-2-furfurylmethyliden)-Δ^2-oxazolin-5-one **6** or 2-phenyl-4-(2-furfurylmethyliden)-Δ^2-oxazolin-5-one 7 were prepared in one step from **2-3** and 5-nitro-2-furfuraldehyde or 2-furfuraldehyde respectively in presence of glacial acetic acid and solid sodium acetate in good yield. The general method for the preparation of 2-aminobenzothiazole derivatives involve a reaction of a suitable substituted aniline with thiocyanogen (generated in situ), and the subsequent cyclization of the intermediate formed giving compounds **8b-g**. In to 3,4-dichloroaniline, both ortho position are disposable, allowing the formation of both of the observed products 5,6 and 6,7-dichlorobenzothiazole **8e, f**. These isomers were separated by using the different solubilities of the hydrochloride salt in water respectively (12). Initially it was expecting mixture of both geometrical isomers E and Z. However, only isomer Z was confirmed according with results obtained by NMR. The isomers Z was determined by their chemical shift of protons at C3 and C4 in the furan ring, 6.64-7.18 and 7.70-7.75 ppm as doublet respectively. This clear indicate good agreement with some derivatives reported in the literature (13). The compounds **9a-k** were tested against five different strains of bacteria representing both Gram-positive and Gram-negative bacteria and a yeast (*C. albicans*). Nitrofurantoin was chosen as a reference substance 100μg, 50μg and 5μg per disc. Compounds **9a**, **9c-g** (100μg) were found effective to inhibit the growth of Gram-positive and Gram-negative bacteria, except compounds **9b** was effective only against *B. subtilis* and *S. aureus*. None of the compounds studied here showed antimicrobial activity against *P. aeruginosa*. Compounds **9c, d** (50μg and 5μg per disc) were found effective to inhibit the growth of *B. subtilis* and *S. aureus*. Comporids **9h-k** were not found active against the bacterial and fungal strains used in this study, even at a concentration of 100 μg/disc. Compounds **9a**, **9c-g** (100μg) showed a high activity against *C. albicans*. From the data obtained in this work, this suggest that 2-aminobenzothiazole and acetamido on 5-nitrofurans could play an important role in the antibacterial and antifungal activities and replacement of the nitro with a hydrogen group on the furan results in loss of antibacterial and antifungal activities.

Scheme 1. Synthesis of derivatives **9a-k.**



R_1: CH_3 or Ph, R_2: H or NO_2, R_3: 6-H; 6-F; 5,6-F; 6-Cl; 5,6-Cl; 6,7-Cl; 6-OCH_3

a. Ac_2O, H_2O, rt; **b**. Ac_2O, NaAc,Δ, **c**. 1,4-dioxane, Δ.

Experimental

Melting points were determined on a Thomas micro hot stage apparatus and are uncorrected. Infrared spectra were determined as KBr pellets on a Shimadzu model 470 spectrophotometer. The ^{1}H-NMR spectra were recorded using a JEOL Eclipse 270 MHz spectrometer and are reported in ppm downfield from $CHCl_3$ or DMSO residual. Mass spectra were obtained a Varian Saturn GC/MS 2080 workstation. Elemental analysis were performed by (Atlantic Microlab, Norcross, GA, USA), and analytical results were within ±0.4% of the calculate values. All solvent were distilled and dried with the usual desiccants. 5-nitro-2-furfuraldehyde, 2-furfuraldehyde and 2-aminobenzothiazole were obtained from Aldrich Chemical Co. Black paper discs, 6mm diameter 231039, Becton Dickinson Co.

Synthesis of acetylglycine 2 and benzoylglycine 3

Compounds **2** and **3** were similarly prepared as reported in the literature (10).

General procedure for the synthesis of 2-furfurylmethyliden-Δ^2-oxazolin-5-one 6-7

A mixture of acetylglycine **2** or benzoylglycine **3** (3.45 mmol), 5-nitro-2-furaldehyde **4** or 2-furaldehyde **5** (3.54 mmol), sodium acetate (3.6 mmol) and acetic anhydride (6 ml, was heated under

reflux for 2 h. The solution was allowed to cool until reach to room temperature and isopropanol (20 ml) was added and cool into a freezer for 24 h. The solid was filtered and then recrystallized from EtOH.

2-methyl-4-(5-nitro-2-furfurylmethyliden)-Δ^2-oxazolin-5-one 6

Yield: 0.58g, 53 %; m.p.:135-137°C; IR cm ^{1}: 1664 CO, 1526 NO_2; ^{1}H NMR δ ppm $CDCl_3$: 2.43(s, 3H, CH_3); 7.00(d, 1H, HC=C, J:0.49Hz); 7.41(dd, 1H, H_3, J: 3.96, 0.49Hz); 7.55(d, 1H, H_4, J:3.96Hz). EM m/z: 223 M^++1 (100%).

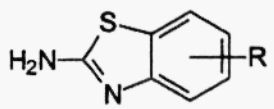
2-phenyl-4-(2-furfurylmethyliden)-Δ^2-oxazolin-5-one 7

Yield: 0.3g, 56 %; m.p.:169-170°C; IR cm^{-1}: 1670 CO, 1526 NO_2; ^{1}H NMR δ ppm $CDCl_3$: 6.64(dd, 1H, H_4, J:3.71, 1.73Hz); 7.16(s, 1H, HC=C); 7.54(m, 3H, $H_{3',4',5'}$); 7.66(d, 1H, H_5, J:3.96Hz), 8.13(2d, 3H, $H_{4,2',6'}$, J:8.41, 1.73Hz). EM m/z: 240 M^++1 (100%).

General procedure for the synthesis of 2-aminobenzothiazole derivatives 8b-g

A mixture of aniline respective (50 mmol), ammonium thiocyanate (100 mmol) in glacial acetic acid 50 ml, was cooled to 0°C in an ice bath. Bromine (100 mmol) in glacial acetic acid 6 ml was added dropwise on a period of 30 min, the ice bath was then removed and the mixture was stirred at room temperature for 1 h, ice water 50 ml was added, and the resulting solid was filtered off, the solution was neutralized with solid calcium carbonate. The solid formed was removed by filtration, and recrystallized from EtOH, to give the title compounds **8b-g** Table 1.

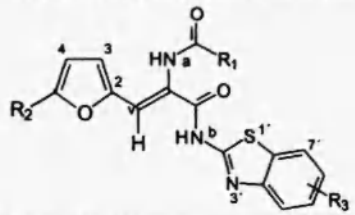
Table 1. Yields, melting points, infrared, and ^{1}H-NMR spectroscopic data for the compounds **8b-g**.



Cmpd	R	Yield%	m.p. °C	IR cm^{-1} (NH_2)	^{1}H NMR
8b	6-F	82	127-128	3264	7.02(td, 1H, H_5, J:8.66, 8.90, 2.72Hz); 7.30(d, 1H, H_4, J:8.66, 4.95Hz); 7.44(s, 2H, NH_2); 7.56(dd, 1H, H_7, J:8.90, 2.72Hz).
8c	5,6-F	64	174-175	3260	7.34(dd, 1H, H_4, J:7.42, 7.18Hz); 7.63(s, 1H, NH_2); 7.80(dd, 1H, H_7, J:8.16, 8.16Hz)
8d	6-Cl	72	183-185	3264	7.19(dd, 1H, H_5, J:8.66, 1.98Hz); 7.29(d, 1H, H_4, J:8.66Hz); 7.59(s, 1H, NH_2); 7.76(d, 1H, H_7, J:1.98Hz)
8e	5,6-Cl	26	211-212	3264	7.28(d, 1H, H_4, J:8.66Hz); 7.43(d, 1H, H_5, J:8.66Hz); 7.86(s, 1H, NH_2)
8f	6,7-Cl	33	225-227	3268	7.51(s, 1H, H_4); 7.90(s, 1H, H_7); 7.81(s, 1H, NH_2)
8g	6-OMe	76	149-150	3264	3.89(s, 3H, OCH_3); 6.90(dd, 1H, H_5, J:8.90, 2.47Hz); 7.10(d, 1H, H_7, J:2.47Hz); 7.41(d, 1H, H_4, J:8.90Hz); 7.82(s, 1H, NH_2)

8a. Was obtained from Aldrich Chemical Co. Inc.

Table 2. melting points, Yields, infrared, analytical data and ^{1}H-NMR spectroscopic data with assignments, for the compounds **9a-k**.



No	R_3	R_1	R_2	mp °C	Yield %	IR cm^{-1} (CONH)	ea calc. (found) C H N	^{1}H-NMR
9a	H	CH_3	NO_2	187-188	51	3296, 1690, 1665	49.22 3.91 13.89 (49.10) (3.79) (13.49)	2.04(s, 3H, CH_3); 6.98(td, 1H, H_5, J:8.41, 0.99Hz); 7.05(s, 1H, Hv); 7.06(d, 1H, H_3, J:3.96Hz); 7.19(td, 1H, H_6, J:7.42, 1.24Hz); 7.31(dd, 1H, H_4, J:7.92, 1.24Hz); 7.43(s, 1H, NH); 7.63(dd, 1H, $H_{7'}$, J:7.67, 1.24Hz); 7.75(d, 1H, H_4, J:3.96Hz); 9.72(s, 1H, NH).
9b	6-F	CH_3	NO_2	222-224	55	3296, 1690, 1665	48.10 3.02 14.01 (47.54) (2.99) (13.74)	2.12(s, 3H, CH_3); 7.10(s, 1H, Hv); 7.18(d, 1H, H_3, J:3.96Hz); 7.32(td, 1H, H_5, J:2.72Hz), 7.80(d, 1H H_4, J:8.37Hz); 7.81(d, 1H H_4, J:3.96Hz); 7.91(dd, 1H, H_7, J:8.37 2.72Hz); 9.72(s, 1H NH).
9c	5,6-F	CH_3	NO_2	207-209	58	3298, 1692, 1666	45.07 2.60 13.14 (45.26) (2.86) (12.94)	2.05(s, 3H, CH_3); 7.06(s, 1H, Hv); 7.08(d, 1H, H_3, J:3.92Hz); 7.34(dd, 1H, H_4, J:7.42, 7.42Hz); 7.63(s, 1H, NH); 7.76(d, 1H, H_3 J:3.92Hz); 7.80(dd, 1H H_7, J:8.16, 8.16Hz); 9.74(s, 1H NH).
9d	6-Cl	CH_3	NO_2	160-162	52	3294, 1688, 1663	43.39 2.41 12.65 (43.67) (2.31) (12.48)	2.06(s, 3H CH_3); 7.02(s, 1H, Hv); 7.14(d, 1H, H_3, J:3.96Hz); 7.19(dd, 1H, H_5, J:8.41, 2.23Hz); 7.29(d, 1H, H_4, J:8.41Hz); 7.60(s, 1H, NH); 7.76(d, 1H, H_7, J:2.41Hz); 7.78(d, 1H, H_4, J:3.96Hz); 9.87(s, 1H, NH).
9e	6,7-Cl	CH_3	NO_2	205-207	48	3286, 1690, 1665	42.26 2.36 12.38 (42.30) (2.76) (11.88)	2.05(s, 3H, CH_3); 7.06(s, 1H, Hv); 7.08(d, 1H, H_3, J:3.96Hz); 7.28(d, 1H, H_5, J:8.66Hz); 7.43(d, 1H, H_4, J:8.66Hz); 7.75(d, 1H, H_4, J:3.96Hz); 7.85(s, 1H, NH); 9.74(s, 1H, NH).
9f	5,6-Cl	CH_3	NO_2	126-127	46	3288, 1685, 1670	42.26 2.36 12.38 (42.60) (2.46) (12.13)	2.05(s, 3H CH_3); 6.71(s, 1H, Hv); 6.84(d, 1H, H_3, J:3.92Hz); 7.50(s, 1H, H_4); 7.68(d, 1H, H_4 J:3.92Hz); 7.81(s 1H, NH); 7.96(s, 1H, H_7); 9.74(s, 1H, NH).
9g	6-OMe	CH_3	NO_2	140-142	77	3296, 1690, 1665	48.05 4.03 13.18 (47.83) (3.95) (12.56)	2.05(s, 3H, CH_3); 3.72(s, 3H, O CH_3); 6.81(dd, 1H, H5, J:8.03, 2.20Hz); 7.06(s, 1H, Hv); 7.09(d, 1H, H_3, J:3.92Hz); 7.21(d, 1H, H_4, J:9.03Hz); 7.24(s, 1H, NH); 7.28(d, 1H, H7, J:2.20Hz); 7.75(d, 1H, H_4 J:3.92Hz); 9.75(s, 1H, NH).
9h	H	Ph	H	195-196	81	3286, 1680, 1665	49.22 3.61 13.89 (49.10) (3.79) (13.49)	5.74(s, 1H, NH); 6.64(dd, 1H, H_4, J:3.96, 5.23Hz); 6.87(d, 1H, H_3, J:3.22Hz); 7.31(t, 1H, H_5, J:7.67Hz; 7.39(s, 1H, Hv); 7.45(t, 1H, H_6, J:7.18Hz); 7.58m, 3H, $H_{3',4',5'}$); 7.72(d, 1H, H_4, J 8.16Hz); 7.85(d, 1H, H_5, J:2.73Hz); 8.00(d, 1H, H_7, J:7.92Hz); 8.01(d, 2H, $H_{2',6'}$, J 6.93Hz); 10.28(s, 1H, NH).
9i	6-F	Ph	H	232-234	82	3290, 1685, 1675	60.56 3.63 10.08 (60.79) (3.46) (10.38)	5.63(s, 1H NH); 6.64(dd, 1H, H_4, J:3.22, 5.42Hz); 6.87(d, 1H, H_3, J:3.22Hz); 7.29(td, 1H, H_5, J:8.3, 2.47Hz); 7.38(s, 1H, Hv); 7.58m, 3H, $H_{3',4',5'}$); 7.74(dd, 1H, H_4, J 8.3, 2.47Hz); 7.85(d, 1H, H_5, J:5.23Hz); 7.93(dd, 1H, H_7 J:8.15, 2.03Hz); 8.09(d 2H, $H_{2',6'}$, J 7.17Hz); 10.07(s, 1H, NH).
9j	5,6-F	Ph	H	253-255	58	3294, 1683, 1662	54.55 3.30 9.19 (54.78) (3.09) (9.43)	5.21(s, 1H, NH); 6.64(dd, 1H, H_4, J:3.32, 5.73Hz); 6.88(d, 1H H_3, J:3.46Hz); 7.37(s, 1H, Hv); 7.58m, 3H, $H_{3',4',5'}$); 7.81(dd 1H, H_4, J:7.18, 7.42Hz); 7.85(d, 1H H_5 J:5.23Hz); 8.10(d 2H, $H_{2,6'}$, J 7.19Hz); 8.18(dd, 1H, H_7, J:8.16Hz); 10.1(s, 1H, NH).
9k	6-Cl	Ph	H	245-247	64	3288, 1688, 1660	59.98 3.32 9.01 (61.30) (3.42) (9.06)	5.56(s, 1H NH); 6.64(dd, 1H, H_4, J:3.22Hz); 6.87(d, 1H, H_3 J:3.26Hz); 7.38(s, 1H, Hv); 7.47(dd, 1H, H_5, J:7.89 2.23Hz); 7.58m, 3H, $H_{3',4',5'}$); 7.70(d, 1H, H_4, J:7.89Hz); 7.85(d, 1H, H_5, J:5.23Hz); 8.10(d, 1H, H_7, J:2.23Hz); 8.14(d, 2H, $H_{2',6'}$, J:7.19Hz); 10.07(s, 1H, NH).

General procedure for the synthesis of 2-[2-acetylaminosubstituted-2-[2-(benzothiazolylamino-carbonyl)]vinyl]furano 9a-k.

A mixture of 2-furfurylmethyliden-Δ^2-oxazolin-5-one **6** and **7** respectively (1.13 mmol), 2-aminobenthiazole derivatives **8a-g** (1.26 mmol) and 8 ml of 1,4-dioxane, was heated under refluxed for 12 h. the solvent was removed under reduced pressure and water 10 ml was added. The precipitate was collected off by filtration and recrystallized from ethyl acetate, to obtain the title compounds **9a-k** Table 2.

Biological activity

Compounds **9a-k** were evaluated for *in vitro* antibacterial activity against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium* by disc diffusion methods (14). Stock solutions of the compounds were prepared in DMSO and sterile paper disc (6 mm diameter) were impregnated with the stock solution of the test compounds to obtain a concentration of 100 μg, 50 μg or 5 μg of the test compounds per disc. Antibacterial screening was performed in triplicate on Muller-Hinton agar and the antibacterial activity profile was evaluated.

Sabouraud dextrose agar medium was used for the antifungal screening (15). Compounds 9a-k were screened for activity against *Candida albicans* at a concentration of 100 μg, 50 μg and 5 μg per disc.

Acknowledgements

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