# Dimephosphone analogs: a pharmacological aspect

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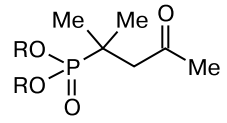
We studied some pharmacological properties of dimephosphone P—C and aza analogs, *viz.*, dimethyl (2-methyl-4-oxopent-2-yl)phosphonate, which is one of the first organophosphorus drugs having no anticholinesterase activity. Replacement of two P—O—C fragments in dialkyl (2-methyl-4-oxopent-2-yl)phosphonates with the P—C ones on going to dialkyl-(2-methyl-4-oxopent-2-yl)phosphine oxides results in a dramatic decrease in the acute toxicity of the latter to warmblooded animals. The toxicities of dimephosphone aza analogs and (γ-oxoalkyl)phosphine oxides under study depend on the nature of aza fragment introduced instead of the oxygen atom. Dimephosphone pyridinoylhydrazones were found to exhibit a high antiinflammatory activity, which increases the interest for this type compounds as promising tuberculostatics.

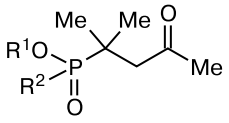
**Key words:** dimephosphone, dimethyl (2-methyl-4-oxopent-2-yl)phosphonate, phosphine oxide, oxime, pyridinoyl hydrazone, nicotinoyl hydrazone, isonicotinoyl hydrazone, Isoniazide, nicotinic acid hydrazide, benzhydrazide, 4-nitrobenzhydrazide, acyl hydrazone, aroyl hydrazone, bioactivity, acute toxicity, antituberculous activity, antiinflammatory activity.

Dimephosphone is one of the first organophophorus drugs possessing no anticholinesterase activity, which is produced by the "Tatchempharmpreparaty" enterprise. It has been being used since 1983 as antiacidotic agent upon acidoses of various etiology, vasoactive agent upon impaired cerebral circulation, and upon respiratory diseases as monotherapy drug and in combination with other drugs.[1—3] Despite well studied pharmacological properties of this drug preparation, its novel properties[4] and dosage forms[5] continue to be studied, which considerably expands its application field.

The medical preparation Dimephosphone is an aqueous solution of dimethyl (2-methyl-4-oxopent-2-yl)phosphonate (ketone **1a**, hereinafter referred to as dimephosphone; also known by author´s name as dimethyl (1,1-dimethyl-3-oxobutyl)phosphonate[1—3]), which relates to -phosphorylated ketones and, therefore, is of interest as the matrix in the synthesis of new potentially bioactive compounds. The drug design by analogy to known drugs is widely used in pharmacology along with other approaches.




**1a—d**




**2a—c**

**1:** R = Me (**a**), Et (**b**), Pr (**c**), Bu (**d**)
**2:** $R^1 = R^2$ = Me (**a**), $R^1$ = Me, $R^2$ = Et (**b**), $R^1 = R^2$ = Et (**c**)

The authors of dimephosphone[1] have also synthesized its first analogs: they varied the nature of substituents in the ester fragment of dimephosphone or introduced the oxime fragment instead of the oxo group. The bioactivities of different alkyl (2-methyl-4-oxopent-2-yl)phosphonates (**1a—d**) have been studied in detail to show that the increase in the length of alkyl chain in the ester fragment leads to the increase in the acute toxicity while preserving the most of useful properties.[1] For example, diethyl ester (**1b**) and dipropyl ester (**1s**) are, respectively, 2.5-and 6-fold more toxic and dibutyl ester (**1d**) is by an order of magnitude more toxic than dimephosphone. At that time, it has been shown[1] that the toxicities of alkyl (2-methyl-4-oxopent-2-yl)phosphonates (**2a—c**) depend in a like man-

† Deceased.

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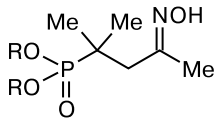
ner on the length of alkyl radical in both the ester and alkylphosphoryl fragments. On going from dialkyl phosphonates **1a—c** to phosphinates **2a—c**, the toxicity decreases appreciably (Table 1). The observed effect is caused by replacement of the P—O—C fragment with the P—C one and can be explained not only by a decrease in the size of substituent at the phosphorus atom, but also by less reactivities of esters **2a—c** in phorphorylation.

As the first N-analogs of dimephosphone and esters **1a—c**, we studied dialkyl (2-methyl-4-oxopent-2-yl) phosphonate oximes (**3a—c**). Their toxicities also increases with increasing the length of alkyl chain in the ester fragment (see Table 1).[6,7] The oxime analog **3a** is almost 2-fold more toxic than dimephosphone, although their other pharmacological properties are close. Like dimephosphone, oximes **3a—c** cause the central nervous system depression and reflectory hyperexcitability. The anti-inflammatory and antidotal properties of oxime **3a** (Methime) and dimephosphone are comparable.[1,6]



**3a—c**

R = Me (**a**), Et (**b**), Pr (**c**)

Taking into account the data given above, we assumed that replacement of two P—O—C fragments in esters **1a—d** with the P—C one will reduce to an even greater degree the toxicities of dimephosphone P—C analogs under design, *viz.*, dialkyl (2-methyl-4-oxopent-2-yl)phosphine oxides (**4a—j**). To synthesize phosphine oxides **4a—j**, we used the convenient methodology developed in our laboratory[8—13] for the synthesis of functionalized phosphine oxides from phosphorus heterocycles containing at least one P—C bond and one P—O bond. For example, a series of novel (γ-oxoalkyl)phosphine oxides (**4**), including the analogs of dialkyl (2-methyl-4-oxopent-2-yl)phosphonates **1**, were obtained using 3,3,5-trimethyl-2-R-1,2-oxaphospholene-2-oxides (**5a**,**b**) as the starting derivatives (Scheme 1); for some of these compounds, the acute toxicities to warm-blooded animals was determined first of all (see Table 1).

Depending on the nature of substituent $R^1$ in oxaphospholenes **5** used, (γ-oxoalkyl)phosphine oxides **4** with both different (when in the starting phosphacycle there is an additonal endocyclic P—C bond and $R^1$ = Alk, Ar, or heterocycle)[9,11] and identical substituents ($R^1$ = Cl)[8—10,12—14] can be synthesized. This method afforded dialkyl (**4a—f**),[9,10,13] dibenzyl (**4g**),[13] diaryl (**4h**,**i**),[13] diallyl, dithienyl, and miscellaneous (alkyl)(aryl)(γ-oxoalkyl)phosphine oxides,[9] including compound **4j**, in good yields.

The acute toxicities of dialkyl (2-methyl-4-oxopent-2-yl)phosphine oxides (**4a—e**) studied by us on white outbred mice depend on the nature of alkyl fragment at the phosphorus atom and increase rapidly with increasing the

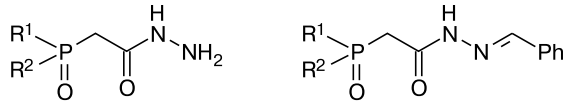
**Scheme 1**

**5a,b** **4a—j**

| Compound | $R^1$ | $R^2$ | Compound | $R^1$ | $R^2$ |
|---|---|---|---|---|---|
| **4a** | Et | Et | **4g** | Bn | Bn |
| **4b** | Pr | Pr | **4h** | Ph | Ph |
| **4c** | Bu | Bu | **4i** | 2-$MeOC_6H_4$ | 2-$MeOC_6H_4$ |
| **4d** | $C_5H_{11}$ | $C_5H_{11}$ | **4j** | Et | 2-$MeOC_6H_4$ |
| **4e** | $C_6H_{13}$ | $C_6H_{13}$ | **5a** | Cl | — |
| **4f** | $C_8H_{17}$ | $C_8H_{17}$ | **5b** | Et | — |

size of this fragment (see Table 1). No symbasis of properties in the corresponding representatives of both groups containing the identical alkyl fragment was observed. While diethylphosphine oxide **4a** is almost 2.5-fold less toxic than the corresponding diethyl ester **1b**, the propyl analogs **1c** and **4b** have approximately identical toxicities and dibutylphosphine oxide **4c** is more than 2-fold toxic than dibutyl ester **1d**.

Replacement of even one alkyl group in dialkyl (2-methyl-4-oxopent-2-yl)phosphine oxides with the aryl one increases the toxicity even more. For example, the toxicity increases almost 20-fold on going from diethylphosphine oxide **4a** to phosphine oxide **4j** where one ethyl group is replaced with the 2-methoxyphenyl one. Such effect of the nature of substituent at the phosphorus atom on the toxic properties has been noted earlier for other group of functionalized phosphine oxides of type **6** and **7** containing functional groups with a similar electronic nature.[15,16]



**6a—c** **7a,b**

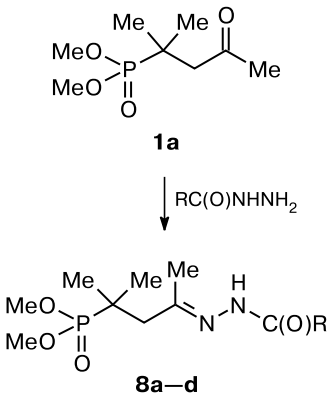
$R^1$ = $R^2$ = Ph (**6a**, **7a**), Et (**6b**),
$R^1$ = $ClCH_2CH_2O$, $R^2$ = 4-$MeC_6H_4$ (**6c**, **7b**)

The decrease in the toxicity on going from hydrazides to hydrazones agrees well with known approaches to decrease the toxicity of commonly used antituberculous medicament Isoniazide (Tubazide and other synonyms), which by its chemical nature is pyridine-4-carboxylic (isonicotinic) acid hydrazide. Many isonicotinoyl hydrazones, such as Ftivazide, Saluzidum, INHA-17, and Larusanum have been proposed earlier[17,18] as antituberculous drugs with toxicity less than that of Isoniazide, although their antimycobacterial activities were inferior to that of Isoniazide. For example, the minimum inhibitory concentration (MIC) for Larusanum is 1.57 μg $mL^{-1}$ compared to 0.1—0.6 μg $mL^{-1}$ for Isoniazide. The MIC

of Ftivazide was estimated to be about 1 μg $mL^{-1}$ and its toxicity is less than 100 μg $kg^{-1}$ (see Ref. 17).

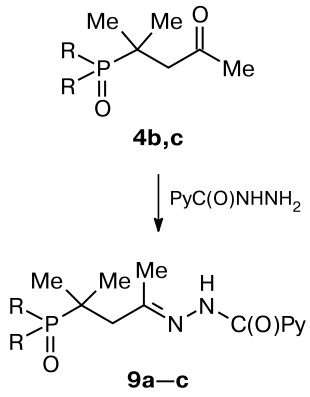
Isoniazide relates to sufficiently toxic drugs, while Ftivazide, Saluzidum, INHA-17, and Larusanum like Isoniazide have some adverse side effects; therefore, compounds with antimycobacterial activity continue to be searched also at the present time among various isonicotinoyl hydrazones with different degree of success.[19] This trend was advanced by the synthesis of aryl and acyl hydrazones based on dimephosphone **1a** (Scheme 2), as well as new pyridinoyl hydrazones **9a—c** based on phosphine oxides **4b**,**c** (Scheme 3). (2-Methyl-4-oxopent-2-yl)diethylphosphine oxide **4b** was chosen for the synthesis of pyridinoyl hydrazones **9a**,**c** to compare its properties with those of dimephosphone derivatives **8a**,**b**, since the steric sizes of methoxy and ethyl groups are close.

**Scheme 2**




**8:** R = 4-Py (**a**), 3-Py (**b**), Ph (**c**), 4-$C_6H_4NO_2$ (**d**)

**Scheme 3**




**4:** R = Et (**b**), Pr (**c**)
**9:** R = Et (**a**, **b**), Pr (**c**); Py = 4-Py (**a**, **c**), 3-Py (**b**)

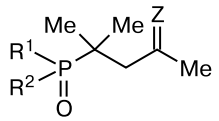
As expected, the toxicity of dimephosphone isonicotinoyl hydrazone (**8a**) was found to be much less than that of Isoniazide and comparable with that of Dimephosphone, which can be assigned to the third hazard class, *viz.*, moderately hazardous compounds, and even to low-toxicity compounds[20] (see Table 1). The toxicity of hydrazone **8a** is 13-fold less upon intraperitoneal administration to mice or rats and only 10-fold less upon peroral administration than that of Isoniazide. The increase in its toxicity upon peroral administration compared to intraperitoneal administration is explained by the fact that this compound like all acyl hydrazones[21] can undergo partial hydrolysis in the acidic medium of stomach into the starting dimephosphone and more toxic Isoniazide. On going from nicotinic acid hydrazide to dimephosphone nicotinoyl hydrazone (**8b**), the acute toxicity also decreases, although to a lesser degree (5-fold) as compared with the 4-pyridinoyl analog **8a** (see Table 1).

Dimephosphone pyridinoyl hydrazones **8a**,**b** in contrast to **1a** have muscle relaxation effect and exhibit anticonvulsant activity,[22] but cause no symptoms of neurotoxic action upon acute intoxication of experimental animals and possess no hepatotoxic properties. In contrast to the effect of dimephosphone toxic doses, they do not lead to a narcosis-like state of animals. Consequently, they have a pharmacological effect as single compound without decomposition into initial components.

One of the adverse properties of Isoniazide is its high hepatotoxicity, on which the first data appeared as early as in 1969.[23] The incidence of clinically significant hepatitis due to the Isoniazide monotherapy in adults is on average 0.6%. The combined therapy with Isoniazide and Rifampin increases the incidence of side effect up to 2.73% (see Ref. 24). To decrease this effect hepatoprotectors are administered together with Isoniazide. It is desirable to search for new compounds having antimycobacterial activity comparable with that of reference medications. For this reason, we studied additionally the effects of pyridinoyl hydrazones **8a**,**b** and Isoniazide on the hepatic function upon their introduction according to treatment schedule for 30 days.

During the first 20 days of daily introduction of pyridinoyl hydrazones **8a**,**b** and Isoniazide (concomitantly with each hydrazone), no changes in the animal behavior were observed. Starting from Day 21, rats showed a slight anxiety and agression, which is likely due to the toxic effect of introduced agents on the central nervous system.

Scheduled introduction of all products under study to rats revealed no statistically significant differences between the body weights in all four groups at the same time points. Calculation of the relative weights of liver, kidney, and suprarenal bodies showed no significant differences in the groups under test. An increase in the relative weight was observed only for the spleen in the group of rats received isonicotinoyl hydrazone **8a** (the weight coefficient is 3.90±0.86 g) and Isoniazide (3.59±0.76 g) compared to the control (2.58±0.41 g).



**1a—d, 2a—c, 3a—c, 4a—e, 8a—d, 9a—c**

**Table 1.** Toxicities ($LD_{50}$/mg kg$^{-1}$) of dimephosphone and its analogs[a]

| Compound | $R^1$ | $R^2$ | Z | $LD_{50}$ | Reference |
|---|---|---|---|---|---|
| **1a** | OMe | OMe | O | 3000±155, 2300—2500[b], 2475[c] | **1** |
| **1b** | OEt | OEt | O | 1050±45 | **1** |
| **1c** | OPr | OPr | O | 520±25 | **1** |
| **1d** | OBu | OBu | O | 390±20 | **1** |
| **2a** | OMe | Me | O | 4800±157 | **1** |
| **2b** | OMe | Et | O | 3200±45 | **1** |
| **2c** | OEt | Et | O | 1450±80 | **1** |
| **3a** | OMe | OMe | NOH | 1600±74 | **6**, **7** |
| **3b** | OEt | OEt | NOH | 785±32 | **6**, **7** |
| **3c** | OPr | OPr | NOH | 260±17 | **6**, **7** |
| **4a** | Et | Et | O | 2514±82 | —[b] |
| **4b** | Pr | Pr | O | 661±31 | —[b] |
| **4c** | Bu | Bu | O | 167±7 | —[b] |
| **4j** | Et | 2-$MeOC_6H_4$ | O | 130±16 | —[b] |
| **6a** | Ph | Ph | — | 315±25 | **15**, **16** |
| **6b** | Et | Et | — | 5400±235 | **15**, **16** |
| **6c** | $ClCH_2CH_2O$ | 4-$Me_2C_6H_4$ | — | 960±35 | **15**, **16** |
| **7a** | Ph | Ph | — | 5200±310 | **16** |
| **7b** | $ClCH_2CH_2O$ | 4-$Me_2C_6H_4$ | — | 4960±210 | **16** |
| **8a** | OMe | OMe | NNHC(O)Py-4 | 2005±59, 2064±64, 1550[d] | **22** —[b] |
| **8b** | OMe | OMe | NNHC(O)Py-3 | 1788±31, 2326±48[b] | **22** |
| **9a** | Et | Et | NNHC(O)Py-4 | 3073.7±87 | —[d] |
| **9b** | Et | Et | NNHC(O)Py-3 | 2164.2±43 | —[d] |
| **9c** | Pr | Pr | NNHC(O)Py-4 | 1702.7±81.8 | —[d] |
| Isoniazide, 4-PyC(O)$NHNH_2$ | | | | 151±7, 172.3, 133 | **25**, **26** —[d] |
| Isoniazide against the backdrop of dimephosphone | | | | 235.5±8 | —[d] |
| 3-PyC(O)$NHNH_2$ | | | | 300±7 | —[d] |

[a] The medicament was administered intraperitoneally to male and female scrub mice.
[b] Perorally. [c] Rats were used. [d] The data of the present work.

In the rate group upon scheduled administration of hydrazone **8a** and Isoniazide, there was a tendency for a decrease in the red blood count and hemoglobin level. No statistically significant differences in other red blood parameters (platelet count and erythrocyte sedimentation rate) were observed upon introduction of hydrazones **8a**,**b**.

Among indices of biochemical blood analysis, of special interest are total bilirubin, alanin transaminase (ALT), aspartate transaminase (AST), and γ-glutamyl transpeptidase (GGT), since their levels reflect direct effect on the hepatic function or its absence.

The biochemical blood assay showed a significant increase in the AST level only in the group received isonicotinoyl hydrazone **8a** (203.1±25.9 U L$^{-1}$) relative to the control (142.6±22 U L$^{-1}$). For the isomeric hydrazone **8b**, there was only a tendency for an increase in the AST level, although such a tendency was comparable with that for Isoniazide. Both hydrazones **8a**,**b** have no effect on the ALT level in contrast to Isoniazide, which decreases significantly its content. The increase in the GGT level up to 6.6±1.4 U L$^{-1}$ upon exposure to hydrazone **8a** is more pronounced than that upon administration of Isoniazide

being 5.2±1.3 U $L^{-1}$ (for the control, it was 2.6±1.7 U $L^{-1}$), while the GGT level upon administration of hydrazone **8b** was less than that for the control by ~1.0 U $L^{-1}$.

The biochemical blood analysis revealed a statistically significant increase in the total bilirubin in the groups of Isoniazide (5.2±0.9 μmol $L^{-1}$) and nicotinoyl hydrazone **8b** (60±0.8 μmol $L^{-1}$) with regard to the control group (2.9±0.8 μmol $L^{-1}$). Isonicotinoyl hydrazone **8a** has no effect on the total bilirubin and glucose metabolism and hydrazone **8b** increases significantly only the glucose content relative to the control group (6.2±0.7 vs. 5.3±0.5 mmol $L^{-1}$, respectively, $\rho < 0.05$). The creatinine level was the same in all groups. Isoniazide and pyridinoyl hydrazones **8a**,**b** cause a comparable decrease in the urea level compared to the control (for **8a** 6.5±0 and 9.4±1.2 mmol $L^{-1}$, respectively). There was a statistically significant decrease in the total protein in the Isoniazide group compared to the control (61.1±4.6 vs. 67.9±2.4 g $L^{-1}$, respectively, $\rho < 0.05$).

Based on the experiments performed, one can make a preliminary conclusion on that dimephosphone nicotinoyl hydrazone **8b** have no hepatotoxic properties in contrast to Isoniazide.

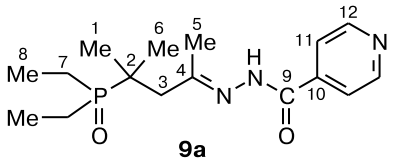
We also estimated the anti-inflammatory activities of hydrazone **8a** and its presumable metabolites, *viz.*, Isoniazide and Dimephosphone, and their combination while observing the same experimental conditions.

The comparison data for the anti-inflammatory activities of Dimephosphone, hydrazone **8a**, and Isoniazide at the half maximum tolerated dose (MTD) showed that the efficiencies of these substances towards carrageenan-induced oedema are different: Dimephosphone at the dose of 1000 mg $kg^{-1}$ arrested the progress of rat foot oedema by 39.1%, hydrazone **8a** (800 mg $kg^{-1}$) inhibited the oedema by 66.3%, and Isoniazide exhibited no such activity. It is interesting to note that, with decreasing the dose of hydrazone **8a**, its antiinflammatory activity decreases in a non-symbate mode: it was only 31.5% at the dose of 400 mg $kg^{-1}$ and 55.4% at the dose of 200 mg $kg^{-1}$. To explain the effect observed, an additional study needs to be performed.

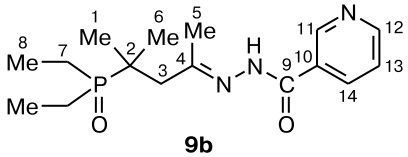
Since the animal body can create conditions suitable for the hydrolysis of hydrazone **8a** into the starting compounds, we studied separately the antiinflammatory activity of the Isoniazide (160 mg $kg^{-1}$) and Dimephosphone (240 mg $kg^{-1}$) combination. This ratio of the drugs can emerge upon decomposition of hydrazone **8a** introduced at the dose of 400 mg $kg^{-1}$. The application of solutions of the above-mentioned Isoniazide—Dimephosphone mixture provides even a higher antiinflammatory effect (66.3%) compared to Dimephosphone (52.2%) taken alone.

Dialkyl(2-methyl-4-oxopent-2-yl)phosphine oxide pyridinoyl hydrazones (**9a—c**) were synthesized analogously to dimephosphone acyl hydrazones **8a—d** (see Scheme 3).

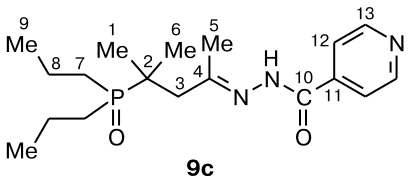
The purities of compounds obtained were controlled by TLC on Silufol plates and their structures were established by several physicochemical methods: IR and NMR spectroscopy and mass spectrometry. Hydrazones **9a—c** like dimephospone hydrazones **8a**,**b** are well crystallizable and water-soluble substances.




**9a**




**9b**




**9c**

[4-(2-Isonicotinoylhydrazono)-(2-methylpent-2-yl)]-diethylphosphine oxide (**9a**) was found to be the least toxic among acyl hydrazones of types **8** and **9** obtained by us. It is 20-fold less toxic than Isoniazide and less toxic than the initial phosphine oxide **4b** (see Table 1). Its dipropyl analog **9c** due to elongation of the alkyl chain is almost 2-fold more toxic, but it is 25-fold less toxic than its ketone precursor **4c**. A similar pattern is observed for the isomeric nicotinoyl hydrazone **9b**. The latter is 7-fold less toxic than the starting nicotinic acid hydrazide and only sligthly more toxic than its ketone **4a** (see Table 1).

(2-Methyl-4-pent-2-yl)phosphine oxide pyridinoyl hydrazones **9a—c** as dimephosphone hydrazones **8a**,**b** differ slightly from each other in their toxic effects. 4—6 min after injection, the animals showed a decrease in the motor activity and labored breathing. The animals died during asphyxia.

In contrast to Isoniazide,[25,26] dialkyl-(2-methyl-4-(pyridinoylhydrazono)pent-2-yl)phosphine oxides **9a—c** by their toxicity pattern, as well as dimephosphone hydrazones **8a**,**b** cause no symptoms of a neurotoxic effect upon acute intoxication of the experimental animals.

The data of Ref. 1 and the data obtained in the study of the toxicities of (2-methyl-4-oxopent-2-yl)phosphonates, dialkyl(2-methyl-4-oxopent-2-yl)phosphine oxides and their N analogs (oximes and acyl hydrazones), and other functionalized phosphine oxides[15,16] allow us to make conclusions reflecting the structure-property relationship as follows: (1) the increase in the length of alkyl chain in the phosphoryl fragment of functionalized phosphine oxides increases their toxicity; (2) the size of a substituent at the

phosphorus atom in such compounds appears to play a more important role compared to their electronic and chemical structure (for example, dimephosphone **1a** and (2-methyl-4-oxopent-2-yl)diethylphosphine oxide **4b** each having two groups, which are approximately equal by the stereochemical requirements (OMe and Et, respectively), possess similar properties as the corresponding pair of their N analogs); (3) introduction of the ylidene fragment of dimephosphone or its P—C analogs to the molecule of toxic compound (by the example of carboxylic acid hydrazides) results in a dramatic decrease in the acute toxicities of aroyl hydrazones obtained.

The bacteriostatic activities of nicotinoyl hydrazones **8a**,**b** and **9a**—**c** towards the mycobacterium tuberculosis strain $H_{37}Rv$ were studied using a BACTEC MGIT 960 standard growth system (Becton Dickinson). A Middlebrook 7 H9 digest medium containing a BACTEC MGIT enrichment additive was used.

As Table 2 shows, introduction of the dimephosphone ylidene fragment to the Isoniazide molecule (going to hydrazone **8a**) does not increase the antimycobacterial activity, although Dimephosphone itself increases the efficacy of tuberculosis treatment due to stimulation of the animal immune response and its ability to decrease the progress of mycobacterium tuberculosis resistance to the action of main antituberculous agents, such as Rifampin.[1] A considerably stronger effect of introduction of the dimephosphone ylidene fragment was observed by the example of the low-activity nicotinic acid hydrazide or inactive benz- and 4-nitrobenzhydrazides. While nicotinic acid hydrazide is inferior to Isoniazide, it possesses an activity towards the *V. tuberculosis* strain $H_{37}Rv$: according to the data of Ref. 27, the activity is 6.25 μg $mL^{-1}$ compared to 1.25 μg $mL^{-1}$ for Isoniazide; towards the strain under study the activity is 10.0 and 0.5 μg $mL^{-1}$, respectively (see Table 2). For the pair of nicotinoyl hydrazide and its hydrazone **8b**, there was a symbate change in the properties: a dramatic decrease in the toxicity (see Table 1) and the increase in the antituberculous activity by an order of magnitude. The effect found is considerably superior to that of the isomeric pair of Isoniazide and its hydrazone **8a** (see Tables 1 and 2), although a low toxicity and good water solublity of the latter also makes it promising for subsequent studies.

**Table 2.** Minimum inhibitory concentrations (MIC/μg $mL^{-1}$) of dimephosphone pyridinoyl hydrazones **8a**,**b**, dimephosphone aroyl hydrazones **8c**,**d**, and dialkyl(2-methyl-4-oxopent-2-yl)phosphine oxide pyridinoyl hydrazones **9a**—**c**

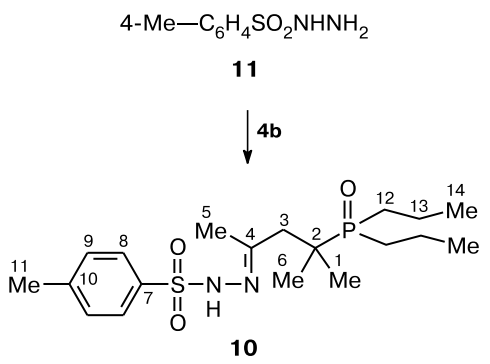
| Compound | $R^1$ | $R^2$ | $R^3$ | MIC |
|---|---|---|---|---|
| **8a** | OMe | OMe | 4-Py | 10.0 |
| **8b** | OMe | OMe | 3-Py | 1.0 |
| **8c** | OMe | OMe | Ph | 10.0 |
| **8d** | OMe | OMe | 4-$O_2NC_6H_4$ | 10.0 |
| **9a** | Et | Et | 4-Py | 1.0 |
| **9b** | Et | Et | 3-Py | 5.0 |
| **9c** | Pr | Pr | 4-Py | 5.0 |
| **10** | Pr | Pr | $NNHSO_2C_6H_4Me$ | 20.0 |
| Isoniazide, 4-$PyC(O)NHNH_2$ | | | | 0.5 |
| 3-$PyC(O)NHNH_2$ | | | | 10.0 |
| $H_2NNHSO_2C_6H_4Me$ | | | | >20 |

*Note*. A BACTEC MGIT 960 instrument was used.

Benzoic and 4-nitrobenzoic acid hydrazides are known[28] to possess no antituberculous activity. In our experiments, dimephosphone benzoyl hydrazone (**8c**) and 4-nitrobenzoyl hydrazone (**8d**) showed noticeable antimycobacterial activities (the MICs of both compounds are 10 μg $mL^{-1}$). Consequently, one can conclude that introduction of the dimephosphone fragment of the carboxylic acid hydrazide molecule allows the resulted hydrazone to exhibit antimycobacterial properties, *i.e.* allows the whole molecule to exhibit properties as a single pharmacophore.

It should be noted that this conclusion seems to be true only for the hydrazones based on carboxylic acid hydrazides. For example, the antimycobacterial activity of dipropyl(2-methyl-4-oxopent-2-yl)phosphine oxide tosyl hydrazone (**10**) prepared from tosyl hydrazide (**11**) (Scheme 4) was found to be quite low as that of the starting hydrazide (see Table 2).

**Scheme 4**




Dialkyl(2-methyl-4-oxopent-2-yl)phosphine oxide pyridinoyl hydrazones **9a**—**c** by their antimycobacterial activies are also inferior to Isoniazide whose activity was determined with regard to the same samples of *M. tuberculosis* $H_{37}Rv$ strain (see Table 2). The most promising compounds for subsequent studies in terms of low acute toxicity are diethyl- and dipropylphosphine oxide isonicotinoyl hydrazones **9a**,**c**. The activity of the former (the MIC is 1 μg $mL^{-1}$) is quite close to that of Isoniazide.

For comparison, we studied the antibacterial and antifungal activities of some dialkyl(2-methyl-4-oxopent-2-yl)phosphine oxides using strains as follows: *Staphylococcus aureus*, *Bacillus cereus* (Gram-positive spore-forming aerobic bacterium), *Escherichia coli*, *Pseudomonas aerugi-*

*nosa*, *Enterococcus faecalis*, *Aspergillus niger* (higher mold fungi from *Aspergillus* sp.), *Trichophyton mentagrophytes* (fungus, dermatophyte) and *Candida Albicans* (diploid fungus, yeast-like fungal form). Chloramphenicol (Laevomycetin) was used as the reference sample, which is a pluripotential antibiotic and highly efficient in treatment of enteric fever, bacterial food poisoning, rickettsioses, bloody flux, and other diseases. Dialkyl-, dibenzyl-, and diaryl(2-methyl-4-oxopent-2-yl)phosphine oxides **4c—j** were found to possess no bactericidal or fungicidal activity. The bacteriostatic and fungistatic activities of these compounds are also very low. For diaryl- (**4i**,**j**), dibenzyl- (**4h**), and even dipropyl- and dibutylphosphine oxides **4c**,**d**, the MICs are considerably higher than 500 mg $L^{-1}$. Only the increase in the length of the phosphorus-bound alkyl chain in compounds **4e—g** results in the emergence of bacteriostatic activity (Table 3). And only when the $C_8H_{17}$ radicals are introduced into the molecule (phosphine oxide **4g**), its bacteriostatic activity towards the Gram-positive bacteria *Staphylococcus aureus* and *Bacillus cereus* is 8-fold higher than that of Chloramphenicol (whose MIC is 62.5 mg $mL^{-1}$, Table 3).

The microbiological tests of pyridinoyl hydrazones **9a—c** and tosyl hydrazone **10** for antibacterial and antifungal activties showed that these compounds as the corresponding starting γ-phosphorylketones have no antimicrobial effect (the MICs are > 500 μg $L^{-1}$) towards the microbes under study (see Table 3).

Thus, the high antimycobacterial activities of pyridinoyl hydrazones **8a**,**b** and **9a—c** (see Table 2) towards tuberculosis mycobacteria is specific just for *Mycobacterium tuberculosis*.

To obtain comparative data, we further performed the comparative *in vitro* study (under the same conditions) of the antimicrobial activities of Dimephosphone, Isoniazide, dimephosphone isonicotinoyl and nicotinoyl hydrazones (**8a**,**b**) towards *Pseudomonas aeruginosa*, *Escherichia coli, Salmonella Paratypi B*, and *Staphylococcus aureus* bacteria and *Candida Albicans* fungi. 10% initial solutions of all compounds were used. The resulted data are given in Table 4.

When the compounds under study contacted with microorganisms for 1 h, only Dimephosphone in the 1 : 50 dilution of the initial 10% solution had a bactericidal effect on *Ps. Aeruginosa* and *St. Aureus* and Isoniazide in the 1 : 50 dilution had a bactericidal effect only towards *C. albicans*. Upon 1 day treatment, in the 1 : 50 and 1 : 100 dilutions all compounds under study had a disastrous effect on all species under test, excluding nicotinoyl hydrazone **8a**, which in the 1 : 100 dilution was found to be inefficient towards *C. albicans*. In the 1 : 1000 dilution, only Dimephospone exhibited antimicrobial activity towards *E.coli, Ps. Aeruginosa, St. Aureus* bacterial strains and *C. albicans* fungal strain. However, at the above-mentioned concentration Dimephosphone was inefficient towards *S. paratyphi B*.

Thus, at high concentrations all compounds (**8a**,**b**, Dimephosphone, and Isoniazide) exhibited contact antimicrobial activity. The formation of such blood concentrations of **8a**,**b** and Dimephosphone is possible in theory. For example, the blood concentration of 1 : 100 can be created upon administration of drugs at the dose of 1000 mg $kg^{-1}$. Such dose is tolerable for these drugs. Moreover, their application as antimicrobial agents to achieve a resporptive effect is unlikely. However, a promising outlook for application of dimephosphone pyiridinoyl hydrazones **8a**,**b** for washing cavernous cavities in order to have an antimicrobial effect and to remedy a coincident common infection is a quite promising, although it requires further studies.

The above-mentioned results allow conclusions as follows: (1) Isoniazide at the *in vitro* concentrations of 1 : 50 and 1 : 100 has bactericidal effect on the *S. paratyphi B*, *E. Coli*, *Ps. Aeruginosa*, *St. aureus*, and *C. albicans* microorganisms upon 24 h action; (2) dimephosphone isonicotinoyl and nicotinoyl hydrazones (**8a** and **8b**) are comparable with Isoniazide by their antimicrobial activity; (3) the antimicrobial activity of Dimephosphone is higher than

**Table 3.** Minimum inhibitory concentrations (MIC) of dialkyl(2-methyl-4-oxopent-2-yl)phosphine oxides **4b—f**[a]

| Compound | MIC/μg $mL^{-1}$ | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Sa*[b] | *Ba*[c] | *Ec*[d] | *Pa*[e] | *Ef*[f] | *An*[g] | *Tm*[h] | *Ca*[i] |
| **4b** | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 |
| **4c** | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 |
| **4d** | 250 | 250 | >500 | >500 | >500 | >500 | >500 | >500 |
| **4e** | 250 | 250 | >500 | >500 | >500 | >500 | >500 | >500 |
| **4f** | 7.8 | 7.8 | >500 | >500 | >500 | >500 | >500 | >500 |
| Chloro-amphenicol | 62.5 | 62.5 | 125 | 250 | — | — | — | — |

*Note.* [a] For diaryl- and dibenzyl(2-methyl-4-oxopent-2-yl)phosphine oxides **4h—j**, the MIC towards all microbes is more than 500 mg $L^{-1}$. [b] *Staphylococcus aureus*. [c] *Bacillus cereus*. [d] *Escherichia coli*. [e] *Pseudomonas aeruginosa*. [f] *Enterococcus faecalis*. [g] *Aspergillus niger*. [h] *Trichophyton mentagrophytes*. [i] *Candida Albicans*.

**Table 4.** Microbial proliferation (+ sign) or its absence (0 digit) after incubation of a culture with aqueous solutions of dimephosphone **1a**, dimephosphone pyridinyl hydrazones **8a**,**b**, and Isoniazide for 2 and 24 h (within parentheses); the control medium is water

| Microorganism | Dilution of a 10% solution | Process | | | | Water (control) |
|---|---|---|---|---|---|---|
| | | Dimephosphone (**1a**) | **8a** | **8b** | Isoniazide | |
| *S. paratyphi B* | 1 : 50 | + (0) | + (0) | + (0) | + (0) | + (+) |
| | 1 : 100 | + (0) | + (0) | + (0) | + (0) | + (+) |
| | 1 : 1000 | + (+) | + (+) | + (+) | + (+) | + (+) |
| | 1 : 10000 | + (+) | + (+) | + (+) | + (+) | + (+) |
| *E. coli* | 1 : 50 | + (0) | + (0) | + (0) | + (0) | + (+) |
| | 1 : 100 | + (0) | + (0) | + (0) | + (0) | + (+) |
| | 1 : 1000 | + (0) | + (+) | + (+) | + (+) | + (+) |
| | 1 : 10000 | + (+) | + (+) | + (+) | + (+) | + (+) |
| *Ps. aeruginosa* | 1 : 50 | 0 (0) | + (0) | + (0) | + (0) | + (+) |
| | 1 : 100 | + (0) | + (+) | + (0) | + (0) | + (+) |
| | 1 : 1000 | + (0) | + (+) | + (+) | + (+) | + (+) |
| | 1 : 10000 | + (+) | + (+) | + (+) | + (+) | + (+) |
| *St. aureus* | 1 : 50 | 0 (0) | + (0) | + (0) | + (0) | + (+) |
| | 1 : 100 | + (0) | + (0) | + (0) | + (0) | + (+) |
| | 1 : 1000 | + (0) | + (0) | + (0) | + (0) | + (+) |
| | 1 : 10000 | + (+) | +/(**?**) | + (+) | + (+) | + (+) |
| *C. albicans* | 1 : 50 | + (0) | + (0) | + (0) | 0 (0) | + (+) |
| | 1 : 100 | + (0) | + (0) | + (+) | + (0) | + (+) |
| | 1 : 1000 | + (0) | +/(**?**) | + (+) | + (+) | + (+) |
| | 1 : 10000 | + (+) | + (+) | + (+) | + (+) | + (+) |

those of Isoniazide and dimephosphone pyridinoyl hydrazones **8a**,**b**; (4) dimephosphone pyridinoyl hydrazones **8a**,**b** and pyridinoyl hydrazones **9a**—**c** of the dimephosphone P—C analogs exhibit specific antimicrobial activity: they are quite active towards tuberculosis mycobacteria, but inactive towards a large group of other Gram-positive and Gram-negative bacteria and some fungi; (5) the 2-dialkylphosphoryl- or 2-dialkylphosphoryl-(2-methylpent-4-ylidene) fragments are new pharmacophore groups and their introduction to the molecules of pyridinoyl and aroyl hydrazides results in an increase in the antimycobacterial activity or its emergence, which is accompanied by a dramatic decrease in the toxicity.

## Experimental

NMR spectra were recorded on a Bruker Avance-400 instrument (400 MHz, $^1$H; 161.0 MHz, $^{31}$P; and 100.6 MHz, $^{13}$C) in $CDCl_3$ relative to the residual signals of the solvent. IR spectra were obtained on a Bruker Vector-22 instrument for suspensions of substances in Nujol or for a thin film between KBr plates.

**[4-(2-Isonicotinoylhydrazono)-(2-methylpent-2-yl)]diethylphosphine oxide (9a).** A mixture of (1-methyl-4-oxopent-2-yl)diethylphosphine oxide (**4b**) (5.9 g, 0.0292 mol) and isonicotinoyl hydrazide (4 g, 0.0292 mol) in benzene (50 mL) was refluxed in a round-bottom flask equipped with a Dean-Stark head and magnetic stirrer for 1.5 h and then the solvent was evaporated. The resulted oil transformed slowly into a powdery mass, which was washed with dry diethyl ether. The powder was filtered off and dried *in vacuo* (12 Torr, 60 °C) to yield isonicotinoyl hydrazone **9a** (8.8 g, 93%) as a white powder, m.p. 144—146 °C. Found (%): C, 59.57 (59.32); H, 7.96; N, 12.36. $C_{10}H_{21}N_3O_2P$. Calculated (%): C, 59.43; H, 8.10; N, 12.99. IR, $\nu$/cm$^{-1}$: 412, 453, 483, 509, 538, 617, 656, 671, 702, 748, 762, 791, 845, 887, 916, 954, 990, 1031, 1064, 1136, 1171, 1191, 1215, 1245, 1301, 1324, 1370, 1408, 1436, 1465, 1538, 1593, 1609, 1666, 1964, 2821, 2885, 2948, 2976, 3036, 3145, 3428. $^1$H NMR (major isomer), $\delta$: 1.20 (br.d.t, 6 H, C(8)H$_3$, $^3J_{PCCH}$ = = 15.0 Hz, $^3J_{HCCH}$ = 7.8 Hz); 1.34 (d, 6 H, C(1)H$_3$, C(6)H$_3$, $^3J_{PCCH}$ = 14.0 Hz); 1.64—1.84 (m, 4 H, C(7)H$_2$, $^2J_{PCH}$ = 15.6 Hz, $^3J_{HCCH}$ = 7.9 Hz); 2.25 (s, 3 H, C(5)H$_3$); 2.58 (d, 2 H, C(3)H$_2$, $^3J_{PCCH}$ = 14.5 Hz); 8.07 (br.d, 2 H, C(11)H, AA part of the AA´BB spectrum, $^3J_{AB}$ = $^3J_{A'B'}$ =6.0 Hz); 8.76 (br.d, 2 H, C(12)H, BB part of the AA´BB spectrum, $^3J_{AB}$ = $^3J_{A'B'}$ = 6.0 Hz, $^3J_{HCCH}$ = = 6.0 Hz). $^{13}$C NMR (150.9 MHz, $CDCl_3$, 32 °C, given hereinafter in parentheses is the signal form in $^{13}$C—{$^1$H} NMR), $\delta$: 6.58 (qm (d), C(8), $^1J_{HC}$ = 129.5 Hz, $^2J_{PCC}$ = 5.3 Hz); 17.65 (tdm (d), C(7), $^1J_{HC}$ = 126.9 Hz, $^1J_{PC}$ = 62.0 Hz); 24.80 (qm (s), C(1), C(6), $^1J_{HC}$ = 128.4 Hz, $^3J_{HCCC}$ = 4.3 Hz); 27.00 (qt (s), C(5), $^1J_{HC}$ = 127.8 Hz, $^3J_{HC}$ = 4.0 Hz); 36.70 (d.m (d), C(2), $^1J_{PC}$ = 63.1 Hz); 41.45 (tm (s), C(3), $^1J_{HC}$ = 129.4 Hz); 122.10 (ddd (s), C(12), $^1J_{HC}$ = 166.0 Hz, $^2J_{HC}$ = 8.7 Hz, $^3J_{HC}$ = 5.5 Hz); 140.82 (t (s), C(10), $^2J_{HCC}$ = 6.5 Hz); 150.41 (ddd (s), C(11), $^1J_{HC}$ = 179.3 Hz, $^3J_{HCNC}$ = 10.5 Hz, $^2J_{HCC}$ = 2.4 Hz); 157.40 (m (s), C(4)); 163.31 (d.t (s), C(9), $^2J_{HNC}$ = 10.8 Hz, $^3J_{HCCC}$ = = 3.9 Hz). $^{31}$P—{$^1$H} NMR, $\delta_P$: 62.7 (major isomer) and 62.5.

**[2-Methylpent-2-yl-4-(2-nicotinoylhydrazono)]diethylphosphine oxide (9b)** was prepared under the conditions of the previous example from (2-methyl-4-oxopent-2-yl)diethylphosphine

oxide (**4b**) (5.0 g, 0.0245 mol) and nicotinoyl hydrazide (4 g, 0.0245 mol) in a yield of 7.5 g (94%) as a white powder, m.p. 144—146 °C. Found (%): C, 59.71; H, 8.27; N, 12.45. $C_{16}H_{26}N_3O_2P$. Calculated (%): C, 59.43; H, 8.10; N, 12.99. IR, ν/cm$^{-1}$: 540, 616, 644, 707, 739, 756, 789, 839, 919, 940, 980, 1028, 1058, 1134, 1166, 1199, 1262, 1281, 1322, 1378, 1463, 1525, 1586, 1624, 1668, 2725, 2854, 2923, 3195, 3439. $^1$H NMR (major isomer), δ: 1.18 (d.t, 6 H, C(8)H$_3$, $^3J_{PCCH}$ = 15.7 Hz, $^3J_{HCCH}$ = 7.7 Hz); 1.34 (d, 6 H, C(1)H$_3$, C(6)H$_3$, $^3J_{PCCH}$ = = 14.0 Hz); 1.72 (m, 4 H, C(7)H$_2$, $^2J_{PCH}$ = 7.7 Hz, $^3J_{HCCH}$ = = 7.7 Hz); 2.26 (s, 3 H, C(5)H$_3$); 2.59 (d, 2 H, C(3)H$_2$, $^3J_{PCCH}$ = 14.5 Hz); 7.38 (dd, 1 H, C(13)H, $^3J_{HC(14)C(13)H}$ = 8.0 Hz, $^3J_{HC(12)C(13)H}$ = 5.0 Hz); 8.48 (ddd, 1 H, C(14)H, $^3J_{HC(13)C(14)H}$ = = 8.0 Hz, $^4J_{HC(11)CC(14)H}$ = 1.7 Hz, $^4J_{HC(12)CC(14)H}$ = 1.6 Hz); 8.70 (dd, 1 H, C(12)H, $^3J_{HC(13)C(12)H}$ = 5.0 Hz, $^4J_{HC(14)CC(12)H}$ = = 1.6 Hz); 9.37 (br.d, 1 H, C(11)H, $^4J_{HC(14)CCC(11)H}$ = 1.7 Hz). $^{13}$C NMR (major isomer, 32 °C), δ: 6.63 (q.d.t (d), C(8), $^1J_{HC}$ = = 129.1 Hz, $^2J_{PCC}$ = 5.5 Hz, $^2J_{HCC}$ = 5.0 Hz); 17.69 (tdm (d), C(7), $^1J_{HC}$ = 127.1 Hz, $^1J_{PC}$ = 62.0 Hz, $^2J_{HC(8)C(7)}$ = 4.1 Hz, $^3J_{HC(7)PC(7)}$ = 3.1 Hz); 24.86 (qm (br.s), C(1), C(6), $^1J_{HC}$ = = 128.2 Hz, $^3J_{HCCC}$ = 4.2—4.3 Hz, $^3J_{HCCC}$ = 4.1—4.2 Hz); 27.07 (qt (s), C(5), $^1J_{HC}$ = 127.7 Hz, $^3J_{HCCC}$ = 4.0 Hz); 36.49 (d.m (d), C(2), $^1J_{PC}$ = 63.1 Hz); 41.43 (tm (s), C(3), $^1J_{HC}$ = = 126.3 Hz); 123.37 (ddd (s), C(13), $^1J_{HC}$ = 164.6 Hz, $^2J_{HC(12)C(13)}$ = 8.3 Hz, $^2J_{HC(14)C(13)}$ = 1.0 Hz); 129.62 (dd (s), C(10), $^2J_{HC(11)C(10)}$ = 7.5 Hz, $^3J_{HC(13)CC(10)}$ = 6.8 Hz, $^2J_{HC(14)C(10)}$ = 1.5 Hz); 136.04 (ddd (s), C(14), $^1J_{HC}$ = 165.6 Hz, $^3J_{HC(12)CC(14)}$ = 6.1 Hz, $^3J_{HC(11)CC(14)}$ = 5.4 Hz); 149.88 (ddd (s), C(11), $^1J_{HC}$ = 183.1 Hz, $^3J_{HC(12)NC}$ = 11.5 Hz, $^3J_{HC(14)CC(11)}$ = = 5.5 Hz); 152.02 (ddd (s), C(12), $^1J_{HC}$ = 179.3 Hz, $^3J_{HC(11)NC(12)}$ = = 7.2 Hz, $^3J_{HC(14)CC(12)}$ = 3.4 Hz); 156.75 (m (d), C(4), $^3J_{PCCC}$ = = 1.8 Hz); 163.56 (m (s), C(9), $^2J_{HNC}$ = 9.3 Hz, $^3J_{HCCC}$ = = 2.9—3.0 Hz, $^3J_{HCCC}$ = 1.8—2.0 Hz). $^{31}$P—{$^1$H} NMR, $\delta_P$: 62.9 (major isomer).

**[4-(2-Isonicotinoylhydrazono)-2-methylpent-2-yl]dipropylphosphine oxide (9c)** was prepared under the conditions of the previous example from (2-methyl-4-oxopent-2-yl)dipropylphosphine oxide (**4c**) (1.7 g, 0.0073 mol) and isonicotinoyl hydrazide (1 g, 0.0073 mol) in a yield of 1.9 g (74%) as a white powder, m.p. 122—124 °C. Found (%): C, 61.57; H, 8.54; N, 12.01. $C_{10}H_{21}N_3O_2P$. Calculated (%): C, 61.52; H, 8.60; N, 11.96. MS, *m/z*: 301 $[M + H]^{+\bullet}$, 300 $[M]^{+\bullet}$, 285 $[M - CH_3]$, 257 $[M - C_2H_3O]$, 244 $[M - C_3H_4O]$, 243 $[M - C_3H_5O]$, 219 $[M - C_6H_9]$, 202 $[M - C_6H_{10}O]$, 201 $[M - C_6H_{11}O]$, 155 $[C_{12}H_{11}]$, 154 $[C_{12}H_{10}]$, 125 $[C_6H_7OP]$, 124 $[C_6H_6OP]$, 99.0 $[C_6H_{11}O]$, 81 $[C_6H_9]$, 77, 57, 55, 43, 29. IR, ν/cm$^{-1}$: 670, 722, 756, 782, 846, 1078, 1128, 1166, 1216, 1247, 1271, 1297, 1377, 1462, 1527, 1552, 1598, 1632, 1680, 2726, 2852, 2925, 2955, 3036, 3234, 3417. $^1$H NMR (major isomer), δ: 1.00 (t, 6 H, C(9)H$_3$, $^3J_{HCCH}$ = = 7.1 Hz); 1.28 (d, 6 H, C(1)H$_3$, C(6)H$_3$, $^3J_{PCCH}$ = 14.2 Hz); 1.58—1.64 (m, 8 H, C(7)H$_2$, C(8)H$_2$); 2.21 (s, 3 H, C(5)H$_3$); 2.53 (d, 2 H, C(3)H$_2$, $^3J_{PCCH}$ = 14.4 Hz); 8.00 (m, 2 H, C(12)H, AA´ part of the AA´BB´ spectrum, $^3J_{AB}$ = $^3J_{A'B'}$ = 6.0 Hz); 8.69 (m, 2 H, C(13)H, BB´ part of the AA´BB´ spectrum, $^3J_{AB}$ = $^3J_{A'B'}$ = = 6.0 Hz). $^1$H NMR (minor isomer), δ: 0.98 (t, 6 H, C(9)H$_3$, $^3J_{HCCH}$ = 7.1 Hz); 1.22 (d, 6 H, C(1)H$_3$, C(6)H$_3$, $^3J_{PCCH}$ = = 14.2 Hz); 1.58—1.64 (m, 8 H, C(7)H$_2$, C(8)H$_2$); 2.13 (s, 3 H, C(5)H$_3$); 2.63 (d, 2 H, C(3)H$_2$, $^3J_{PCCH}$ = 8.0 Hz); 7.69 (m, 2 H, C(12)H, AA´ part of the AA´BB´ spectrum, $^3J_{AB}$ = $^3J_{A'B'}$ = 6.0 Hz); 8.66 (m, 2 H, C(13)H, BB´ part of the AA´BB´ spectrum, $^3J_{AB}$ = = $^3J_{A'B'}$ = 6.0 Hz). $^{13}$C NMR (major isomer, 32 °C), δ: 15.99 (qdm (d), C(9), $^1J_{HC}$ = 126.3 Hz, $^3J_{PCCC}$ = 14.0 Hz, $^2J_{HCC}$ = 4.5 Hz, $^3J_{HCCC}$ = 3.6—4.3 Hz); 16.15 (tm (d), C(8), $^1J_{HC}$ = 127.7 Hz, $^2J_{PCC}$ = 4.3 Hz); 24.62 (qm (s), C(1,6), $^1J_{HC}$ = 128.2 Hz, $^3J_{HCCC}$ = 4.4 Hz, $^3J_{HCCC}$ = 4.2—4.4 Hz); 26.92 (qt (s), C(5), $^1J_{HC}$ = 127.8 Hz, $^3J_{HCCC}$ 3.8—3.9 Hz); 27.32 (tdm (d), C(7), $^1J_{HC}$ = 127.0—128.0 Hz, $^1J_{PC}$ = 60.7 Hz); 36.58 (d.m (d), C(2), $^1J_{PC}$ = 63.1 Hz); 41.12 (tm (s), C(3), $^1J_{HC}$ = 128.3 Hz); 122.06 (dddd (s), C(12), $^1J_{HC}$ = 165.9 Hz, $^2J_{HC(13)C(12)}$ = 8.7 Hz, $^3J_{HC(12')CC(12)}$ = 5.5 Hz, $^4J_{HC(13')CCC(12)}$ = 1.1 Hz); 150.27 (ddd (s)); 140.81 (t (s), C(11), $^3J_{HC(13)CC(11)}$ = 6.5 Hz); C(13), $^1J_{HC}$ = = 180.0 Hz, $^3J_{HC(13')NC(13)}$ = 11.5 Hz, $^2J_{HC(12)C(13)}$ = 2.3 Hz); 157.63 (m (d), C(4), $^3J_{PCCC}$ = 2.3 Hz); 163.33 (br.d.t (s), C(10), $^2J_{HNC(10)}$ = 8.2 Hz, $^3J_{HC(12)CC(10)}$ = 3.8 Hz). $^{13}$C (minor isomer, 32 °C), δ: 16.17 (qdm (d), C(9), $^1J_{HC}$ = 126.3 Hz, $^3J_{PCCC}$ = 13.8 Hz, $^2J_{HCC}$ = 4.5 Hz, $^3J_{HCCC}$ = 3.6—4.3 Hz); 16.11 (tm (d), C(8), $^1J_{HC}$ = 127.7 Hz, $^2J_{PCC}$ = 4.2 Hz); 22.46 (br.qm (br.s), C(1,6), $^1J_{HC}$ = 128.2 Hz); 20.93 (qt (s), C(5), $^1J_{HC}$ = 127.8 Hz, $^3J_{HCCC}$ = 3.8 Hz); 26.75 (tdm (d), C(7), $^1J_{HC}$ = 127.0—128.0 Hz, $^1J_{PC}$ = 61.0 Hz); 35.38 (d.m (d), C(2), $^1J_{PC}$ = 64.0 Hz); 47.01 (tm (s), C(3), $^1J_{HC}$ = 128.1 Hz); 121.26 (dddd (s), C(12), $^1J_{HC}$ = 165.9 Hz, $^2J_{HC(13)C(12)}$ = 8.7 Hz, $^3J_{HC(12')CC(12)}$ = 5.5 Hz, $^4J_{HC(13')CCC(12)}$ = 1.1 Hz); 140.23 (t (s), C(11), $^3J_{HC(13)CC(11)}$ = = 6.5 Hz); 150.39 (ddd (s), C(13), $^1J_{HC}$ = 180.0 Hz, $^3J_{HC(13')NC(13)}$ = 11.5 Hz, $^2J_{HC(12)C(13)}$ = 2.3 Hz); 161.53 (m (d), C(4), $^3J_{PCCC}$ = 2.3 Hz); 166.00 (m (s), C(10)). $^{31}$P—{$^1$H} NMR, $\delta_P$: 60.1 (major isomer) and 56.0.

**{2-Methylpent-2-yl-4-[2-(4-tolyl)sulfonylhydrazono]}dipropylphosphine oxide (10).** To a solution of phosphine oxide **5b** (1.00 g, 0.0043 mol) in benzene (50 mL), 4-toluene sulfenyl hydrazide **11** (0.80 g, 0.0043 mol) was added and the mixture was refluxed until hydrazide **11** was dissolved. Benzene was evaporated *in vacuo* (80 °C, 30 Torr). The resulted viscous oil was washed with ethyl acetate. The formed tosyl hydrazone **10** (0.60 g, 35%) was separated as a white finely divided powder, m.p. 152—154 °C. IR, ν/cm$^{-1}$: 432, 450, 461, 481, 547, 582, 660, 706, 723, 778, 812, 856, 902, 929, 947, 971, 1023, 1055, 1122, 1168, 1241, 1305, 1336, 1378, 1463, 1493, 1597, 1619, 2670, 2855, 2927, 3442. $^{31}$P—{$^1$H} NMR ($CDCl_3$), $\delta_P$: 54.9 and 59.4. $^1$H NMR ($CDCl_3$), δ: 0.88 (t, 6 H, H(14), $^3J_{HCCP}$ = 7.1 Hz); 1.02 (d, 6 H, H(1), H(6), $^3J_{HCCP}$ = 14.1 Hz); 1.04 (t, 6 H, H(14), $^3J_{HCCP}$ = 7.0 Hz); 1.21 (d, 6 H, H(1), H(6), $^3J_{HCCP}$ = = 14.1 Hz); 1.34—1.55 (4 H, H (13)); 1.56—1.78 (m, 4 H, H(13)); 1.87 (s, 3 H, H(5)); 2.03 (s, 3 H, H(5)); 2.19 (s, 3 H, H(11)); 2.40 (s, 3 H, H(11)); 2.42 (d, 2 H, H(3), $^3J_{HCCP}$ = 12.5 Hz); 2.42 (d, 2 H, H(3), $^3J_{HCCP}$ = 14.0 Hz); 7.26 (br.d, 2 H, H(9), $^3J_{HCCH}$ = 8.1 Hz); 7.31 (br.d, 2 H, H(9), $^3J_{HCCH}$ = 8.1 Hz); 7.38 (s, 1 H, NH); 7.84 (br.d, 2 H, H(8), $^3J_{HCCH}$ = 8.3 Hz); 7.92 (br.d, 2 H, H(8), $^3J_{HCCH}$ = 8.2 Hz). Found (%): C, 57.00; H, 8.28; N, 6.97; P, 7.75; S, 8.00. $C_{19}H_{33}N_2O_3PS$. Calculated (%): C, 56.98; H, 8.30; N, 6.99; P, 7.73; S, 8.01.

**Acute toxicities of compounds 4a—e, 8a,b, and 9a—c** were determined on male and female white outbread mice upon intraperitoneal administration.[29] The research data were treated by the Behrens method.[30] Aqueous solutions of the drugs prepared under aseptic conditions just prior to introduction were injected to mice of six groups (six animals in each group). γ-Phorphorylated ketones **4a—e** and pyridinoyl hydrazones **8a**,**b** and **9a—c** are very soluble in water. The compounds were administered at equal doses (250 mg) at regular intervals between them. After dosing, the animal behavior was monitored and the number of died animals was recorded. The result-

ed data were treated by the graphic method. The results are given in Table 1.

Toxication symptoms in mice upon introduction of dialkyl(2-methyl-4-oxopent-2-yl)phosphine oxides **4—c** and mixed-type alkyl(aryl)phosphine oxide **4j** were of the same type: hurried breathing and suppression of motor activity. Dialkyl(2-methyl-2-oxopent-2-yl)phosphine oxides have no effect on orientating response and motor activity.

By the mode of toxic action, dimephosphone pyridinoyl hydrazones **8a**,**b** and, respectively, dialkyl(2-methyl-4-oxopent-2-yl)phosphine oxide pyridinoyl hydrazones **9a—c** differ slightly from each other. Several minutes after injection of **8a**,**b** at toxic letal doses, the mice showed respiratory depression and, then, decreased motor activity, hypomyotonia, and their limbs lost ability to hold the body, but animals did not change their position to the lateral one. In contrast to the above-mentioned compounds, dimephosphone upon action at the toxic doses is known[1—3] to cause the narcosis-like conditions of animals. In the case of hydrazones **9a—c**, there was also a dramatic decrease in the motor activity, rough breathing, and hypertonicity of tail. There was also no change in the position to the lateral one. In these cases, the animals also died during asphyxia.

**Comparative study of the toxicity of Isoniazide** (possible hydrolysis product of hydrazones **8a** and **9a,c)** was performed on six mice upon intraperitoneal administration at the dose of 150 mg $kg^{-1}$, since its $LD_{50}$ is 151±6.9 mg $kg^{-1}$ according to the data of Ref. 18. At the above-mentioned concentration, Isoniazide has a topical irritant effect causing wamble symptoms (stomach suction and periodic body bendings) and after 55—65 min there was a progress of clonicotonic contractions in mice.

To reveal the most promising compound among pyridinoyl hydrazones **9a—c**, we compared the ratio of their toxicities and acitivites, *i.e.* $LD_{50}$/MIC (see Tables 1 and 2). This ratio for Isoniazide used as the control is 356. For compounds **9c** and **9b**, this ratio is 340 and 430, respectively, *i.e.* very close to that of Isoniazide. The leader compound, *viz.*, (2-methyl-4-oxopent-2-yl)diethyl phosphine oxide isonicotinoyl hydrazone (**9a**) has a $LD_{50}$/MIC ratio of more than 3000. Hydrazones **9a—c** like dimephosphone pyridinoyl hydrazones **8a**,**b** in contrast to Isoniazide cause no symptoms of central nervous system malfunction upon acute intoxication of experimental animals.

**Materials and methods for the study of hepatotoxicity**. Compounds **8a**,**b** under study and Isoniazide were introduced to 30 white outbread mice with a weight of 120—180 g by the intraperitoneal route daily at the same time of the day (18-00—19-00) for 30 days. For each pyridinoyl hydrazone **8a**,**b**, the animals were divided into three groups with 10 rats in each group: group 1 received the corresponding hydrazone **8a** or **8b** at the dose of 250 mg $kg^{-1}$, group 2 received Isoniazide (100 mg $kg^{-1}$), and group 3 received distilled water (control). The solutions of drugs were prepared just prior to administration by dissolving crystals in sterile distilled water. The choice of the Isoniazide dose is caused by the analysis of literature regarding simulation of toxic Isoniazide hepatitis, where the above-mentioned dose (100 mg $kg^{-1}$)[31] is the most commonly used. The dose of 250 mg $kg^{-1}$ for hydrazones **8a**,**b** is equimolar with regard to the pyridinoylhydrazone fragment (possible formation of Isoniazide upon hydrolysis of pyridinoyl hydrazones). We performed continous monitoring for the general state of animals and their behavior. The weights of test animals were recorded every five days. At the end of experiment, the animals were sacrificied by bloodletting. The blood was sampled from each animal in a sequential order into two test tubes: centrifugal one (followed by centrifugation at 3000 rpm for 10 min to obtain serum) and vacutainers* with dry anticoagulating agent. The serum was used for biochemical blood assay and the blood with anticoagulant was used for a hematologic study. Once animals were sacrificed, their innards (liver, kidneys, spleen, and suprarenal bodies) were isolated to determine their relative weights (the ratio of organ weight (in mg) to the body weight (in g)). For paired organs, their average weight was calculated.

Among blood indices evaluated on a blood analyzer were red blood count, white blood count, platelet count, Hb level, and erythrocyte sedimentation rate. Among biochemical indices to be estimated were the activities of hepatic enzymes, such as alanine aminotransferase (ALT), aspartate transaminase (AAT), and γ-glutamyl transpeptidase (GTP), and levels of glucose, urea, creatinine, total bilirubin, and total protein. Statistical processing of the experimental data was performed using Student´s t-test.[30] The results obtained are given upon discussion of the hematological properties of pyridinoyl hydrazones **8a**,**b**.

**The study of mycobacteriostatic activities** of dialkyl(2-methyl-4-oxopent-2-yl)phosphine oxide pyridinoyl hydrazones **9a—c** towards the *M. tuberculosis* $H_{37}Rv$ strain was performed in the Kazan Tuberculosis Dispensary of the Ministry of Healthcare of the Republic of Tatarstan using a BACTEC MGIT 960 standard radiometric growth system (Becton Dickinson).

The content of a MGIT tube is a nutrient broth, which accelerates the growth of mycobacteria. The tube contains 7 mL of a Middlebrook 7H9 sterile digest broth, which is supplemented prior to its use by a BACTEC MGIT OADC enrichment additive (oleic acid, albumin, dextrose, and catalase). To avoid contamination, MGIT PANTA needs to be added.

In addition to the liqiud medium, the tube contains oxygen-free fluorochrome, *viz.*, ruthenium tris(4,7-diphenyl-1,10-phenanthroline) chloride pentahydrate, which is placed to the tube bottom and coated with silicone. During the bacterial growth, the free oxygen inside the tube is absorbed and replaced with carbon dioxide. As the free oxygen is consumed, inhibition of the fluorochrome terminates. The fluorescence becomes visible upon UV irradiation of the tube and recorded automatically by photodetectors integrated to the instrument. The fluorescence intensity is directly proportional to the oxygen consumption rate and recorded in growth units (GU). The BACTEC MGIT 960 system records the tube as positive if the number of survived microorganisms therein is 100 000 per 1 mL of the medium (GU > 75). The test tube was incubated at 37 °C followed by the analysis.

To determine the bacteriostatic effects of compounds **8—10** and reference substances (Isoniazide, nicotinoyl hydrazide, and tosyl hydrazide), the initial solutions were prepared in water and added to the MGIT tubes in the amounts providing the final concentration of 0.1 μg $mL^{-1}$ of the medium. The experiments were performed on the *M. tuberculosis* $H_{37}Rv$ strain by the serial dilution method. The test strain culture was weighed on an electronic balance, the weighing of 10 mg was placed to a porcelain mortar and grinded thoroughly, and a suspension of the culture was prepared according to the bacterial turbidity standard of 100 mln microbial bodies per 1 mL (10 units). The resulted suspen-

* Siliconized plastic (polyethyleneterephthalate) tubes with a dosed vacuum, for blood serum studies.

sions (0.1 mL) were inoculated to BBL tubes containing a liquid digest medium and a solution (5.0 mL) of the compound under study (for each dilution) and the tubes were placed into the instrument. The instrument recorded the presence of mycobacterial growth or its absence every day for the period of 11 or 41 days. The minimum inhibitory concentration (MIC) was determined as the least concentration at which the growth of *M. Tuberculosis* was delayed by 1 day compared to Isoniazide.

The individual tubes with a BACTEC MGIT 960 radiometric system medium containing a germ culture without chemical compounds and the tubes with saline solution were used as the control In all comparative experiments, the analogous studies using Isoniazide were performed. The MIC of Isoniazide towards the strain used is 0.5 μg $mL^{-1}$. All tubes were incubated at 37 °C in the instrument. The results are given in Table 2.

**Comparative experiments on the study of antimicrobial activities** of dimephosphone pyridinoyl hydrazones **8a**,**b**, Dimephosphone, and Isoniazide were performed *in vitro* on the bacterial strains *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella paratyphi B* and the fungus from *Candida* sp., *viz.*, *Candida albicans*. The compounds were diluted so that their final concentrations were 1 : 50, 1 : 100, 1 : 1000, and 1 : 10000 in tubes each containing 1 mL of a suspension of daily germ culture after mixing with 1 mL of each test solution. After 2 and 24 h, the cultures were inoculated using a platinum loop to tubes each containing 5 mL of a digest medium. The test tubes were incubated at 37 °C. The absence of growth in the test tube served as an indicator of the antimicrobial activity. The control was the inoculate of microbial cultures contacted with the analogous volume of distilled water. The resulted data are given in Tables 3 and 4

**Acute inflammatory response** was reproduced by subplantar injection of a 1% solution (0.1 mL) of carragheenin (sulfated polysaccharide from Irish sea moss).[32] The intensity of inflammatory response was estimated 3 h after induction of inflammation by the change in the pad volume (by plethysmometry). 80 white rats (seven test groups and one control group, 10 animals in each group in each experiment) were used. Aqueous solutions of the substances were injected intraperitoneally 1 h prior to injection of carragheenin at doses as follows: **8a** at 800, 400, and 200 mg $kg^{-1}$; dimephosphone at 240 mg $kg^{-1}$; solution containing the mixture of dimephosphone (240 mg $kg^{-1}$) and Isoniazide (160 mg $kg^{-1}$); Isoniazide at 42.5 mg $kg^{-1}$, and control (distilled water, 0.1 mL). The antiinflammatory effect estimated by the decrease in oedema was expressed as a percentage of control.

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