Synthesis, characterization and an extensive biological evaluation of 5-[2-(methylthio)ethyl]-3-(2-propen-1-yl)-2-thioxo-4-imidazolidinone

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Abstract:

Ever since their discovery, hydantoins have attracted huge attention due to their intriguing properties, vast chemical diversity and potential, as well as their broad spectra of biological activity. The wide set of their biological activity includes antimicrobial, antitumor, antiandrogen, anticonvulsant, antiteratogenic activity, etc. They are also used in the treatment of cachexia, psoriasis, wounds in general and also as muscle relaxants.

There are many synthetic routes to hydantoins and some of them involve amino acids. As rigorous chemical conditions are not required, these reactions can be manifested in physiological conditions too, especially in the cases when protein consumption is increased and thus hydantoins have been isolated from urine. With all this in mind, elucidation of biological implications of hydantoins gains importance.

In this study, an amino acid derived 2-thiohydantoin, 5-[2-(methylthio)ethyl]-3-(2-propen-1-yl)-2-thioxo-4-imidazolidinone, has been synthesized and fully characterized by NMR and IR spectroscopy, as well as X-ray crystallography. An extensive antimicrobial study has been carried out on ten bacterial isolates (Gram-positive and Gram-negative), as well as on five fungal isolates. Cytotoxicity has been tested on the cell lines of the normal lung fibroblasts, as well as breast, colon and lung tumor cell lines. Ultimately, a fish embryo toxicity (FET) assay has been carried out in vivo on the zebrafish model, testing for lethal and teratogenic effects and cardiotoxicity. Based on the found biological activity in previously mentioned assays, a determination of therapeutic potential has been carried out to show whether the compound is toxic in antimicrobial and anticancer doses.

Keywords: thiohydantoins; synthesis; biological activity; zebrafish model
Introduction

Hydantoins represent an interesting class of biologically active heterocyclic molecules. They are five-membered cyclic ureides in which various substituents in the N1, N3 and C5 positions lead to great structural diversity.\[^1\]

This structural diversity has attributed many different and interesting chemical and biological properties to compounds containing the hydantoin moiety.\[^2\] Some of the attributed biological properties include:

- antimicrobial, antitumor, antiandrogen, antiteratogenic activity
- treatment of cachexia, psoriasis, wound healing, muscle relaxant
- hypnotic, anti-epileptic, anticonvulsant activity, treatment of chorea
- treatment of anoxia, tuberculosis and some infectious diseases
Several hydantoins, which have shown strong bioactivity, have been commercialized and marketed as pharmaceuticals, mostly as anticonvulsants.

Phenytoin (Dilantin© Pfizer, 1951) anticonvulsant
Fosphenytoin (Cerebryx© Pfizer, 2013) anticonvulsant
Ethotoin (Peganone© Recordati, 1957) anticonvulsant

Nilutamide (Anandron© Sanofi-Aventis, 1996) anti-androgen
Nitrofurantoin (Furadantin© Shionogi, 1953) anti-bacterial
Dantrolene (Dantrium© Norwich Eaton, 1979) muscle relaxant
Results and discussion

There are many synthetic routes to hydantoins. A lot of them include carbonyl compounds such as ketones and aldehydes, as is the case of the Bucherer–Bergs reaction.\[^3\] In a physiological point of view, the synthesis of hydantoins from amino acids, which are ever-present in the food chain, is more important. One such synthesis includes urea/thiourea.\[^4\] This reaction is responsible for the occurrence of hydantoins in urine when protein consumption is increased.

\[
\text{NH}_2\text{S} \text{Me}
\]

Another synthetic route to hydantoins involving amino acids includes alkyl and aryl isocyanates or isothiocyanates. 5-[2-(methylthio)ethyl]-3-(2-propen-1-yl)-2-thioxo-4-imidazolidinone, a 2-thiohydantoin derived from methionine, was synthesized by standard procedure.\[^5\]
5-[2-(methylthio)ethyl]-3-(2-propen-1-yl)-2-thioxo-4-imidazolidinone has been fully characterized by NMR and IR spectroscopy, as well as X-ray crystallography.
Antimicrobial activity was tested via standard CLSI-2012 procedure[6] on a panel of 15 different microorganisms, including five Gram-negative bacteria, five Gram-positive bacteria and five fungi of the Candida genus. Antimicrobial activity is expressed as a minimal inhibitory concentration (MIC) for each of the tested microorganism strains.

| Microorganism                  | MIC (μg/ml) |
|-------------------------------|-------------|
| **Gram-negative bacteria**    |             |
| Acinetobacter baumannii (ATCC 19608) | 250         |
| Escherichia coli (ATCC 25922) | 500         |
| Pseudomonas aeruginosa (PAO1)  | 500         |
| Salmonella Typhimurium (ATCC 14028) | 500       |
| Serratia marcescens (ATCC 27117) | >500       |
| **Gram-positive bacteria**    |             |
| Bacillus subtilis (ATCC 6633) | 500         |
| Listeria monocytogenes (NCTC 11994) | 500        |
| Micrococcus luteus (ATCC 379)  | 500         |
| Staphylococcus aureus (ATCC 25923) | 125        |
| Staphylococcus aureus MRSA (ATCC 43300) | 62.5       |
| **Candida strains**           |             |
| Candida albicans (ATCC 10231) | >500        |
| Candida albicans (SC5314)     | >500        |
| Candida krusei (ATCC 19608)   | >500        |
| Candida glabrata (ATCC 19608) | >500        |
| Candida parapsilosis (ATCC 19608) | >500     |

The compound exhibited no antifungal activity and weak antibacterial activity. Only in the case of the Gram-positive bacteria, Staphylococcus aureus, it exhibited moderate activity.
**Cytotoxicity** of the compound was tested via MTT method.\textsuperscript{[7]} Cells from the cell lines MRC-5 (human lung fibroblasts), A549 (human lung carcinoma cells), HCT-116 (human colorectal carcinoma cells) and MDA-MB-231 (human breast carcinoma cells) were sown and treated with increasing concentrations of the compound over 48 hours, after which a survival percent was determined relative to untreated control cell lines.

The compound did not exhibit *in vitro* cytotoxicity on the tested cell lines in concentrations up to 200 μM.
*In vivo toxicity* was tested on wild type zebrafish embryos (*Danio rerio*) not older than five days\(^8,9\) in seven different concentrations (5, 10, 25, 50, 100, 150 and 200 μM), while DMSO (0.25 %) was used as a negative control. Survival of the embryos was monitored daily, as well as teratogenic malformations and cardiovascular functions.

| Category          | Developmental endpoints                                      | Exposure time (hpf) |
|-------------------|--------------------------------------------------------------|---------------------|
|                   |                                                              | 24  | 48  | 72  | 96 / 120 |
| Lethal effect     | Coagulated eggs                                              | ●   | ●   | ●   | ●        |
|                   | Lack of somite formation                                     | ●   | ●   | ●   | ●        |
|                   | Non-detachment of the tail                                   | ●   | ●   | ●   | ●        |
|                   | Lack of the heart beating                                    | ●   | ●   | ●   | ●        |
| Teratogenic effect| Malformation of head                                         | ●   | ●   | ●   | ●        |
|                   | Malformation of eyes                                         | ●   | ●   | ●   | ●        |
|                   | Malformation of sacculi/otoliths                              | ●   | ●   | ●   | ●        |
|                   | Malformation of chorda                                       | ●   | ●   | ●   | ●        |
|                   | Malformation of tail                                         | ●   | ●   | ●   | ●        |
|                   | Scoliosis                                                    | ●   | ●   | ●   | ●        |
|                   | Yolk edema                                                   | ●   | ●   | ●   | ●        |
|                   | Yolk deformation                                             | ●   | ●   | ●   | ●        |
|                   | Growth retardation                                           | ●   | ●   | ●   | ●        |
|                   | Hatching                                                     | ●   | ●   | ●   | ●        |
| Cardiotoxicity     | Pericardial edema                                            | ●   | ●   | ●   | ●        |
|                   | Heart morphology                                             | ●   | ●   | ●   | ●        |
|                   | Heart beating rate (beat/min)                                 | ●   | ●   | ●   | ●        |
The compound was not toxic in concentrations up to 100 μM and did not show negative effects on the survival and development of embryos in the 120 hpf timeframe. Also, the compound did not show any undesirable cardiotoxic effects (pericardial edema, bradycardia/tachycardia and changes of the heart morphology) in concentrations up to 100 μM. However, embryos treated with 150 μM had slightly stunted development on the fifth day relative to the control group treated with 0.25 % DMSO (slight teratogenic effect, already visible after 48 hpf). Embryos treated with 200 μM were seriously lagging behind in development and had deformities of the whole body and notochord (*spina bifida*).
Additionally, the compound has led to decreased pigmentation of the embryos in concentration from 25 μM to 100 μM and the depigmentation of melanocytes was complete at 100 μM, which indicates potential anti-melanogenic activity.
Conclusions

- A methionine derived 2-thiohydantoin, 5-[2-(methylthio)ethyl]-3-(2-propen-1-yl)-2-thioxo-4-imidazolidinone, has been synthesized and fully characterized by NMR and IR spectroscopy, as well as X-ray crystallography.
- Antimicrobial activity was tested on a panel of 15 different microorganisms, including five Gram-negative bacteria, five Gram-positive bacteria and five fungi of the *Candida* genus. The compound exhibited no antifungal activity and weak antibacterial activity. Only in the case of the Gram-positive bacteria, *Staphylococcus aureus*, it exhibited moderate activity. It is most effective against *Staphylococcus aureus* MRSA, a strain resistant to methicillin.
- Cytotoxicity of the compound was tested via MTT method on the MRC-5 (human lung fibroblasts), A549 (human lung carcinoma cells), HCT-116 (human colorectal carcinoma cells) and MDA-MB-231 (human breast carcinoma cells) cell lines. The compound did not exhibit *in vitro* cytotoxicity on the tested cell lines in concentrations up to 200 μM.
- *In vivo* toxicity was tested on wild type zebrafish embryos (*Danio rerio*). The compound was not toxic in concentrations up to 100 μM and did not show negative effects on the survival and development of embryos and any undesirable cardiotoxic effects. However, embryos treated with 150 μM had slightly stunted development relative to the control group. Embryos treated with 200 μM were seriously lagging behind in development and had deformities of the whole body and notochord (*spina bifida*).
- The compound has led to decreased pigmentation of the embryos in non-toxic concentrations from 25 μM to 100 μM and the depigmentation of melanocytes was complete at 100 μM, which indicates potential anti melanogenic activity. As it inhibits melanogenesis, it could potentially be used as a skin-lightening agent for cosmetic purposes and also clinically for treatment of hyperpigmentary disorders.
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