Design, Microwave-Assisted Synthesis and Biological Activities of 1,2,4-Triazol-3-Yl-Thiazolidin-4-Ones

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Abstract

A new 3-(5-alkyl-2-phenyl-2H-1,2,4-triazol-3-yl)thiazolidin-4-ones derivatives were obtained by condensation of 5-amino-1,2,4-triazoles, mercaptoacetic acid with aromatic aldehydes and catalyzed by Sm(SO3CF3)3, using microwave irradiation. The prepared compounds were tested for their antioxidant, antibacterial and antifungal proprieties. Some of these compounds displayed significant activities. Among them, compound 2e exhibited remarkable activity against a broad spectrum of Gram positive, negative bacteria and pathogenic fungal strains with low MIC values. The investigation of the mode of action of the most potent antifungal compounds on the fungus *Pythium panidermatum* showed a membrane alteration and distortions of hyphal morphology. The newly synthesized compounds exhibited also promising radical scavenging activity.

Keywords: Aminotriazoles; Triazolothiazolidinones; Antimicrobial activity; Antioxidant activity

Introduction

Bacterial infections are a common problem in hospitals and clinical setting worldwide and have become an increasing public health problem. The indiscriminate and the overuse of antibiotics has led to the emergence of antibiotic-resistant bacteria such as Methicillin-resistant *Staphylococcus aureus* [1]. Thus, designing and developing new antimicrobial agents having new modes of action are being a big challenge for scientific. Triazolothiazolidinones have received intensive research interests due to their biological activities, and found a wide range of applications in pharmaceutical and agrochemical field. Thiazolidin-4-one is a versatile scaffold for designing potential bioactive agents. In fact, some derivatives of thiazolidin-4-one showed an antioxidant, anticancer [2], antitumor [3], anti-inflammatory [4], antimicrobial [5], anti-HYV [6], antiviral [7], anticonvulsant [8] and antihypertensive [9] activities. Moreover, Reactive Oxygen Species (ROS) are various forms of activated oxygen. A disproportion of the reactive oxygen species and the absence of their scavenging systems in cells leads to oxidative stress and increases the risk of several human chronic diseases [10].

In previous papers [11,12] we reported that derivatives of aminotriazoles can be used such as starting materiel for obtaining polyheterocyclic compounds having interest biological activities. In continuation, we present here our study of three-component reaction of aminotriazoles, aromatic aldehydes and mercaptoacetic acid and valorization of biological activities of some triazolothiazolidinones obtained.

Materials and Methods

Chemistry

All microwave-assisted reactions were carried out in synthetic microwave: Monowave 300 with a maximum power of 300 W. Microwave irradiation. The mixture was stirred at 180°C in sealed tube by irradiating microwave for 15 min. Subsequently, thiglycic acid (1.3 mmol) was added and was irradiated in a microwave at 140°C for 15 min. After, *N,N,N*-dicyclocexylcarbodiimide (DCC) (1.3 mmol) and additional dry toluene were added. The mixture was stirred at 140°C in sealed tube by irradiating microwave for 15 min reaction.

Then, after cooled to room temperature, *1,3-dicyclohexylurea* (DCU) was removed by filtration and the residue was purified by chromatography on silica gel (petroleum ether/CH2Cl2, 6:4). Biological activities

Microorganisms and growth conditions: The synthesized compounds were tested against a panel of microorganisms including eight bacteria and six fungal strains obtained from American Type Culture Collection (ATCC), local culture collection of Tunisian Microorganisms “CTM” of the Centre of Biotechnology of Sfax, Collection of the Institut Pasteur (CIP) and Plant Pathology Experimental Institute (ISPAVE). The tested pathogenic bacteria are: *Bacillus cereus* ATCC 14579, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Micrococcus luteus* ATCC 1880, *Esherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 10031, *Sabronella enteitdis* (food isolate 824), *Listeria monocytogenes* (food isolate 2132). The fungi tested are *Rhizopus nigricans* (LPAP26); *Alternaria alternata* CMT 10239; *Pythium panidermatum* (LPAP32); *Fusarium culmorum* ISPAVE 21W; *Fusarium graminearum* ISPAVE 271; *Aspergillus flavus* (food isolate). Bacteria were cultivated in Muller-Hinton agar (MH)

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Antimicrobial assays: Antibacterial and antifungal assays were performed by agar well diffusion method as described by Trigui [13] and broth micro dilution assay in sterile 96-well micro plate according to Eloff [14]. For agar well diffusion assay, the surface of agar plates were streaked by a freshly cell suspension adjusted to 10⁶ CFU/mL for bacterial strains and 10⁶ spores/ml for fungi. Then, wells (6 cm) were punched into the inoculated agar and compounds were added to each well. DMSO (20%), used to dissolve the compounds, was used as negative controls. Gentamicin (10 µg/wells) and Amphotericin B (20 µg/well) were used as positive control for bacterial and fungal strains respectively. After diffusion of the compounds at 4°C for 2 h, plates were incubated at 37°C for 24 h for bacterial strains and 72 h for fungi at 28°C. The activity was evaluated by measuring the zones of inhibition around the well. All tests were repeated three times.

The broth micro dilution method aimed to determine the Minimum inhibitory concentrations (MICs) of each compounds by a twofold serial dilution. The range of compounds concentration tested in the micro plate is from 0.01-5.5 mg/mL. After dilution, 10 µL of cell suspension was added to each test well. The plates were then covered and incubated at the appropriate temperature for the microorganisms under investigation. Gentamicin and Amphotericin B were used as positive drug controls against bacterial and fungal strains respectively. After diffusion of the compounds at 4°C for 2 h, plates were incubated at 37°C for 24 h for bacterial strains and 72 h for fungi at 28°C. The activity was evaluated by measuring the zones of inhibition around the well. All tests were repeated three times.

Antioxidant activity: Radical scavenging activity of synthesized compounds was determined using DPPH as a reagent according to the method of Kirby and Schmidt [15] with slight modifications. Compounds were diluted in methanol to a final concentration of 0.5 mg/ml and then 500 µL were added to 1 mL of DPPH radical solution in methanol 4% (w/v). The mixture was vigorously shaken and incubated in the dark at room temperature for 30 min. The absorbance was measured at 517 nm against a blank and activity was compared to the ascorbic acid used as positive control. Gentamicin (10 µg/wells) and Amphotericin B (20 µg/well) were used as positive control for bacterial and fungal strains respectively. After diffusion of the compounds at 4°C for 2 h, plates were incubated at 37°C for 24 h for bacterial strains and 72 h for fungi at 28°C. The activity was evaluated by measuring the zones of inhibition around the well. All tests were repeated three times.

Antifungal: The in vitro antibacterial activity of compounds (2b-h, 2n and 2p) were carried out against a panel of five Gram-positive and three Gram-negative bacteria and compared to the gentamicin used as standard antibiotic. The results of antibacterial testing, using well diffusion method and broth micro dilution technique, are presented in Table 2. Out of the nine newly synthesized compounds, the compound 2e exhibited the strongest antibacterial activity with inhibition zone ranged from 11 to 23 mm and very low minimum inhibitory concentration (MIC) values. It was also noticed that the Gram-positive bacteria Bacillus cereus and Staphylococcus aureus are the most sensitive bacteria to the all synthetized compounds whereas Gram negative ones are resistant to these compounds except for 2e. Gram-negative bacteria are generally less susceptible to antibiotics than the Gram-positive bacteria, since they have an outer membrane which plays the role of a barrier to the biomolecules [16].

Results and Discussion

Chemistry

The synthetic strategy adopted to obtain the target compounds is presented in Scheme 1. 5-amino-1,2,4-triazoles 1a-c reacts with aromatic aldehydes catalyzed by samarium (III) trifluoromethanesulfonic Sm(SO₂CF₃)₃ in toluene under microwave irradiation followed by addition of excess of mercaptoacetic acid. After that, we added dicyclohexylcarbodiimide (DCC). The dicyclohexylurea (DCU), which was precipitated, was removed by filtration.

According with experimental protocol we supposed initially the formation of imine due to the action of aminotriazoles with aromatic aldehydes. In the second step, the nucleophilic sulfur atom of the mercaptoacetic acid, attack iminic carbon, then intramolecular cyclization followed by elimination of water molecule affords the thiazolidin-4-one 2a-p.

The dehydrating agent DCC accelerates the intramolecular cyclization process and increases the yield of the reaction as well. The obtained products were isolated by conventional workup in satisfactory yields (Table 1).

The spectral data and HRMS of the new compounds reported in this study correlate with the proposed structures. The ¹H NMR spectra of compounds C₅-H signals were observed at δ 3.65 - 3.9 as double doublets due to chiral center at C₅.

The developed synthetic protocol was used to access a series of triazol-3-yl-thiazolidinones 2a-p, which were obtained in moderate yields ranging from 45% to 56%.

Biological activities

Antibacterial: The in vitro antibacterial activity of compounds (2b-h, 2n and 2p) were carried out against a panel of five Gram-positive and three Gram-negative bacteria and compared to the gentamicin used as standard antibiotic. The results of antibacterial testing, using well diffusion method and broth micro dilution technique, are presented in Table 2. Out of the nine newly synthesized compounds, the compound 2e exhibited the strongest antibacterial activity with inhibition zone ranged from 11 to 23 mm and very low minimum inhibitory concentration (MIC) values. It was also noticed that the Gram-positive bacteria Bacillus cereus and Staphylococcus aureus are the most sensitive bacteria to the all synthetized compounds whereas Gram negative ones are resistant to these compounds except for 2e. Gram-negative bacteria are generally less susceptible to antibiotics than the Gram-positive bacteria, since they have an outer membrane which plays the role of a barrier to the biomolecules [16].

Antifungal: The synthesized compounds were also evaluated for their in vitro antifungal activity against various phytopathogenic

![Scheme 1: Synthesis of 3-(5-alkyl-2-phenyl-2H-1,2,4-triazol-3-yl)thiazolidin-4-ones. Reagents and conditions: (i) Sm(SO₂CF₃)₃ (10% mol), toluene, MW, 180°C, 15 min. (ii) SHOH, COOH, MW, 140°C, 15 min. (iii) DCC, MW, toluene, 140°C, 15 min.](image-url)
fungus using the agar well diffusion method and the minimal inhibitory concentrations (MIC, mg/ml) by the two fold broth dilution technique in liquid plate count agar (PDA). The results, presented in Table 3, showed that all the synthesized 3-(5-alkyl-2-phenyl-2H-1,2,4-triazol-3-yl)thiazolidin-4-ones derivatives exhibit broad-spectrum antifungal activity towards several phytopathogenic fungi. Compound 2e showed the greatest antifungal activity against all tested fungi with low MIC values ranging from 0.172 to 1.375 mg/ml. To a lesser extent, compounds 2c and 2h were active against 83% of the tested fungi with MIC values higher than 2e. Compounds 2p followed by 2b were inactive. The other compound showed a moderate antifungal activity.

Table 1: Yields of new 3-(5-alkyl-2-phenyl-2H-1,2,4-triazol-3-yl)thiazolidin-4-ones.
Understanding the mechanism of action of antifungal compounds is desirable. To the antifungal investigate activity of compounds 2e, 2c and 2g in liquid medium and their mode of action, Pythium phanidermatum was used as positive control. This fungal strain is a cosmopolitan pathogen with a wide host range causing damping off, root and stem rots, and blights of grasses and fruit. It is of economic concern on most annuals, cucurbits, and grasses. It is considered one of the water molds because it survives and grows best in wet soils. Pythium

![Figure 1: Light microphotograph of mycelium growing of Pythium phanidermatum (LPAP32) on PDB with or without compounds 2e, 2c and 2g. A: Control mycelium of Pythium phanidermatum; B and C: Mycelium collected from cultures supplemented with 687 µg/ml of 2e and 2c; D: Mycelium collected from cultures supplemented with 2.75 µg/ml of 2g.](image)

![Figure 2: Free radical scavenging activity.](image)

Table 2: The antibacterial activity in vitro of the target compounds.

| Compounds | Activity | Bacterial strains | Gram positive bacteria | Gram negative bacteria | Activity(%) |
|-----------|----------|-------------------|------------------------|------------------------|------------|
|           |          |                   |                        |                        |            |
| 2b        | IZ       | Bc                | Sa                     | Ef                     | Ml         | Ec       | Se       | Kp       |            |
|           |          | 16                | 10                     | 0                      | 0          | 0        | 0        | 0        | 25         |
|           | MIC      | > 5.5             | -                      | -                      | -          | -        | -        | -        |            |
| 2c        | IZ       | 23                | 12                     | 0                      | 0          | 0        | 0        | 0        | 25         |
|           | MIC      | 2.75              | -                      | -                      | -          | -        | -        | -        |            |
| 2d        | IZ       | 23                | 12                     | 0                      | 0          | 0        | 0        | 0        | 25         |
|           | MIC      | 2.75              | -                      | -                      | -          | -        | -        | -        |            |
| 2e        | IZ       | 23                | 17                     | 12                     | 13         | 21       | 11       | 11       | 12         |
|           | MIC      | 0.021             | 0.021                  | 0.687                  | 0.172      | 0.687    | 2.75     | 2.75     | 1.375      |
| 2f        | IZ       | 20                | 13                     | 0                      | 0          | 20       | 0        | 0        | 0          |
|           | MIC      | 0.172             | 0.172                  | -                      | -          | 0.344    | -        | -        |            |
| 2g        | IZ       | 16                | 10                     | 0                      | 0          | 0        | 0        | 0        | 0          |
|           | MIC      | 0.172             | 5.5                    | -                      | -          | -        | -        | -        |            |
| 2h        | IZ       | 18                | 10                     | 0                      | 0          | 0        | 0        | 0        | 0          |
|           | MIC      | 1.375             | > 5.5                  | -                      | -          | -        | -        | -        |            |
| 2n        | IZ       | 20                | 0                      | 0                      | 0          | 0        | 0        | 0        | 0          |
|           | MIC      | 0.687             | -                      | -                      | -          | -        | -        | -        |            |
| 2p        | IZ       | 17                | 0                      | 0                      | 0          | 0        | 0        | 0        | 0          |
|           | MIC      | 0.172             | -                      | -                      | -          | -        | -        | -        |            |
| Gentamicin | IZ       | 20                | 20                     | 20                     | 18         | 20       | 25       | 25       | 22         |
|           | MIC      | 0.004             | 0.004                  | 0.004                  | 0.004      | 0.001    | 0.002    | 0.002    | 0.002      |
| DMSO 20%  | IZ       | 0                 | 0                      | 0                      | 0          | 0        | 0        | 0        | 0          |

Bacterial strains: Bc: Bacillus cereus ATCC14579; Sa: Staphylococcus aureus ATCC25923; Ef: Enterococcus faecalis ATCC 29212; Ml: Micrococcus luteus ATCC 1880; Lm: Listeria monocyogenes (FI 2132); Ec: Escherichia Coli ATCC 25922; Se: Salmonella enteritidis (food isolate); Kp: Klebsiella pneumonia CIP 32147. (a) Diameter of inhibition zones including diameter of well 6 mm. (b) MIC: The Minimum Inhibitory Concentrations in mg/mL. (c) Gentamicin: Gentamicin was used as a standard antibiotic at a concentration of 15 µg/Well. (-) Inactive.
The tested compounds showed variable antioxidant activity at a final concentration of 0.5 mg/ml. Among the nine tested compounds, the most potent radical scavenger effect was obtained with 2f which showed an inhibition of 91.57% compared to 98% using ascorbic acid. The compounds 2d followed by 2e showed also an antioxidant activity with respectively 69.62 and 56.41% of inhibition (Figure 2). A lower activity was obtained in the case of 2g, 2b and 2p.

**Conclusion**

In conclusion, this paper presented an improved microwave-assisted combining with the use of DCC for synthesizing 3-(5-alkyl-2-phenyl-2H-1,2,4-triazol-3-yl)thiazolidin-4-ones in moderates yields. The research subscribed in this paper indicates a wide spectrum of biological activities exhibited by 1,2,4-triazol-3-yl-thiazolidin-4-one derivatives. The biological profiles of these new generations of compounds would represent a fruitful matrix for further development of better antifungal, antibacterial and antioxidant agents.

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