Synthesis and characterization of acetic acid functionalized poly (4-vinylpyridinium) salt as new catalyst for the synthesis of spiropyran derivatives and their biological activity

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Abstract:

Acetic acid functionalized poly(4-vinylpyridinium) bromide (APVPB), as a new and heterogeneous catalyst, was designed and successfully tested on the synthesis of 2-aminospiro[indoline-3,4'pyran]-2-one and spiro[indeno[1,2-b]quinoxaline-11,4-pyran]-2'-amine derivatives. Also, infrared spectroscopy (FT-IR), thermal gravimetric analysis (TGA), differential thermal gravimetric (DTG), scanning electron microscope (SEM), X-ray diffraction analysis (XRD) and energy dispersive X-ray analysis (EDX) of the catalyst has been studied in the presented work. The prepared spiro-pyrans were solved in DMSO and subjected to antioxidant activity screening by DPPH free radical scavenging assay and antifungal activity against *Fusarium oxysporum*. Results showed that the synthesized spiro-pyran possess strong antioxidant activity (IC₅₀; 0.161 ± 0.009 to 1.509 ± 0.007 mg/ml) and also good inhibition activity against *Fusarium oxysporum* (65.8 ± 1.4% to 88.3 ± 2.2% at 500 ppm concentration and

89.2 $\pm$ 3.2% to 100 $\pm$ 0.0% at 1000 ppm concentration). For their biological activities, **the prepared** spiro-pyran compounds may be suggested to apply as alternative systems in medicine and agriculture.

Key words: Acetic acid functionalized poly(4-vinylpyridinium) bromide (AFPVPB), Heterogeneous catalyst, 2-amino spiro[indoline-3,4'pyran]-2-one, Spiro [indeno[1,2-b]quinoxaline-11,4-pyran]-2'-amine, Antioxidant, Antifungal.

1. Introduction

During the last decade multi component reactions (MCRs) have been attracted the attention of organic chemists for combinatorial chemistry in toward the prepare target compounds including heterocyclics scaffold with a significant role in biologically activity, greater efficiency and atomic economy by generating in a single step from three or more reactants. Also, MCRs offer some advantages such as simplicity and synthetic efficiency over conventional chemical reactions [1-5].

Free radical such as reactive oxygen or nitrogen species (ROS or RNS) are generated either naturally in living cells or due to some biological dysfunction such as stress. These compounds have important roles in cell signaling but high amount of these compounds are harmful to the cells and their major components including DNA, enzymes, cell membrane lipids and proteins that play an important role in the development of diseases such as cancer and other disorders [6, 7]. Antioxidants are compounds that may prevent from the free radical damages in biological systems. Among antioxidants, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) as synthetic antioxidants are used as additives in foods but they have toxic and carcinogenic effects [8]. Studies focuses on antioxidant agents that can protect cell components from oxidative damage without harmful side effects [9]. Spiro compounds have also been

recently used as antioxidants [10, 11]. Today most of the microbes showed resistance to classical antibiotics [12, 13] and the discovery of new compounds with novel mechanisms is a matter of urgency [14, 15]. Recently, spiro compounds used as antimicrobial agents in the agricultural and industrial fields [16, 17].

The tetrahydrobenzo[b]pyrans are an important class of the **heterocyclic compounds**. These ring systems have been subjected of various studies and successfully applied as anti-coagulant, diuretic, spasmolytic, anti-cancer, and anti-anaphylactic [18]. Also, these compounds possess the physical properties and they can be widely used as photoactive materials [19]. Moreover, indenoquinoxalines exhibit different pharmacological activity such as anti metabolism and antitubercular [20] and also spirooxindols are the core of a large pharmaceutical and natural alkaloids (Fig. 1) [21-25].

Figure 1. The structure of some biologically active spirooxindole derivatives containing natural products.

Ionic liquids **have been considered** as eco-friendly solvents, catalysts, and reagents in organic transformation because of their unique properties, including low volatility, non-flammability, high thermal stability, negligible vapor pressure and the their ability to dissolve of various materials [26–34]. Among them, Brønsted acidic ionic liquids, with useful characteristics of both solid acids and mineral liquid acids, have been prepared to replace traditional mineral liquid acids, such as sulfuric acid and hydrochloric acid, in chemical procedures [35-53].

Having above facts, we have designed acetic acid functionalized poly(4-vinylpyridinium) bromide (APVPB) (Figure 2) as new, highly efficient and reusable catalyst for the preparation of 2-aminospiro[indoline-3,4'pyran]-2-one and spiro[indeno[1,2-b]quinoxaline-11,4-pyran]-2'-amine derivatives by the multi-component condensation reaction of various carbonyl compounds (**A**) and α -methylen carbonyl compounds (**B**) as an activated carbonyl compound and malononitrile **in water under reflux conditions** (Scheme1). Also, we tried to synthesize some new spiro-compounds with potential antimicrobial or antioxidant activities.

Figure 2. The structure of acetic acid functionalized poly(4-vinylpyridinium) bromide (APVPB).

Scheme 1. The synthesis of spiro-pyran catalyzed by APVPB.

2. Experimental

2.1. Materials

All chemicals were purchased from Merck or Fluka Chemical Companies. The known products were identified by comparison of their melting points and spectral data with those reported in the literature. Progress of the reactions was monitored by TLC using silica gel SIL G/UV 254 plates. The ¹H NMR (400 or 300 MHz) and ¹³C NMR (100 or 75 MHz) were recorded on a Bruker Avance DPX FT-NMR spectrometer (δ in ppm). Melting points were recorded on a Büchi B-545 apparatus in open capillary tubes. Differential thermal gravimetric (DTG) were analysed by a Perkin Elmer (Model: Pyris 1). TG/DTG analysis (25 to 600 °C, temperature increase rate of 10

°C. min⁻¹, nitrogen atmosphere). The structure of synthesized materials was determined by an X-ray diffractometer (Italstructure ADP2000 XRD diffractometer) at ambient temperature.

2.2. Catalyst preparation

A mixture of bromoacetic acid (1.39 g, 10 mmol), sulfuric acid (2 ml, 98%) and ethanol (10 ml, 98%) in a 50 mL round-bottomed flask connected to a reflux condenser, was stirred for 24 hours under reflux conditions. Then the mixture was washed with distilled water (3×15 mL) to obtain 1.42 g of an oily ethyl bromoacetate with 85% yield. In the next step mixture of ethyl bromoacetate (1.67 g, 10 mmol), was added to a suspension of powdered poly(4-vinylpyridine) (1.05 g) [poly(4-vinylpyridine) cross-linked with 2% DVB ~60 mesh, MW: 60,000; Fluka Chemical] in over a period of 5 min. Then, diethyl ether (30 mL) was added to **desire** product in a round-bottomed flask (50 mL) connected to a reflux condenser and stirred for 24 hours under reflux conditions. After removing solvent the residue was triturated with diethyl ether (3×15 mL), and dried at 50 °C for 1 h. Afterwards, hydrobromic acid (1 ml, 48%) and water (2 mL) was added to reaction mixture and stirred for 2 hours under reflux conditions. The solvent was removed and the obtained product was triturated with diethyl ether (3×15 mL) and dried under powerful vacuum at 50 °C. Finally, acetic acid functionalized poly(4-vinylpyridinium) bromide (APVPB) was obtained as a white powder.

2. 3. General procedure for the synthesis spiro[indeno[1,2-b]quinoxaline-11,4'-pyran]-2'-aminoderivatives:

Ninhydrin (1 mmol), 1,2-diaminobenzenes (1 mmol), and APVPB (10 mg) were added in a 25-mL round-bottomed flask contained H₂O (15 mL) and connected to a reflux condenser for 10 min, Then, alkylmalonates (1 mmol) and malononitril (1 mmol) were added to the reaction mixture. The mixture was stirred under reflux conditions for the appropriate time. Afterwards,

the reaction mixture was cooled to room temperature and was allowed to attain at 25 °C for about 1 h. Then, the solvent was removed under vacuum and the prepared solid was collected by filtration. The mentioned solid was washed with ethanol and diethyl ether (2×20 mL) and dried to remove starting materials. Finally, acetone (10 mL) was added, stirred and refluxed for 3 minutes. Then, the resulting mixture was centrifuged for the separation of the catalyst from the pure product (the product was soluble in warm acetone and the catalyst was insoluble in this solvent).

2.4. General procedure for the synthesis 2'-aminospiro[indoline-3,4'-pyran]-2-one derivatives:

Isatin (1 mmol), alkylmalonates (1 mmol), malononitril (1 mmol) and APVPB (10 mg) were added in a 25-mL round-bottomed flask contained H₂O (15 mL) and connected to a reflux condenser. The reaction mixture was stirred under reflux conditions for the appropriate time. Afterwards, the reaction mixture was cooled to room temperature and was allowed to attain at 25 °C for 1h. Then, the solvent was removed under vacuum. Then, the prepared solid was collected by filtration and washed with ethanol and diethylether (2×15 mL) and dried to remove starting materials. Finally, acetone (10 mL) was added, stirred and refluxed for 3 minutes. Then, the resulting mixture was centrifuged for the separation of the catalyst from the pure product (the product was soluble in warm acetone and the catalyst was insoluble in this solvent).

Biological activity

Antioxidant capacity

Method A: The DPPH radical scavenging activity of the spiro-pyran derivatives were evaluated according to Mensor *et al* [54]. Initially, 2.5 ml of the samples at concentration of 0.2 to 1 mg/ml were prepared in methanol and mixed with 1 ml of 0.3 mM DPPH that was dissolved in methanol. The reaction mixture was incubated in the dark for 30 min at 25 °C. Bleaching of

DPPH was read at 517 nm against a blank (methanol) using the UV-VIS spectrophotometer. The inhibition in percent for each concentration was calculated according to the following formula:

$$\text{DPPH free radical scavenging (\%)} = [1 - (A_s - A_b)/A_c] \times 100$$

Where, A_s is absorbance of the reaction mixture containing 2.5 ml of samples + 1 ml of DPPH, A_b is absorbance of the reaction mixture containing 2.5 ml of samples + 1 ml methanol and A_c is absorbance of control sample containing 1 ml of DPPH + 2.5 ml methanol. Also IC_{50} value, which represented the concentration of the sample that caused 50% inhibition, was determined. Tests were carried out in triplicate and ascorbic acid was used as a positive control.

Method B: Blood samples were obtained from rats by cardiac puncture and collected in falcon tubes. Then, they were immediately centrifuged at 4000 rpm for 10 min to obtain plasma. 2.4 ml of the samples (1 mg/ml) were mixed with 1 ml of 0.3 mM DPPH dissolved in methanol. The DPPH radical scavenging activity of the samples was evaluated according to method A and above formula. Where, A_s is absorbance of the reaction mixture containing 2.4 ml of samples (plasma + spiro-pyran or plasma without spiro-pyran and or spiro-pyran without plasma) + 1 ml of DPPH, A_b is absorbance of the reaction mixture containing 2.4 ml of samples+ 1 ml methanol and A_c is absorbance of control sample containing 1 ml of DPPH + 2.4 ml methanol.

Antifungal activity

Antifungal activity of spiro-pyran derivatives was *in vitro* assessed against *Fusarium oxysporum* cultivated in Potatoes Dextrose Agar (PDA) medium. Some spiro-pyran derivative samples (C_1 , C_{13} and C_{19}) were added to cultivation medium at two concentrations (500 and 1000 ppm). In order to make the control groups, double distilled water and DMSO were added to the plates. After a 7-days incubation of fungus on culture

medium containing samples, radial growth of fungal mycelium was recorded. The following formula was used for calculation of the inhibition rate (%):

$$\text{Inhibition rate (\%)} = (R-r/R) \times 100$$

Where, R is the radial growth of fungal mycelia on the control plate and r is the radial growth of fungal mycelia on the plate treated with spiro-pyran derivatives. All data are the average of triplicate analyses.

2.5. Statistical analysis

Statistical analysis of variance was performed using Student's t-test by SPSS program and *p* value < 0.05 was regarded as significant. Data are expressed as means $\pm$ standard deviation.

3. Results and Discussion

Acetic acid functionalized poly(4-vinylpyridinium) bromide (APVPB) was prepared in three steps. Initially, bromo ethylacetate was prepared by the reaction of bromoacetic acid with sulfuric acid **I**. In the second step, bromo ethylacetate reacted with poly(4-vinylpyridine) in diethyl ether under reflux conditions for 24 hours to afford **II**. Afterwards, hydrobromic acid (1 mL, 48%) and water (2 mL) was added to reaction mixture and stirred for 2 hours under reflux conditions to hydrolyze and prepare acetic acid functionalized poly(4-vinylpyridinium) bromide (APVPB) (Scheme 2).

Scheme 2. The preparation of acetic acid functionalized poly (4-vinylpyridinium) bromide (APVPB).

The structure of APVPB was identified by IR, XRD, TG, DTG, EDX, as well as SEM analysis.

The IR spectrum of the catalyst showed a broad peak at $2700\text{--}3600\text{ cm}^{-1}$ which could be corresponded to O-H stretching of COOH group and the peak observed at 1729 cm^{-1} corresponds to stretching mode of C=O bond of this group. The IR spectrum of APVPB in comparison with poly (4-vinylpyridine) was shown in Figure 3.

Figure 3. The FT-IR spectrum of APVPB in comparison with poly(4-vinylpyridine).

The XRD pattern of the catalyst (APVPB) was studied and exhibited a broadened pattern due to its non-crystalline nature at $2\theta = 14\text{--}35^\circ$ (Figure 4).

Figure 4. The XRD diagram of APVPB in comparison with poly(4-vinylpyridine).

Thermal gravimetric analysis (TGA) of APVPB was also investigated. The corresponding diagrams are displayed in Figure 5. The thermal gravimetric (TG) and differential thermal gravimetric (DTG) of the catalyst showed weight losses in two step. Acetic acid functionalized poly(4-vinylpyridinum) bromide (APVPB) was decomposed after 231 °C in two step. This study clearly showed that APVPB can be used at temperatures up to 231 °C.

Figure 5. Thermal gravimetric (TG) and derivative thermal gravimetric (DTG) analysis of APVPB at range of 40–490 °C.

Energy-dispersive X-ray spectroscopy (EDX) of desired catalyst (APVPB) showed the existence of the expected elements in the structure of the catalyst, namely carbon, oxygen, nitrogen and bromine (Figure 6).

Figure 6. Energy-dispersive X-ray spectroscopy (EDX) of APVPB.

Accordingly, the structure of the catalyst was completely confirmed by EDX analyze. In another investigation, the SEM micrograph of the catalyst showed that the particles have not completely agglomerated. The particles of the catalyst were observed in nano scales (Figure 7).

Figure 7. SEM micrograph of APVPB.

After the preparation and characterization of the catalyst, the catalytic application of APVPB was investigated on the synthesis of spiropyran derivatives. To optimize the reaction conditions, as a model reaction, the condensation between isatin (1 mmol), dimedon(1 mmol) and malononitrile (1 mmol) was studied in the presence of different amounts of APVPB under reflux conditions. The reaction was efficiently performed using 10 mg of the catalyst under reflux condition to give the desired product in high yield within short reaction times.

In the next step, the model reaction was examined in the persence of several solvents using 10 mg of APVPB under reflux conditions. **As Table 1, indicates that** water was the best solvent in

this reaction (Table 1, entry 2). Although, the reaction was studied in the absence of solvent, but the yield of product was very low (Table 1, entry 1).

Table 1. The reaction of isatin (1 mmol), dimedon (1 mmol) and malononitrile (1 mmol) was studied using APVPB (10 mg) in different solvents (15 mL) under reflux conditions.

Entry	Solvent	Time (h)	Yield ^a (%)
1 ^b	-	1	trace
2	Water	1	92
3	Ethanol	1	89
4	Acetonitrile	1	89
5	Dichloromethane	1	26
6	Ethyl acetate	1	50
7	Toluene	1	37

^aIsolated yield. ^bThe reaction was carried out at 80 °C.

To assess the efficacy and generality of APVPB in the **preparation** of 2-aminospiro[indoline-3,4'-pyran]-2-one and spiro[indeno[1,2-b]quinoxaline-11,4-pyran]-2'-amine derivatives, various α -methylencarbonyl compounds as an activated carbonyl compound (B) were reacted with carbonyl compounds (A) and malononitrile in water under reflux conditions to afford the corresponding products in high yields and short reaction times. **The results are depicted** in Tables 2, 3 and 4.

Table 2. The synthesis of spiro[indeno[1,2-b]quinoxaline-11,4-pyran]-2'-amine catalyzed by APVPB.

<div style="text-align: center;"> <p>$R_1=H, Me \quad R_2=H, Me$</p> <p>$R_1=H, Me \quad R_2=H, Me$</p> </div>				
Entry	Product	Yield ^a (%)	Time (min)	Mp. °C (Lit.) ^{Ref}
C ₁		95	5	282 dec (280 dec) ⁵⁵
C ₂		90	5	243dec (240dec) ⁵⁵
C ₃		92	5	282dec (278dec) ⁵⁵
C ₄		90	5	299dec (297dec) ⁵⁵
C ₅		90	5	283 dec.
C ₆		92	5	262 dec.

Table 2. Continued.

Entry	Product	Yield ^a (%)	Time (min)	Mp. °C (Lit.) ^{Ref}
C ₇		88	5	274dec(274dec) ⁵⁵
C ₈		89	5	248dec(248dec) ⁵⁵

^aIsolated yield.

Table 3. The synthesis of 2-aminospiro[indoline-3,4'pyran]-2-one catalyzed by APVPB.

<div style="text-align: center;"> <p>R=H, R=Br, R=I</p> <p>R=H, R=Br, R=I</p> </div>				
Entry	Product	Yield ^a (%)	Time (h)	Mp. °C (Lit.) ^{Ref}
C ₉		92	1	290-292 (289-291) ⁵⁶
C ₁₀		90	1	289-291(297-299) ⁵⁶
C ₁₁		91	1	294-296(285-286) ⁵⁷
C ₁₂		90	1	280-281 (283-285) ⁵⁸
C ₁₃		90	1	290dce(295dce) ⁵⁹
C ₁₄		92	1	289dec. (289dec.) ⁵⁶

Table 3. Continued.

Entry	Product	Yield ^a (%)	Time (h)	Mp. °C (Lit.) ^{Ref}
C ₁₅		95	1	309-311 (304-306) ⁵⁶
C ₁₆		90	1	228-230 (227-229) ⁵⁶
C ₁₇		89	1	315 dec. (310 dec.) ⁵⁶
C ₁₈		92	1	>300 (>300) ⁶⁰

^aIsolated yield.

Table 4. The synthesis of 2-aminospiro [indoline-3, 4'pyran]-2-one catalyzed by APVPB.

Entry	Product	Yield ^a (%)	Time (h)	Mp. °C (Lit.) ^{Ref}
C ₁₉		92	1	266-268(262-264) ⁵⁶
C ₂₀		90	1	287dec. (289 dec.) ⁵⁶

^aIsolated yield.

In a proposed mechanism [17b], **at first**, ninhydrin and 1,2-phenylenediamine reacted together **to give** the corresponding indenoquinoxaline A in the presence of APVPB (Scheme 3). Indenoquinoxaline and isatin derivatives as carbonyl compounds A (**I**), which is **activated by** APVPB, was reacted with malononitrile **to furnish** intermediate **II** after removing of one molecule of H₂O. Methylene carbonyl compounds (B) is converted **to enol form** after tautomerisation and attacked to cyanoolefin compound (**II**), as a Michael acceptor, to give **III**. Finally, cyclocondensation reaction of **III** prepared **IV**, which is converted to the corresponding product.

Scheme 3. The proposed mechanism for the synthesis of spiropyrans.

The recyclability of APVPB was examined upon the condensation reaction of isatin (1 mmol), dimedon (1 mmol) and malononitrile (1 mmol). After the completion of the reaction, the reaction mixture was filtered and the solvent of the filtrate (H_2O) was removed under reduced pressure. The reaction mixture was washed with ethanol and diethyl ether (2×20 mL) and dried to remove starting materials. Afterwards, acetone was added, stirred and refluxed for 3 minutes. Then, the mixture product and catalyst was centrifuged for the separation of the catalyst from the product. The remained catalyst was washed with warm acetone (10 mL) and dried to reuse for the next run. Catalytic activity of APVPB was restored within the limits of the experimental errors for 4 successive recycle runs (Figure 8).

Figure 8. The condensation of isatin with dimedon and malononitrile in the presence of reused catalyst.

Biological activity

Antioxidant capacity

Antioxidants are as a health-protection factor in foods and scientific evidence suggests that these compounds are very important due to their role in reducing the risk of chronic diseases such as cancer and inflammation diseases [61]. Antioxidants may act as scavenger, reducing, quencher agents and/or can strongly interact with proteins and inhibit activity of some enzymes involved in ROS and RNS generation, such as various cytochrome P₄₅₀ isoforms, lipoxygenases, cyclooxygenase, xanthine oxidase and also activators of cellular antioxidant enzymes to prevent from the free radical damages in biological systems [62-64]. Many studies focus on new antioxidant agents without harmful side effects. Among new compounds, spiro compounds have also been recently used as antioxidants [65-67]. **In the presented research**, DPPH free radical scavenging activity of the new spiro-pyran derivatives was determined (Table 5, 2 and Fig 9). Our results showed that most of the synthesized spiro-pyran derivatives showed excellent dose-dependent (0.2-1 mg/ml) antiradical properties (24.59-64.07%) (Table 5). Antioxidant activity is reflected in a lower IC₅₀ value. Ascorbic acid (Vitamin C) as a synthetic antioxidant represented lower IC₅₀ value (IC₅₀; 0.145 ± 0.009 mg/ml) than those of the most compounds studied here (IC₅₀; 0.161 ± 0.009 to 1.509 ± 0.007 mg/ml), (Fig 9). Generally, most of the free radicals generated are scavenged by endogenous defense system such as catalase, superoxide dismutase and peroxidase-glutathione system [68]. Antiradical properties of synthesized spiro-pyran derivatives (C₁, C₁₅ and C₃₃) increased by adding blood plasma of rats (53.16 ± 2.6% to 76.00 ± 2.5%) and were more compared to plasma as negative control (22.61 ± 2.3%). However, ascorbic acid as a positive control (82.04 ± 1.2%) showed the most antiradical activity (Table 6).

Table 5. DPPH radical scavenging activity (%) of spiro-pyran derivatives (C₁₋₂₀) at different concentrations and ascorbic acid as standard.

Compounds	Concentration (mg/ml)					Average
	0.2	0.4	0.6	0.8	1	
C ₁	62.00 ± 2.1 ^a	65.50 ± 1.6 ^a	63.11 ± 3.1 ^a	63.24 ± 1.5 ^a	66.51 ± 3.5 ^a	64.07
C ₂	61.18 ± 2.3 ^a	60.32 ± 1.2 ^a	63.21 ± 3.2 ^{ab}	62.33 ± 3.8 ^{ab}	66.28 ± 1.3 ^b	62.66
C ₃	45.25 ± 1.6 ^a	43.56 ± 2.9 ^a	49.27 ± 2.2 ^b	47.35 ± 3.3 ^b	52.21 ± 1.5 ^c	47.53
C ₄	45.32 ± 2.1 ^a	48.12 ± 1.3 ^a	51.33 ± 2.2 ^{ab}	55.14 ± 1.1 ^b	54.46 ± 2.2 ^b	50.87
C ₅	58.21 ± 1.2 ^a	57.13 ± 3.3 ^a	64.43 ± 2.7 ^b	67.22 ± 1.4 ^b	66.32 ± 2.2 ^b	62.66
C ₆	43.12 ± 1.1 ^a	41.32 ± 1.5 ^a	55.11 ± 1.9 ^b	53.23 ± 2.9 ^b	55.25 ± 3.1 ^b	46.60
C ₇	28.05 ± 1.8 ^a	34.16 ± 1.2 ^b	33.71 ± 3.1 ^b	41.15 ± 2.6 ^c	46.45 ± 2.2 ^d	36.70
C ₈	36.18 ± 3.2 ^a	43.51 ± 1.1 ^b	41.23 ± 3.4 ^b	47.28 ± 1.7 ^c	46.39 ± 2.1 ^c	42.92
C ₉	29.31 ± 1.5 ^a	35.33 ± 3.3 ^b	39.24 ± 1.3 ^b	44.12 ± 3.2 ^{ab}	50.10 ± 3.1 ^c	39.62
C ₁₀	16.29 ± 2.2 ^a	21.34 ± 1.2 ^{ab}	35.23 ± 3.3 ^b	33.15 ± 2.8 ^b	38.63 ± 2.2 ^c	29.93
C ₁₁	18.19 ± 1.8 ^a	21.32 ± 1.2 ^a	26.19 ± 0.9 ^a	24.12 ± 0.8 ^a	33.13 ± 3.4 ^b	24.59
C ₁₂	22.41 ± 2.6 ^a	19.11 ± 2.4 ^a	33.33 ± 2.8 ^b	37.15 ± 3.1 ^b	42.29 ± 2.2 ^b	30.86
C ₁₃	33.22 ± 1.1 ^a	30.14 ± 1.7 ^a	31.10 ± 0.6 ^a	36.09 ± 1.1 ^b	36.12 ± 1.2 ^b	33.33
C ₁₄	34.14 ± 1.1 ^a	38.25 ± 2.6 ^a	48.33 ± 1.4 ^b	55.21 ± 3.7 ^b	60.16 ± 0.9 ^b	47.22
C ₁₅	18.33 ± 1.8 ^a	24.13 ± 3.1 ^a	21.27 ± 2.3 ^a	29.22 ± 1.6 ^b	40.43 ± 1.6 ^c	26.68
C ₁₆	24.53 ± 1.5 ^a	21.65 ± 1.3 ^a	27.32 ± 3.2 ^a	38.77 ± 2.3 ^b	46.51 ± 2.6 ^c	31.76
C ₁₇	46.61 ± 2.4 ^a	49.31 ± 3.3 ^a	58.26 ± 3.1 ^b	57.22 ± 2.5 ^b	55.15 ± 1.4 ^b	53.31
C ₁₈	29.52 ± 1.5 ^a	38.38 ± 0.7 ^b	44.33 ± 1.3 ^c	60.32 ± 2.8 ^d	58.55 ± 2.7 ^d	45.02
C ₁₉	19.33 ± 4.1 ^a	33.41 ± 2.2 ^b	40.16 ± 3.3 ^c	49.52 ± 3.2 ^d	62.11 ± 0.8 ^e	40.91
C ₂₀	22.64 ± 1.5 ^c	27.67 ± 3.3 ^c	35.72 ± 2.4 ^c	39.25 ± 2.1 ^c	48.44 ± 1.5 ^c	34.74
Ascorbic acid	68.74 ± 1.7 ^a	75.16 ± 2.4 ^b	81.71 ± 0.9 ^c	80.22 ± 1.1 ^c	82.04 ± 1.2 ^c	77.57

Experiment was performed in triplicate and expressed as mean ± SD. Values along column with different superscripts are significantly different ($P < 0.05$).

Table 6: Comparison of DPPH radical scavenging activity of spiro-pyran derivatives, plasma of rats and ascorbic acid as a standard.

Samples	Antioxidant activity (%)	
	Plasma + DPPH	DPPH
C ₁	76.00 ± 2.5	66.51 ± 3.5
C ₁₅	53.16 ± 2.6	36.12 ± 1.2
C ₁₉	58.66 ± 3.3	62.11 ± 0.8
Ascorbic acid	82.13 ± 2.1	82.04 ± 1.2
Plasma	-	22.61 ± 2.3

Experiment was performed in triplicate and expressed as mean ± SD. Values along column with different superscripts are significantly different ($P \leq 0.05$).

Na: No active

Figure 9. Comparison of DPPH radical scavenging ability (IC₅₀ Value) of the studied spiro-pyran derivatives with ascorbic acid as a positive control.

Antifungal activity

Many crops throughout the world are host plants for *Fusarium* species causing soil-borne diseases of great economic importance. *F. oxysporum* is one of the major soil-borne pathogens that attacks its host by entering through the root and grows in the plant xylem, eventually blocking the vascular system and causes severe losses of crop and ultimately death of the plants of great economic importance [69, 70]. The inhibition zone values were determined for some random selected compounds synthesized here (C₁, C₁₃, and C₁₉) against *F. oxysporum* (Table 7). In this research, it was observed that DMSO as negative control had no activity against *F. oxysporum*, but samples showed excellent antifungal activity (Table 7). Most of old synthetic

fungicides usually are difficult to degrade and are toxic to humans. Hence, the examined samples may be used to treat plant diseases caused by *F. oxysporum* and also to increase resistance in a wide variety of crop plants.

Table 7. Antifungal activity of some synthesized spiropyran derivatives.

Compounds	Concentration (PPM)	Inhibition radial growth of fungal mycelium (%)
C ₁	500	88.3 ± 2.2
	1000	94.6 ± 3.0
C ₁₄	500	75.8 ± 2.1
	1000	100 ± 0.0
C ₁₉	500	65.8 ± 1.4
	1000	89.2 ± 3.2
DMSO	-	Na

Experiment was performed in triplicate and expressed as mean ± SD.

NA: No active

4. Conclusion

In summary, we have synthesized acetic acid functionalized poly(4-vinylpyridinium) bromide (APVPB) as type of Bronsted acidic catalyst and fully characterized by several techniques including fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), thermal gravimetric analysis (TGA), differential thermal gravimetric (DTG), scanning electron microscope (SEM), and energy dispersive X-ray analysis (EDX). APVPB was successfully tested as a reusable, stable, general and heterogeneous catalyst for the synthesis of 2-aminospiro[indoline-3,4'pyran]-2-one and spiro[indeno[1,2-b]quinoxaline-11,4-pyran]-2'-amine derivatives *via* four-component or one-pot three-component condensation reaction of various carbonyl compounds (**1–3**) and α -methylencarbonyl compounds (**4a-e**) and malononitrile in water under reflux conditions. Short reaction times, solvent-free and mild reaction conditions and reusability of catalyst are some advantages of this work. Also, results from present study

clearly demonstrated that studied compounds exhibit biological properties that might be helpful in preventing the progress of development of novel therapeutic agents.

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Graphical Abstract

Synthesis and characterization of acetic acid functionalized poly (4-vinylpyridinium) salt as new catalyst for the synthesis of spiropyran derivatives and their biological activity

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