Synthesis of New Bisphosphonate and Bisphosphonic Acid Derivatives and Heterocyclic and Dialkylcarbamoyl Oxazolone Derivatives with Anticancer and Antischistosomal Activity

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The reaction of 5(4*H*)-oxazolones with tetraethyl methanediylbis(phosphonate) afforded new 1,1-bisphosphonate and 1,1-bisphosphonic acid derivatives. Moreover, 5(4*H*)-oxazolones reacted with Wittig reagents to give the corresponding heterocyclic products. Treatment of 5(4*H*)-oxazolones with trisdialkylaminophosphanes gave the corresponding *N*-(1-dialkylcarbamoyl)-2-phenylvinylbenzamides. Mechanisms accounting for the formation of the new products are discussed. The biological activity of the newly synthesized compounds was also examined.

Key words: 1,1-Bisphosphonate, 1,1-Bisphosphonic Acid, Wittig Reagents, Oxazolones, Anticancer Activity, Antischistosomal Activity

Introduction

Bisphosphonates, especially the nitrogen-containing analogs, have been reported to be potent inhibitors of bone resorption and highly selective bone targeting agents [1 – 3]. Moreover, nitrogen-, sulfur- or oxygen-containing five- and six-membered heterocyclic compounds have enormous significance in the field of medicinal chemistry. Oxazolones play a very vital role in the manufacturing of various biologically active drugs with analgesic [4], anti-inflammatory [5], antidepressant [6], anticancer [7], antimicrobial, antidiabetic, and anti-obesity activity [8, 9]. In view of this, and in continuation of our work in organophosphorus chemistry [10 – 16], it was of considerable interest to synthesize new 1,1-bisphosphonate, 1,1-bisphosphonic acid and heterocyclic oxazolone derivatives to obtain potent biologically active compounds.

The present study deals with the reaction of 5(4*H*)-oxazolones **1a** – **e** with tetraethyl methanediylbis(phosphonate) (**2**), phosphonium ylides **3a** – **c**, and trisdialkylaminophosphanes **4a** – **b** (Fig. 1).

Results and Discussion

Chemistry

4-Benzylidene-2-phenyl-5(4*H*)-oxazolone (**1a**) was treated with two molar equivalents of tetraethyl methanediylbis(phosphonate) (**2**) in anhydrous dimethylformamide (DMF) solution using excess NaH as a base. After the reaction took place, dilute aqueous HCl was added to the reaction mixture at −5 °C. The crude
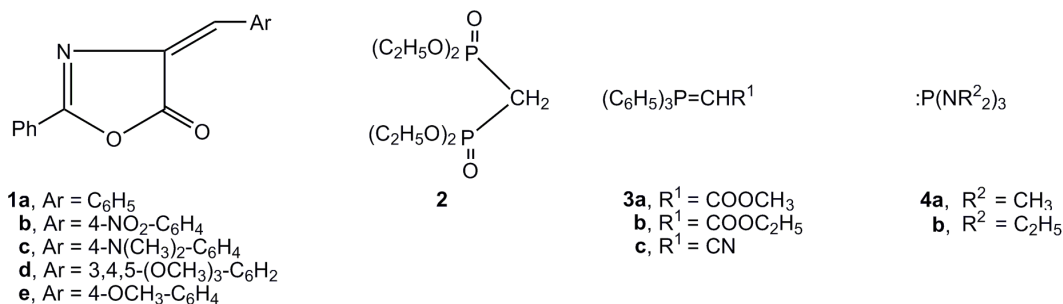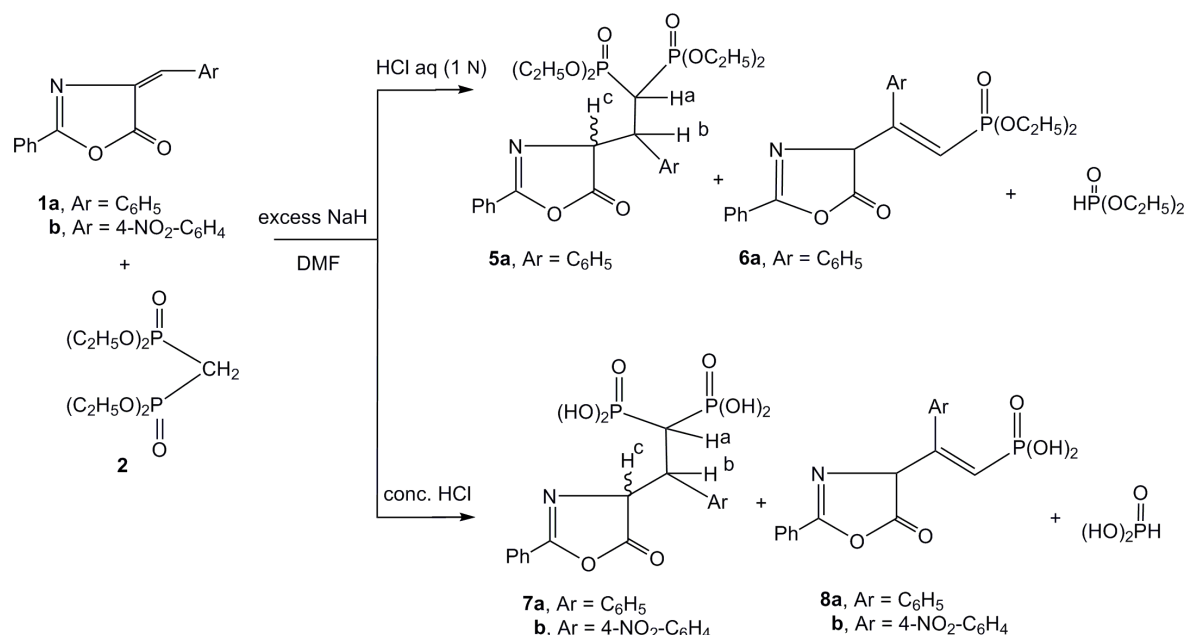

1a, Ar = C_6H_5
 b, Ar = 4-NO_2-C_6H_4
 c, Ar = 4-N(CH_3)$_2$-C_6H_4
 d, Ar = 3,4,5-(OCH_3)$_3$-C_6H_2
 e, Ar = 4-OCH_3-C_6H_4

2

3a, R^1 = $COOCH_3$
 b, R^1 = $COOC_2H_5$
 c, R^1 = CN

4a, R^2 = CH_3
 b, R^2 = C_2H_5

Fig. 1.

Scheme 1.

product was easily separated by solvent extraction and purified by column chromatography to give 1,1-bis-phosphonate **5a** as the major product (45 % yield) together with the phosphonate adduct **6a**. Diethylphosphite was also identified (by TLC) (Scheme 1).

The most important feature of structure **5a** is the presence of two signals at δ_P = 23.16 (s) and 25.16 ppm (s) in its ^{31}P NMR spectrum. Moreover, the ^{1}H NMR spectrum of **5a** (500 MHz) revealed three types of methine protons with different chemical shifts at δ_H = 1.92 (ddd, *J* = 7 and 21.6 Hz, 1H, CH-P$_2$), 3.45, 3.47 (2 dd each set with J_{HH} = 7.8 Hz, $^3J_{HP}$ = 12 Hz, 1H, *CH*CH-P$_2$) and 4.38 ppm (dd, *J* = 7 and 6 Hz, 1H, cyclic CH). Also, ^{13}C NMR spectroscopy confirmed that both phosphorus atoms are attached to the same quaternary carbon atom [δ_C = 25.3 (dd, J_{CP} = 130 and 132 Hz, C-P$_2$)]. The structure of **5a** has also been assigned on the basis of elemental analysis and mass spectral data (*cf.* Experimental Section). Compound **6a** (minor product, 25 % yield) was identified as diethyl 2-(4,5-dihydro-5-oxo-2-phenyloxazol-4-yl)-2-phenylvinylphosphonate on the basis of its IR, ^{1}H, ^{13}C, ^{31}P NMR and mass spectral data (*cf.* Experimental Section).

On the other hand, performing the reaction of **1a** with **2** in concentrated hydrochloric acid instead of dilute acid led to the formation of bisphosphonic acid **7a** as the main product (55 % yield), vinylphosphonic

acid **8a** (25 % yield) and phosphorus acid (Scheme 1). The structural assignments for compounds **7a** and **8a** are based upon elemental and spectroscopic data (IR, ^{1}H, ^{13}C, ^{31}P NMR and mass spectra) (*cf.* Experimental Section).

Similarly, when 1,3-oxazol-5(4*H*)-one **1b** was reacted with bis(phosphonate) **2** using concentrated hydrochloric acid, bisphosphonic acid **7b** (the major product) and phosphonic acid **8b** were obtained. Phosphorus acid was also identified (TLC) in the reaction medium (Scheme 1). Structure elucidation of **7b** and **8b** was derived from their spectral data (*cf.* Experimental Section).

A possible explanation for the course of the reaction of **1a** and **1b** with **2** is shown in Scheme 1. 1,1-bis-phosphonate **5a** is possibly formed through a Michael addition [3, 17] of **2** to oxazolone **1a**. Moreover, the phosphonate adduct **6a** can result from a simple acid catalyzed elimination of diethyl phosphite from **5a** (Scheme 1).

On the other hand, the substituted 1,1-bisphosphonic acids **7a,b** and vinylphosphonic acids **8a,b** were easily obtained *via* acid hydrolysis with concentrated HCl, which enabled the phosphonate ester to be removed to give directly **7a,b** and **8a,b**, respectively, with elimination of phosphorous acid (Scheme 1). Previous work has reported the synthesis of substituted 1,1-bisphosphonic acid through hydrolysis of the cor-
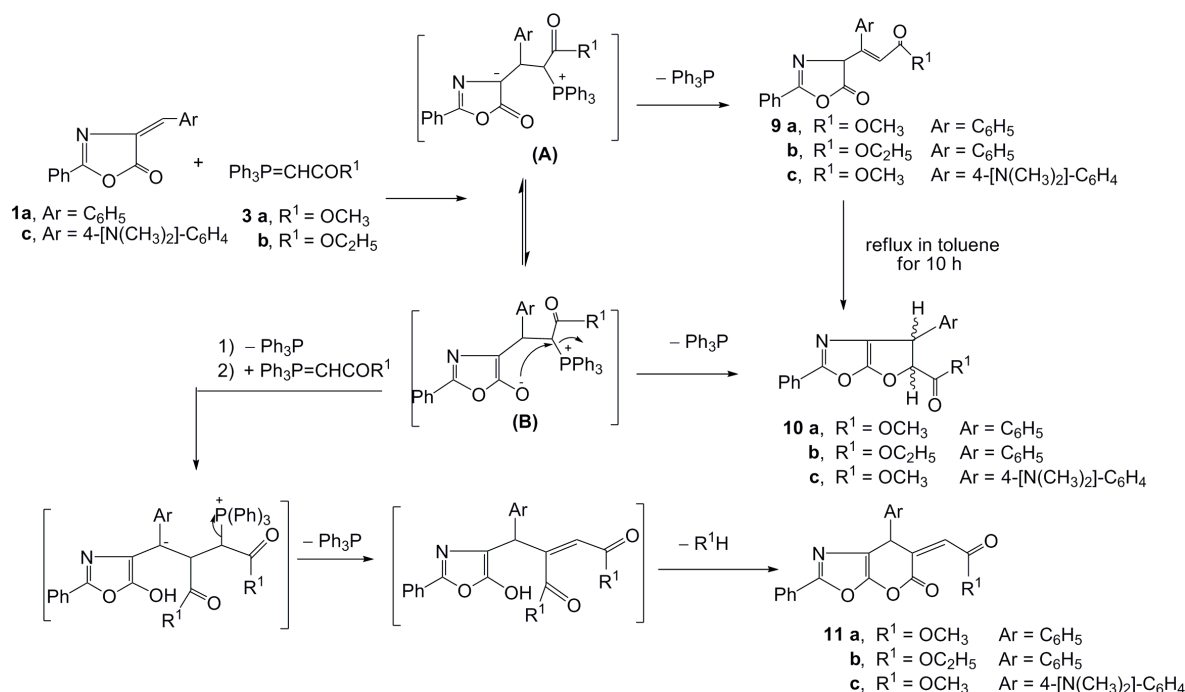
Scheme 2.

responding bisphosphonate product with concentrated hydrochloric acid [18]. The present study showed that substituted 1,1-bisphosphonic acids can be synthesized directly from the reaction of 4-arylidene-2-phenyl-5(4H)-oxazolone derivatives **1a, b** with bisphosphonate **2** using concentrated hydrochloric acid (*cf.* Experimental Section).
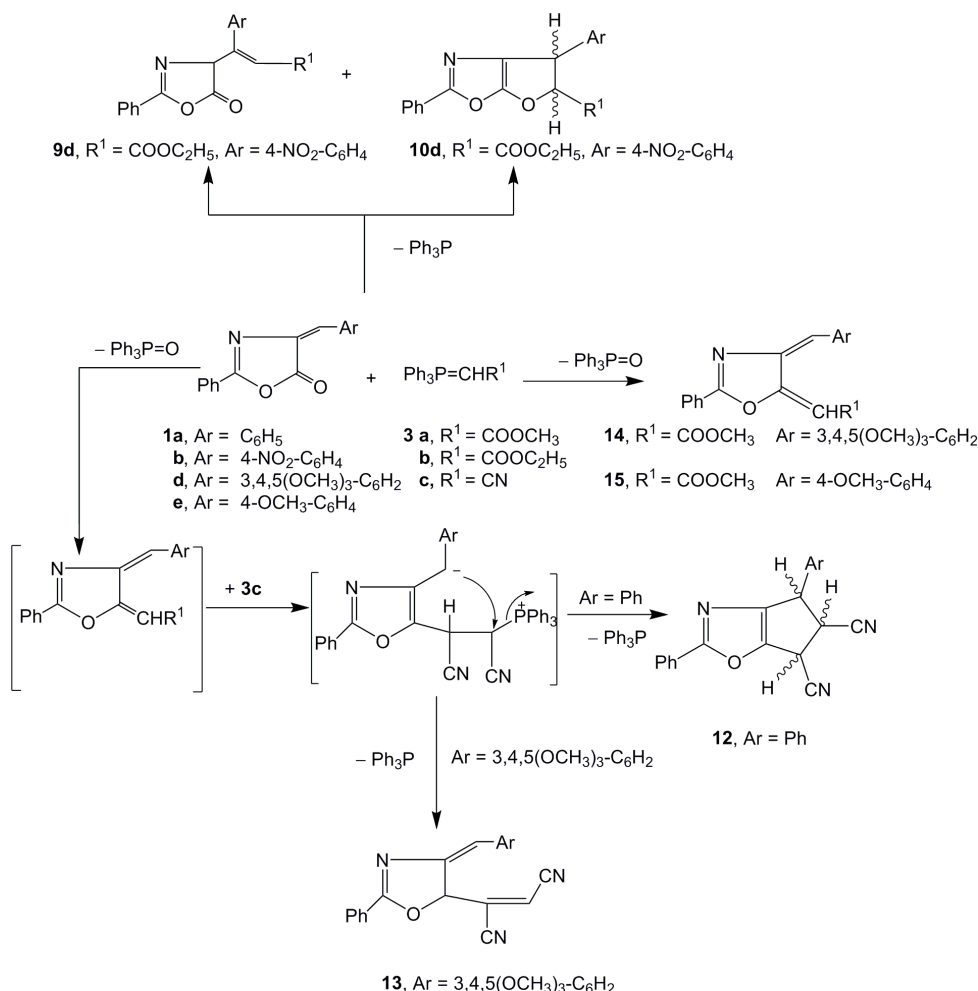
Worthy to mention is that only one isomer of bisphosphonate **5a** and the bisphosphonic acids **7a, b** was isolated, suggested as *syn* configuration. The assigned *syn* configuration for these products, although not established with certainly, is supported by an inspection of a model drawn by Newman projection as well as by the chemical shifts and coupling constants of the exocyclic methine protons, and especially CH^b-CH^a-P, in their ^1H NMR spectra. The observed coupling constants of H^b ($J_{H^bH^a} = J_{H^bH^c}$, *ca.* 6–8 Hz) indicate the *syn* form, rather than the *anti* configuration which would give rise to larger coupling constants (9–15 Hz).

Furthermore, this study was extended to include the behavior of oxazolones **1** towards phosphonium ylides **3a–c** and tris(dialkylamino)phosphanes **4a, b** to determine the preferential site of attack. We have found that when oxazolone **1a** was allowed to react with two molar equivalents of ylides **3a** and **3b** in re-

fluxing toluene, products **9a, b**, **10a, b**, **11a, b**, triphenylphosphane, and unchanged oxazolone **1a** were isolated. Carrying out the reaction using three instead of two molar equivalents of phosphonium ylides **3a, b** under the same reaction conditions led to the formation of compounds **9a, b**, **10a, b**, **11a, b** in good yields. Triphenylphosphane was also isolated in each case (Scheme 2).

Similarly, oxazolone **1c** reacted with three molar equivalents of **3a**, to give products **9c, 10c** and **11c**, respectively. Triphenylphosphane was also isolated (Scheme 2). Compounds **9a–c**, **10a–c** and **11a–c** were obtained chromatographically pure by silica gel column chromatography and possess sharp melting points. Their structures were assigned based on elemental analysis, IR, ^1H, ^{13}C NMR and mass spectral data (*cf.* Experimental Section).

A possible explanation for the course of the reaction of **1a** and **1c** with the Wittig reagents **3a, b** is shown in Scheme 2. Formation of products **9a–c** presumably occurs *via* Michael addition of the ylide species **3a, b** to the active methine carbon atom in oxazolones **1a** and **1c** to afford the intermediate **A**. Since triphenylphosphane is a good leaving group [19, 20], Hofmann elimination [21] of triphenylphosphane followed by proton transfer leads to the final prod-

9d, R^1 = COOC$_2$H$_5$, Ar = 4-NO$_2$-C$_6$H$_4$ **10d**, R^1 = COOC$_2$H$_5$, Ar = 4-NO$_2$-C$_6$H$_4$

1a, Ar = C$_6$H$_5$
  b, Ar = 4-NO$_2$-C$_6$H$_4$
  d, Ar = 3,4,5(OCH$_3$)$_3$-C$_6$H$_2$
  e, Ar = 4-OCH$_3$-C$_6$H$_4$

3 a, R^1 = COOCH$_3$
  b, R^1 = COOC$_2$H$_5$
  c, R^1 = CN

14, R^1 = COOCH$_3$ Ar = 3,4,5(OCH$_3$)$_3$-C$_6$H$_2$
15, R^1 = COOCH$_3$ Ar = 4-OCH$_3$-C$_6$H$_4$

12, Ar = Ph
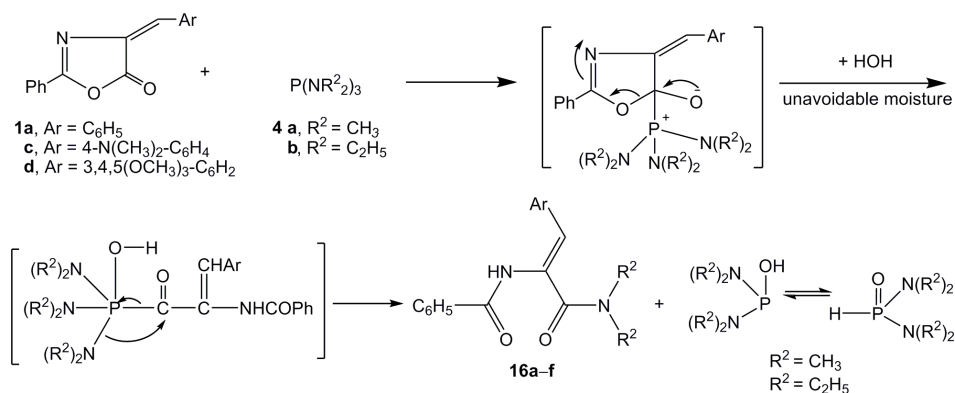
13, Ar = 3,4,5(OCH$_3$)$_3$-C$_6$H$_2$

Scheme 3.

ucts **9a–c**. Moreover, compounds **10a–c** can be obtained by protonation of **1a, c** at the carbanion center of ylides **3a, b** to give the transient intermediate (**B**) followed by elimination of triphenylphosphane to afford **10a–c**. It is worthy to mention that when compounds **9a–c** were heated in toluene for 10 h, the bicyclic compounds **10a–c** were obtained in 85 % yield. Compounds **11a–c** may be regarded as products of an intramolecular Wittig reaction. Such addition-cyclization products apparently result from the addition of another ylide molecule **3a, b** to the intermediate **B** followed by elimination of triphenylphosphane and the appropriate alcohol molecule under the applied reaction conditions to give the cyclic products **11a–c** (Scheme 2).

Next, when oxazolone **1b** was allowed to react with two molar equivalents of **3b**, products **9d** and **10d**

were obtained in good yield. Triphenylphosphane was also isolated and identified (Scheme 3). Moreover, when **1a** and **1d** were allowed to react with two molar equivalents of (triphenyl-λ^5-phosphanylidene)acetonitrile (**3c**) in refluxing toluene, bicyclic dicarbonitrile **12**, and 2-[2-phenyl-4-but-2-enedinitrile **13** were obtained. Triphenylphosphane and triphenylphosphane oxide were also isolated from the reaction mixture (Scheme 3). The structures of **9d**, **10d**, **12**, and **13** have been confirmed on the basis of the full set of their spectral data (*cf.* Experimental Section). Also, when compound **1d** reacted with one molar equivalent of **3a** in refluxing toluene, methyl oxazol-5(4*H*)-ylidene)acetate **14** was obtained in 85 % yield. Triphenylphosphane oxide was also isolated.

Similarly, oxazolone **1e** reacted with one molar equivalent of **3a** in refluxing toluene, to give methyl

1a, Ar = C$_6$H$_5$
c, Ar = 4-N(CH$_3$)$_2$-C$_6$H$_4$
d, Ar = 3,4,5(OCH$_3$)$_3$-C$_6$H$_2$

4 a, R^2 = CH$_3$
b, R^2 = C$_2$H$_5$

+ HOH
unavoidable moisture

16a–f

R^2 = CH$_3$
R^2 = C$_2$H$_5$

Scheme 4.

16a, Ar = C$_6$H$_5$ R^2 = CH$_3$
b, Ar = C$_6$H$_5$ R^2 = C$_2$H$_5$
c, Ar = 4-N(CH$_3$)$_2$-C$_6$H$_4$ R^2 = CH$_3$
d, Ar = 4-N(CH$_3$)$_2$-C$_6$H$_4$ R^2 = C$_2$H$_5$
e, Ar = 3,4,5(OCH$_3$)$_3$-C$_6$H$_2$ R^2 = CH$_3$
f, Ar = 3,4,5(OCH$_3$)$_3$-C$_6$H$_2$ R^2 = C$_2$H$_5$

Fig. 2.

Table 1. Effect of the tested compounds on MCF7 tumor cell lines (breast cancer).

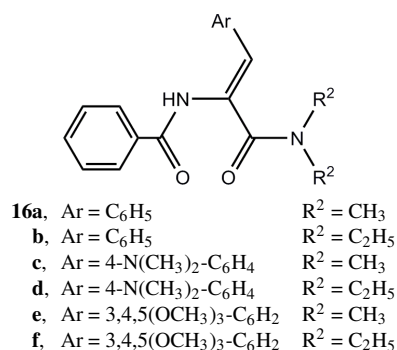| Compound	IC$_{50}$ (μg mL^{-1})
Doxorubicin (st.)	2.97
9a	21.1
9b	21.4
10c	19.3
12	19.3
13	19.6

oxazol-5(4*H*)-ylidene)acetate **15** in 82 % yield besides triphenylphosphane oxide (Scheme 3). The structure elucidation of compounds **14** and **15** was based on their analytical and spectral data (*cf.* Experimental Section).

The reactions of 4-arylidene-2-phenyl-5(4*H*)-oxazolones **1a, c, d** with tris(dialkylamino)phosphanes **4a** and **4b** were also investigated. We have found that the reaction of **1a, c, d** with one molar equivalent of each **1** and **4** in dry dichloromethane at r. t., affords the pure adducts formulated as **16a–f** (Fig. 2).

Products **16a–f** were presumably formed *via* nucleophilic attack of **4a, b** on the lactone carbonyl group in **1a, c, d** followed by azalactone ring opening. Since dialkylaminophosphite is a good leaving group, compounds **16a–f** can be formed through rapid hydrolysis due to the presence of unavoidable moisture (Scheme 4). The structures of compounds **16a–f** were attested by their elemental analysis, IR, ^{1}H, ^{13}C NMR and mass spectral data (*cf.* Experimental Section).

It is worth mentioning that when 5(4*H*)-oxazolones **1a, c, d** were allowed to react with a secondary amine, the same compounds **16a–f** were obtained in good yields (*cf.* Experimental Section).

Pharmacological evaluation

Cancer diseases are a serious threat to health and development of mankind, and the search for effective anti-cancer agents is a continuing challenge. Considerable progress has been made in recent years in the field of drug development against different types of cancer. Chemotherapy is a major approach for both localized and metastasized cancers [22], and for many years oxazolone-related compounds have proved to have significant therapeutic potential [23]. Based on these considerations, five of the newly synthesized compounds were screened for their *in vitro* cytotoxic and growth inhibitory activities against human breast carcinoma cell line (MCF7), in comparison with the activity of the anticancer agent Doxorubicin (DXR) as a reference drug. The cytotoxic activities of the tested compounds were expressed as the median growth inhibitory concentration (IC$_{50}$) which is the dose that reduces survival to 50 %. The screening results are compiled in Table 1. According to the American National Cancer Institute guidelines [24], drugs with IC$_{50}$ < 30 μg mL^{-1} are active. From Table 1, it is evident that most of the tested compounds show anti-tumor activities with IC$_{50}$ values ranging from 19.3 – 21.4 μg mL^{-1}.

The comparison of the cytotoxicity against MCF7 cells (Fig. 3) of the prepared compounds shows that the cell killing potency follows the order **10c** = **12** >

Table 2. *In vitro* evaluation of compounds for schistosomicidal activity at 10 μg mL^{-1}.

Formula numbers	1st day		2nd day		3rd day		4th day	
			No. of worms (12)					
	Dead	Mortality (%)	Dead	Mortality (%)	Dead	Mortality (%)	Dead	Mortality (%)
16a	0	0	0	0	0	0	2	16.7
16b	0	0	0	0	1	8.3	5	41.7
16c	0	0	0	0	0	0	4	33.3
16d	0	0	0	0	2	16.7	2	16.7
16e	0	0	0	0	7	58.3	12	100
16f	0	0	0	0	0	0	4	33.3
PZQ (+ve) Control[a]	12	100	12	100	12	100	12	100
DMSO (−ve) Control	0	0	0	0	0	0	0	0

[a] Praziquantel (PZQ) is the mainstay of schistosomiasis control programs worldwide.
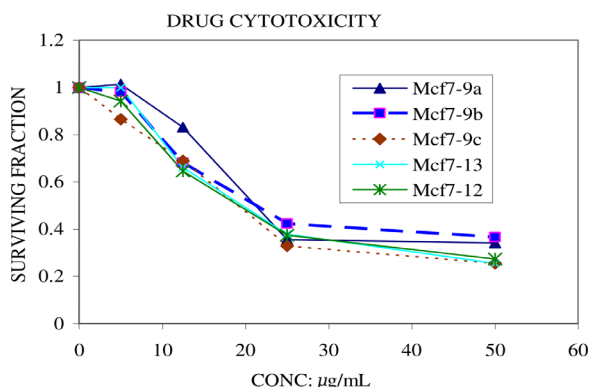

Fig 3. Effect of the tested compounds on MCF7 tumor cell lines.

13 > **9a** > **9b**. This may be attributable to the presence of the oxazolone moiety by contributing to the cytotoxic activity through interaction with DNA by intercalation and inhibition of the macromolecular biosynthesis. This inhibits the progression of the enzyme transcription topoisomerase II, which unwinds DNA for transcription and stabilizes the topoisomerase II complex after it has broken the DNA chain for replication, preventing the DNA double helix from being resealed. This process stops the replication in the same way as the Doxorubicin [25] suppressing agent, thus inhibiting the formation and growth of tumors from initiated cells [26].

Antischistosomal activity of some compounds

It is well known that organophosphorus compounds are the most important group of pesticides due to their rapid metabolism [27]. On the other hand, oxazolone derivatives serve a wide range of pharmaceutical and biological purposes [28], and they also possess insecticidal effects [29]. Therefore, this work aimed to prepare some oxazolone derivatives and to test

Table 3. *In vitro* antischistosomal effect (IC$_{50}$ and IC$_{90}$) of compound **16e** compared to PZQ.

Compound	IC$_{50}$ (μg mL^{-1})	IC$_{90}$ (μg mL^{-1})
16e	7.04	9.01
PZQ (+ve) control	0.4	0.64

them for antischistosomal activity *in vitro* on *Schistosoma mansoni* worms. The results revealed that benzamide **16e** possesses *in vitro* antischistosomal activity (100 % mortality). Compounds **16a** – **d** and **16f** showed various degrees of lethal efficacy rang (17 – 42 % mortality) on worms at 10 μg mL^{-1} after 4 d of exposure (Table 2).

Compound **16e** was further subjected to the determination of its IC$_{50}$ and IC$_{90}$ values (Table 3). The results have shown that compound **16e** possesses the strongest antischistosomal activity (IC$_{50}$ values equal to or less than 10 μg mL^{-1}).

Conclusion

Based on the results of the present investigations, it could be concluded that tetraethyl methanediylbis-(phosphonate) (**2**), phosphonium ylides and tris(dialkylamino)phosphanes behave differently towards 2-aryl-5(4*H*)-oxazolones **1**. While the reaction of **2** with oxazolones **1a**, **b** resulted in the formation of the new 1,1-bisphosphonate, 1,1-bisphosphonic acid, and phosphonate derivatives, a different course was observed in the reaction of **1** with stabilized ylides **3** and tris(dialkylamino)phosphanes **4**, depending on the nature of the addition products. A simple and direct method for the synthesis of substituted 1,1-bisphosphonic acids is also reported.

Some of the newly synthesized compounds were screened for their anticancer and antischistosomal activities. The compounds **9a**, **9b**, **10c**, **12**, and **13** revealed pronounced *in vitro* antitumor activities when

tested against human breast cancer carcinoma cell lines (MCF7) in comparison with the anticancer agent Doxorubicin (DXR) as a reference. The most promising result against breast carcinoma (MCF7) was obtained by oxazoledicarbonitrile **12**, oxazolemaleonitrile **13**, and oxazole dicarboxylate **10c**. They showed antitumor activities ranging from $19.3 - 19.6 \, \mu \text{g mL}^{-1}$. On the other hand, the antischistosomal activity of the compounds **16a – d**, **16f** showed that *N*-((1-dimethylcarbamoyl)-2-(3,4,5-trimethoxyphenyl)vinyl) benzamide **16e** possesses *in vitro* antischistosomal activity (mortality 100 %, IC_{50} values equal to or less than $10 \, \mu \text{g mL}^{-1}$). Compound **16e** therefore is considered a promising bioactive compound which deserves further investigation.

Notably, clinical trials showed that high activity is observed for methylene-bisphosphonates with nitrogen-containing heteroaromatic substituents. Data on pharmacological potency of the new bisphosphonate will be published elsewhere.

Experimental Section

Melting points were determined in open glass capillaries using an Electrothermal IA 9100 series digital melting point apparatus (Electrothermal, Essex, UK), and IR spectra were measured from KBr pellets with a Perkin-Elmer Infracord spectrophotometer model 157. The ^{1}H and ^{13}C NMR spectra were recorded in CDCl$_3$ or [D$_6$]DMSO as solvent on a Jeol 500 MHz spectrometer, and the chemical shifts were recorded in δ values (ppm) relative to TMS as internal reference. The ^{31}P NMR spectra were taken on a Varian CFT-20 spectrometer in δ values (ppm) *vs.* external 85 % H$_3$PO$_4$ as a standard. The mass spectra were performed at 70 eV on a Shimada GCS-OP 1000 Ex Spectrometer equipped with a data system. Elemental analyses were performed using an Elementar Vario E1 instrument. The values agreed favorably with calculated ones.

General procedure for reactions of 4-arylidene-2-phenyl-5(4H)-oxazolone (1a, 1b) with tetraethyl methanediylbis-(phosphonate) (2)

0.30 g (10 mmol) 80 % sodium hydride in 15 mL of anhydrous DMF was added slowly to a stirred solution of **2** (1.40 g, 5 mmol) in anhydrous DMF at 0 °C. After the addition was completed (1 h), a solution of **1a** or **1b** (2.5 mmol) in anhydrous DMF (10 mL) was added, and the resulting mixture was allowed to warm to room temperature and stirred for additional 0.5 – 5 h (TLC), and then cooled again to −5 °C. HCl (1N) was added until pH was acidic; the mixture was extracted with CHCl$_3$ (3 × 50 mL). The combined organic phase was dried over anhydrous Na$_2$SO$_4$. After removal of the solvent under vacuum, the residue was chromatographed

to give the products **5a** and **6a**. Also diethyl phosphite was identified (TLC).

Carrying out this reaction under the same conditions except adding conc. HCl instead of HCl (1N), **7a**, **b** as the major products and **8a**, **b** were separated. Also phosphorous acid was identified (TLC).

Tetraethyl [2-(5-oxo-2-phenyl-4,5-dihydro-1,3-oxazol-4-yl)-2-phenylethane-1,1-diyl]bisphosphonate (5a)

Eluent: petroleum ether-acetone (80/20, v/v). Product **5a** was separated as yellow crystals, yield 45 %. – M. p. 242 °C. – IR (KBr): ν = 1690 (C=O), 1635 (C=N), 1210 (P=O), 1120 (P-O) cm^{-1}. – ^{1}H NMR (500.14 MHz, CDCl$_3$): δ = 1.34, 1.36, 1.39, 1.41 (12 H, 4 t, J = 7.0 Hz, 4 CH_3CH$_2$O), 1.90 (1H, ddd, J = 7.0, 21.6 Hz, CH-P$_2$), 3.45, 3.47 (1H, 2 dd each set with J_{HH} = 7.8 Hz, $^{3}J_{HP}$ = 12 Hz, *CH*-CH-P), 4.1 – 4.25 (8H, m, br, 4 CH_2CH$_3$), 4.40 (1H, dd,J_{HH} = 7.0, 6.0 Hz, cyclic CH), 7.1 – 7.9 (m, 10H, H$_{\text{arom}}$). – ^{13}C NMR (125.76 MHz, CDCl$_3$): δ = 14.7 – 15.2, 16.3 (3s, 4 CH$_2$*CH$_3$*), 18.7 (t, $^{2}J_{CP}$ = 8.4 Hz, *CH*-CH-P), 25.3 (dd, J_{CP} = 130, 132 Hz, C-P$_2$), 62.3, 62.4, 62.6, 62.8 (4s, 4CH$_2$O), 71.8 (cyclic CH), 126.2 – 148.6 (aromatic C-H)), 154.8 (C=N), 165.6 (C=O). – ^{31}P NMR: δ = 23.16, 25.16. – MS (EI, 70 eV): *m/z* (%) = 537 (20) [M–1]$^{+}$, 536 (25) [M–2]$^{+}$, 535 (12) [M–3]$^{+}$, 401 (55) [M–137, (P(O)(OC$_2$H$_5$)$_2$]$^{+}$. – C$_{25}$H$_{33}$NO$_8$P$_2$ (537.17): calcd. C 55.87, H 6.19, N 2.61, P 11.53; found C 55.66, H 6.18, N 2.82, P 11.23.

Diethyl [2-(4,5-dihydro-5-oxo-2-phenyloxazol-4-yl)-2-phenylvinyl]phosphonate (6a)

Eluent: petroleum ether-acetone (95/5, v/v). Product **6a** was separated as pale-yellow crystals, yield 25 %. – M. p. 145 – 146 °C. – IR (KBr):ν = 1690 (C=O), 1640 (C=N), 1610 (C=C), 1237 (P=O), 1127 (P-O) cm^{-1}. – ^{1}H NMR (500.14 MHz, CDCl$_3$): δ = 1.35, 1.37 (6H, 2 dt, J = 6.7, 4.9 Hz, 2 CH_3CH$_2$O), 4.0 (1H, s, cyclic CH), 4.30, 4.35 (4H, 2 q, $^{3}J_{HP}$ = 11.7 Hz, 2 CH$_3$*CH$_2$*-O-P), 5.3 (1H, d, $^{2}J_{HP}$ = 18.6 Hz, CH-P), 7.2 – 7.8 (m, 10H, H$_{\text{arom}}$). – ^{13}C NMR (125.76 MHz, CDCl$_3$): δ = 14.90, 15.30 (2 CH_3CH$_2$O), 62.10, 62.70 (2 d, $^{2}J_{CP}$ = 28.25 Hz, CH$_3$*CH$_2$*-O-P), 75.8 (cyclic CH), 103.8 (d, J_{CP} = 93.2 Hz, C-P), 126.4 – 139.4 (aromatic C-H), 154.2 (C=N), 165.2 (C=O). – ^{31}P NMR: δ = 20.34. – MS (EI, 70 eV): *m/z* (%) = 399 (15) [M]$^{+}$, 262 (100) [M–137, (P(O)(OC$_2$H$_5$)$_2$]$^{+}$. – C$_{21}$H$_{22}$NO$_5$P (399.12): calcd. C 63.15, H 5.55, N 3.51, P 7.76; found C 63.20, H 5.42, N 3.72, P 8.02.

[2-(5-Oxo-2-phenyl-4,5-dihydro-1,3-oxazol-4-yl)-2-phenylethane-1,1-diyl]bis(phosphonic acid) (7a)

Eluent: petroleum ether-acetone (90/10, v/v). Product **7a** was separated as colorless crystals, yield 55 %. – M. p. 120 – 122 °C. – IR (KBr): ν = 3320 (OH), 1690 (C=O),

1640 (C=N), 1257 (P=O), 1130 (P-O) cm^{-1}. – ^{1}H NMR (500.14 MHz, CDCl$_3$): δ = 1.88 (1H, ddd, J_{HH} = 7.0 Hz, $^2J_{HP}$ = 30.0 Hz, CH-P$_2$), 3.34, 3.36 (1H, 2 dd each set with J_{HH} = 7.6 Hz, J_{HP} = 11.5 Hz *CH*-CH-P$_2$), 4.3 (1H, d, J_{HH} = 7.0, 5.0 Hz, cyclic CH), 6.30 (4H, s, 4 OH, exchangeable with D$_2$O), 7.4 – 7.8 (m, 10H, H$_{arom}$). – ^{13}C NMR (125.76 MHz, CDCl$_3$): δ = 17.5 (d, $^2J_{CP}$ = 28.5 Hz, *CH*-CH-P$_2$), 34.2, 34.2 (dd, J_{CP} = 130 Hz, CH-P$_2$), 71.9 (cyclic CH), 126.4 – 148.8 (aromatic CH), 157.8 (C=N), 165.6 (C=O). – ^{31}P NMR: δ = 23.7. – MS (EI, 70 eV): m/z (%) = 424 (50) [M]$^+$. – C$_{17}$H$_{17}$NO$_8$P$_2$ (425.04): calcd. C 48.01, H 4.03, N 3.29, P 14.57; found C 48.40, H 4.01, N 3.20, P 13.25.

2-[(4,5-Dihydro-5-oxo-2-phenyloxazol-4-yl)-2-phenyl-vinyl]phosphonic acid (8a)

Eluent: petroleum ether-acetone (95/5, v/v). Product **8a** was separated as yellow crystals, yield 25 %. – M. p. 120 °C. – IR (KBr): ν = 3325 (OH), 1690 (C=O), 1635 (C=N), 1610 (C=C), 1220 (P=O) cm^{-1}. – ^{1}H NMR (500.76 MHz, [D$_6$]DMSO): δ = 4.65 (1H, s, cyclic CH), 5.1 (1H, d, $^2J_{HP}$ = 20.0 Hz, CH-P), 6.1 (2H, s, 2 OH, exchangeable with D$_2$O), 7.2 – 8.1 (m, 10H, H$_{arom}$). – ^{13}C NMR (125.76 MHz, [D$_6$]DMSO): δ = 75.8 (cyclic CH), 103.8 (d, J_{CP} = 93.2 Hz, C-P), 121.0 – 147.6 (aromatic C-H), 157.8 (C=N), 166.0 (C=O). – ^{31}P NMR: δ = 20.5. – MS (EI, 70 eV): m/z (%) = 343 (50) [M]$^+$. – C$_{17}$H$_{14}$NO$_5$P (343.27): calcd. C 59.48, H 4.11, N 4.08, P 9.02; found C 59.40, H 4.10, N 4.10, P 9.04.

[2-(4-Nitrophenyl)-2-(5-oxo-2-phenyl-4,5-dihydro-1,3-oxazol-4-yl)ethane-1,1-diyl]bis(phosphonic acid) (7b)

Eluent: petroleum ether-acetone (70/30, v/v). Product **7b** was separated as yellow crystals, yield 50 %. – M. p. 159 – 160 °C. – IR (KBr): ν = 3320 (OH), 1690 (C=O), 1640 (C=N), 1344 (NO$_2$), 1249 (P=O), 1130 (P-O) cm^{-1}. – ^{1}H NMR (500.76 MHz, [D$_6$]DMSO): δ = 1.85, 1.89 (1H, ddd, J_{HH} = 7 Hz, $^2J_{HP}$ = 30 Hz CH-P$_2$), 3.43, 3.45 (1H, 2 dd each set with J_{HH} = 7.6 Hz, J_{HP} = 21.5 Hz, *CH*-CH-P), 4.3 (1H, dd, J_{HH} = 7, 5 Hz, cyclic CH), 7.4 – 8.2 (9H$_{arom}$, m, 9CH), 10.1 (4H, s, 4-OH, exchangeable with D$_2$O). – ^{13}C NMR (125.76 MHz, [D$_6$]DMSO): δ = 17.50, 17.53 (d, $^2J_{CP}$ = 30 Hz, *CH*-CH-P$_2$), 34.2, 34.3 (dd, J_{CP} = 130 Hz, CH-P$_2$), 71.9 (cyclic CH), 120.8 – 154.1 (aromatic CH), 157.8 (C=N), 165.6 (C=O). – ^{31}P NMR: δ = 23.8. – MS (EI, 70 eV): m/z (%) = 470 (55) [M]$^+$. – C$_{17}$H$_{16}$N$_2$O$_{10}$P$_2$ (470.17): calcd. C 43.42, H 3.43, N 5.96, P 13.17; found C 43.80, H 3.42, N 5.90, P 13.50.

[2-(4-Nitrophenyl)-2-(5-oxo-2-phenyl-4,5-dihydro-1,3-oxazol-4-yl)ethenyl]phosphonic acid (8b)

Eluent: petroleum ether-acetone (95/5, v/v). Product **8b** was separated as yellow crystals, yield 20 %. –

M. p. 130 °C. – IR (KBr): ν = 3320 (OH), 1690 (C=O), 1640 (C=N), 1610 (C=C), 1344 (NO$_2$), 1225 (P=O) cm^{-1}. – ^{1}H NMR (500.14 MHz, CDCl$_3$): δ = 4.65 (1H, s, cyclic CH), 5.4 (1H, d, $^2J_{HP}$ = 20 Hz, CH-P), 6.2 (2H, s, 2 OH, exchangeable with D$_2$O), 7.2 – 8.1 (m, 9H, H$_{arom}$). – ^{13}C NMR (125.76 MHz, [D$_6$]DMSO): δ = 75.8 (cyclic CH), 109.8 (d, J_{CP} = 93.2 Hz, C-P), 125.0 – 147.6 (aromatic C-H), 154.8 (C=N), 165.0 (C=O). – ^{31}P NMR: δ = 22.5. – MS (EI, 70 eV): m/z (%) = 388.27 (40) [M]$^+$. – C$_{21}$H$_{21}$N$_2$O$_7$P (388.27): calcd. C 52.59, H 3.37, N 7.21, P 7.98; found C 52.60, H 3.21, N 7.20, P 7.93.

General procedure for reactions of 4-arylidene-2-phenyl-5(4H)-oxazolones (1a, 1c) with carbalkoxymethylene triphenylphosphoranes (3a, 3b)

One mmol of **1a** or **1c** was added to 3 mmol of **3a** or **3b**, and the mixture was refluxed in 30 mL of dry toluene for 5 – 7 h. The volatile materials were evaporated under reduced pressure. The residue was subjected to silica gel column chromatography to give the products **9a – c**, **10a – c** and **11a – c**. Triphenylphosphane was also isolated from the reaction medium and identified (mixed m. p., MS). Products **9a – c** were refluxed in dry toluene to give products **10a – c** (cyclic form) in 85 % yield.

Methyl 3-(4,5-dihydro-5-oxo-2-phenyloxazol-4-yl)-3-phenylacrylate (9a)

Eluent: petroleum ether-acetone (85/15, v/v). Product **9a** was separated as colorless crystals, yield 27 %. – M. p. 90 °C. – IR (KBr): ν = 1741 (ester C=O), 1690 (O-C=O), 1654 (C=N) cm^{-1}. – ^{1}H NMR (500.14 MHz, CDCl$_3$): δ = 3.5 (3H, s, CH$_3$), 4.65 (1H, s, cyclic CH), 5.88 (1H, s, *CH*COOCH$_3$), 7.0 – 8.0 (m, 10H, H$_{arom}$). – ^{13}C NMR (125.76 MHz, CDCl$_3$): δ = 52.7 (CH$_3$), 73.9 (cyclic CH), 143.2, 119.5 (C=CH), 126.5 – 135.9 (aromatic CH), 153.9 (C=N), 166.0 (ester C=O), 176.1 (O-C=O). – MS (EI, 70 eV): m/z (%) = 321 (5) [M]$^+$, 306 (100) [M–15]$^+$. – C$_{19}$H$_{15}$NO$_4$ (321.10): calcd. C 71.02, H 4.71, N 4.36; found C 70.80, H 4.81, N 4.30.

Methyl 2,6-diphenyl-5,6-dihydrofuro[3,2-d][1,3]oxazole-5-carboxylate (10a)

Eluent: petroleum ether-acetone (95/5, v/v). Product **10a** was separated as colorless crystals, yield 35 %. – M. p. 198 – 200 °C. – IR (KBr): ν = 1741 (ester C=O), 1654 (C=N), 1610 (C=C-O) cm^{-1}. – ^{1}H NMR (500.14 MHz, CDCl$_3$): δ = 3.5 (3H, s, CH$_3$), 5.1 (1H, d, CH-Ar), 5.29 (1H, d, cyclic CH), 7.1 – 7.9 (m, 10H, H$_{arom}$). – ^{13}C NMR (125.76 MHz, CDCl$_3$): δ = 45.5 (CH-Ar), 54.0 (CH$_3$), 84.4 (cyclic CH), 125.4, 138.1 (C=C), 126.5 – 144.2 (aromatic CH), 153.6 (C=N), 167.05 (ester C=O). – MS (EI, 70 eV): m/z (%) = 321 (25) [M]$^+$, 306 (100) [M–15]$^+$. – C$_{19}$H$_{15}$NO$_4$ (321.10):

calcd. C 71.02, H 4.71, N 4.36; found C 70.80, H 4.81, N 4.20.

Methyl (5-oxo-2,7-diphenyl-5H-pyrano[3,2-d][1,3]oxazol-6(7H)-ylidene)ethanoate (11a)

Eluent: petroleum ether-acetone (70/30, v/v). Product **11a** was separated as pale-yellow crystals, yield 22 %. – M. p. 268 – 270 °C. – IR (KBr): ν = 1742 (ester C=O), 1690 (O-C=O), 1653 (C=N) cm^{-1}. – ^{1}H NMR (500.14 MHz, CDCl$_3$): δ = 3.7 (3H, s, CH$_3$), 4.7 (1H, s, cyclic CH), 6.2 (1H, s, CH), 7.0 – 7.9 (m, 10H, H$_{arom}$). – ^{13}C NMR (125.76 MHz, CDCl$_3$): δ = 38.7 (cyclic CH), 52.2 (CH$_3$), 125.4, 138.1 (C=C), 126.4 – 135.5 (aromatic CH), 153.2 (C=N), 166.5 (ester C=O), 176.0 (O-C=O). – MS (EI, 70 eV): m/z (%) = 361 (55) [M]$^+$. – C$_{21}$H$_{15}$NO$_5$ (361.10): calcd. C 69.80, H 4.18, N 3.88; found C 68.99, H 4.60, N 4.12.

Ethyl 3-(5-oxo-2-phenyl-4,5-dihydro-1,3-oxazol-4-yl)-3-phenylprop-2-enoate (9b)

Eluent: petroleum ether-ethyl acetate (90/10, v/v). Product **9a** was separated as colorless crystals, yield 30 %. – M. p. 99 °C. – IR (KBr): ν = 1742 (ester C=O), 1690 (O-C=O), 1653 (C=N) cm^{-1}. – ^{1}H NMR (500.14 MHz, CDCl$_3$): δ = 1.1 (3H, t, CH$_3$), 4.1 (2H, q, CH$_2$), 4.7 (1H, s, cyclic CH), 5.8 (1H, s, CH), 7.1 – 8.0 (m, 10H, H$_{arom}$). – ^{13}C NMR (125.76 MHz, CDCl$_3$): δ = 14.1 (CH$_3$), 61.4 (CH$_2$), 73.7 (cyclic CH), 126.6 – 136.4 (aromatic CH), 152.2, 119.2 (C=CH), 154.9 (C=N), 166.2 (ester C=O), 176.1 (O-C=O). – MS (EI, 70 eV): m/z (%) = 335 (10) [M]$^+$, 318 (75) [M–15]$^+$. – C$_{20}$H$_{17}$NO$_4$ (335.12): calcd. C 71.63, H 5.11, N 4.18; found C 71.89, H 5.67, N 4.02.

Ethyl 2,6-diphenyl-5,6-dihydrofuro[3,2-d][1,3]oxazole-5-carboxylate (10b)

Eluent: petroleum ether-ethyl acetate (95/5, v/v). Product **10a** was separated as colorless crystals, yield 37 %. – M. p. 210 – 212 °C. – IR (KBr): ν = 1740 (ester C=O), 1690 (C=C-O), 1654 (C=N) cm^{-1}. – ^{1}H NMR (500.14 MHz, CDCl$_3$): δ = 1.1 (3H, t, CH$_3$), 4.1 (2H, q, CH$_2$), 5.1 (1H, d, CH-Ar), 5.3 (1H, d, cyclic CH), 7.1 – 8.0 (m, 10H, H$_{arom}$). – ^{13}C NMR (125.76 MHz, CDCl$_3$): δ = 14.0 (CH$_3$), 44.7 (CH-Ar), 61.6 (CH$_2$), 84.1 (cyclic CH), 125.4 (C=C), 126.6 – 140.3 (aromatic CH), 154.5 (C=N), 169.5 (ester C=O). – MS (EI, 70 eV): m/z (%) = 335 (10) [M]$^+$, 319 (100) [M–15]$^+$. – C$_{20}$H$_{17}$NO$_4$ (335.12): calcd. C 71.63, H 5.11, N 4.18; found C 70.96, H 5.60, N 4.08.

Ethyl (5-oxo-2,7-diphenyl-5H-pyrano[3,2-d][1,3]oxazol-6(7H)-ylidene)ethanoate (11b)

Eluent: petroleum ether-ethyl acetate (70/30, v/v). Product **11b** was separated as yellow crystals, yield 20 %. – M. p. 240 – 242 °C. – IR (KBr): ν = 1742 (ester C=O), 1690

(O-C=O), 1652 (C=N) cm^{-1}. – ^{1}H NMR (500.14 MHz, CDCl$_3$): δ = 1.2 (3H, t, CH$_3$), 4.1 (2H, q, CH$_2$), 4.7 (1H, s, cyclic CH), 6.2 (1H, s, CH), 7.0 – 8.0 (m, 10H, H$_{arom}$). – ^{13}C NMR (125.76 MHz, CDCl$_3$): δ = 14.3 (CH$_3$), 38.8 (cyclic CH), 61.5 (CH$_2$), 145.4, 119.1 (C=CH), 126.5 – 138.5 (aromatic CH), 153.9 (C=N), 166.4 (ester C=O), 176.4 (O-C=O). – MS (EI): m/z (%) = 375 (10) [M]$^+$, 359 (70) [M–15]$^+$. – C$_{22}$H$_{17}$NO$_5$ (375.11): calcd. C 70.39, H 4.56, N 3.73; found C 70.02, H 4.75, N 3.32.

Methyl 3-[4-(dimethylamino)phenyl]-3-(5-oxo-2-phenyl-4,5-dihydro-1,3-oxazol-4-yl)prop-2-enoate (9c)

Eluent: petroleum ether-acetone (95/5, v/v). Product **9c** was separated as colorless crystals, yield 35 %. – M. p. 228 – 230 °C. – IR (KBr): ν = 1741 (ester C=O), 1690 (O-C=O), 1653 (C=N) cm^{-1}. – ^{1}H NMR (500.14 MHz, CDCl$_3$): δ = 2.85, 2.86 (6H, 2 s, N(CH$_3$)$_2$), 3.7 (3H, s, CH$_3$), 4.6 (1H, s, cyclic CH), 5.8 (1H, s, CHCOO), 7.0 – 7.9 (m, 9H, H$_{arom}$). – ^{13}C NMR (125.76 MHz, CDCl$_3$): δ = 40.3 (N(CH$_3$)$_2$), 53.2 (CH$_3$), 73.3 (cyclic CH), 126.9 – 138.1 (aromatic CH), 150.7, 120 (C=CH), 154.0 (C=N), 166.2 (ester C=O), 176.2 (O-C=O). – MS (EI, 70 eV): m/z (%) = 364 (50) [M]$^+$. – C$_{21}$H$_{20}$N$_2$O$_4$ (364.14): calcd. C 69.22, H 5.53, N 7.69; found C 70.30, H 5.28, N 8.01.

Methyl 6-[4-(dimethylamino)phenyl]-2-phenyl-5,6-dihydro-furo[3,2-d][1,3]oxazole-5-carboxylate (10c)

Eluent: petroleum ether-acetone (95/5, v/v). Product **10c** was separated as pale-yellow crystals, yield 30 %. – M. p. 290 – 291 °C. – IR (KBr): ν = 1741 (ester C=O), 1690 (C=C-O), 1654 (C=N) cm^{-1}. – ^{1}H NMR (500.14 MHz, CDCl$_3$): δ = 2.84, 2.85 (6H, 2 s, N(CH$_3$)$_2$), 3.6 (3H, s, CH$_3$), 5.2 (1H, d, CH-Ar), 5.3 (1H, d, CH), 7.0 – 8.0 (m, 9H, H$_{arom}$). – ^{13}C NMR (125.76 MHz, CDCl$_3$): δ = 40.3 (N(CH$_3$)$_2$), 45.0 (CH-Ar), 53.6 (CH$_3$), 91.9 (cyclic CH), 125.4 (C=C), 126.6 – 138.9 (aromatic CH), 153.2 (C=N), 166.9 (ester C=O). – MS (EI, 70 eV): m/z (%) = 364 (15) [M]$^+$, 348 (100) [M–15]$^+$. – C$_{21}$H$_{20}$N$_2$O$_4$ (364.14): calcd. C 69.22, H 5.53, N 7.69; found C 69.30, H 5.28, N 8.01.

Methyl {7-[4-(dimethylamino)phenyl]-5-oxo-2-phenyl-5H-pyrano[3,2-d][1,3]oxazol-6(7H)-ylidene}ethanoate (11c)

Eluent: petroleum ether-acetone (65/35, v/v). Product **11c** was separated as pale yellow crystals, yield 30 %. – M. p. 250 – 253 °C. – IR (KBr): ν = 1740 (ester C=O), 1690 (O-C=O), 1652 (C=N) cm^{-1}. – ^{1}H NMR (500.14 MHz, CDCl$_3$): δ = 2.84, 2.85 (6H, 2 s, N(CH$_3$)$_2$), 3.6 (3H, s, CH$_3$), 4.7 (1H, s, cyclic CH), 6.4 (1H, s, CH), 7.0 – 8.0 (m, 9H, H$_{arom}$). – ^{13}C NMR (125.76 MHz, CDCl$_3$): δ = 38.1 (cyclic CH), 40.6 (N(CH$_3$)$_2$), 52.6 (CH$_3$), 126.6 – 138.3 (aro-

matic CH), 143.2, 119.1 (C=CH), 153.3 (C=N), 166.4 (ester C=O), 170.4 (O-C=O). – MS (EI, 70 eV): m/z (%) = 404 (25) $[M]^+$. – $C_{23}H_{20}N_2O_5$ (404.14): calcd. C 68.31, H 4.98, N 6.93; found C 69.02, H 4.39, N 6.90.

Reaction of 4-(4-nitrobenzylidene)-2-phenyl-1,3-oxazol-5(4H)-one (*1b*) with ethyl (triphenyl-λ^5-phosphanylidene)-acetate (*3b*)

0.29 g (1 mmol) of **1b** was added to 0.7 g (2 mmol) of **3b**, and the mixture was allowed to reflux in 30 mL dry toluene for 10 h (TLC). The volatile materials were evaporated under reduced pressure. The residue was chromatographed to give the products **9d** and **10d**. Triphenylphosphane was also isolated from the reaction medium and identified (mixed m. p., MS).

Ethyl 3-[3-(4-nitrophenyl)-3-(4,5-dihydro-5-oxo)-2-phenyl-oxazol-4-yl]acrylate (*9d*)

Eluent: petroleum ether-acetone (90/10, v/v). Product **9d** was separated as yellow crystals, yield 25 %. – M. p. 195 – 196 °C. – IR (KBr): ν = 1740 (ester C=O), 1690 (O-C=O), 1654 (C=N), 1344 (NO$_2$) cm^{-1}. – ^{1}H NMR (500.14 MHz, CDCl$_3$): δ = 1.02 (3H, t, CH$_3$), 4.1 (2H, q, CH$_2$), 4.7 (1H, s, cyclic CH), 5.8 (1H, s, CHCOO), 7.4 – 8.2 (m, 9H, H$_{arom}$). – ^{13}C NMR (125.76 MHz, CDCl$_3$): δ = 14.3 (CH$_3$), 61.3 (CH$_2$), 74.2 (cyclic CH), 149.9, 119.2 (C=CH), 121.2 – 147.6 (aromatic CH), 154.2 (C=N), 166.0 (ester, C=O), 176.9 (O-C=O). – MS (EI, 70 eV): m/z (%) = 380 (35) $[M]^+$. – $C_{20}H_{16}N_2O_6$ (380.17): calcd. C 63.16, H 4.24, N 7.37; found C 63.00, H 4.63, N 6.89.

Ethyl 6-(4-nitrophenyl)-2-phenyl-5,6-dihydrofuro[3,2-d]-[1,3]oxazole-5-carboxylate (*10d*)

Eluent: petroleum ether-acetone (95/5, v/v). Product **10d** was separated as yellow crystals, yield 40 %. – M. p. 183 – 185 °C. – IR (KBr): ν= 1740 (ester C=O), 1654 (C=N), 1620 (C=C-O), 1344 (NO$_2$) cm^{-1}. – ^{1}H NMR (500.14 MHz, CDCl$_3$): δ = 1.2 (3H, t, CH$_3$), 5.2 (1H, d, CH-Ar), 4.18 (2H, q, CH$_2$), 5.3 (1H, d, cyclic CH), 7.4 – 8.3 (m, 9H, H$_{arom}$). – ^{13}C NMR (125.76 MHz, CDCl$_3$): δ = 14.3 (CH$_3$), 45.5 (CH-Ar), 61.4 (CH$_2$), 73.9 (*CH*COO), 125.2 (C=C), 128.9 – 147.6 (aromatic CH), 154.8 (C=N), 166.0 (ester C=O). – MS (EI, 70 eV): m/z (%) = 380 (40) $[M]^+$. – $C_{20}H_{16}N_2O_6$ (380.17): calcd. C 63.16, H 4.24, N 7.37; found C 63.00, H 4.63, N 6.89.

General procedure for reactions of 4-arylidene-2-phenyl-1,3-oxazol-5(4H)-ones (*1a, d*) with (triphenyl-λ^5-phosphanylidene)acetonitrile (*3c*)

A mixture of **1a, d** (1 mmol) and **3c** (0.60 g, 2 mmol) in 30 mL of dry toluene was refluxed for 7 – 10 h (TLC).

The volatile materials were evaporated under reduced pressure. The residue was chromatographed to give products **12** and **13**. Triphenylphosphane and triphenylphosphane oxide were also isolated from the reaction mixture and identified (mixed m. p., MS).

2,4-Diphenyl-5,6-dihydro-4H-cyclopenta[d][1,3]oxazole-5,6-dicarbonitrile (*12*)

Eluent: petroleum ether-ethyl acetate (90/10, v/v). Product **12** was separated as yellow crystals, yield 65 %. – M. p. 153 – 155 °C. – IR (KBr): ν = 2210, 2215 (2 C≡N), 1653 (C=N), 1610 (C=C) cm^{-1}. – ^{1}H NMR (500.14 MHz, CDCl$_3$): δ = 4.57 (1H, dd, CHCN), 4.9 (1H, d, CHCN), 5.5 (1H, d, CH-Ar), 7.1 – 7.9 (m, 10H, H$_{arom}$). – ^{13}C NMR (125.76 MHz, CDCl$_3$): δ = 25.6, 26.9 (2 CHCN), 37.9 (CH-Ar), 119.2, 120.3 (2 C≡N), 129.3 – 138.8 (aromatic CH), 153.3 (C=N). – MS (EI, 70 eV): m/z (%) = 311 (100) $[M]^+$. – $C_{20}H_{13}N_3O$ (311.11): calcd. C 77.16, H 4.21, N 13.50; found C 76.80, H 4.20, N 13.91.

2-[2-Phenyl-4-(3,4,5-trimethoxybenzylidene)-4,5-dihydro-1,3-oxazol-5-yl]but-2-enedinitrile (*13*)

Eluent: petroleum ether-ethyl acetate (92/8, v/v). Product **13** was separated as colorless crystals, yield 75 %. – M. p. 143 – 145 °C. – IR (KBr): ν = 2220, 2225 (2 C≡N), 1654 (C=N), 1610 (C=C) cm^{-1}. – ^{1}H NMR (500.14 MHz, [D$_6$]DMSO): δ = 3.8 (9H, s, 3CH$_3$), 4.5 (1H, s, cyclic CH), 6.1 (1H, s, CHCN), 6.6 (1H, s, C=CH), 7.2 – 8.1 (m, 7H, 7H$_{arom}$,). – ^{13}C NMR (125.76 MHz, [D$_6$]DMSO): δ = 56.4 (CH$_3$), 65.0 (cyclic CH), 108.6 (CHCN), 119.2, 120.0 (2 C≡N), 125.6 – 150.7 (aromatic CH), 154.4 (C=N). – MS (EI, 70 eV): m/z (%) = 401 (35) $[M]^+$. – $C_{23}H_{19}N_3O_4$ (401.41): calcd. C 68.82, H 4.77, N 10.47; found C 69.02, H 4.67, N 11.02.

General procedure for reactions of 4-arylidene-2-phenyl-1,3-oxazol-5(4H)-ones (*1d, e*) with methyl(triphenyl-λ^5-phosphanylidene)acetate (*3a*)

A mixture of **1d, e** (0.30 g, 1 mmol) and **3a** (0.30 g, 1 mmol) was refluxed in dry toluene (30 mL) for 7 h; the volatile materials were evaporated under reduced pressure. The residue was chromatographed to give product **14** or **15**. Triphenylphosphane oxide was also isolated from the reaction mixture and identified (mixed m. p. MS).

Methyl [2-phenyl-4-(3,4,5-trimethoxybenzylidene)-1,3-oxazol-5(4H)-ylidene]ethanoate (*14*)

Eluent: petroleum ether-acetone (90/10, v/v). Product **14** was separated as colorless crystals, yield 85 %. – M. p. 210 – 212 °C. – IR (KBr): ν = 1742 (ester C=O), 1653 (C=N), 1610 (C=CH) cm^{-1}. – ^{1}H NMR (500.14 MHz, CDCl$_3$): δ =

3.6 (3H, s, CH$_3$), 3.9 (9H, s, 3 OCH$_3$), 6.2 (1H, s, C=CH), 6.6 (1H, s, CH-Ar), 7.3 – 8.0 (m, 7H, H$_{arom}$). – ^{13}C NMR (125.76 MHz, CDCl$_3$): δ = 52.9 (COO*CH$_3$*), 56.3 (OCH$_3$), 95.7 (CH-Ar), 98.6 (CHCOO), 120.9 – 150.7 (aromatic CH), 153.6 (C=N), 195.1, 103.1 (C=CH), 166.2 (ester C=O). – MS (EI, 70 eV): *m/z* (%) = 395 (30) [M]$^+$. – C$_{22}$H$_{21}$NO$_6$ (395.14): calcd. C 66.83, H 5.35, N 3.54; found C 67.02, H 5.20, N 4.01.

Methyl 2-(4-(4-methoxybenzylidene)-2-phenyloxazol-5(4H)-ylidene)acetate (15)

Eluent: petroleum ether-ethyl acetate (93/7, v/v). Product **15** was separated as colorless crystals, yield 80 %. – M. p. 245 – 248 °C. – IR (KBr): ν = 1742 (ester C=O), 1653 (C=N), 1615 (C=CH) cm^{-1}. – ^{1}H NMR (500.14 MHz, CDCl$_3$): δ = 3.6 (3H, s, CH$_3$), 3.7 (3H, s, OCH$_3$), 6.5 (1H, s, C=CH), 6.6 (1H, s, CH-Ar), 7.1 – 8.0 (m, 9H, H$_{arom}$). – ^{13}C NMR (125.76 MHz, CDCl$_3$): δ = 52.7, 55.1 (2 OCH$_3$), 95.2 (CH-Ar), 98.6 (CHCOO), 113.4 – 159.6 (aromatic CH), 153.5 (C=N), 169.9 (ester C=O). – MS (EI, 70 eV): *m/z* (%) = 335 (20) [M]$^+$. – C$_{20}$H$_{17}$NO$_4$ (335.12): calcd. C 71.63, H 5.11, N 4.18; found C 70.22, H 5.63, N 4.52.

General procedure for reactions of 4-arylidene-2-phenyl-1,3-oxazol-5(4H)-ones (1a, c, d) with tris(dialkylamino)-phosphanes (4a, b)

A mixture of **1a, c, d** (1 mmol) and tris(dialkylamino)-phosphanes **4a, b** (1 mmol) in dry dichloromethane (30 mL) was stirred at r. t. for 5 – 10 h (TLC). The volatile materials were evaporated under reduced pressure. The residue was washed with boiled cyclohexane to give the products **16a – f** in pure form.

General procedure for reactions of 4-arylidene-2-phenyl-1,3-oxazol-5(4H)-ones (1a, c, d) with secondary amines

4-arylidene-2-phenyl-1,3-oxazol-5(4*H*)-ones **1a, c, d** (1 mmol) and diethylamine or dimethylamine (1 mmol) were warmed for few seconds until complete fusion. The reaction mixture was washed with boiled cyclohexane to give the products **16a – f** in good yields (m. p., mixed m. p. and comparative IR).

N-[(1-dimethylcarbamoyl)-2-phenylvinyl]benzamide (16a)

Product **16a** was separated as colorless needles, yield 85 %. – M. p. 127 – 128 °C (ethyl acetate). – IR (KBr): ν= 3367 (NH), 1660, 1650 (2 C=O) cm^{-1}. – ^{1}H NMR (500.14 MHz, CDCl$_3$): δ = 3.1, 3.2 (6H, 2 s, N(CH$_3$)$_2$), 6.1 (1H, s, CH), 7.3 – 7.8 (m, 10H, H$_{arom}$,), 8.5 (1H, s, NH exchangeable with D$_2$O). – ^{13}C NMR (125.76 MHz, CDCl$_3$): δ = 37.1, 37.2 (2 s, N(CH$_3$)$_2$), 115.7, 127.8 (CH=C),

127.9 – 131.8 (aromatic CH), 165.7, 168.2 (2 C=O). – MS (EI, 70 eV): *m/z* (%) = 294 (50) [M]$^+$. – C$_{18}$H$_{18}$N$_2$O$_2$ (294.14): calcd. C 73.45, H 6.16, N 9.52; found C 72.98, H 6.70, N 9.20.

N-[(1-Diethylcarbamoyl)-2-phenylvinyl]benzamide (16b)

Product **16b** was separated as colorless needles, yield 90 %. – M. p. 130 – 133 °C (ethyl acetate). – IR (KBr): ν = 3368 (NH), 1660, 1650 (2 C=O) cm^{-1}. – ^{1}H NMR (500.14 MHz, CDCl$_3$): δ = 1.2 (6H, 2 t, 2CH$_3$), 4.0, 4.1 (4H, 2 q, 2 CH$_2$), 6.3 (1H, s, CH), 7.1 – 7.9 (m, 10H, H$_{arom}$,), 8.3 (1H, s, NH exchangeable with D$_2$O). – ^{13}C NMR (125.76 MHz, CDCl$_3$): δ = 14.7, 14.8 (2 CH$_3$), 61.8 (2 CH$_2$), 107.4, 122.7 (CH=C), 128.2 – 132.4 (CH aromatic), 165.6, 168.4 (2 C=O). – MS (EI, 70 eV): *m/z* (%) = 322 (55) [M]$^+$. – C$_{20}$H$_{22}$N$_2$O$_2$ (322.17): calcd. C 71.51, H 6.88, N 8.69; found C 72.02, H 6.53, N 9.32.

N-[(1-Dimethylcarbamoyl)-2-(4-(dimethylamino)phenyl)-vinyl]benzamide (16c)

Product **16c** was separated as colorless needles (diethyl ether), yield 90 %. – M. p. 132 – 133 °C. – IR (KBr): ν = 3360 (NH), 1662, 1650 (2 C=O) cm^{-1}. – ^{1}H NMR (500.14 MHz, CDCl$_3$): δ = 3.0 – 3.2 (12H, 2 s, N(CH$_3$)$_2$), 6.0 (1H, s, CH), 6.6 – 7.8 (m, 9H, H$_{arom}$,), 8.5 (1H, s, NH exchangeable with D$_2$O). – ^{13}C NMR (125.76 MHz, CDCl$_3$): δ = 44.9 – 45.1 (2N(CH$_3$)$_2$), 117.0, 127.1 (CH=C), 131.0 – 136.9 (CH aromatic), 165.5, 168.1 (2 C=O). – MS (EI, 70 eV): *m/z* (%) = 337 (50) [M]$^+$. – C$_{20}$H$_{23}$N$_3$O$_2$ (338.18): calcd. C 71.19, H 6.87, N 12.45; found C 71.80, H 6.44, N 12.02.

N-[(1-Diethylcarbamoyl)-2-(4-dimethylamino)phenyl)-vinyl]benzamide (16d)

Product **16d** was separated as colorless needles (ethyl acetate), yield 90 %. – M. p. 232 – 233 °C. – IR (KBr): ν = 3354 (NH), 1659, 1650 (2 C=O) cm^{-1}. – ^{1}H NMR (500.14 MHz, CDCl$_3$): δ = 0.8 – 1.1 (6H, 2 t, N(CH$_2$*CH$_3$*)$_2$), 2.9 (6H, s, N(CH$_3$)$_2$), 4.1, 4.3 (4H, 2 q, N(*CH$_2$*CH$_3$)$_2$), 6.9 (1H, s, CH), 7.1 – 7.8 (m, 9H, H$_{arom}$), 8.3 (1H, s, NH exchangeable with D$_2$O). – ^{13}C NMR (125.76 MHz, CDCl$_3$): δ = 12.9 (N(CH$_2$*CH$_3$*)$_2$), 40.1 (N(CH$_3$)$_2$), 61.2 (N(*CH$_2$*CH$_3$)$_2$), 107.4, 122.7 (CH=C), 128.2 – 148.4 (aromatic CH), 163.6, 168.4 (2 C=O). – MS (EI, 70 eV): *m/z* (%) = 367 (15) [M]$^+$. – C$_{22}$H$_{27}$N$_3$O$_2$ (367.21): Calcd. C 72.30, H 7.45, N 11.50; found C 71.89, H 7.40, N 12.01.

N-[(1-dimethylcarbamoyl)-2-(3,4,5-trimethoxyphenyl)-vinyl]benzamide (16e)

Product **16e** was separated as colorless needles, yield 75 %. – M. p. 150 – 152 °C (ethyl acetate). – IR (KBr): ν = 3360 (NH), 1656, 1650 (2 C=O) cm^{-1}. – ^{1}H NMR

(500.14 MHz, CDCl$_3$): δ = 2.9 – 3.2 (6H, 2 s, N(CH$_2$*CH*$_3$)$_2$,), 3.8 (9H, 2 s, (3O*CH*$_3$), 6.0 (1H, s, CH), 6.6 – 7.8 (m, 7H, H$_{arom}$), 8.5 (1H, s, NH exchangeable with D$_2$O). – ^{13}C NMR (125.76 MHz, CDCl$_3$): δ = 37.2 (N(CH$_2$*CH*$_3$)$_2$), 56.0, 60.5 (3 O*CH*$_3$), 107.4, 127.1 (CH=C), 128.2 – 132.4 (CH aromatic), 165.6, 168.4 (2 C=O). – MS (EI, 70 eV): m/z (%) = 384 (35) [M]$^+$. – C$_{21}$H$_{24}$N$_2$O$_5$ (384.17): calcd. C 65.61, H 6.29, N 7.29; found C 66.01, H 6.27, N 7.83.

N-[(1-Diethylcarbamoyl)-2-(3,4,5-trimethoxyphenyl)-vinyl]benzamide (*16f*)

Product **16f** was separated as colorless needles, yield 85 %. – M. p. 149 – 150 °C (cyclohexane). – IR (KBr): ν = 3368 (NH), 1660, 1650 (2 C=O) cm^{-1}. – ^{1}H NMR (500.14 MHz, CDCl$_3$): δ = 1.22 – 1.24 (6H, 2 t, N(2CH$_2$*CH*$_3$), 3.8 – 3.9 (9H, 2s, 3O*CH*$_3$), 3.92, 3.95 (4H, 2q, N(2*CH*$_2$CH$_3$), 6.2 (1H, s, CH), 6.6 – 8.3 (m, 7H, H$_{arom}$.), 8.4 (1H, s, NH exchangeable with D$_2$O). – ^{13}C NMR (125.76 MHz, CDCl$_3$): δ = 14.3 (N(CH$_2$*CH*$_3$)$_2$), 65.0 (3 O*CH*$_3$), 60.5 (N(2*CH*$_2$CH$_3$), 107.4, 122.7 (CH=C), 128.2 – 153.1 (aromatic CH), 165.3, 167.4 (2 C=O). – MS (EI, 70 eV): m/z (%) = 412 (45) [M]$^+$. – C$_{23}$H$_{28}$N$_2$O$_5$ (412.2): calcd. C 66.97, H 6.84, N 6.79; found C 66.45, H 6.92, N 6.97.

Pharmacological activity

Materials and methods

Chemicals: All chemicals and reagents used in this study were of analytical grade and purchased from Sigma Chemical Co., St. Louis, Mo (USA).

Cell culture: MCF7 human breast cancer cells were maintained and grown in RPMI-1640 medium supplemented with 10 % heat-inactivated fetal borine serum (Sigma Chemical Co., St. Louis, USA), penicillin and streptomycin at 37 °C in humidified atmosphere containing 5 % CO$_2$.

In vitro cytotoxicity assay: For *in vitro* short-term cytotoxicity evaluation of prepared compounds, MCF7 cells were placed at a concentration of $5 \times 10^4 – 10^5$ cells per well, in complete culture medium in 96 well flat-bottomed culture plates (Falcon) for 24 h to ensure total attachment. Then various concentrations of test compounds were added to the cells suspended in 0.10 mL of phosphate buffered saline (FBS) (0.20 M, pH 7.4). The control cells without the test compounds were also cultured, then the plate was incubated for 24 h at 40 °C and 72 h at 37 °C, in a humidified 5 % CO$_2$ atmosphere. Cell survival was evaluated at the end of the incubation period with the Sulphorhodamine-B (SRB) colorimetric assay according to Skehan *et al.* [24]. This test is based on the sensitivity of the human tumor cell lines to thymoquinone as determined by the SRB assay. SRB is a bright pink aminoxanthrene dye with two sulfonic groups. It is a protein stain that binds to the amino groups of intra-cellular proteins under mildly acidic conditions to provide a sensitive index of cellular protein content. After incubation, media were removed and 50 μL of 0.4 % SRB dissolved in 1 % acetic acid solution was added and then washed 4 times with 1 % acetic acid. The absorbance was determined photometrically at 564 nm with an ELISA microplate reader (Meter tech. Σ 960, USA).

Calculation: The percentage of cell survival was calculated as follows: Survival fraction = O. D. (treated cells) / O. D. (control cells) where (O. D.) is the optical density. The IC$_{50}$ values are the concentrations of thymoquinone required to produce 50 % inhibition of cell growth. The experiment was repeated 3 times for each cell line.

Evaluation of antischistosomal activity of some compounds

Materials: Adult worms of *Schistosoma mansoni* (Egyptian strain) are obtained by infecting Syrian golden hamsters (*Mesocricetus auratus*) by percutaneous infection of 350 cercariae / animal, freshly shed from infected *Biomphalaria alexandrina* snails [30]. The animal experiments were carried out according to the internationally valid guidelines in an institution coping with biological ethics (Theodor Bilharz Research Institute) [31]. Worms are obtained by portomesenteric perfusion, 45 d post infection using citrated saline (7.5 g sodium citrate +8.5 g sodium chloride per liter).

The worms in a small sterilized sieve were washed three times with phosphate buffer (pH = 7.4), then three times with a 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.

The heterocyclic compounds were tested at a concentration of 10 μg mL^{-1} for *in vitro* bioactivity on viable *Schistosoma mansoni* mature worms in a 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 (DMSO) and positive (Praziquantel) controls. Praziquantel (PZQ) is the mainstay of schistosomiasis control programs worldwide.

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