



## Anti-cancer, Anti-inflammatory, Cytotoxic and Biochemical Activities of a Novel Phosphonotripeptide Synthesized from Formyl Pyrazolofuran using TUBU as Condensing Agent

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### ABSTRACT

A novel and mild protocol for the synthesis of phosphonotripeptide **16** were achieved from novel formylpyrazolofuran **3**, benzyl carbamate **5**, triphenyl phosphite **6** by Birum-Oleksyszyn reaction using copper (II) triflate as Lewis acid catalyst in dry dichloromethane at room temperature which synthesis of novel N-benzyloxy- $\alpha$ -aminophosphonate **7**. A mechanism for this condensation reaction is proposed. Cleavage of the N-benzyloxy carbonyl group under acid hydrolysis afford the free  $\alpha$ -aminophosphonate **9** in quantitative yield. This in turn, by reaction with N-phthaloylglycine **12** via TUBU to phthalimido group **13**. Cleavage of phthalimido group under hydrazinedihydrochloride afford free peptide **14**. The result phosphonotripeptide **16** obtained by the reaction of free peptide with bioactive cyclic moiety-COCl **15**. The structures of all new compounds were established by IR, <sup>1</sup>HNMR and mass spectral data. Also anti-cancer, anti-inflammatory and biochemical activities of all these compounds were reported.

### Indexing terms/Keywords

$\alpha$ -aminophosphonate; N-phthaloylglycine; TUBU; hydrazinedihydrochloride; antiinflammatory activity

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## Introduction

Phosphonotripeptides have been found to exhibit a wide range of biological activities. To the best of our knowledge there are no papers reports on the preparation of Phosphonotripeptide biconjugates. We focused on formyl pyrazolofuran because it is important class of compounds and attracted widespread. Attention due to their pharmacological properties, being reported to have a large spectrum of biological effects, anti-cancer [1-3]. The present work was aimed to synthesize novel phosphonotripeptide containing pyrazolofuran and N-protected phthaloyl glycine in order to find new anticancer agent. The general method for synthesis of diphenyl  $\alpha$ -aminophosphate is the three component reaction of formyl pyrazolo furan, carbamate and triphenylphosphite in organic solvent by one pot Birum-Oleksyszyn reaction [4] according to **Scheme 1**.  $\alpha$ -aminophosphate converted by the deprotection with the appropriate acid condition into the respective free  $\alpha$ -aminophosphonate, this is in turn, by reaction with N-protected phthaloyl glycine [5] via TUBU to phthalimido group [6] which by the cleavage of phthalimido group turn to free peptide via hydrazinedihydrochloride [7]. The novel phosphinotripeptide afforded by the coupling of N-protected acid chloride with phosphonodipeptide [8].

## 1-Results and Discussion

### 1.1 Chemistry

In our efforts to prepare organophosphorous compounds to be employed as novel biologically active agents, we focused on development of a new three-component synthesis of N-protected  $\alpha$ -aminophosphate from novel 4-formyl-1-phenyl-3-furyl pyrazole, benzyl carbamate triphenylphosphite and Lewis acid. The synthesis of aldehyde needed for this study is achieved according to the following synthetic protocol using Vilsmeier-Haack (VH) reagent. The wide biological application of 4-formyl-1-phenyl-3-furyl pyrazole prompted to undertake the synthesis of  $\alpha$ -aminophosphate bearing pyrazole moiety.

The synthesis of 4-formyl pyrazole from the double formylation of hydrazone **3** with Vilsmeier-Haack (VH) reagent. To this end the intermediate phenyl hydrazone **3** were in situ-synthesized in almost quantitative yields by the reaction of phenyl hydrazine **1** and acetyl furan **2**. The reaction of **3** was carried out at 70°C for 6 h. using three equivalents of POCl<sub>3</sub> and dry DMF (VH) reagent affording novel 1-phenyl-3-furyl pyrazole-4-carboxaldehyde **4** in very good yield. It should be noted that **4** cannot be obtained if the DMF is not anhydrous. We started the next step to apply one-pot three component synthesis of diphenyl  $\alpha$ -aminophosphonate **7** via nucleophilic addition of triphenyl phosphite **6** into an amine formed from condensation of 1-phenyl-3-furyl pyrazole-4-carboxaldehyde **4** with carbamate **5** in dry solvent as dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>) at room temperature in the presence of cuppor triflate according to (**Scheme 1**). The removal of nitrogen protecting group is well known in the art of organic synthesis. The ammonium hydrobromide **8** were obtained after deprotection of benzyloxy carbonyl group of benzyl carbamate with solution of hydrogen bromide in acetic acid at room temperature in a short time (0.5-2h). Transformation of ammonium bromide into free amine **9** was achieved. Triethyl amine was chosen, in this case, to preferentially saponify the corresponding salt even though completion of the reaction required a little longer time. Thus, the desired free diphenyl  $\alpha$ -aminophosphonate were obtained by treating hydrobromide with triethyl amine in anhydrous THF. Filtration of the salt followed by evaporation of low boiling substance under reduced pressure resulted in the free amine **9**. Synthesis of the phosphinopeptide began with condensation of phthalic anhydride **10** with glycine **11** at high temperature  $\geq 180^\circ\text{C}$  under solvent free conditions it should be noted that both of the reactants should be well ground and mixed evenly to ensure entire transformation of starting materials into the N-phthaloyl product **12**. At room temperature N-protected glycine **12** is readily coupled with free diphenyl  $\alpha$ -aminophosphonate **9** in the presence of 2(1H-benzotriazol-1-yl)1,1,3,3-tetramethyl uroanium tetrafluoroborate (TBTU) as a peptide coupling agent to afford the N-protected phosphonodipeptide **13** in high yield. The phthaloyl group in the protected dipeptide **13** was subsequently subjected to cleavage by hydrazinolysis in absolute methanol overnight at room temperature to deliver the free amine containing phosphonopeptide **14**.

To prepare phosphonotripeptide, chlorination of N-protected glycine **12** by thionyl chloride was achieved under solvent-free condition at 60°C to afford **15** in high yields as illustrated in **Scheme 1**. The excess of thionyl chloride was removed at 55°C. It was found that high temperature would cause partial decomposition of the product. The crude acid chloride generated was used immediately in the next step after the dissolution in anhydrous CH<sub>2</sub>Cl<sub>2</sub>. The coupling of acid chloride **15** with phosphonodipeptide **14** proceeded smoothly on ice-path in the presence of triethyl amine. The pure product **16** was obtained in higher yield after recrystallization from a mixture of dichloromethane/diethyl ether.

### 1.2 Biological studies

#### 1.2.1 Anticancer Activity

**It was observed through analysis that:** The dipeptide **16** had the highest cytotoxic activity against breast carcinoma (MCF7) cell line (IC<sub>50</sub>) = (0.534  $\mu\text{g/ml}$ ) and also against colon carcinoma cell line (IC<sub>50</sub>) = (0.629  $\mu\text{g/ml}$ ) as shown in **table 1**. Compound **4** was active against colon carcinoma cell line (IC<sub>50</sub> = 0.781  $\mu\text{g/ml}$ ) had IC<sub>50</sub> similar to that of standard drug and showed high activity against breast carcinoma cell line (IC<sub>50</sub> = 0.743  $\mu\text{g/ml}$ ). Compounds **7, 9, 13, 14** showed high activity against breast carcinoma cell line and colon carcinoma cell line (IC<sub>50</sub> = 0.865, 0.858 and 0.896  $\mu\text{g/ml}$ ) respectively for breast carcinoma and (IC<sub>50</sub> = 0.868, 890 and 750  $\mu\text{g/ml}$ ) for colon carcinoma.

#### 1.2.2-Anti-inflammatory activity

From **Table 2** it appeared that compound **16** at doses of 40, 60 and 80 mg % has significant anti-inflammatory activity compared with indomethacin as a reference drug at 1, 2, 3 and 4 hours.



### 1.2.3-Acute toxicity studies (LD<sub>50</sub>)

The acute toxicity of compound **16** in adult mice were tested. The results are given in **Table 3** shows that i.p. injection of compound **16** in doses of 80, 120, 200, 250 and 350 and 400 mg/100g b.w. resulted in mortalities of 0, 1, 2, 4, 8 and 10 respectively.

### 1.2.4- Biochemical studies

Administration of compound **16** orally to the rats at dose of 300 mg/kg b.w. for 15 days showed non-significant differences in liver enzymes AST, ALT, ALP, LDH,  $\gamma$ -GT and serum MDA as compared with the control group **Table 4**.

## 2- Experimental

### 2-1. Chemistry

All melting points were taken on Electrothermal IA 9000 series digital melting point apparatus. The IR spectra (KBr) were recorded on a PYEUNICAM spectrophotometer. The <sup>1</sup>H NMR spectra was recorded at 300 MHz on a Perkin Elmer R12B Spectrometer using TMS as an internal standard. The mass spectra were performed using VG2AB-3F spectrometer (70 eV). All reactions were followed by TLC (silica gel, aluminum sheets 60 F254; Merck).

#### 2.1.1. General procedure for the preparation of 1-phenyl-3-furyl pyrazol 4-Carboxaldehyde

To (0.01 mol) methylfuryl ketone hydrazone, (0.01 mol) Vilsmeier reagent (14.6 ml DMF and 19.1 ml POCl<sub>3</sub>) was added drop wise with stirring for one hour. The reaction mixture was refluxed for 6 hours at 70-80°C. then hydrolyzed on ice-water mixture, neutralized by 5% NaOH solution till pH = 4, the solid formed was filtered off, washed with water, dried and recrystallized from isopropanol.

#### 2.1.2 General procedure for the preparation of $\alpha$ -aminophosphonate.

benzyl carbamates (1 mmol), aldehyde (1.2 mmol) and P(OPh)<sub>3</sub> (1 mmol) were dissolved in well dried CH<sub>2</sub>Cl<sub>2</sub> (2 ml). The Lewis acid Cu(OTf)<sub>2</sub> (10 mol%) was added in one portion as 1 M solution in anhydrous CH<sub>2</sub>Cl<sub>2</sub>. This mixture was stirred at room temperature until TLC analysis showed the complete consumption of benzyl carbamate. then CH<sub>2</sub>Cl<sub>2</sub> was evaporated and the residue dissolved in MeOH (10 ml). The product was precipitated from this solution by storing at -20°C for 3- 6 h., followed by the collection of the precipitate by the filtration. Recrystallization was carried out by dissolving the product in CHCl<sub>3</sub> (5 ml), Concentration the CHCl<sub>3</sub> under reduced pressure, and adding MeOH (8 ml) to the residue. This solution was stored at -20°C for 3-6 h., the precipitated product was filtered off and dried under reduced pressure.

#### 2.1.3 General procedure for cleavage of benzyloxy carbonyl group.

To (0.01 mol) benzyloxy carbonyl group, (0.01 mol) HBr/HOAc was added drop wise with stirring. After complete addition, the reaction mixture was left to stirred at r.t. for 1 h. The solid formed was filtered off, washed with methanol (10 ml) and dried under reduced pressure.

#### 2.1.4 preparation of phthaloylglycine

Phthalic anhydride (0.01 mol), glycine (0.1 mol) was heated in sand bath for 3 h. After the reaction completed added (10 ml) ethanol and filtered the white solid, dried, recrystallization was carried out by using H<sub>2</sub>O.

#### 2.1.5 General procedure for the preparation of phthalimido group

Phthaloylglycine (1 mmol) was dissolved in dry dichloromethane (15 ml), triethylamine (4 mmol) was added, followed by TBTU (1.1 mmol). After the mixture was stirred for 5 min, the appropriate amine (1.1 mmol) dissolved in 2 ml of dichloromethane was dropped into the solution and stirring was continued for 3 h., The solid formed was filtered off, dried, recrystallization was carried out using ethanol.

#### 2.1.6 General procedure for cleavage of the phthalimido group.

To a solution of **13** (0.6 mmol) and hydrazinedihydrochloride (0.83g, 2.7 mmol) in dry methanol (8 ml), a solution of triethyl amine (0.7 ml, 5.4 mmol) in dry ethanol (2 ml) was added drop wise. The mixture was heated at 40°C for 4h. and then cooled, add water (30 ml) and extracted with ethyl acetate. The solvent was evaporated and the residue was chromatographed on silica gel column with dichloromethane/ethyl acetate 1:1 give **14**.

#### 2.1.7 General procedure for the preparation of Carbonyl chloride of Phthaloylglycine

Phthaloylglycine (0.1 mol) and toluene (50 ml) was placed in 250 ml flask, equipped with magnetic stirrer. After dissolving of Phthaloylglycine, (0.3 mol) of thionyl chloride was added to the reaction mixture. The mixture was refluxed for 6-7 h., with stirring. The volatile compound was removed by rotary evaporation and gave the desired, crude carbonyl chloride which was immediately used to the next step without further purification.

#### 2.1.8 General procedure for synthesis of phosphonotriptide

(0.1 mol) of carbonyl chloride was dissolved in (15 ml) dry dichloromethane and placed in the flask (100 ml). The reaction mixture was cooled to 0°C. and (0.1 ml) of free amino phosphonotriptide **14** was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> and added very slowly with stirring to the carbonyl chloride. Added few drop of Et<sub>3</sub>N to the mixture. The mixture was stirring until TLC



analysis showed the complete of the reaction. The volatile compound were removed by rotary evaporation and gave the desired product, filter the precipitate, dried and crystallized from ETOH.

#### 2.1.9-phenyl-3-furyl pyrazol-4-Carboxaldehyde (4)

m.p.= 150-151°C, IR(KBr)  $\nu$ =1600, 1510, 820, 730  $\text{Cm}^{-1}$ ,  $^1\text{HNMR}$  (400 MHz,  $\text{CDCl}_3$ ),  $\delta$ =7.4-8.1(m, 8H, Harom), 8.7 (s, 1H, CHpyrazole), 10.25 (s, 1H, CHO), MS: m/z = 238( $\text{M}^+$ ), 209, 161 (base peak).

#### 2.1.10-Diphenyl[(BenzyloxyCarbonyl) amino](1-phenyl 3-furyl pyrazole) methyl phosphonate (7)

m.p.= 230-231°C, IR(KBr)  $\nu$ =3271(NH), 1600(C=N), 1500(C=C), 1232 (P-O), 1062(P-O-C)  $\text{Cm}^{-1}$ ,  $^1\text{HNMR}$  (400MHz,  $\text{CDCl}_3$ )  $\delta$ =5.05-5.22 (m, 2H,  $\text{CH}_2\text{OC}$ ), 5.5-5.69 (m, 1H, CHpyrazole), 8.6 (s, 1H, CHpyrazole).  $\delta$ =6.81-6.98(s, 1H, NH), 6.78-6.89 (m, 5Harom), 7.05-7.43 (m, 3Hfuran). MS: m/z = 605( $\text{M}^+$ ).

#### 2.1.11-Diphenyl-1-amino[1-phenyl-3-furyl pyrazole) methyl phosphonate (9)

m.p.= 200-201°C, IR(KBr)  $\nu$ =3404(NH), 1600(C=N), 1500(C=C), 1232(P-O), 1062(P-O-C)  $\text{Cm}^{-1}$ ,  $^1\text{HNMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$ =5.5-3.5 (m, 1H, CHpyrazole), 5.8-6.0 (d, 2H,  $\text{NH}_2$ ), 7.05-7.2 (m, 15Harom), 7.41-7.6(m, 3Hfuran), 8.61(s, 1H, CHpyrazole), MS: m/z = 470( $\text{M}^+$ ).

#### 2.1.12-Phthaloyl glycine (12)

m.p.= 190-191°C, IR(KBr)  $\nu$ =1500, 1610  $\text{Cm}^{-1}$ ,  $^1\text{HNMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$ =4.2 (s, 2H,  $\text{CH}_2$ ), 3.3 (s, 1H, OH), 7.8-7.9 (m, 4Harom). MS: m/z = 206( $\text{M}^+$ ).

#### 2.1.13-Diphenyl-2 [(N-phthaloyl glycine) amino]-1-phenyl-3-furyl pyrazole) methyl phosphonate(13)

m.p.= 320-321°C, IR(KBr)  $\nu$ =3296, 1234, 1027  $\text{Cm}^{-1}$ ,  $^1\text{HNMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$ =4.4-4.42 (s, 2H,  $\text{CH}_2$ ), 5.1-5.15 (m, 1H, CHP), 5.7-5.9 (d, 1H, NH), 7.00-7.34 (m, 15Harom), MS: m/z = 657( $\text{M}^+$ )

#### 2.1.14-Diphenyl-2 [(glycinamino)]-1-phenyl-3-furyl pyrazole) methyl phosphonate(14):

m.p.= 290-291°C, IR(KBr)  $\nu$ =3400, 3295, 1234, 1027  $\text{Cm}^{-1}$ ,  $^1\text{HNMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$ =3.4-3.44 (t, 2H,  $\text{CH}_2$ ), 5.0-5.1 (m, 1H, CHP), 5.7-5.86 (t, 2H,  $\text{NH}_2$ ), 7.05-7.2 (m, 15Harom), 7.041-7.6(m, 3Hfuran), 8.61(s, 1H, CHpyrazole) MS: m/z = 527 ( $\text{M}^+$ ).

#### 2.1.15-Phthaloyl glycinacyl chloride (15)

m.p.= 152-154°C, IR(KBr)  $\nu$ =1500, 1820  $\text{Cm}^{-1}$ ,  $^1\text{HNMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$ =4.21-4.33 (s, 2H,  $\text{CH}_2$ ), 7.8-7.9 (m, 4Harom). MS: m/z = 223( $\text{M}^+$ ).

#### 2.1.16-Diphenyl-3 [(N-phthaloyl glycine)-2-glycinamino]-1-phenyl-3-furyl pyrazole) methyl phosphonate(16):

m.p.= 360-361°C, IR(KBr)  $\nu$ =3271, 1820, 1600, 1500, 1232, 1062  $\text{Cm}^{-1}$ ,  $^1\text{HNMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$ =3.04-3.1 (s, 2H,  $\text{CH}_2$ ), 3.5-3.51 (d, 2H,  $\text{CH}_2$ ), 5.7-5.9 (d, 1H, NH), 8.3-8.31 (s, 1H, NH), 8.60(s, 1H, CHpyrazole), 7.05-7.1 (m, 10Harom), 7.41-7.42(m, 3Hfuran). MS: m/z = 715( $\text{M}^+$ ).

## 2.2-Biological studies

### 2.2.1- Cytotoxic activity evaluation

The Sulporhodamine B (SRB) assay of Skehan [10] was used to evaluate the cytotoxic activity of the newly synthesized compounds 4, 7, 9, 13, 14, 16 against two cell lines, breast carcinoma (MCF7) and colon carcinoma (HCT116). Cells were plated in 96-multiwell plates ( $10^4$  cells/well) for 24 h. before treatment with the compound to allow attachment of the cells to the wall of the plate.

Different concentrations of the compounds under test (0, 1, 2.5, 5 and 10 mg/ml) were added to the cell monolayer. Triplicate wells were prepared for each dose. Monolayer cells were incubated with the compounds under test for 48h. at 37°C and atmosphere of 5%  $\text{CO}_2$ . After 48 h., cells were fixed, washed and stained with The Sulporhodamine B stain. Excess stain was washed with acetic acid and then after attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader.  $\text{IC}_{50}$  value was determined for each tumor cell line for the specified compounds, calculated by an available computerized program, which was defined as the concentration of drug to produce 50% reduction in the viability relative. The control given compound were recorded in Table 1. Given compounds were considered significantly inactive when their  $\text{IC}_{50}$  values are higher than 10  $\mu\text{g/ml}$ .

### 2.2.2- Anti-inflammatory activity

Male albino mice weight around 150- 200 g were purchased from Faculty of Science, Tanta University, Egypt. They were acclimatized to animal house conditions. Animals were provided with standard diet. The anti-inflammatory activity was



carried out following the method of *Domenjoz, R* [11]. Rats were divided into 5 different groups each of 10 animals. At the beginning, the thickness of the left paw was measured. They were treated orally with the tested compounds, at 40 mg/ kg body weight or indomethacin 600 mg/kg as a reference standard. After one hour of administration, the inflammation was induced by S.C. injection of 0.1 ml of 6 % formalin solution in normal saline. The right hind paw was injected with unequal volume of saline. The difference in thickness between the two paws gave the swelling induced by formalin. The anti-inflammatory efficacy was estimated by comparing the swelling of the treated with the control. The difference in thickness was recorded after 1, 2, 3 and 4 hours.

### 2.2.3-Acute Toxicity Studies (LD<sub>50</sub>)

Preliminary experiments were carried out on 6 main groups (10 mice/each dose/each group). Compound **16** was injected in different doses to find out the range of doses which cause zero and 100 % mortality of animals. LD<sub>50</sub> was determined as indicated in **Tables 2**.

The LD<sub>50</sub> was then calculated by the application of the following formula:

$$LD_{50} = \frac{D_m - \sum(Z.d)}{n}$$

D<sub>m</sub> = The dose by which killed all the mice in the group.

Z = Half the sum of the dead rats from 2 successive groups.

d = The difference between 2 successive doses.

n = number of animals in each group.

### 2.2.4- Biochemical studies

Administration of compound **16** orally to the rats at dose of 300 mg/kg b.w. for 15 days showed non-significant differences in liver enzymes AST, ALT, ALP, LDH, γ-GT and serum MDA as compared with the control group (**Table 3**).

#### 2.2.4.1- Experimental Design

This experiment was carried out to examine the effect of compound **16** on liver enzymes. A solution of 6 g % for compound **16** in DMSO was prepared and administrated to rats. Groups of animals each consisting of 6 rats in each were treated daily for 15 days as follows:

**Group I:** Control (was given similar volume of saline)

**Group II:** Normal (was given similar volume of DMSO)

**Group III:** Was treated with compound **16** (300 mg/kg b.w.) dissolved in DMSO orally in a single daily dose [12].

After 10 days of treatment, animals were sacrificed, blood samples were withdrawn of each animal. The separated blood was used for the estimation of AST, ALT, γ-GT, ALP, LDH and MDA.

#### 2.2.4.2- Biochemical Assays

The separated blood was used for the estimation of Serum levels of Aspartate transaminase (AST), Alanine transaminase (ALT) [13], alkaline phosphatase (ALP) [14], Gamma Glutamyltransferase γ-GT [15], lactate dehydrogenase [16] and Malondialdehyde as TBARS in serum [17].

## Conclusion

The main objective of this study was to synthesize novel derivatives and exploration their potential cytotoxic activity and measuring some biochemical parameters to assess their safety as anticancer drugs. The results in table 1 showed that compound **16** was the most active one against breast carcinoma (MCF7) cell line and colon carcinoma (HCT116). Also results at table 2 showed that all examined compounds has non-significant differences in liver enzymes AST, ALT, ALP, LDH, γ-GT and serum MDA as compared with the control group.

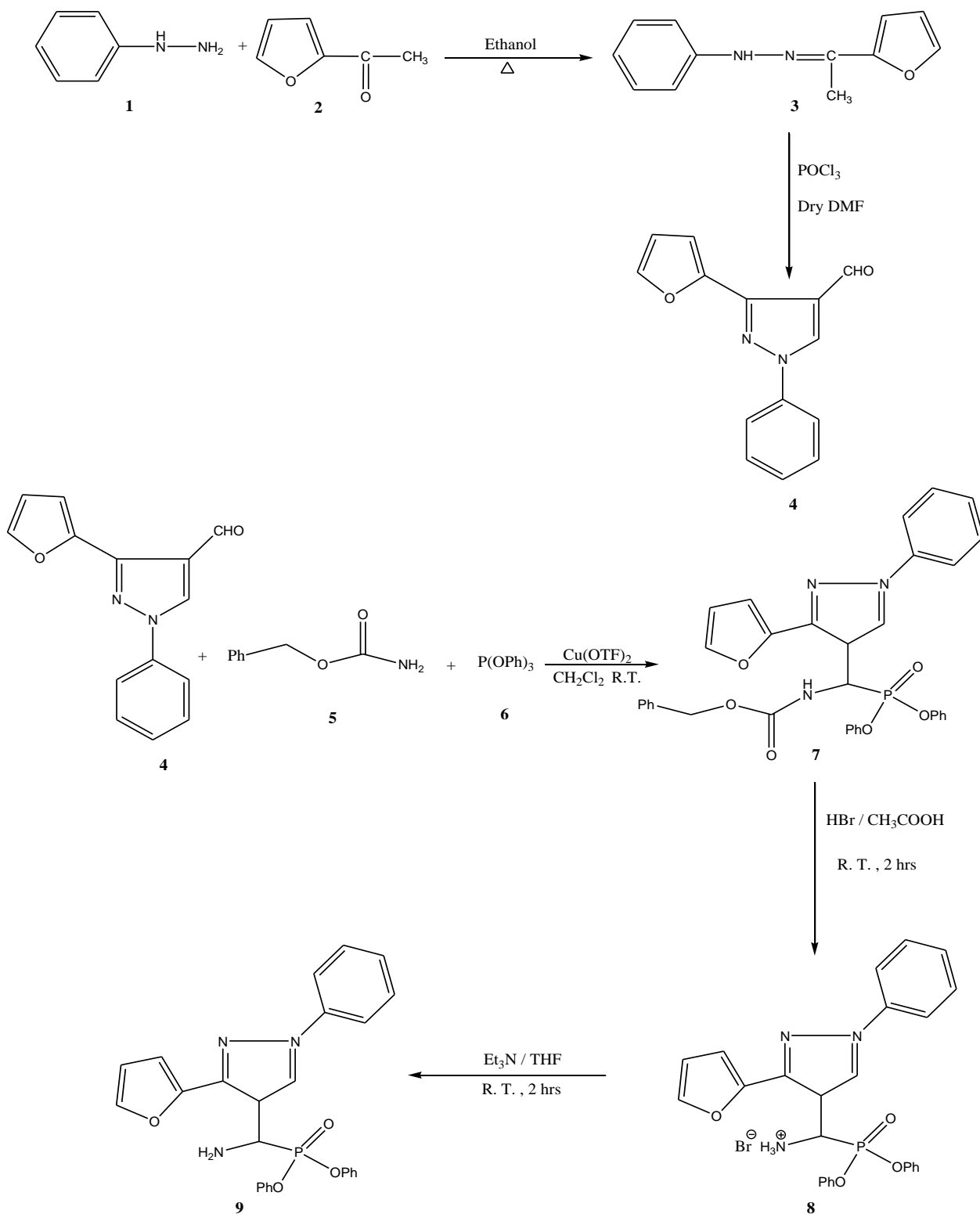
## References

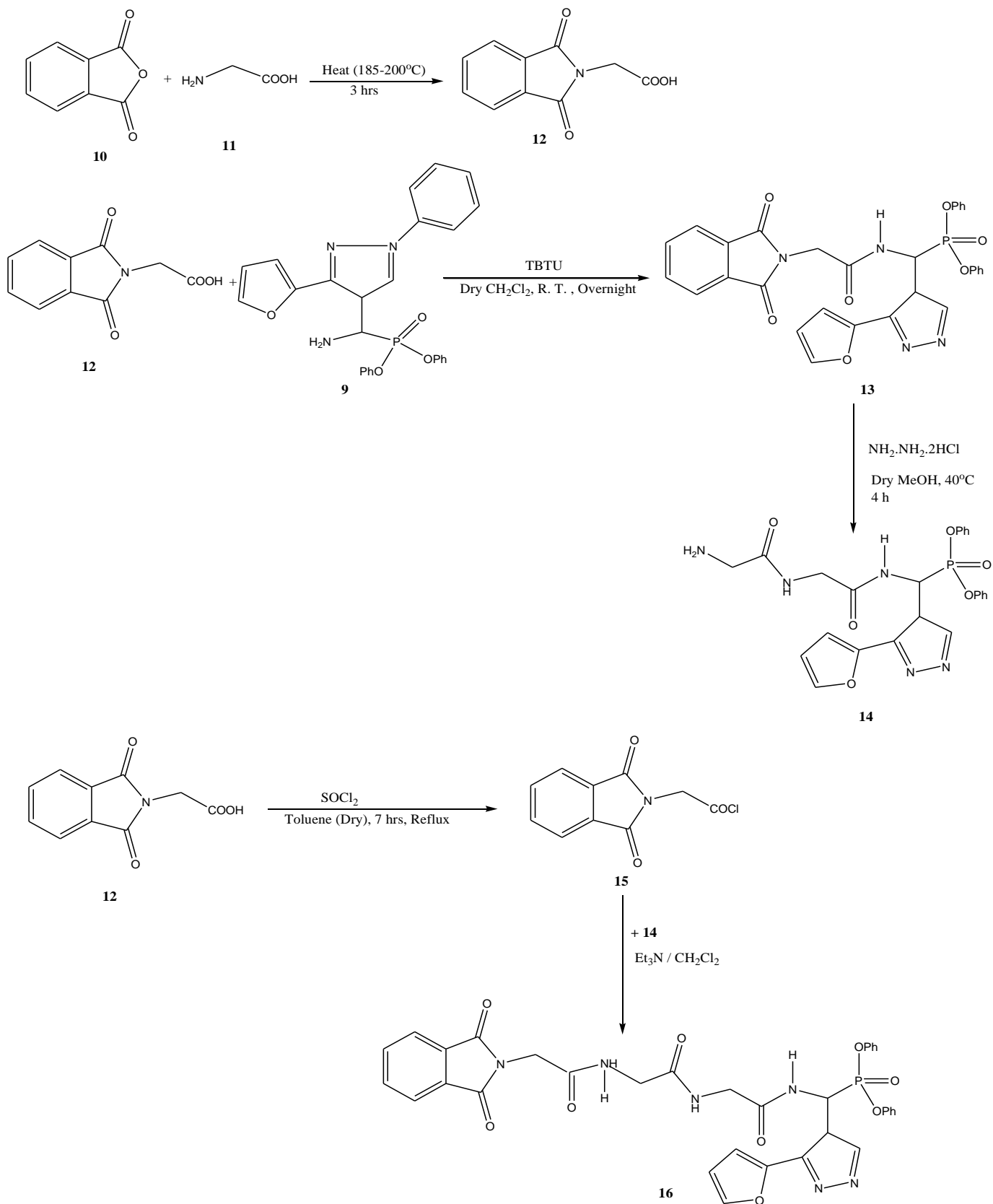
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Scheme (1)





**Table (1): Cytotoxic activity of the synthesized compounds against breast carcinoma (MCF7) and colon carcinoma (HCT116) cell lines.**

Compound No.	IC <sub>50</sub>	
	Breast carcinoma(MCF7) cell line	Colon carcinoma(HCT116) cell lines.
4	0.743	0.781
7	0.743	0.896
9	0.865	0.858
13	0.858	0.868
14	0.896	0.890
16	0.534	0.750
Doxorubicin	0.629	0.743

IC<sub>50</sub> is a dose required to inhibit the cell growth by 50%

**Table (2) : The Anti-inflammatory activity of compound 16 and indomethacin in dose of 0.4, 0.6 and 0.8 mg / 100 gm body weight of rats on the inflamed rat paw (n=5 rats). (Mean+ S.E).**

Groups	Initial thickness	Thickness of Rat Paw (mm) after			
	(zero time)	1 hour	2 hours	3 hours	4 hours
Control	2.0±0.0	5.8±0.23	6.72±0.31	7.7±0.31	7.8±0.32
Indomethacin	2.2±0.17	2.33±0.23 <sup>a</sup>	2.71±0.31 <sup>a</sup>	3.7±0.28 <sup>a</sup>	3.9±0.24 <sup>a</sup>
0.4 mg/100 g b.w	2.3±0.0	3.5±0.21 <sup>ab</sup>	4.8±0.31 <sup>ab</sup>	5.6±0.23 <sup>ab</sup>	5.3±0.22 <sup>ab</sup>
0.6 mg/100 g b.w	2.2 ±0.0	3.3±0.23 <sup>ab</sup>	4.6±0.28 <sup>ab</sup>	5.5±0.24 <sup>ab</sup>	5.2±0.24 <sup>ab</sup>
0.8 mg/ 100g b.w	2.2 ±0.21	3.0±0.21 <sup>ab</sup>	4.4±0.22 <sup>ab</sup>	5.2±2.2 <sup>ab</sup>	5.0±0.21 <sup>ab</sup>

Compared with control, <sup>a</sup> p< 0.001

Compared with indomethacin <sup>b</sup> p< 0.01

**Table ( 3): Determination of LD<sub>50</sub> of compounds 14b, 14d, 15 and 17 given i.p. in adult mice**

Compound	Dose(mg/kg)	animals/group	Dead animals	(Z)	(d)	(Z.d)
16	80	10	0	0.5	40	20
	120	10	1	8.0	50	40
	200	10	2	2	70	140
	250	10	4	4	80	320
	350	10	8	8	90	720
	400	10	10	10	0	00



$$LD_{50} = \frac{Dm - \sum(Z.d)}{n}$$

LD<sub>50</sub> of compound 16 = 276 mg/100 g. b.w.

Table(4): Serum Levels of AST, ALT, ALP, γ-GT, LDH and MDA in normal and experimental groups of rats

Group	AST U/l	ALT U/l	ALP U/l	γ-GT U/l	LDH U/l	MDA nmol/ml
Normal Saline	8.6± 1.3	32± 2.5	35.0±2.6	5.3± 1.8	223.0± 6.22	5.22±1.31
Control(DMSO)	9.22± 1.75 N.S.	34± 2.7 N.S.	38.3±2.9 N.S.	5.55±1.65 N.S.	232.1± 6.8 N.S.	5.52±1.5 N.S.
Compound 16 (300 mg/kg.b.w)	10.1± 1.3 N.S.	36± 2.44 N.S.	40.5±2.62 N.S.	5.9± 1.8 N.S.	240±6.4 N.S.	5.72±1.4 N.S.

Compared with control, <sup>a</sup>p< 0.001

N.S. Non- significant