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CLOSELY RELATED ISOXAZOLES MAY EXHIBIT OPPOSITE IMMUNOLOGICAL ACTIVITIES

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Advances of modern medicine require constant supply of new drugs displaying specific therapeutic actions. Bio-accessibility and lack of side-effects are highly desired features of new therapeutics. Modifications of the isoxazole structure offer a possibility of generating potential drugs exhibiting a wide array of biological activities, as described by us during the last decade (1-10). A majority of compounds from the isoxazole family are immunosuppressive or anti-inflammatory (8, 10-12). However, even a minor modification of the isoxazole structure may lead to unexpected biological activity (5). Among compounds, synthesized recently in the Department of Organic Chemistry, two isoxazoles require special attention and were the subject of more detailed investigations (5, 8, 10).

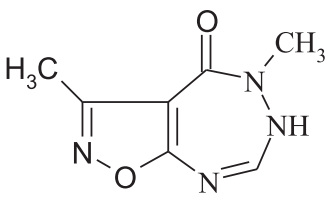
These compounds exhibited strong, directional, immunotropic actions and may be potentially applied for various therapeutic goals.

RESULTS AND DISCUSSION

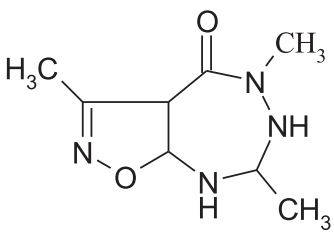
The immunosuppressive activities of an isoxazolo[5,4-e]triazepine (**RM-33**) were described very recently (8, 10) and the summary of its actions is presented in Table 1. In general, the compound exhibited differential immunosuppressive actions. The inhibition of the pro-inflammatory cytokine-TNF-α and a lack of inhibition of the anti-inflammatory cytokines, such as IL-6 and IL-10, indicate that **RM-33** selectively affects the cytokine network. In addition (data not presented), we demonstrated that the compound ameliorated clinical symptoms of experimental autoimmune encephalomyelitis in Lewis rats, suggesting that **RM-33** may also suppress the autoimmune disorders. Of note, **RM-33** was also active when given *per os* and its toxicity was very low.

RM-11 represents, in turn, an example of the isoxazole derivative, with an activity quite opposite as compared with **RM-33**. The compound (5) is distinctly immunostimulatory and its immunological activities are summarized in Table 2.

The unpublished results indicate that **RM-11** strongly up-regulated lipopolysaccharide (LPS)-induced production of TNF-α by cultures of human mononuclear peripheral blood cells. That finding does not mean that **RM-11** is a pro-inflammatory compound since pretreatment of LPS-injected mice with **RM-11** significant-



RM11



RM33

Figure 1.

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Table 1. The immunological activities of **RM-33**

Model	Effect
Humoral immune response *in vivo*	64% inhibition
Cellular immune response *in vivo*, induction phase	69% inhibition
Cellular immune response *in vivo*, effector phase	46% inhibition
Local administration of RM-33 with antigen	72% inhibition
Adjuvant-induced foot pad edema	64% inhibition*
LPS-induced TNF-α production *in vivo*	63% inhibition
LPS-induced IL-6 production *in vivo*	17% inhibition
LPS-induced IL-10 production *in vitro*	no effect
Carrageenan-induced foot pad inflammation	26% inhibition**
Carrageenan-induced TNF-α serum level	47% inhibition

The carrageenan-induced inflammation was performed in a rat model, other experiments were performed on mice. **RM-33** was administered intraperitoneally.
* The effect of **RM-33** after one day was shown. The inhibition of the foot pad edema after 3 and 4 days was complete (100%).
** Three 500μg doses were applied. In all other tests the **RM-33** dose was 100 μg.
LPS – lipopolysaccharide, TNF-α – tumor necrosis factor alpha, IL – interleukin.

Table 2. Immunostimulatory activities of **RM-11**

Model	Effect
Humoral immune response *in vivo*	4.1-fold stimulation
Cellular immune response *in vivo*	1.7-fold stimulation
ConA-induced splenocyte proliferation *in vitro*	1.6-fold stimulation*

The experiments were performed on mice. The dose of **RM-11** *in vivo* was 100 μg.
*The *in vitro* dose was 0.1 μg/mL which already resulted in a maximal stimulation of the proliferative response.
Con A – concanavalin A.

ly reduced serum TNF-α level. **RM-11** was also active when administered *per os* and was more stimulatory than the reference drug levamisole in the investigated models. We suggested (5) that **RM-11** may even replace levamisole in some therapeutic interventions. Our very recent studies revealed also other important feature of **RM-11**, namely an ability of the compound to reconstitute humoral and cellular immune response in immunocompromised mice (13). Such a property opens a possibility of clinical application of **RM-11**.

Since the described compounds were recently protected by patents, we reveal in this article their chemical structures. The structures are very close, thus the compounds may serve as examples of isoxazole derivatives where minor changes in the basic structure (a difference in one substituent) may lead to acquirement of opposite biological activities.

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