Reactions of 5(4*H*)-Oxazolones with Wittig-Horner Reagents: Novel Synthesis of Dioxopyrrolidinephosphonates and Phosphonoalkanoates with Anticipated Schistosomicidal Activity

Leila S. Boulos[a], Mona H. N. Arsanious[a], Ewies F. Ewies[a], and Fatem Ramzy[b]

[a] National Research Centre, Dokki, Cairo, Egypt
[b] Parasitology Department, Theodor Bilharz Research Institute, Imbaba, Egypt

Reprint requests to Prof. Dr. Leila S. Boulos. E-mail: leilagoubran@yahoo.com

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4-Benzylidene-2-phenyl-5(4*H*)-oxazolones react with Wittig-Horner reagents in the presence of alcoholic sodium alkoxide to give novel dioxopyrrolidinephosphonates, diethyl [3-(benzoylamino)-1-cyano-2-oxo-4-phenylbut-3-en-1-yl]phosphonate and phosphonoalkenoate derivatives. Both the phosphonate adducts and the ester products were also isolated from the reaction of oxazolones with triethyl phosphonoacetate using alcoholic sodium ethoxide and/or sodium hydride as a base. Possible reaction mechanisms are considered, and the structural assignments are based on analytical and spectroscopic results. The biological evaluation of the new compounds is reported.

Key words: 5(4*H*)-Oxazolones, Wittig-Horner Reagents, Dioxopyrrolidinephosphonates

Introduction

Oxazolones exhibit a wide spectrum of pharmacological activities including anticancer, antimicrobial, antifungal, and antagonistic [1 – 3] activity. 5(4*H*)-Oxazolones that are internal anhydrides of acyl amino acids make an important class of five-membered heterocycles which are also used for the synthesis of several organic molecules, including amino acids, peptides [4 – 6], antimicrobial or antitumor agents [7, 8]. This together with our interest in organophosphorus chemistry [9 – 14] triggered the synthesis of new phosphorus compounds incorporating such important units that may possibly lead to biological activity.

The present study deals with the reaction of Wittig-Horner reagents **1a** – **c** with 5(4*H*)-oxazolones **2a** – **e** (Fig. 1). The purpose of this study was to determine the preferential site of attack by these reagents and to synthesize new phosphonate adducts with anticipated schistosomicidal activity.

Results and Discussion

When 4-benzylidene-2-phenyl-5(4*H*)-oxazolone (**2a**) was treated with one equivalent of triethyl phosphonoacetate (**1a**) in the presence of alcoholic sodium ethoxide solution at r. t. for 4 h, adduct **3a** was isolated (Scheme 1). The structure of diethyl [1-benzoyl-5-benzylidene-2,4-dioxopyrrolidin-3-yl]-phosphonate (**3a**) is deduced from its spectroscopic data (Scheme 1, Experimental Section).

Similarly, compound **2a** reacts with trimethyl phosphonoacetate (**1b**), in the presence of methanolic sodium methoxide solution, to give a colorless crystalline compound formulated as dimethyl [1-benzoyl-5-benzylidene-2,4dioxopyrrolidin-3-yl]-phosphonate (**3b**) (Scheme 1). Structure **3b** was established on the basis of its spectral data (*cf.* Experimental Section). A possible explanation for the reaction course of **2a** with Wittig-Horner reagents **1a** and **1b** is shown in Scheme 1. Initial attack of the phosphonate carbanion **1a** and/or **1b** on the lactone carbonyl group in **2a** followed by azlactone ring opening (under the influence of the alkoxide present in the reaction medium) [15, 16] accompanied by elimination of the appropriate alcohol molecule from the intermediate **A**, gives the cyclic products **3a** and **3b** (Scheme 1).

Next, when **2a** was allowed to react with one equivalent of diethyl (cyanomethyl)phosphonate (**1c**), in the presence of alcoholic sodium ethoxide solution, adduct **4** was isolated in 75 % yield (Scheme 1). The structure of diethyl [3-(benzoylamino)-1-cyano-2-oxo-4-phenylbut-3-en-1-yl]phosphonate (**4**) is derived from its spectral data (*cf.* Experimental Section).

Furthermore, this study has been extended to include the reaction of triethyl phosphonoacetate (**1a**)

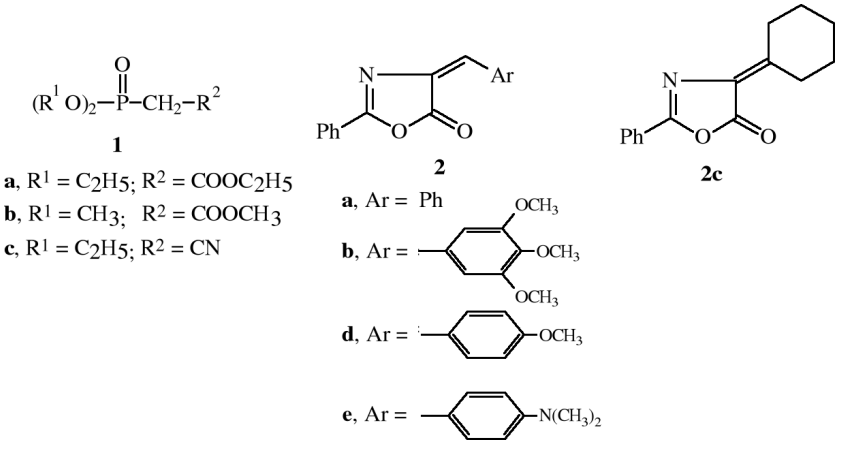
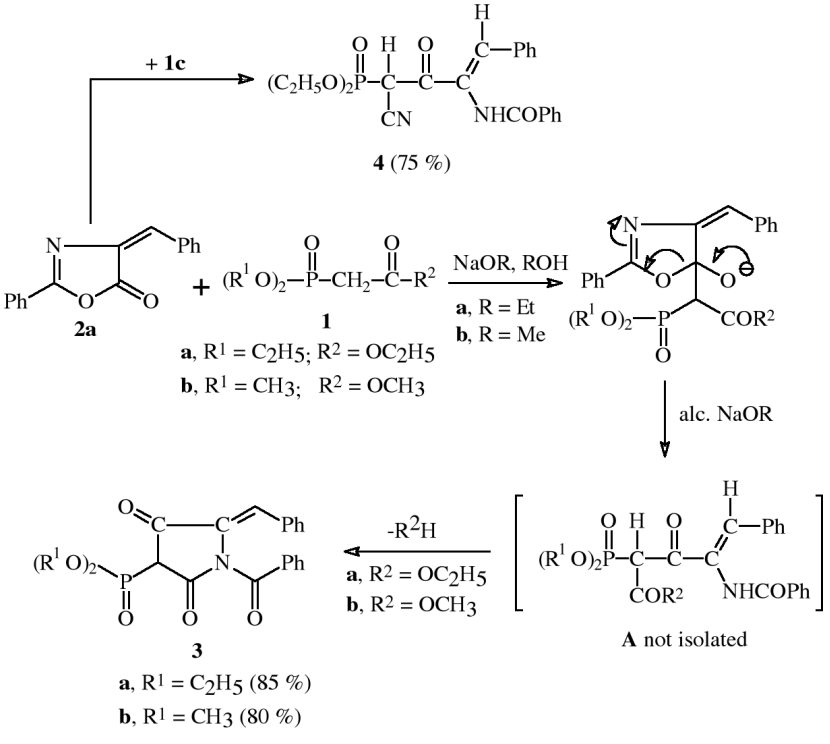


Fig. 1.

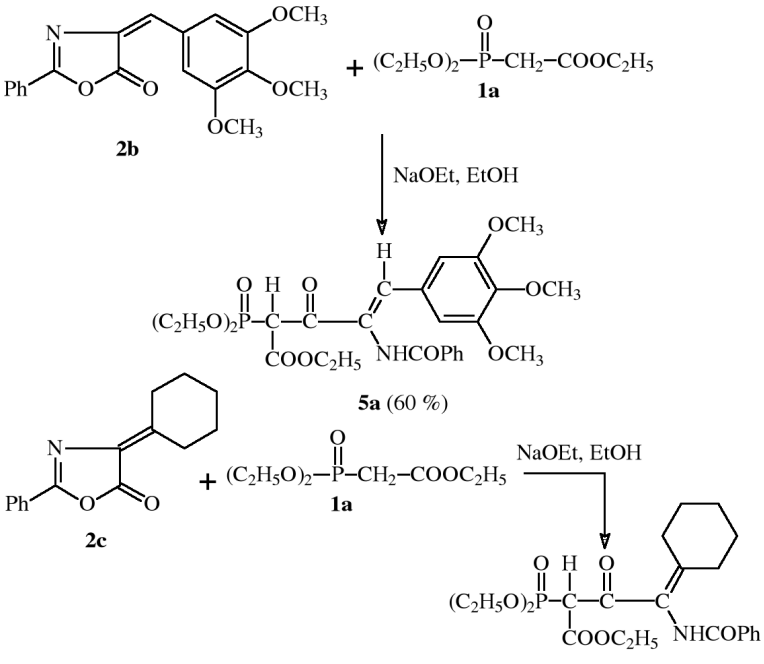


Scheme 1.

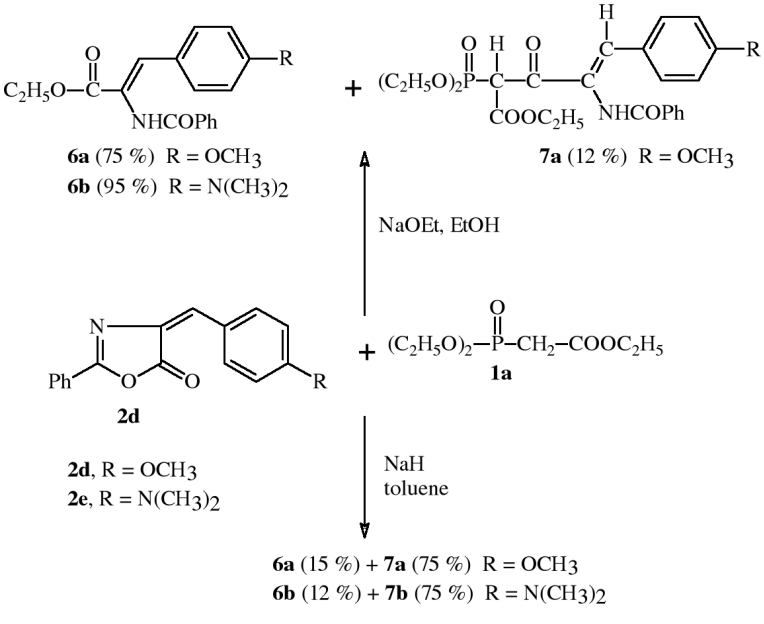
with **2b**–**c**. When **2b** was allowed to react with one equivalent of **1a**, in the presence of alcoholic sodium ethoxide solution at r. t. for 8 h, adduct **5a** was isolated (Scheme 2, Experimental Section).

Similarly, **2c** reacts with triethyl phosphonoacetate (**1a**) in alcoholic sodium ethoxide solution to give a colorless crystalline compound formulated as ethyl 1-(ethoxyphosphono)-3-benzamido-3-cyclohexyl-2-oxopropanoate (**5b**) (Scheme 2). The structure of the new compound **5b** is assigned on the basis of the full set of its spectral data (*cf.* Experimental Section). Compounds **4**, **5a**, and **5b** are possibly obtained through addition of the carbanion reagents **1c** and **1a** to the lactone carbonyl group of the azlactone ring followed by ring opening [15] due to the presence of a base to give the final products **4**, **5a**, and **5b**, respectively.

When 4-[(4-methoxyphenyl)methylidene]-2-phenyl-1,3-oxazol-5(4*H*)-one (**2d**) was allowed to react with one mole equivalent of **1a** in alcoholic sodium



Scheme 2.



Scheme 3.

ethoxide solution at r. t. for 5 h, two adducts formulated as compounds **6a** and **7a** were obtained (Scheme 3). Compound **6a** (major product) was formulated as the known ethyl 3-(4-methoxyphenyl)-2-[(phenylcarbonyl)amino]prop-2-enoate [16]. Carrying out the reaction using sodium hydride in dry toluene as a base instead of alcoholic sodium ethoxide, led to the formation of ethyl 1-(ethoxyphosphono)-3-benzamido-4-(4-methoxyphenyl)-2-oxobut-3-enoate (**7a**) in good yields together with ethyl 3-(4-methoxyphenyl)-2-[(phenylcarbonyl)amino]prop-2-enoate **(6a**) as the minor product (Scheme 3, Experimental Section).

Table 1. *In vitro* evaluation of compounds for schistosomicidal activity at 10 μg mL^{-1}.

Compound	1st day		2nd day		3rd day		4th day	
	No. of worms (12)		No. of worms (12)		No. of worms (12)		No. of worms (12)	
	Dead	Mortality (%)	Dead	Mortality (%)	Dead	Mortality (%)	Dead	Mortality (%)
3a	4	33.3	9	75	10	100	12	100*
3b	0	0	0	0	7	58.3	12	100*
5a	0	0	0	0	2	16.7	2	16.7
5b	0	0	0	0	3	25	3	25
6a	0	0	0	0	0	0	1	8.3
6b	0	0	0	0	1	8.3	5	41.7
PZQ (+ve) control	12	100	12	100	12	100	12	100
DMSO (−ve) control	0	0	0	0	0	0	0	0

We found that, when **2e** reacts with triethyl phosphonoacetate (**1a**) in alcoholic sodium ethoxide solution, ethyl-2-(benzoylamino)-3-[4-(dimethylamino)-phenyl]acrylate (**6b**) is the sole reaction product. When **2e** reacts with **1a** using sodium hydride in toluene instead of alcoholic sodium ethoxide, two products formulated as **6b** (12 %) and **7b** (75 %) were obtained (Scheme 3).

From the results of the present investigation, it can be concluded that the reactions of oxazolones **2** with Wittig-Horner reagents **1** lead to different products, depending on the nature of the phosphonate anion, the base used, as well as on the reactivity of the corresponding benzylidene moiety. We have noted that, when electron donating groups are attached to the arylidene ring as in compounds **2b** and **2c** the reaction was directed to form the phosphonate products **5a** and **5b**. However, when only an electron-donating system is attached to the arylidene ring as in compounds **2d** and **2e**, both the phosphonate adducts (**7a**, **7b**) and the ester products (**6a**, **6b**) were isolated. The yields of products **6a**, **b** and **7a**, **b** depend markedly on the substituents of the arylidene moiety as well as on the base used. The significance of these findings is not only the discovery of a new reactivity pattern for the Wittig-Horner reagents but also the establishment of a novel method for the synthesis of dioxopyrrolidine-phosphonate, phosphonopropanoate, and phosphonobutenoate derivatives.

Biological evaluation

The antischistosomal activity of the newly prepared organophosphorus and ester compounds was tested *in vitro* on *Schistosoma mansoni* worms. Schistosomiasis is a disease related to water contact in agriculture fields and is affecting millions of people in developing countries in tropical and subtropical parts of Africa, Asia, Central and South America [19]. It is a parasitic uncontrolled disease and represents an international as well as an important national health problem in Egypt. One of the most important methods for controlling schistosomiasis is through its intermediate host by using a chemical molluscidal agent. Therefore, the present work aimed to introduce oxazolone derivatives and test them for new antischistosomal activity *in vitro* on *Schistosoma mansoni* worms.

Compound	IC_{50}	IC_{90}
3a	5.74	7.78
3b	7.04	9.01
PZQ(+ve) control	0.4	0.64

Table 2. *In vitro* antischistosomal effect (IC_{50} and IC_{90}) of compounds compared to PZQ.

The organophosphorus and ester compounds were tested in a concentration of 10 μg mL^{-1} for *in vitro* bioactivity on viable *Schistosoma mansoni* mature worms in culture medium (RPMI 1640). Three replicates were used for each compound, and three pairs of worms, males and females equally represented, were placed in each vial containing the medium and the compound. The worms were considered dead when they did not show mobility for one minute. The viability ratio of worms was determined by calculating the number of dead worms relative to the total number of worms. The results were compared with negative dimethylsulfoxide (DMSO) and positive (praziquantel) controls. Praziquantel (PZQ) is the mainstay of schistosomiasis control programs worldwide, in other words an enormous investment in terms of money, manpower and training rests on the efficacy of a single synthetic compound.

The present data revealed that both compounds **3a** and **3b** induced *in vitro* antischistosomal activity (100 % mortality). Compounds **4a**, **4b**, **5a** and **5b** showed various degrees of lethal effect (8.3 – 41.7 % mortality) on worms at 10 μg mL^{-1} of the compounds after 4 d of exposure (Table 1).

The two active compounds were further subjected to determination of their inhibitory concentration (IC_{50} and IC_{90}) values (Table 2). The compounds **3a** and **3b** possess the strongest antischistosomal activity (IC_{50} values equal to or less than 10 μg mL^{-1}) (Table 2), and they are considered as promising bioactive compounds which deserve further investigation. Therefore, the present study is a trial to throw light on some new chemotherapeutic antischistosomal drugs.

Experimental Section

General remarks

Melting points were determined in open glass capillaries using an Electrothermal IA 9000 Series digital melting point apparatus (Electrothermal, Essex, U. K.) and are uncorrected. Elemental analyses were performed with an Elementar Vario EL, (Microanalytical Unit, National Research Centre, Cairo, Egypt) and were in good agreement (±0.2 %) with the calculated values. The IR spectra (KBr) were recorded on an FT IR-8201 PC spectrophotometer (Schimadzu, Japan). The ^{1}H and ^{13}C NMR spectra were measured on a Jeol 500 MHz spectrometer in [D$_6$]DMSO or $CDCl_3$. The chemical shifts were recorded relative to TMS. The ^{31}P NMR spectra were taken with a Varian CFT-20 instrument *vs*. external 85 % H_3PO_4 as the standard. The mass spectra (EI) were run at 70 eV on a Finnegan SSQ 7000 spectrometer (Thermo-instrument System Incorp., USA), *m/z* values are given in Dalton. TLC (silica gel, aluminum sheets 60 F_{254}, Merck, Darmstadt, Germany) was used for tracing the reactions. Wittig-Horner reagents **1a**–**c** and 5(4*H*)-oxazolones **2a**–**e** were prepared according to reported procedures [20–22].

*Reaction of triethyl phosphonoacetate (**1a**) with 4-benzylidene-2-phenyl-5(4H)-oxazolone (**2a**)*

A solution of sodium ethoxide (0.068 g, 1 mmol) in absolute ethanol (30 mL) was treated with an equimolar amount of triethyl phosphonoacetate (**1a**), and then the oxazolone **2a** (0.16 g, 1 mmol) was added. The resulting reaction mixture was stirred at r. t. for 4 h. Then the reaction mixture was poured into a small amount of water, extracted with ethyl acetate, and the extracts were dried over anhydrous sodium sulfate and the solvents evaporated under reduced pressure. The residual material was recrystallized from ethyl acetate to give **3a**. M. p. 130–132 °C (ethyl acetate). – IR (film): ν = 1100 (P–O), 1245 (P=O), 1622 (C=C, exocyclic), 1645, 1650 (2 amide C=O), 1712 (C=O) cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.23, 1.29 (2dt, 6H, 2 × CH_3, J_{HH} = 6.8, $^4J_{HP}$ = 4.1 Hz), 2.90 (d, 1H, J_{HP} = 21.20, CH–P), 4.16, 4.28 (2dq, 4H, 2 × CH_2–O–P, J_{HH} = 6.8, $^3J_{HP}$ = 5.9 Hz), 7.29 (s, 1H, C=CH exocyclic), 7.30–7.99 (m, 10H, 2 × Ph-H). – ^{13}C{H} NMR (125.76 MHz, $CDCl_3$): δ = 14.1, 14.3 (2 × CH_3, $^3J_{CP}$ = 17.9 Hz), 33.7, 34.8 (d, J_{CP} =133.53 Hz, C–P), 61.8, 62.7 (2 × CH_2, $^2J_{CP}$ = 27.5 Hz), 127.6–133.0 (Ar-C), 132.1, 133.9 (C=CH), 165.5 (NCOPh), 166.8 (COCHP, $^2J_{CP}$ = 25.5 Hz), 187.5 (C=O) [23]. – ^{31}P NMR: δ = 20.34 [24]. – MS (EI, 70 eV): *m/z* (%) = 427 (20) [M]$^+$, 295 (95) [M–CO–Ph–CO]$^+$. – $C_{22}H_{22}NO_6P$ (427.38): calcd. C 61.83, H 5.19, N 3.28, P 7.25; found C 61.90, H 5.25, N 3.35, P 7.30.

*Reaction of trimethyl phosphonoacetate (**1b**) with 4-benzylidene-2-phenyl-5(4H)-oxazolone (**2a**)*

A solution of sodium methoxide (0.054 g, 1 mmol) in absolute methanol (30 mL) was treated with an equimolar amount of **1b**, and then the oxazolone compound **2a** (0.16 g, 1 mmol) was added. The resulting reaction mixture was stirred at r. t. for 4 h and then poured into a small amount of water (2 mL) and extracted with ethyl acetate. The extracts were dried and the solvents evaporated under reduced pressure. The residue was crystallized from diethyl ether to give **3b**. M. p. 133–135 °C (diethyl ether). – IR (film): ν = 1135 (P–O), 1269 (P=O), 1579 (C=C, exocyclic), 1627 (amide C=O), 1715 (C=O) cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.77, 3.84 (2d, 6H, J_{HH} = 7.2, $^3J_{HP}$ = 10.9 Hz, 2 × CH_3O), 2.95, 3.03 (d, 1H, J_{HP} = 21.6 Hz, CH–P), 7.26 (s, 1H, exocyclic CH), 7.32–7.88 (m, 10H, 2 × Ph-H). – ^{13}C{H} NMR (125.76 MHz, $CDCl_3$): δ = 52.7, 52.8 (2 × CH_3O, $^2J_{CP}$ = 28.5 Hz), 54.3, 56.4 (d, J_{CP} = 130.4 Hz, C–P), 128.0–132.5 (Ar-C), 133.5–133.8 (C=C), 164.3 (NCOPh), 165.4 (d, $^2J_{CP}$ = 26.35 Hz, amide C=O), 190.3 (d, CO, $^2J_{CP}$ = 30.54 Hz). – ^{31}P NMR: δ = 22.95. – MS (EI, 70 eV): *m/z* (%) = 399 (5) [M]$^+$, 339 (100) [M–$(CH_3O)_2$]$^+$. – $C_{20}H_{18}NO_6P$ (399.33): calcd. C 60.15, H 4.54, N 3.51, P 7.76; found C 60.25, H 4.60, N 3.56, P 7.82.

*Reaction of diethyl (cyanomethyl)phosphonate (**1c**) with **2a***

A solution of sodium ethoxide (0.068 g, 1 mmol) in absolute ethanol (30 mL) was treated with **1c** (0.18 g, 1 mmol). Compound **2a** (0.16 g, 1 mmol) was added, and the reaction mixture was stirred at r. t. for 5 h, then poured into a small amount of water (2 mL). The mixture was extracted with chloroform, dried, and the solvents were evaporated under reduced pressure. The residue was washed with petroleum ether (b. p. 40–60 °C) and recrystallized from diethyl ether to give **4**. M. p. 136–137 °C (diethyl ether). – IR (film): ν = 1129 (P–O), 1252 (P=O), 1590 (C=CH), 1690 (*CO*Ph), 2230 (CN), 3235 (NH) cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.36, 1.39 (2dt, 6H, J_{HH} = 4.9, $^4J_{HP}$ = 4.85 Hz, 2 × CH_3), 2.85–2.89 (d, 1H, J_{HP} = 25 Hz, CH–P), 4.32, 4.35 (2dq, 4H, J_{HH} = 7.6, $^3J_{HP}$ = 5.15 Hz, 2 × *CH_2*–O–P), 6.89 (s, 1H, C=*CH*), 8.15 (s, 1H, NH, exchangeable with D_2O),

7.28 – 7.95 (m, 10H, Ph-H). – $^{13}C\{H\}$ NMR (125.76 MHz, [D_6]DMSO): δ = 14.7 (s, 2 × CH_3), 39.8 (d, J_{CP} = 133 Hz, C–P), 61.4 (s, 2 × CH_2), 109.5 (C=CH), 114.9 (CN), 128.2 – 133.7 (Ar-C), 165.4 (amide CO) 196.5 (d, C=O, $^2J_{CP}$ = 31.05 Hz). – ^{31}P NMR: δ = 14.96. – MS (EI, 70 eV): *m/z* (%) = 426 (8) $[M]^+$, 306 (15) $[M–NHCOPh]^+$, 295 (60) $[M–PhCO–CN]^+$, 249 (15) $[M–(C_2H_5O)_2P(O)–CHCN]^+$, 105 (100) $[PhCO]^+$. – $C_{22}H_{22}NO_6P$ (427.38): calcd. C 61.97, H 5.44, N 6.57, P 7.26; found C 62.03, H 5.49, N 6.62, P 7.32.

*General procedure for the reaction of triethyl phosphonoacetate (**1a**) with **2b**, **c***

A solution of sodium ethoxide (0.068 g, 1 mmol) in absolute ethanol (30 mL) was treated with an equimolar amount of the phosphonate **1a** (0.22 g,1 mmol). After 5 min, **2b** or **2c** (1 mmol) was added, and the reaction mixture was stirred at r. t. for 8 h. The solution was poured into water (1 – 2 mL), the mixture extracted with ethyl acetate (3 × 20 mL), the solution was dried, and the solvents were evaporated under reduced pressure. The residue was recrystallized to give adducts **5a**, **b**.

*Adduct **5a**:* M. p. 105 – 107 °C (ethyl acetate). – IR (film) ν = 1127 (P–O), 1239 (P=O), 1654, 1660 (amide C=O), 1711 (C=C), 1730 (CO), 3271 (broad band, NH) cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.29, 1.30 (2 × dt, 6H, J_{HH} = 6.8, $^4J_{HP}$ = 3.75 Hz, 2 × CH_3), 1.31 (t, 3H, ester CH_3), 2.88, 2.93 (d, 1H, J_{HP} = 26.15 Hz, CH–P), 3.7 (s, 9H, 3 × OCH_3), 4.26, 4.27 (2 × dq, 4H, J_{HH} = 6.8, $^3J_{HP}$ = 5.1 Hz, 2 × CH_2–O–P), 6.74 (s, 1H, C=*CH*), 7.20 – 7.52 (m, 7H, 2 × Ph-H), 7.86 (s, 1H, NH, exchangeable with D_2O). – $^{13}C\{H\}$ NMR (125.76 MHz, $CDCl_3$): δ = 14.2 (s, 3 × CH_3), 32.5, 33.0 (d, J_{CP} = 125 Hz, C–P), 55.6 (s, 3 × OMe), 60.0, 61.9 (s, 3 × CH_2), 125.9, 138.7 (C=CH), 127.5 – 133.5 (m, Ar-C), 164.9, 165.6, 166.0 (3 × C=O, $^2J_{CP}$ = 28.27 Hz). – ^{31}P NMR: δ = 22.3 ppm. – MS (EI, 70 eV): *m/z* (%) = 563 (10) $[M]^+$, 486 (40) $[M–Ph]^+$, 458 (20) $[M–COPh]^+$, 443 (15) $[M–NHCOPh]^+$, 386 (85) $[M–(3OCH_3C_6H_2)]^+$, 105 (100) $[PhCO]^+$. – $C_{27}H_{34}NO_{10}P$ (563.53): calcd. C 57.55, H 6.08, N 2.49, P 5.50; found C 57.60, H 6.14, N 2.55, P 5.56.

*Adduct **5b**:* M. p. 106 – 108 °C (ethyl acetate). – IR (film): ν = 1032 (P–O), 1219 (P=O), 1640, 1648 (amide C=O), 1620 (C=C), 1722 (ester CO), 3324 (broad band NH) cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.09, 1.13, 1.57 (cyclohexylidene, CH_2), 1.17, 1.19 (2 × dt, 6H, J_{HH} = 7, $^4J_{HP}$ = 4 Hz, 2 × CH_3), 1.20 (t, 3H, ester CH_3), 3.04, 3.15 (d, 1H, J_{HP} = 21.4 Hz, CH–P), 4.04, 4.05 (2 × dq, 4H, J_{HH} = 7, $^3J_{HP}$ = 5.2 Hz, 2 × CH_2–O–P), 4.1 (q, 2H, ester CH_2), 7.48 – 7.91 (m, 5H, Ar-H), 9.7 (s, 1H, NH, exchangeable with D_2O). – $^{13}C\{H\}$ NMR (125.76 MHz, [D_6]DMSO): δ = 13.8, 14.0 (2 × CH_3, $^3J_{HP}$ = 14.3 Hz), 16.09 (ester CH_3), 27.5 – 32.4 (cyclohexylidene C), 33.0, 34.1 (d, J_{CP} = 137.5 Hz, C–P), 59.7, 60.9 (ester CH_2), 61.8, 61.9 (2 × CH_2–O–P, $^2J_{CP}$ = 12.5 Hz), 120.3, 146.2 (C=CH), 127.4 – 133.7 (Ar-C), 164.7 (amide, C=O), 165.6 (ester C=O), 165.7 (COCH). – ^{31}P NMR: δ = 23.02. – MS (EI, 70 eV): *m/z* (%) = 465 (15) $[M]^+$, 388 (10) $[M–Ph]^+$, 345 (30) $[M–NHCOPh]^+$, 287 (100) $[(C_2H_5)_2P(O)CHCO]^+$. – $C_{23}H_{32}NO_7P$ (465.47): calcd. C 59.35, H 6.93, N 3.01, P 6.65; found C 59.44, H 7.10, N 3.07, P 6.71.

*General procedure for the reaction of triethyl phosphonoacetate (**1a**) with **2d**, **2e***

a) In the presence of alcoholic sodium ethoxide

Sodium ethoxide (0.068 g, 1 mmol) in absolute alcohol (30 mL) was added to a solution of an equimolar amount of **1a**, and then the starting material (**2d**, **2e**) (1 mmol) was added. The reaction mixture was stirred for 8 h at r. t., then poured into water (1 – 2 mL). The mixture was extracted with ethyl acetate (3 × 20 mL), and the extracts were dried over anhydrous sodium sulfate and the solvents evaporated under reduced pressure. The residue was subjected to silica gel column chromatography to give the products **6a**, **7a** and **5b**.

*Adduct **6a**:* M. p. 99 – 101 °C (ethyl acetate), eluent (acetone : petroleum ether, 5 : 95, v : v). – IR (film) ν = 1609 (C=C), 1644 (amide CO), 1707 (ester CO), 3290 (broad band, NH) cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.31 (t, 3H, CH_3), 3.74 (s, 3H, OMe), 4.29 (q, 2H, CH_2), 6.82 (s, 1H, C=CH), 7.24 – 7.86 (m, 9H, 2 × Ph-H), 8.2 (s, 1H, NH, exchangeable with D_2O). – $^{13}C\{H\}$ NMR (125.76 MHz, $CDCl_3$): δ = 13.91 (Me), 40.6 (OCH_3), 61.2 (CH_2), 127.9 – 131.6 (Ar-C), 164.3 (amide CO), 165.2 (ester CO), 121.1, 134.2 (C=CH). – MS (EI, 70 eV): *m/z* (%) = 325 (20) $[M]^+$, 280 (35) $[M–C_2H_5O]^+$, 220 (10) $[M–COPh]^+$, 105 (100) $[PhCO]^+$. – $C_{19}H_{19}NO_4$ (325.35): calcd. C 70.14, H 5.89, N 4.31; found C 70.21, H 5.95, N 4.38.

*Adduct **7a**:* M. p. 198 – 201 °C, eluent (acetone : petroleum ether) (20 : 80, v/v). – IR (film): ν = 1109 (P–O), 1252 (P=O), 1622 (C=C), 1640, 1645 (amide C=O), 1720 (ester, CO), 3310 (broad band, NH) cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.03 – 1.6 (2 × dt, 6H, J_{HH} = 6.9, $^4J_{HP}$ = 5.2 Hz, 2 × CH_3), 1.11 (t, 3H, ester CH_3), 2.6, 2.7 (d, 1H, J_{HP} = 20.1 Hz, CH–P), 3.6 (s, 3H, OMe), 3.65, 3.85 (2 × dq, 4H, J_{HH} = 6.9, $^3J_{HP}$ = 6 Hz, 2 × CH_2), 3.85 (q, 2H, ester CH_2), 6.2 (s, 1H, C=CH), 7.1 – 7.8 (m, 9H, 2 × Ph-H), 9.04 (s, 1H, NH exchangeable with D_2O). – $^{13}C\{H\}$ NMR (125.76 MHz, $CDCl_3$): δ = 7.6, 14.8 (3 × CH_3), 54.4 (d, J_{CP} = 132 Hz, C–P), 61.6 (3 × CH_2), 40.6 (OMe), 121.3, 134.6 (C=CH), 127.4, 133.7 (Ar-C), 163.6 (amide C=O), 196.5, 207.1 (2 × CO). – ^{31}P NMR: δ = 21.53. – MS (EI, 70 eV): *m/z* (%) = 503 (15) $[M]^+$, 426 (10) $[M–Ph]^+$, 398 (25) $[M–COPh]^+$, 383 (20) $[M–NHCOPh]^+$, 279 (65) $[M–(C_2H_5O)_2P(O)CH–COOC_2H_5]^+$, 105 (100) $[PhCO]^+$. – $C_{25}H_{30}NO_8P$ (503.48): calcd. C 59.64, H 6.01,

N 2.78, P 6.15; found C 59.73, H 6.07, N 2.85, P 8.21.

*Adduct **6b**:* M. p. 96 – 98 °C (ethyl acetate). – IR (film): ν = 1609 (amide CO), 1644 (C=C), 1707 (ester CO), 3295 (broad band NH) cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 1.23 (t, 3H, CH_3), 2.27 (s, 6H, $N(CH_3)_2$), 4.21 (q, 2H, CH_2), 6.67 (s, 1H, C=CH), 7.39 – 7.81 (m, 9H, 2 × Ph-H), 8.1 (s, 1H, NH, exchangeable with D_2O). – $^{13}C\{H\}$ NMR (125.76 MHz, $CDCl_3$): δ = 14.15 (Me), 39.6 ($N(CH_3)_2$), 60.26 (CH_2), 120.50, 134.8 (C=CH), 121.3 – 131.6 (Ar-C), 165.3, 165.8 (2 × CO). – MS (EI, 70 eV): *m/z* (%) = 338 (5) $[M]^+$, 309 (10) $[M–C_2H_5]^+$, 292 (30) $[M–OC_2H_5]^+$, 264 (15) $[M–COOC_2H_5]^+$, 105 (100) $[PhCO]^+$. – $C_{20}H_{22}N_2O_3$ (338.40): calcd. C 70.99, H 6.55, N 8.28; found C 71.06, H 6.60, N 8.34.

b) In the presence of sodium hydride

Triethyl phosphonoacetate (**1a**) (0.068 g, 1 mmol) was dissolved in very dry toluene (25 mL), and then sodium hydride (0.024 g, 1 mmol) was added carefully with stirring. Then, the starting material (**2d**, **e**) (1 mmol) was added to the mixture which was refluxed for 8 h. The volatile materials were evaporated under reduced pressure to give **6a**, **b** and **7a**, **b**. Compounds **6a**, **b** and **7a** are the same products as those obtained in the presence of sodium ethoxide.

*Adduct **7b**:* M. p. 136 – 138 °C. Eluent (acetone : petroleum ether, 20 : 80, v : v). – IR (film): ν = 1137 (P–O), 1223 (P=O), 1609 (C=C), 1640, 1644 (amide C=O), 1707 (ester CO), 3309 (broad band, NH) cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 1.23, 1.24 (2 × t, 6H, J_{HH} = 7.45, $^4J_{HP}$ = 3.7 Hz, 2 × CH_3), 1.31 (t, 3H, ester CH_3), 2.26 (s, 6H, $N(CH_3)_2$), 2.71, 2.72 (d, 1H, J_{HP} = 20.19 Hz, CH–P), 4.13, 4.18 (2 × q, 4H, J_{HH} = 7.45, $^3J_{HP}$ = 4.5 Hz, 2 × CH_2), 4.21 (q, 2H, ester CH_2), 6.6 (s, 1H, C=CH), 6.84 – 7.79 (m, 9H, 2 × Ph-H), 7.91 (s, 1H, NH exchangeable with D_2O). – $^{13}C\{H\}$ NMR (125.76 MHz, $CDCl_3$): δ = 14.6, 14.8 (3 × CH_3), 39.8 ($N(CH_3)_2$), 52.8 (d, J_{CP} = 133.2 Hz, C–P), 61.6, 61.8 (3 × CH_2), 114 (C=C), 164.6, 165.3 (amide C=O), 196 (ester CO), 120.59 – 134.80 (Ar-C). – ^{31}P NMR: δ = 23.2. – MS (EI, 70 eV): *m/z* (%) = 516 (10) $[M]^+$, 472 (15) $[M–N(CH_3)_2]^+$, 439 (30) $[M–Ph]^+$, 411 (25) $[M–COPh]^+$, 396 (40) $[M–NHCOPh]^+$, 292 (30) $[M–(C_2H_5O)_2P(O)CHCOO\ C_2H_5]^+$, 105 (100) $[PhCO]^+$. – $C_{26}H_{33}N_2O_7P$ (516.52): calcd. C 60.46, H 6.44, N 5.42, P 6.00; found C 60.53, H 6.48, N 5.48, P 6.07.

Biological evaluation of the tested compounds

Adult worms of *Schistosoma mansoni* (Egyptian strain) were obtained by infecting Syrian golden hamsters (*Mesocricetus auratus*) by percutaneous infection of 350 cercariae per animal, freshly shed from infected *Biomphalaria alexandrina* snails [17]. The animal experiments were carried out according to the internationally valid guidelines in an institution coping with biological ethics (Theodor Bilharz Research Institute) [18]. Worms were obtained, by portomesenteric perfusion, 45 d post infection using citrated saline (7.5 g L^{-1} Na citrate + 8.59 g L^{-1} NaCl).

The worms in small sterilized sieves were washed three times with phosphate buffer (pH = 7.4), then three times with RPMI-1640 medium with L-glutamine containing antibiotics (300 μg Streptomycin, 300 units Penicillin and 160 μg Gentamycin) + 20 % foetal calf serum, inside a sterilization laminar flow. Then the worms were poured into a small petri dish. Compound samples were kept at −20 °C in the dark.

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