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Antibacterial and Antioxidant Activity Evaluation of Bis-Substituted İsovanillin Derivatives

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Abstract: Herein, a series of twelve bis-hydrazone substituted isovanilline derivatives **3(a-l)**, were freshly re-synthesized by the reaction of bis-aldehydes with substituted hydrazide derivatives since these compounds previously showed potent aldose reductase inhibition properties. The obtained compounds were tested for their potential antibacterial and antioxidant activities. In the present study, four different bacterial strains were used, including Gram-positive (*Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212) and Gram-negative (*Pseudomonas aeruginosa* ATCC 10231, *Escherichia coli* ATCC 25912). On the other hand, the antioxidant capacities of freshly re-synthesized bis-hydrazone substituted isovanilline derivatives were determined by using several antioxidant methods, including DPPH free radical scanvenging, TEAC cupric reducing (CUPRAC) and metal chelating activity methods. Several lead molecules were discovered as a potential bacterial inhibitors against S. *aureus* and *E. coli* bacterial strains. More specifically, compounds **3g** (R=-H) and **3j** (R= -4Cl) showed great inhibition properties against *E. coli* bacterial strains by having MIC values of 1.56 and 6.25 µg/mL, respectively. Moreover, none of the compounds showed potent antioxidant activity against tested methods with respect to compared standards.

Keywords: Hydrazone, Isovanillin, Antioxidant, Antibacterial.

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1. INTRODUCTION

Bacterial infections are considered one of the most important infectious diseases, although generally only a small percentage of bacteria cause infection and disease, but their impact on public health is significant and serious. In recent years, researchers have been able to make great progress in the development of antibacterial drugs, but the need remains urgent to discover new antibacterial drugs because of the rapid growing of multidrug-resistant bacteria with potentially devastating consequences (1–4).

Historically, natural products (NPs) were a rich source of bioactive molecules and they are one of the most important sources of inspiration for developing many therapeutic drugs (5). Isovanillin (3-hydroxy-4-methoxybenzaldehyde) is a phenolic aldehyde isolated from a number of plants including *Pycnocycla spinose*, *Alpinia oxyphylla* extract which is used in particular in the pharmaceutical cosmetics, the flavors and fragrance industry, and

agrochemical (6-10). In addition to its potency as a selective inhibitor of aldehyde oxidase, it is also shown to be an important intermediate for the synthesis and production of some functional catechol derivatives such as methyldopa (11). On the other hand, it is considered as a cornerstone in the synthesis of many important pharmaceutical compounds such as morphine, and galantamine (12-14). Moreover, isovanillin-containing plants have been traditionally used in folk medicine for treatment of many diseases such as cancer, depression, hypertension, hyperglycemic and diarrhea (15-18).

Hydrazones containing an azometine -NHN=CH- group found an important scaffold for new drug design and development studies. Many compounds containing hydrazone group in their structure attracted medicinal chemists interest due to its biological importance as antimicrobial, antimalarial, antitubercular, antifungal, antiviral, anticonvulsant, antidepressant, anti-inflammatory, analgesic, antiplatelet, anticancer, cardio protective etc. (19-22).

As has been observed in previous studies (23-27), the bis-type compounds may have many advantages including, ditopic interactions in the active site of receptor and more binding affinities with amino acids on the enzyme active sites and that can play an important role in improving the pharmacological activity of this type of compounds. Based on this, in our past work, we have designed and developed a new group of bis-hydrazone compounds bearing isovanillin components and screened these novel derivatives for their activity towards Aldose reductase (ALR2) (28). All the tested compounds demonstrated good activity in nanomolar range as AR inhibitors. In the current study, we decided to re-synthesize and evaluate a series of twelve bis-hydrazone compounds bearing isovanillin derivatives for their biochemical activities towards antibacterial and antioxidant properties with the hope of discovering more effective and safe antibacterial agents.

2. EXPERIMENTAL SECTION

2.1. Materials and Methods

All reagents and solvents that commercially available were purchased from several companies including, Alfa Aesar, Merck, Sigma-Aldrich, and TCI and used without further purification. The FT-IR spectrums of the synthesized compounds were recorded by using Perkin Elmer Spectrum 100 FT-IR spectrometer. Melting points (mp) were determined with SMP20 melting point apparatus and are uncorrected. Nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectra of compounds were obtained using Agilent 500 MHz NNMRS spectrometer in DMSO-d_6 with TMS as an internal standard. Thin layer chromatography (TLC) was applied on Merck silica gel 60 F_{254} plates.

2.2. Chemistry

In order to assess the antibacterial and antioxidant activities of bis-hydrazone substituted isovanilline derivatives **3(a-l)**, the compounds were freshly re-synthesized as previously described by our research group (28). The general synthetic route for the preparation of bis-hydrazone substituted isovanilline derivatives **3(a-l)** were demonstrated in Figure 1. Physicochemical and spectroscopic analysis of re-synthesized compounds **3(a-l)** have been previously performed and the results were described by us (28).

2.3. Antibacterial assay

The antibacterial analyzes were achieved by modified method according to Al-Blewi et al. and our previous studies (29-31). Briefly, the freshly re-synthesized bis-hydrazone substituted isovanilline derivatives **3(a-l)** were dissolved in DMSO and 200 µg/mL stock solution was formed and were determined at ten different concentrations diluted nine times. The four different bacterial strains were added in 100 µL to each microplate well with approximately 10^6 CFU/mL bacteria. The bacterial density was adjusted with a McFarland densitometer. Microplates were incubated for 24 h at 37°C and then optical densities were measured at 600 nm (OD600) using Thermo 3001 ELISA microplate reader. The Minimal inhibitory concentration (MIC) tests were repeated three times for each microorganism that used in the present study and all the bis-hydrazone substituted isovanilline derivatives **3(a-l)**. MICs were obtained after 24 h incubation time. Additionally, control experiments with standard antibacterial agents (Ceftriaxone and ampicillin were used as a positive control) and unvaccinated media (negative control) were performed in parallel and in the same manner as the test compounds.

2.4. DPPH Free radical scavenging assay

In this study, the radical scavenging activity of compounds were determined in 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical assays applied according to Blois (32). In brief, 1.0 mL sample were added to 0.1 mM DPPH solution. The mixture was kept for 30 min in darkness at room temperature, and the absorbance was then measured at 517 nm. Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and α-tocopherol were used as positive controls.

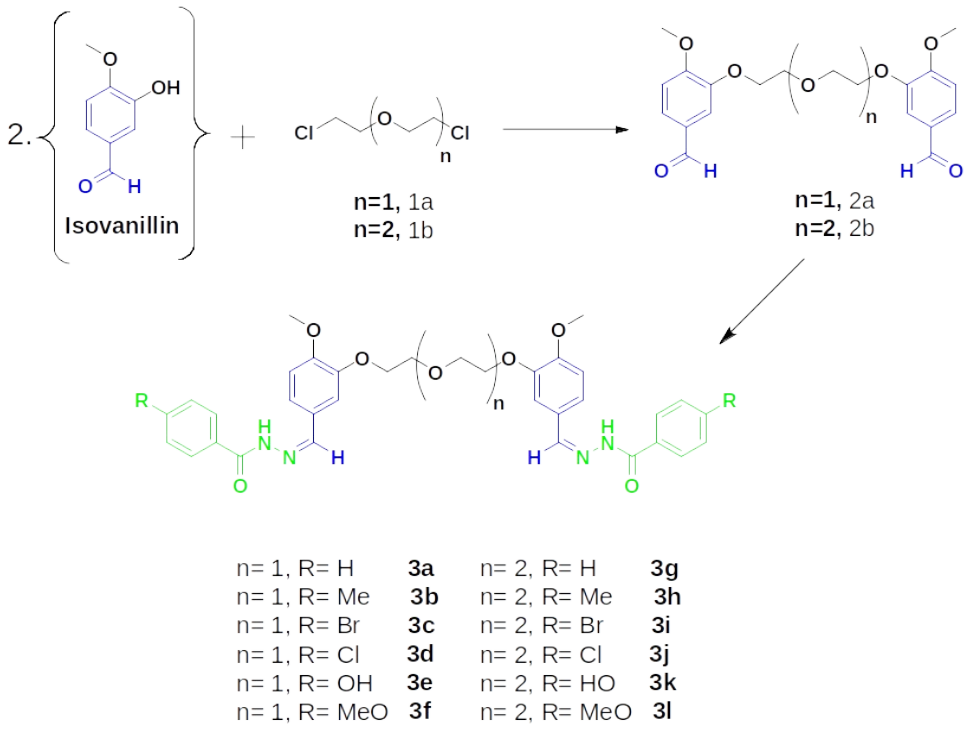

Figure 1: General synthetic route for the synthesis of bis-hydrazone substituted isovanilline derivatives **3(a-l)** (28).

2.5. Metal Chelating Activity

The chelating activity of ferrous iron was determined using the colorimetric method proposed by Carter (33). 50 µL of 2.0 mM $FeCl_2$ were added to compounds of different concentrations. After 10 min incubation at room temperature, 200 µL 5.0 mM ferrozine solution is added. Measure the absorbance of all samples at 562 nm after 25 min incubation. EDTA was used as a positive control.

2.6. Cupric Reducing Antioxidant Capacity (CUPRAC) Assay

Total antioxidant capacity of synthesized compounds were determined using CUPRAC method performed by Apak et al. (34). 1.0 mL of each of the 0.01 M $CuCl_2$, $7.5x10^{-3}$ M neocuproine, NH_4Ac buffer solution (pH 7.00; 0.1 M) were added into the test tube. 50 µL sample or standard solution (BHA, BHT, α-TOC) and 1.05 mL distilled water added to the initial mixture. Measure the absorbance of all samples at 465 nm after 30 min incubation at room temperature.

3. RESULTS AND DISCUSSION

In the current study, we are presenting the synthesis, antibacterial and antioxidant activity of bis-hydrazone substituted isovanilline derivatives **3(a-l)**. These produced compounds were previously synthesized and fully characterized as a potent aldose reductase inhibitors by our research team. By having important biological properties of isovanilline derivatives, in the current work, the antibacterial and several antioxidant assays were focused.

The antibacterial activity assays were applied against four pathogenic bacterial strains, including Gram-negative bacteria and Gram-positive. On the other hand, the antioxidant capacities of freshly re-synthesized bis-hydrazone substituted isovanilline derivatives are determined by using several antioxidant methods, including, TEAC, CUPRAC, metal-chelating, and DPPH free radical scanvenging activity methods.

In general, the re-synthesized compounds showed varying range of antibacterial activity against both Gram-positive as well as Gram-negative tested bacterial strains depending on the chemical nature and substitution on derivatives as the minimum inhibitory concentration values (MIC) were illustrated in Table 1. More specifically, as compared with their linker length, the longer ones have had better activity than their shorter counterparts, in general. The highest antibacterial activity was determined against *E. coli* with the compounds **3g** (R=-H) and **3j** (R= -4Cl) by having MIC values of 1.56 and 6.25 µg/mL, respectively. These compounds were comparable with standard antibiotic drugs ampicillin and ceftriaxon with MIC values of 15.6 and 2.0 µg/mL, respectively. Whereas compound **3a** with no substitution on the phenyl ring and compound **3f** (methoxy group at para position) showed good potency against *E. coli* strains (12.5 µg/mL), which is better than the commercial antibiotic ampicillin (15.6 µg/mL). One of the Gram-positive bacteria strain *S. aureus* was also potently inhibited by some of the compounds, including **3a**, **3f**, **3g** and **3l** with MIC values of 12.5 µg/mL as compared with Standard drug ceftriaxon (31.3 µg/mL). Interestingly, compounds **3a** and **3g**

have same substitution as well as compounds **3f** and **3l** have p-methoxy substitution on the phenyl ring by having only linker length differences. On the other hand, the compounds were less susceptible to Gram-negative bacteria (*P. aeruginosa*) in which some of the compounds showed any activity, including short linker derivatives **3a**, **3c**, **3d** and the one of the longer linker derivative **3g** with no substitution on the phenyl ring. As a result of antibacterial activities of compounds, the best inhibition results were obtained against *E. coli* and *S. aureus* bacterial strains with compound **3g** which can be chosen as a lead molecule since it has an great activity against both bacterial strains.

Table 1: Antibacterial activity of bis-hydrazone substituted isovanilline derivatives **3(a-l)** assayed against Gram-positive and Gram-negative bacterial strains (Minimal Inhibitory Concentration, µg/mL).

Compounds	Antibacterial activity (MIC, µg/mL)			
	Gram-(+) bacteria strains		Gram-(-) bacteria strains	
	S. aureus	***E. faecalis***	***P. aeruginosa***	***E. coli***
3a	12.5	25	ND	12.5
3b	50	12.5	50	50
3c	100	100	ND	100
3d	25	50	ND	100
3e	100	100	25	100
3f	12.5	100	25	12.5
3g	12.5	12.5	ND	1.56
3h	100	50	25	ND
3i	50	25	100	50
3j	50	100	100	6.25
3k	100	25	25	50
3l	12.5	100	100	50
Ampicillin	3.9	3.9	2.0	15.6
Ceftriaxon	31.3	2.0	15.6	2.0

The antioxidant properties of freshly re-synthesized bis-hydrazone substituted isovanilline derivatives **3(a-l)** were assayed using several antioxidant methods, including DPPH free radical scavenging, TEAC CUPRAC, and metal chelating methods. The DPPH results revealed that none of the compounds showed any activity except the compounds **3k** and **3l** which are also having very low DPPH activity at any tested concentrations as demonstrated in Table 2.

4. CONCLUSIONS

In conclusion, the previously synthesized potent aldose reductase inhibitors of bis-hydrazone substituted isovanilline derivatives **3(a-l)** were freshly re-synthesized for their potential antibacterial and antioxidant properties. The produced compounds were tested against two Gram-positive (*S. aureus*, *E. faecalis*) and two Gram-negative (*P. aeruginosa*, *E. coli*) bacterial strains. Also, several antioxidant methods were applied to these compounds, including DPPH free radical scanvenging, TEAC cupric reducing (CUPRAC) and metal chelating activity methods. Unfortunately, these compounds were not susceptible to any tested antioxidant methods. Moreover, several potent compounds were obtained against tested bacterial strains *S. aureus* and *E. coli*. Specifically, compounds **3g** (R=-H) and **3j** (R= -4Cl) showed great inhibition properties against *E. coli* bacterial strains by having MIC values of 1.56 and 6.25 µg/mL, respectively. These compounds were comparable to standard antibiotic drugs ampicillin and ceftriaxon with MIC values of 15.6 and 2.0 µg/mL, respectively. As a result of the antibacterial potency of these compounds, they might be improved and used as a potential leads for the development of antibacterial agents. For this, further research needed to be done, including toxicity studies.

Table 2: DPPH radical scavenging activities of bis-hydrazone substituted isovanilline derivatives **3(a-l)** and controls BHA, BHT, and α-TOC.

Compounds & Standards	DPPH Free Radical Scavenging Activity, %			
	12.5 µg/mL	25.0 µg/mL	37.5 µg/mL	62.5 µg/mL
3a	ND	ND	ND	ND
3b	ND	ND	ND	ND
3c	ND	ND	ND	ND
3d	ND	ND	ND	ND
3e	ND	ND	ND	ND
3f	ND	ND	ND	ND
3g	ND	ND	ND	ND
3h	ND	ND	ND	ND
3i	ND	ND	ND	ND
3j	ND	ND	ND	ND
3k	1.74 ± 0.35	2.36 ± 0.18	2.50 ± 0.19	2.63 ± 0.00
3l	3.60 ± 0.46	4.49 ± 0.30	4.99 ± 0.18	5.37 ± 0.00
BHA	59.85 ± 0.00	63.60 ± 0.00	64.81 ± 0.46	67.16 ± 0.35
BHT	60.22 ± 1.09	65.68 ± 0.63	69.52 ± 0.30	69.64 ± 0.46
α-TOC	63.69 ± 0.46	68.03 ± 1.05	69.64 ± 0.70	70.51 ± 1.15

The TEAC CUPRAC and metal chelating antioxidant properties of compounds were also assayed. The results show that none of the compounds have better activity than the standards and not comparable with them.

Table 3. TEAC CUPRAC and metal chelating antioxidant capasities of bis-hydrazone substituted isovanilline derivatives **3(a-l)** and controls BHA, BHT, α-TOC, and EDTA.

Compounds & Standards	TEAC $_{CUPRAC}$ (mmol TR g^{-1})	Metal Chelating Activity IC_{50} (µg mL^{-1})
3a	0.413 ± 0.006	27.78 ± 0.22
3b	0.455 ± 0.004	30.32 ± 0.16
3c	0.379 ± 0.009	29.19 ± 0.23
3d	0.311 ± 0.002	17.64 ± 0.05
3e	0.215 ± 0.005	ND
3f	0.220 ± 0.005	ND
3g	0.231 ± 0.008	ND
3h	0.249 ± 0.003	ND
3i	0.253 ± 0.004	ND
3j	0.429 ± 0.002	27.21 ± 0.14
3k	0.475 ± 0.006	32.31 ± 0.08

3l	0.470 ± 0.003	42.56 ± 0.06
BHA	2.632 ± 0.007	-----
BHT	3.358 ± 0.003	-----
α-TOC	2.077 ± 0.004	-----
EDTA	-----	1.71 0.19

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