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Synthesis, characterization and application of chitosan conjugated heterocyclic compounds

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Abstract

Derivatives of formyl pyrazole were synthesized by the reaction of acetophenone, 4-methyl acetophenone, 3-acetyl furan, 3-acetyl thiophen and phenyl hydrazine derivatives. The product was treated with Vilsmeier reagent producing different formyl pyrazole derivatives which were characterized by FT-IR, 1HNMR, Elemental analysis and Mass spectroscopy. The formyl pyrazole derivatives were reacted with chitosan to produce chitosan/ pyrazole Schiff base. These Schiff bases were characterized by FT-IR and TGA.The antimicrobial activity of chitosan/ pyrazol Schiff base (CSB) was evaluated against Gram-positive bacteria (Staphylococcus aureus and Bacillus cereus), gram negative bacterium (Escherichia coli) and fungus (Aspergillus niger). Results indicated agood inhibitory activity for CSB-14 when tested against B.cereus that gave inhibition zone of 7.5 ± 0.6 (mm), however CSB-18 gave a pronounced inhibitory activity against S. aureus and recorded 25 ± 2.0 (mm). All synthesized derivatives have no inhibitory activity against Gram negative E. coli. CSB-14, and CSB-15 exhibited inhibitory activity against tested A. niger that was used as a fungal model which gave 19 ± 0.9 and 18 ± 1.0 inhibition zone (mm) respectively. Thus, these results showed that, functionalization of chitosan with the hetero-cyclic compounds created biological activities of the synthesized derivatives; hence the synthesized pyrazole derivatives have not recorded any inhibitory activity before its immobilization onto chitosan.

Keywords Pyrazole · Chitosan · Schiff base · And Antimicrobial Polymers

Introduction

Pyrazole is one of heterocyclic compounds that has two nitrogen atoms in five member ring [1], the presence of two nitrogen atoms in pyrazole nucleus exhibit its activity, while the nitrogen atom at position 1 can lose its proton during reaction with a base and nitrogen atom at position 2 is reactive towards electrophiles due to presence of a lone pair of electrons [2]. Due to the presence of pyrazole nucleus in different structures which provide it to have many applications such as anticancer, antibacterial, antidepressant, anti-inflammatory, antiviral, antifungal, antituberc-ulosis, antiviral and antioxidant [3, 4]. Chitosan is a natural polymer which consist of β-(1–4)-linked (acetylated) N-acetyl-2-amino-2-deoxy -D-glucose and (deacetylated) 2-amino-2-deoxy -D-glucose subunits [5, 6]. The main source of chitosan is a chitin which is found in abundance in nature [7, 8]. Chitin is obtained from crab and shrimp shell in the form of (N-acetyl glucose amine) polymer which is deacetylated to glucose amine unit with 40–50% conc. of NaOH [9, 10], unless the majority of polysaccharides that are behave as neutral or negatively charged in acidic medium, chitosan polymer behaves cationic nature in the this acidic medium thus, it forms a multilayer and electrostatic structure with negatively charged polymer [11]. The commercial interest toward chitosan and chitin is increased due to their biodegradability, biocompatibility and chelation with ions. It can be used as biomaterial for drug delivery, clinical applications, bioactive dressing, scaffold for the engineering of tissues, and anti-microbial to accelerate wounds healing [12–14]. Schiff bases are the compounds produced by condensation of carbonyl compound with primary amine [15], Schiff bases one

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of the most important classes in organic compounds, it can be used for numerous applications in pharmaceutical and medicinal fields such as anti-inflammatory. Furthermore, Schiff base have an application in analytical and in organic chemistry [16, 17]. It is shown that Schiff bases exhibit antibacterial, anticancer, antidepressant, analgesic anti-mycobacterial properties [18]. The anti-tumor and anti-bacterial activities have been reported to halo and nitro derivatives of Schiff base, Beta-lactam which is considered a derivative of Schiff base also showed antimicrobial activity and antifungal activities [19].

As discussed before, because of the biological activity of pyrazole, chitosan and Schiff base, we aimed to prepare new Schiff bases based on chitosan/pyrazole compounds and investigate the biological activities of the prepared compounds.

Experimental

Materials and methods

Materials

Acetophenone freezes under cool conditions with boiling point 202 °C, 4-methyl acetophenone with boiling point 226 °C, belongs to the class of organic compounds known as alkyl-phenyl ketones. These are aromatic compounds containing a ketone substituted by one alkyl group, and a phenyl group, 3-acetyl thiophen also known as 1-thien-3-ylethanone or 3-acetothienone with boiling point 210 °C and melting point 60 °C which belong to the class of organic compounds known as aryl alkyl ketones.These are ketones have the generic structure RC(=O)R', where R = aryl group and R' = alkyl group. 3-Acetylthiophene was an extremely weak basic (essentially neutral) compound (based on its pKa)., 3-acetyl furan, which was known as 1-furan-3-ylethanone or 3-furanone with boiling point 84 °C and melting point 45- 47 °C which belong to the class of organic compounds known as aryl alkyl ketones. These are ketones have the generic structure RC(=O)R', where R = aryl group and R' = alkyl group, phenyl hydrazine appeared as pale yellow crystals, melting point 66 °F, with flash point 192 °F, auto-ignition temperature 345 °F. Soluble in alcohol, 2,4-Dinitrophenylhydrazine (DNPH) is a red to orange solid with melting point 198–202 °C and boiling point 378.6 °C. High molecular weight chitosan (more than 100 kDa) is soluble only in dilute acid with 82% degree of deacetylation were purchased from Sigma Aldrich, USA, Vilsmeier reagent (POCL$_3$, DMF) while POCL$_3$ that was known as phosphoryl chloride (commonly called phosphorusoxychloride) with the formula POCl$_3$ with boiling point 105.8 °C. DMF that was known as dimethylformamide was an organic compound with the formula HCON(CH$_3$)$_2$, with boiling point 153 °C were purchased from LOBA CHEMI (Mumbai, India).

Instruments

FT-IR spectra were recorded in range 400–4000 cm⁻ using Thermo Nicolet AVATAR 330 spectrophotometer and in presence KBr pellet method.^{1}H-NMR data were recorded on a Varian Mercury VX-300 (300 MHz) NMR spectrometer. Thermo-gravimetric analysis was carried out on TGA-50H thermo-gravimetric analyzer, Shimadzu, Japan. Samples were heated up to 800 °C in a platinum pan with a heating rate of 10°C/min, in N$_2$ atmosphere of flow rate 25 mL/min.

Methods

General procedures for the synthesis of formyl pyrazole derivatives

The general method was exactly described as the following:

(Preparation of phenyl hydrazone derivatives): Acetophenone, 4-methyl acetophenone, 3-acetyl thiophene and 3-acetyl furan (0.1 mol) and phenyl hydrazine derivatives (0.1 mol) in a mixture of ethanol (30 mL) and acetic acid (1 mL) were refluxed in water bath for one h. The reaction mixture was cooled and filtered off. The solid product was washed with cold ethanol to produce the products that were outlined in Scheme 1.

(Synthesis of formyl pyrazole derivatives): Derivatives of hydrazones (0.1 mol) were added to Vilsmeier reagent (DMF and POCl$_3$, 0.2 mol), dropwise with continuous stirring for one h in an ice bath. The reaction mixture was refluxed for 6 h at 70 °C then was poured onto ice/water mixture and neutralized by adding sodium hydroxide solution 5%. The precipitate was filtered off, dried to give the products that were shown in Scheme 1.

(General procedure for the synthesis of Chitosan based Pyrazole Schiff base): Chitosan (0.1 mol) was dissolved in 4% glacial acetic acid (100 mL) with stirring for 2 h, equivalent moles of each prepared formyl pyrazole were dissolved in (30 mL) boiled ethanol then added to chitosan solution drop wise with stirring, the reaction mixtures were refluxed for 6–7 h at 70 °C, cooled, the solid formed filtered off, washed with ethanol to remove unreacted aldehyde and dried to give the corresponding polymers that were outlined in Scheme 1.

Study of the antimicrobial activities:

The antimicrobial activity of formyl pyrazole derivatives and the prepared chitosan conjugated heterocyclic samples is assayed against some types of Gram-positive bacteria

Scheme 1 The reaction pathway for synthesis of formyl pyrazole and chitosan/ pyrazole Schiff base derivatives. $R^1 =$ p-CH$_3$-C$_6$H$_4$, C$_6$H$_5$, 3- C$_4$H$_3$S, 3- C$_4$H$_3$O, and $R^2 =$ C$_6$H$_5$, 1-C$_6$H$_3$N$_2$O$_4$
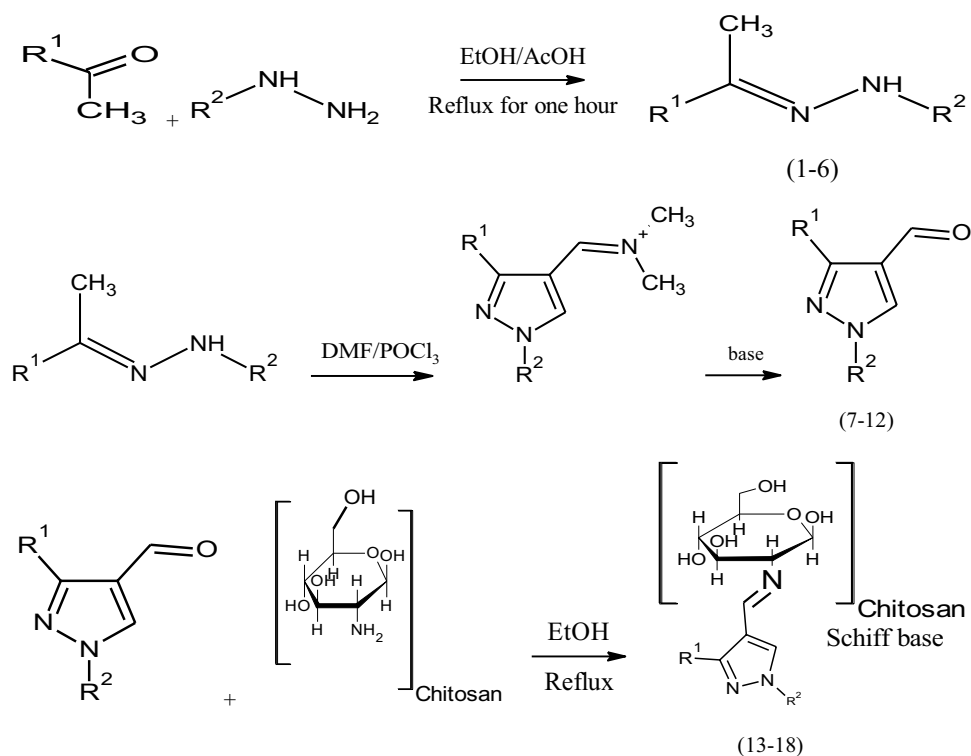
(*Staphylococcus aureus, Bacillus cereus*), or negative bacterium (*Escherichia coli*) and fungus (*Aspergillus niger*). The antimicrobial test was carried out by agar diffusion method, where wells with diameter 6 mm were made. The prepared chitosan conjugated heterocyclic samples were dissolved in dimethyl sulfoxide at concentration of (10%). 50 µl of dissolved CSB derivatives (10 mg/ml) was loaded in medium agar well. The antimicrobial activity of the prepared samples against tested microorganisms was evaluated after 24 h of incubation at 37 °C for 24 h in case of bacterial organisms, however it was recorded in case of *A. niger* after 72 h of incubation at 25 °C. The samples diffuse through the wells, after that the inhibition zones were measured [20]. Amikacin and Nystatin were used as standard antibacterial and antifungal to compare with the synthesized CSB derivative.

Results and discussion

Characterization of phenyl hydrazone derivatives

The hydrazone derivatives were synthesized through refluxing of acetophenone, 4-methyl acetophenone, 3-acetyl thiophene and/or 3-acetyl furan with phenyl hydrazine derivatives in ethanol solution and few drops of acetic acid for one h [21], six different hydrazone derivatives (1–6) were

obtained that were outlined in Scheme 1. These compounds were unstable for long time in air, thus we could not operate any characterization as FT-IR, NMR and elemental analysis for them.

For compound (1), 1-phenyl-2-(1-(*p*-tolyl) ethylidene) hydrazine that was characterized by (m.p. 100–103 °C, yield 83%), compound (2), 1-(2,4-dinitrophenyl)-2-(1-phenylethylidene)hydrazine that was characterized by (m.p. 225–230 °C, yield 81.5%), compound (3), 1-(2,4-dinitrophenyl)-2-(1-(p-tolyl)ethylidene)hydrazine showed (m.p. 250–255 °C, yield 82%), compound (4), 1-phenyl-2-(1-phenylethylidene)hydrazine that was characterized by (m.p. 104–106 °C, yield = 91%), compound (5) 1-phenyl-2-(1-(thiophen-3-yl)-ethylidene)hydrazine characterized by (m.p. 145–155 °C, yield 89%) and compound (6) 1-(1-(furan-3-yl)-ethylidene)-2-phenylhydrazine was characterized by (m.p. 139–141 °C, yield 87%).

Characterization of formyl pyrazole derivatives

Formyl pyrazole derivatives were synthesized through adding Vilsmeier reagent (DMF and POCl$_3$) to hydrazone derivatives by ratio (2:1) respectively dropwise with stirring in ice bath for one h then refluxing at 70 °C for 6–7 h, six different formyl pyrazole derivatives (7–12) were obtained as shown in Scheme 1. The Vilsmeier-Haack reaction mechanism showed how the pyrazole ring was formed as shown in Scheme 2.

Scheme 2 Vilsmeier-Haack reaction mechanism showed the synthetic process for the synthesis of pyrazole nucleus
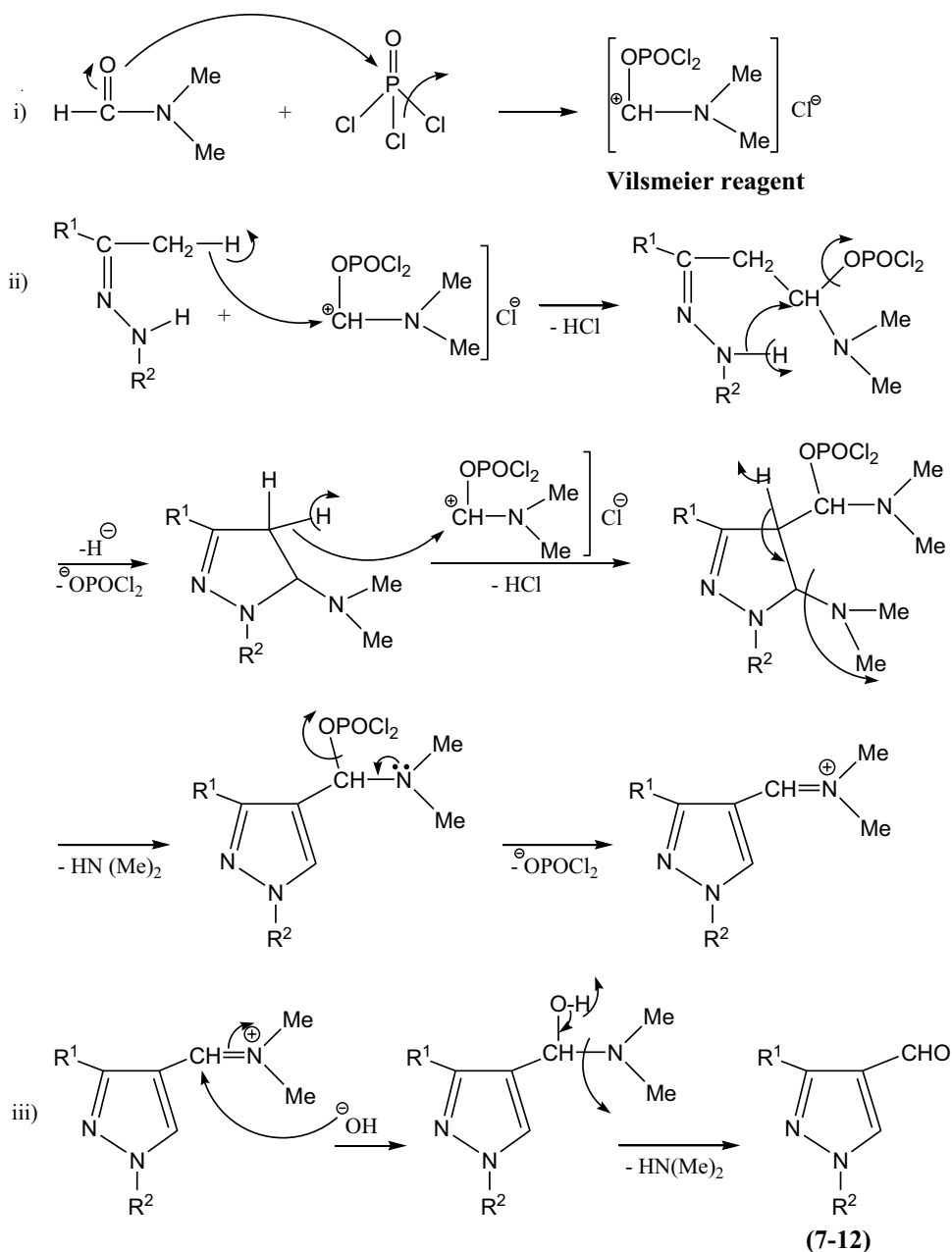
Compound (7), 1-phenyl-3-(p-tolyl)-1H-pyrazole-4-carbaldehyde was characterized by (m.p.118–120 °C, yield 81%) a yellow powder. ¹H NMR (400 MHz, CDCl₃), (δ, ppm) = 2.46 (3H, s, CH₃), 7,3–8 (9H, m, Ar–H of benzene ring), 8.55 (1H, s, CH of pyrazole ring) and 10.079 (1H, s, CHO). FT-IR (KBr) cm⁻¹: (1680 st, C = O, 1605 st, C = N). MS m/z: M = 262; Analysis Calcd. For. C₁₇H₁₄N₂O: C, 77.84; H, 5.38; N, 10.68%; Found: C, 77.86; H, 5.44; N, 10.78%.

For compound (8); 1-(2,4- dinitrophenyl)-3-phenyl-1H-pyrazole-4-carbaldehyde: (m.p.240–243 °C, yield 79%) an orange powder. ¹H NMR (400 MHz, CDCl₃), (δ, ppm) = 7–8 (8H, m, Ar–H of benzene ring), 9.2 (1H, s, CH

of pyrazole ring), 11.4 (1H, s, CHO). FT-IR (KBr) cm⁻¹: (1614 st, C = O, 1507 st, C = N). MS m/z: M = 338; Analysis Calcd. For C₁₆H₁₀N₄O₅: C, 56.80; H, 2.97; N, 16.56%; Found: C, 56.86; H, 2.99; N, 16.59%.

While compound (9); 1-(2,4-dinitrophenyl)-3-(p-tolyl)-1H-pyrazole-4-carbaldehyde (m.p.230–240 °C, yield 75%), a red powder. ¹H NMR (400 MHz, CDCl₃), (δ, ppm) = 2.48 (3H, s, CH₃),7–8 (7H, m, Ar–H of benzene ring), 9.2 (1H, s, CH of pyrazole ring), 11.4 (1H, s, CHO). FT-IR (KBr) cm⁻¹: (1614 st, C = O, 1589 st, C = N). MS m/z: M = 352; Analysis Calcd. For C₁₇H₁₂N₄O₅: C, 57.96; H, 3.40; N, 15.90%; Found: C, 57.88; H, 3.49; N, 15.94%.

Table 1 FT-IR spectral data of formyl pyrazole derivatives (cm^{-1})

Compound	NO$_2$	CH aromatic	C=O	C=N	C=C	C–C	C-H aldehydic	C-N	C-H aliphatic
7	——-	3465	1680	1605	1578–1525	1119	2850	1232	2879–2938
8	1326–1304	3459	1614	1507	1416	1061–1118	2926	1257–1214	3111
9	1305–1331	3420–3306	1614	1589	1420–1503	1104–1130	2918	1223–1259	3109
10	——-	3421	1673	1598	1405–1451	1018–1078	2838–2865	1227–1293	3122
11	———	3419	1675	1597	1407–1505	1159	2364	1214	3070
12	———	3427	1673	1600	1526	1018–1056	2860	1213	3122

For compound (10); 1,3-diphenyl-1H-pyrazole-4-carbaldehyde: (m.p.142–143 °C, yield 91%), a white powder. ^{1}H NMR (400 MHz, CDCl$_3$), (δ, ppm) = 8.4 (1H, CH) of pyrazole, 7–8 (10H, m, Ar–H of benzene ring), 8.4 (1H, s, CH of pyrazole ring) and 9.96 (1H, s, CHO). FT-IR (KBr) cm^{-1}: (1673 st, C=O, 1598, st, C=N). MS *m/z*: M = 248; Analysis Calcd. For C$_{16}$H$_{12}$N$_2$O: C, 77.40; H, 4.87; N, 11.28%; Found: C, 77.45; H, 4.89; N, 11.32%.

For Compound (11); 1-phenyl-3-(thiophen-3-yl)-1H-pyrazole-4-carbaldehyde: (m.p.250–260 °C, yield 83%) a black powder. ^{1}H NMR (400 MHz, CDCl$_3$), (δ, ppm) = 8 (1H, s, CH) of pyrazole, 7–8 (8H, m, Ar–H of benzene ring) benzene ring and 9.3 (1H,s, CH),10 (1H, s, CHO)PPM of pyrazole nucleus. FT-IR (KBr) cm^{-1}: (1675 st, C=O, 1597 st, C=N).Analysis Calcd. For

C$_{14}$H$_{10}$N$_2$OS: C, 70.56; H, 4.20; N, 11.75; S, 13.45%; Found: C, 70.62; H, 4.26; N, 11.78; S, 13.54%.

While compound (12); 3-(furan-3-yl)-1-phenyl-1H-pyrazole-4-carbaldehyde: (m.p.250–255 °C, yield 87%), a green powder. ^{1}H NMR (400 MHz, CDCL3), (δ, ppm) = 8 (1H,CH) of pyrazole, 7–8 (8H, m, Ar–H of benzene ring) and 9.3 (1H, s, CH), 10.133 (1H, s, CHO) ppm of pyrazole nucleus. FT-IR (KBr) cm^{-1}: (1673 st, C=O, 1600 st, C==N).

FT-IR spectral studies

From Table 1 and Fig. 1, the FT-IR spectral data were shown for 1-phenyl-3-(*p*-tolyl)-1H-pyrazole-4-carbaldhyde that were characterized by absorption bands due to aldehyde C-H, C=O and C=N in the regions 2850, 1680 and
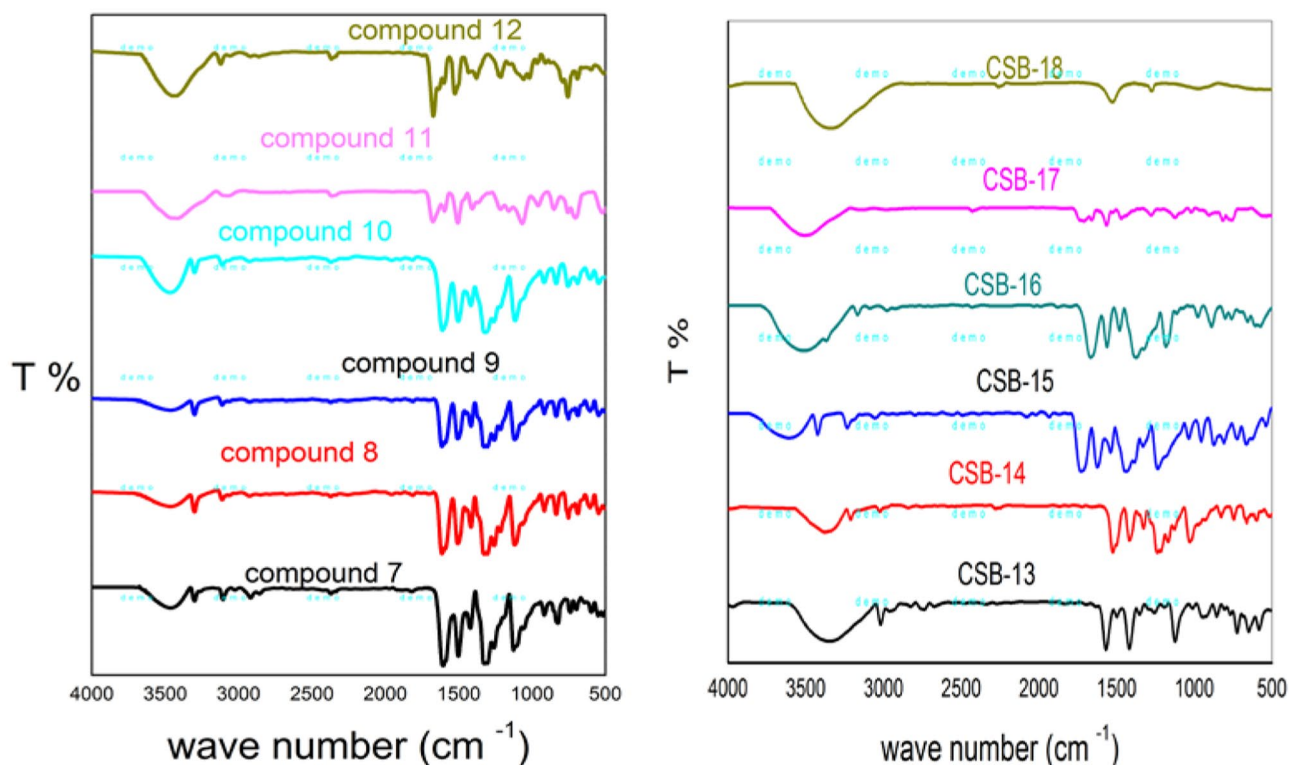
Fig. 1 FT-IR spectra of formyl pyrazole and pyrazole/ chitosan Schiff base derivatives

1605 cm^{-1} respectively, the FT-IR spectra for 1-(2,4-dinitrophenyl)-3-phenyl-1H-pyrazole-4-carbaldehyde were characterized by absorption bands due to aldehydic C-H, C=O and C=N in the regions 2926, 1614 and 1507 cm^{-1} respectively, another spectra of 1-(2,4-dinitrophenyl)-3-tolyl-1H-pyrazole-4-carbaldehyde are characterized by absorption bands due to aldehydic C-H, C=O and C=N in the regions 2918, 1614 and 1589 cm^{-1} respectively. In addition to, there are bands for (1,3) diphenyl-1H-pyrazole-4-carbaldehyde due to aldehydic C-H, C=O and C=N in the regions 2865, 1673 and 1598 cm^{-1} respectively.

The FT-IR spectra for 1-phenyl-3-(thiophen-3-yl)-1H-pyrazole-4-carbaldehyde are characterized by absorption bands due to aldhydic C-H, C=O and C=N in the regions 2364, 1675 and 1597 cm^{-1} respectively. The FT-IR spectra of for 1-phenyl-3-(furan-3-yl)-1H-pyrazole-4-carbaldehyde are characterized by absorption bands due to aldhydic C-H, C=O and C=N in the regions 2860, 1673 and 1600 cm^{-1} respectively.

^{1}H-NMR spectral studies

The structures of formyl pyrazole derivatives were proved by ^{1}H-NMR spectra which showed methyl proton at 2.46 ppm in compound (7), other peaks appear between 7–8 ppm corresponding (9H, m, Ar–H of benzene ring and 10H, m, Ar–H of benzene ring), in addition to it is shown proton single peak corresponding to CH group of pyrazole nucleus at 8.55 ppm for compound (7) and 8.4 ppm for compound (10) and another CHO proton appear as a single peak at 10 ppm for compound (7) and at 9 ppm for compound (10).

Also, by ^{1}H NMR spectra it was proved the structure of compounds (8 and 9). While, a single peak was shown at 2.46 ppm corresponding to methyl proton of compound (9), also, there are multiplet peaks between 7–8 ppm resulted from 8 and 7 H proton for aromatic rings respectively for each compound (8 and 9). In addition to there is a single peak at 9.2 ppm due to H proton of CH of pyrazole nucleus, also another peak appears as a single peak at 11.4 ppm resulted from 1H proton of CHO group, but increasing in de-shielding effect in CHO proton to 11 ppm due to effect of two nitro group in compounds (8 and 9).

For compounds (11, 12), It is reported that, presence of multiple peaks between 7–8 ppm due to 10 proton splitting in aromatic structure for each compound (11,12). Another single peak is shown at 9 ppm resulted from 1H proton of CH group in pyrazole nucleus, in addition to a single peak appear at 10 ppm corresponding to 1H proton of CHO group of compounds (11 and 12).

Characterization of chitosan based pyrazole Schiff base (CSB)

Formyl pyrazole derivatives were grafted to the amino group of chitosan forming different Schiff bases (13–18) that were represented in Scheme 1.

CSB-13 obtained from (conjugation of chitosan with 1-phenyl-3-(*p*-tolyl)-1H-pyrazole-4-carbaldehyde: 88% yield, faint yellow powder. FT-IR (KBr) cm^{-1}: 3448 (st. OH), 1669 (st. CH=N). MS *m/z*: M=421; Analysis Calcd. For $C_{23}H_{23}N_3O_5$: C, 72.49; H, 5.97; N, 11.82%; Found: C, 72.53; H, 5.99; N, 11.86%.

CSB-14 obtained from Conjugation of chitosan with 1-(2,4-dinitrophenyl)-3-phenyl-1H-pyrazole-4-carbaldhyde: 82% yield, orange powder. FT-IR (KBr) cm^{-1}: 3488 (st. OH), 1608 (st. CH=N). MS *m/z*: M=497; Analysis Calcd. For $C_{22}H_{19}N_5O_9$: C, 53.55; H, 4.18; N, 18.76%; Found: C, 53.58; H, 4.23; N, 18.80%.

CSB-15 obtained from (conjugation of chitosan with 1-(2,4-dinitrophenyl)-3-(p-tolyl)-1H-pyrazole-4-carbaldehyde: 86% yield, faint red powder. FT-IR (KBr) cm^{-1}: 3450 (st. OH), 1605 (st. CH=N). MS *m/z*: M=511; Analysis Calcd. For $C_{23}H_{21}N_5O_9$: C, 55.18; H, 4.82; N, 18.47%; Found: C, 55.24; H, 4.86; N, 18.53%.

CSB-16 obtained from (conjugation of chitosan with (1,3)-diphenyl-1H-pyrazole-4-carbaldehyde. 89% yield, faint yellow powder. FT-IR (KBr) cm^{-1}: 3442 (st. OH), 1671 (st. CH=N). MS *m/z*: M=407; Analysis Calcd. For $C_{22}H_{21}N_3O_5$: C, 71.68; H, 5.90; N, 12.13%; Found: C, 71.73; H, 5.15; N, 12.16%.

CSB-17 obtained from (conjugation of chitosan with (1-phenyl-3-thiophen-3-yl)-1H-pyrazole-4-carbaldehyde: 87% yield, brown powder. FT-IR (KBr) cm^{-1}: 3451 (st. OH), 1647 (st. CH=N). MS *m/z*: M=413; Analysis Calcd. For $C_{20}H_{19}N_3O_5$: C, 62.28; H, 4.29; N, 11. 34%; S, 11.94%; Found: C, 62.31; H, 4.34; N, 11.38; S, 11.99%.

CSB-18 obtained from (conjugation of chitosan with (1-phenyl-3-(furan-3-yl)-1H-pyrazole-4-carbaldehyde: 85% yield, black powder. FT-IR (KBr) cm^{-1}: 3441 (st. OH), 1665 (st. CH=N). MS *m/z*: M=397; Analysis Calcd. For $C_{20}H_{19}N_3O_6$: C, 54.88; H, 5.29; N, 10. 44; %; Found: C, 54.91; H, 5.35; N, 10.40%.

From Fig. 1 and Table 2, the FT-IR spectra of chitosan (CS) showed an absorption band from 3431 cm^{-1}, which corresponding to the stretching vibration of -OH and -NH groups, another absorption band appears at 2924 cm^{-1} corresponding to C-H stretching vibration, in addition to there was an absorption band at 1640 cm^{-1} due to acetylated amine or free amine on chitosan chain and another absorption band at 1071 cm^{-1} equal to the absorption frequency of C–O–C group of polysaccharide molecules [22].

Table 2 FT-IR spectral data of Chitosan/pyrazole Schiff base (cm^{-1})

Samples	vOH and v NH (hydroxyl and amino groups)	v CH = N (Imine)	v C = C (aromatic ring)
CS	3431	——	——
CSB -13	3448	1669	1598–1518-1452
CSB-14	3488	1608	1502–1418
CSB-15	3450	1605	1501–1420
CSB-16	3442	1671	1521–1448
CSB-17	3451	1647	1506–1408
CSB-18	3441	1665	1519

The FT-IR spectra of different chitosan Schiff base derivatives (CSBs) showed an absorption band from 3441 to 3488 cm^{-1} corresponding to the stretching vibrations of –OH group for all Schiff base derivatives with increasing in frequency due to withdrawing effect of pyrazole derivatives on chitosan chain, another peaks characterized by absorption band in the regions of 1605 to 1669 cm^{-1} resulting from the stretching vibrations of (-CH = N-) imine group and this absorption band was absent for pure chitosan thus, the appearing of imine emphasizes the formation of Schiff base, in addition to, disappearance the absorption band around 1680 cm^{-1} corresponding to stretching vibration of aromatic aldehyde of formyl pyrazole which ensured the reaction of aldehyde group of formyl pyrazole with free amino group of chitosan. Another peak was observed in the regions ranges from 1405 to

1599 cm^{-1} due to the stretching vibrations of –C = C- of aromatic ring which is absent in pure chitosan. Thus, from the later spectral data the synthesis of Schiff base is emphasized.

TGA studies

Thermo-gravimetric analysis is employed to exhibit the thermal stability of the prepared polymer containing heterocyclic compound derivatives. From the later studies it was shown that, the TGA curve of pure chitosan shows two stages of weight loss is in the range from 47 to 450 °C, the first one in the range of 47–100 °C refers to loss of water molecules. The second degradation of pure chitosan started at 247 °C to completely degradation at about 450 °C [23–25]

From Fig. 2 it was shown that, chitosan/1-phenyl-3-(p-tolyl)-1H-pyrazole-4-carbaldhyde and chitosan/(1,3-diphenyl-1H-pyrazole-4-carbaldhyde respectively, showed a first degradation with total weight loss 90% between 159 till 290 °C and 129 till 325 °C attributed to the thermal decomposition of glucosidic linkage between glucose amine and N-acetyl glucose amine ring and pyranose ring of chitosan followed by a second degradation till 800 °C with weight loss 9.6% due to the degradation of pyrazole ring. while in case of chitosan bearing 1-(2,4-dinitrophenyl)-3-phenyl-1H-pyrazole-4-carbaldhyde, a thermal decomposition occur between 232–311 °C with weight loss 70% due to the thermal decomposition of glucosidic linkage between glucose amine and N-acetyl glucose amine ring and pyranose ring of chitosan followed by a second degradation till 800 °C with weight loss 10.4% due to the degradation
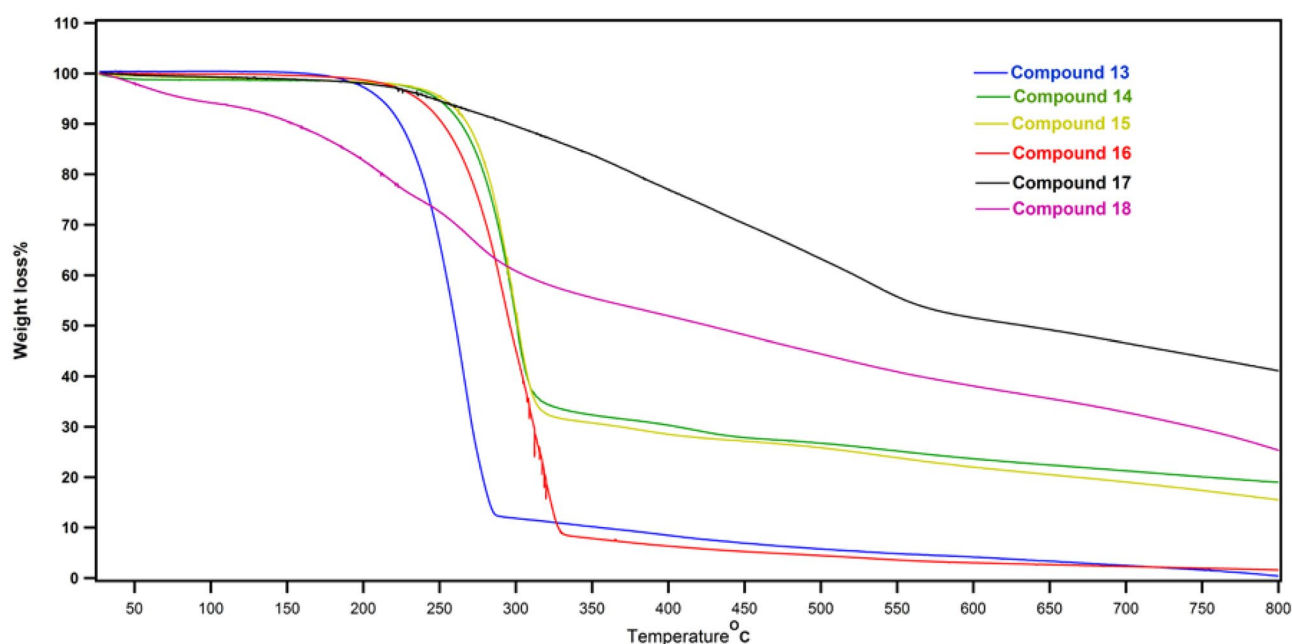
Fig. 2 TGA Studies of Schiff base derivatives

of pyrazole ring. The thermal decomposition of chitosan / 1-(2,4-dinitrophenyl)-3-(p-tolyl)-1H-pyrazole-4-carbaldhyde was occurred between 255–315 °C with weight loss 74% due to the thermal decomposition of glucosidic linkage between glucose amine and N-acetyl glucose amine ring and pyranose ring of chitosan followed by a second degradation till 800 °C with weight loss 10% due to the degradation of pyrazole ring. The thermal degradation of chitosan/1-phenyl-3-(thiophen-3-yl)-1H-pyrazole-4-carbaldhyde occur at two steps, the first step from 27 to 570 °C respectively, with weight loss 46% resulted from adsorbed water loss, thermal decomposition of glucosidic linkage between glucose amine and N-acetyl glucose amine ring and pyranose ring of chitosan, and the second step between 570 to 800 °C with weight loss 12% due to the thermal degradation of pyrazole. While in case of chitosan/1-phenyl-3-(furan-3-yl)-1H-pyrazole-4-carbaldhyde the thermal studies showed.

decomposition occurred at four steps, the first step between 27 till 120 °C with weight loss 6% due evaporation of water molecules adsorbed or hydrogen bonded to chitosan and the second step from 120 till 240 °C with weight loss 18.692% attributed to degradation of glucosidic linkage between glucose amine and N-acetyl glucose amine, the third degradation from 240 to 330 °C with weight loss 17.2% due to degradation of pyranose ring and the last degradation occur from 330 till 800 °C with weight loss 31.881% corresponding to pyrazole moiety.

Antimicrobial activity

Heterocyclic pyrazole derivatives alone have not recorded any inhibitory activity before its immobilization onto chitosan. However, all synthesized Schiff base derivatives were tested in vitro against Gram-positive bacteria (*staphylococcus aureus, Bacillus cereus*) and Gram-negative bacteria (*E. coli*) and the fungus (*Aspergillus niger*). The results showed antibacterial and antifungal activity for all prepared Schiff base due to its inhibition for the two Gram-positive bacteria and poor activity against both *E.coli* bacteria and *Aspergillus niger* fungus. Table 3 data showed that, the higher antibacterial activity of CSB-18 against *S. aureus* other than the other tested compounds, and this might be due to the presence of furan ring. However, it is observed that the least anti-bacterial activity was recorded for CSB-13 and CSB-17 that have toluene and thiophene ring. Additionally, the data showed that the high antibacterial activity of compounds containing nitro groups.

Also, the compounds (CSB-13, CSB-14 and CSB-15) showed higher antibacterial activity that recorded inhibition zone 5 ± 0.4, 17.5 ± 1.2 and 20 ± 2.0 respectively, against *Bacillus cereus* (Gram-positive bacteria) than CSB-16, CSB-17 and CSB-18 that might be due to that CSB-13 and CSB-14 are containing tolyl and Nitro group, while CSB-16 and CSB-18 compounds exhibited medium effect that is lower than CSB-13 and CSB-14 and CSB-17 that containing thiophene group which performed the least effect. Concerning, Escherichia *coli*; all compounds do not give any effect against it that might be due to its high lipids contents and their cell wall chemical structure.

There was another proposal for the antimicrobial effects of the pyrazole-chitosan compounds are that chitosan can penetrate the nucleus of microbes and binds to DNA which inhibit the RNA binding site to DNA, thus the cell wall protein cannot be synthesized [26]. Results showed that these chitosan compounds have antifungal activities while, the studies ensure that, chitosan molecules can diffuse through

Table 3 Inhibition zones (mm) ± SD. of CSBs derivatives against *B. Cereus, S. aureus, E. coli* and *Aspergillus niger*

Compounds	B.cereus	S.aureus	Escherichia Coli	Aspergillus niger
7	2*±0.2	0±0.0	0±0.0	0±0.0
8	4±0.3	0±0.0	0±0.0	0±0.0
9	0±0.0	0±0.0	0±0.0	0±0.0
10	2±0.1	0±0.0	0±0.0	0±0.0
11	4±0.3	0±0.0	0±0.0	0±0.0
12	3±0.2	0±0.0	0±0.0	0±0.0
CSB-13	7±0.4	5±0.4	0±0.0	16.5±1.5
CSB -14	7.5±0.6	17.5±1.2	0±0.0	19±0.9
CSB -15	5±0.3	20±2.0	0±0.0	18±1.0
CSB -16	4±0.2	17.5±1.0	0±0.0	15.5±1.0
CSB-17	3±0.2	2±0.2	0±0.0	8±0.5
CSB-18	4±0.3	25±2.0	0±0.0	6.5±0.5
Standard Antibiotics: Amikacin (30 µg)	31±3.0	20±1.0	22±2.0	20±0.2
Nystatin				

*Reading is the mean value of three replica

fungal hyphae and inhibit enzyme that are responsible for fungal growth [27], in addition to the later has shown that chitosan molecules play an important role in inhibition of germ tube elongation, spore germination [28].

On the other hand, it is shown that the antifungal activity can be affected by chitosan Schiff base heterocyclic compounds derivative. While, it is observed that, both CSB-14 and CSB-15 showed the higher antifungal activity than the other compounds which possess nitro and phenyl group in CSB-14 and nitro and tolyl group in CSB-15, in addition to CSB-13 and CSB-16 compounds bearing phenyl and tolyl group that showed antifungal activity higher than CSB-17 and CSB-18 which bearing thiophenyl and furyl group respectively declaring the least activity as shon in Table 3. Although the synthetized heterocyclic compound derivatives were very reactive as antimicrobial agent but increasing of their hydrophobicity and decreasing their hydrophilic properties prevent them from penetrating the cell membrane of micro-organisms. Therefore, the immobilization of pyrazole derivatives with chitosan that have hydrophobic/ hydrophilic balance enhanced the pyrazole-chitosan derivatives to interact with the cell membrane. Moreover, the hydrophilic character of chitosan increased its selectivity to micro-organism.

Conclusion

Formyl pyrazole derivatives were synthesized through the reaction of acetophenone and phenyl hydrazine derivatives then the product is subjected with Vilsmeier reagent producing different formyl pyrazole derivatives which are characterized by FT-IR, ^{1}H NMR, Elemental analysis and Mass spectroscopy, the later formyl pyrazole derivatives reacted by condensation with chitosan to produce chitosan/pyrazole Schiff base, These Schiff bases are characterized by FT-IR and the thermal properties such as DTA, DSC and TGA were studied, then the antimicrobial activity of chitosan/pyrazole Schiff base was evaluated against some types of Gram-positive bacteria (*staphylococcus aureus, Bacillus cereus*), Gram-negative bacterium (*Escherichia coli*) and fungus (*Aspergillus niger*).

It is shown that, the biological activities of formyl pyrazole and chitosan against some types of Gram-positive bacteria (*staphylococcus aureus, Bacillus cereus*), Gram-negative bacterium (*Escherichia coli*) and fungus (*Aspergillus niger*) were increased by condensation reaction between formyl pyrazole and chitosan forming a Schiff base (-CH=N-) and the later containing a chitosan moiety having long carbon chain, the second moiety was formyl pyrazole carrying biological active derivatives, thus from

the later it was shown that, these Schiff base have long carbon chain which led to increasing the hydrophobicity of molecules, thus, the chance of molecules passage through the cell membrane of microorganism was observed with high efficiency, in addition to decreasing their toxicity.

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