



Synthesis, Characterization and *in vitro* Antitumor Activity of Novel Schiff Bases Containing Pyrazole Group

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Received: 19 December 2013;

Accepted: 3 April 2014;

Published online: 1 December 2014;

AJC-16351

A series of novel Schiff base compounds containing pyrazole group were synthesized with pyrazole aldehyde and benzene hydrazine or pyrazole aldehyde and phenylhydrazine hydrochloride as the starting materials. As-synthesized Schiff base compounds were characterized by means of nuclear magnetic resonance spectroscopy, infrared spectrometry, mass spectrometry and elemental analysis. Moreover the *in vitro* antitumor activity of compounds **B₂** and **B₄** against K562 (human leukemia cell line) was evaluated using MTT assay method. Results show that the compounds can inhibit K562 tumor cell's growth and generation.

Keywords: Schiff base, Synthesis, Characterize, *in vitro* Antitumor activity, Heterocyclic compounds, Pyrazole group.

INTRODUCTION

Schiff base is a kind of organic compounds with special physical and chemical properties¹. For the strong coordination ability, Schiff base can form complexes with metal or rare earth ions and is a good extractant and chelating agent²⁻⁴. At the same time, with strong antibacterial and antiviral ability, Schiff base has been widely used in biology, medicine, metallurgy, dye and photoelectric functional materials fields⁵⁻⁸. On the other hand, as a kind of efficient extractants and chelators of metal ions⁹⁻¹³, pyrazole derivatives have aroused wide concern in biology, medicine, pesticide, analytical applications. Furthermore, the excellent photoelectric properties and carrier transmission capacity of pyrazole derivatives add to their wide use in photoelectric functional materials and organic light-emitting diodes (OLED)^{14,15}. Therefore, research of pyrazole based on Schiff base compounds also appears more and more important and valuable. In this article we report a simple procedure for synthesizing Schiff base containing pyrazole group.

EXPERIMENTAL

Mass spectra (MS) were determined with an Agilent 1100LC-MS mass spectrometer. Infrared (IR) spectra of as-obtained products within 4000-400 cm⁻¹ were recorded with a Nicolet 170 SXFT-IR spectrometer (mixed with KBr and pressed into pellets). Nuclear magnetic resonance (¹H NMR) spectra in (CD₃)₂C=O solvent were recorded with an INOVA-

400 spectrometer in the presence of tetramethylsilane as an internal standard. Elemental analysis was conducted with a PE2400 elemental analysis apparatus.

The *in vitro* antitumor activity of compounds **B₂** and **B₄** was evaluated in K562 (human leukemia cell line) using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay method (amofafide as reference substance, 10 and 30 μmol/L). The inhibition rate was calculated from plotted results using untreated cells as 100 %.

All chemicals and solvents are of commercial reagent grade and used without further purification. Besides, 1-arylpyrazole-4-carbaldehyde was prepared according to procedures available elsewhere. Known structures of as-synthesized products were verified by comparing their data with those reported in the literature^{16,17}.

Synthesis of Schiff base compounds: 5 mmol of 1-arylpyrazole-4-carbaldehyde and 5 mmol of benzene hydrazine or phenylhydrazine hydrochloride were completely dissolved in 35 mL methanol, heated to 80 °C, refluxed for 2 h, cooled to room temperature and placed overnight. After suction filtered and dried, crude mixed products were obtained and purified by recrystallizing in the mixed solvent of EtOH and CH₂Cl₂ (V_{EtOH}:V_{CH₂Cl₂} = 1:3).

3-Methyl-1-phenyl-4-[(2-(*p*-tolyl)hydrazono)methyl]-1H-pyrazol-5(4H)-one (A₁): Yellow solid 0.70 g, yield: 46 %. APCI-MS (*m/z*) calcd. (M⁻) 306.3, found: 304.9. ¹H NMR spectrum (400 MHz, DMSO-*d*₆), δ, ppm (*J*, Hz): 2.19 (3H, s, CH₃), 2.47 (3H, s, CH₃), 6.89 (2H, d, *J* = 8.4, Ar-H), 7.02 (2H,

d, $J = 8.2$, Ar-H), 7.46 (1H, s, N=C-H), 7.51-7.62 (5H, m, Ar-H), 7.79 (1H, s, N-H), 10.22 (1H, s, O-H). IR spectrum (thin layer), ν , cm^{-1} : 3236 (N-H), 1588 (C=N), 1501, 1476 (C=C), 1275 (C-N). Anal. calcd.: C, 69.85; H, 5.52; N, 19.17. Found: C, 69.74; H, 5.61; N, 19.13 %.

4-[[2-(4-Fluorophenyl)hydrazono)methyl]-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one (A₂): Yellow solid 0.85 g, yield: 55 %. APCI-MS (m/z) calcd. (M^+) 310.3, found: 308.8. ¹H NMR spectrum (400 MHz, DMSO- d_6), δ , ppm (J , Hz): 2.48 (3H, s, CH₃), 6.93-7.01 (2H, m, Ar-H), 7.04-7.09 (2H, m, Ar-H), 7.48 (1H, s, N=C-H), 7.52-7.66 (5H, m, Ar-H), 7.82 (1H, s, N-H), 10.30 (1H, s, O-H). IR spectrum (thin layer), ν , cm^{-1} : 3244 (N-H), 1587 (C=N), 1503, 1477 (C=C), 1273 (C-N). Anal. calcd.: C, 65.80; H, 4.87; N, 18.05. Found: C, 65.87; H, 4.95; N, 18.00 %.

4-[[2-(3-Chlorophenyl)hydrazono)methyl]-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one (A₃): Yellow solid 0.80 g, yield: 49 %. APCI-MS (m/z) calcd. (M^+) 326.7, found: 324.9. ¹H NMR spectrum (400 MHz, DMSO- d_6), δ , ppm (J , Hz): 2.49 (s, 3H, CH₃), 6.75 (1H, dd, $J = 7.8, 1.2$, Ar-H), 6.90 (1H, d, $J = 8.2$, Ar-H), 6.99 (1H, t, $J = 1.9$, Ar-H), 7.23 (1H, t, $J = 8.0$, Ar-H), 7.49 (1H, s, N=C-H), 7.53-7.63 (5H, m, Ar-H), 7.86 (1H, s, N-H), 10.53 (1H, s, O-H). IR spectrum (thin layer), ν , cm^{-1} : 3234 (N-H), 1603 (C=N), 1502, 1479 (C=C), 1271 (C-N). Anal. calcd.: C, 62.84; H, 4.63; N, 17.15. Found: C, 62.79; H, 4.71; N, 17.08 %.

4-[[2-(4-Bromophenyl)hydrazono)methyl]-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one (A₄): Yellow solid 0.90 g, yield: 48 %. APCI-MS (m/z) calcd. (M^+) 371.2, found: 369.8. ¹H NMR spectrum (400 MHz, DMSO- d_6), δ , ppm (J , Hz): 2.48 (3H, s, CH₃), 6.91-6.99 (2H, m, Ar-H), 7.33-7.39 (2H, m, Ar-H), 7.48 (1H, s, N=C-H), 7.53-7.61 (5H, m, Ar-H), 7.84 (1H, s, N-H), 10.46 (1H, s, O-H). IR spectrum (thin layer), ν , cm^{-1} : 3230 (N-H), 1591 (C=N), 1492, 1471 (C=C), 1278 (C-N). Anal. calcd.: C, 55.00; H, 4.07; N, 15.09. Found: C, 54.89; H, 4.13; N, 15.05 %.

3-Methyl-4-[[2-(4-nitrophenyl)hydrazono)methyl]-1-phenyl-1H-pyrazol-5(4H)-one (A₅): Deep red solid 0.75 g, yield: 44 %. APCI-MS (m/z) calcd. (M^+) 337.3, found: 335.9. ¹H NMR spectrum (400 MHz, DMSO- d_6), δ , ppm (J , Hz): 2.37 (3H, s, CH₃), 6.98 (2H, d, $J = 8.7$, Ar-H), 7.17-7.26 (1H, m, Ar-H), 7.42 (2H, d, $J = 8.2$, Ar-H), 7.45 (1H, s, N=C-H), 7.80 (2H, d, $J = 7.8$, Ar-H), 8.09 (2H, s, Ar-H), 8.11 (1H, s, N-H), 10.93 (1H, s, O-H). IR spectrum (thin layer), ν , cm^{-1} : 3236 (N-H), 1596 (C=N), 1552, 1372 (NO₂), 1499, 1478 (C=C), 1277 (C-N). Anal. calcd.: C, 60.53; H, 4.48; N, 20.76. Found: C, 60.45; H, 4.52; N, 20.71 %.

5-Chloro-4-[[2-(4-fluorophenyl)hydrazono)methyl]-3-methyl-1-phenyl-1H-pyrazole (B₁): Deep yellow solid 0.55 g, yield: 33 %. APCI-MS (m/z) calcd. (M^+) 328.7, found: 329.1. ¹H NMR spectrum (400 MHz, DMSO- d_6), δ , ppm (J , Hz): 2.60 (3H, s, CH₃), 6.99 (4H, d, $J = 6.7$, Ar-H), 7.41 (1H, s, N=C-H), 7.46-7.53 (3H, m, Ar-H), 7.54-7.57 (2H, m, Ar-H), 7.70 (1H, s, N-H). IR spectrum (thin layer), ν , cm^{-1} : 3230 (N-H), 1597 (C=N), 1502, 1478 (C=C), 1246 (C-N). Anal. calcd.: C, 62.10; H, 4.29; N, 17.04. Found: C, 62.01; H, 4.37; N, 17.00 %.

5-Chloro-4-[[2-(3-chlorophenyl)hydrazono)methyl]-3-methyl-1-phenyl-1H-pyrazole (B₂): Deep yellow solid 1.20

g, yield: 70 %. APCI-MS (m/z) calcd. (M^+) 345.2, found: 346. ¹H NMR spectrum (400 MHz, DMSO- d_6), δ , ppm (J , Hz): 2.48 (3H, s, CH₃), 6.74 (1H, dd, $J = 7.5, 1.6$, Ar-H), 6.89 (1H, d, $J = 7.4$, Ar-H), 6.98 (1H, t, $J = 2.0$, Ar-H), 7.22 (1H, t, $J = 8.1$, Ar-H), 7.48 (1H, s, N=C-H), 7.53-7.60 (5H, m, Ar-H), 7.85 (1H, s, N-H). IR spectrum (thin layer), ν , cm^{-1} : 3234 (N-H), 1598 (C=N), 1501, 1474 (C=C), 1246 (C-N). Anal. calcd.: C, 59.14; H, 4.09; N, 16.23. Found: C, 59.10; H, 4.14; N, 16.20 %.

4-[[2-(4-Bromophenyl)hydrazono)methyl]-5-chloro-3-methyl-1-phenyl-1H-pyrazole (B₃): Deep yellow solid 1.10 g, yield: 56 %. APCI-MS (m/z) calcd. ($M + Na$)⁺ 389.6, found: 412.1. ¹H NMR spectrum (400 MHz, DMSO- d_6), δ , ppm (J , Hz): 2.60 (3H, s, CH₃), 6.94 (2H, d, $J = 7.5$, Ar-H), 7.36 (2H, d, $J = 8.7$, Ar-H), 7.41 (1H, s, N=C-H), 7.47-7.52 (3H, m, Ar-H), 7.54-7.57 (2H, m, Ar-H), 7.70 (1H, s, N-H). IR spectrum (thin layer), ν , cm^{-1} : 3236 (N-H), 1596 (C=N), 1500, 1470 (C=C), 1253 (C-N). Anal. calcd.: C, 52.40; H, 3.62; N, 14.38. Found: C, 52.31; H, 3.71; N, 14.37 %.

5-Chloro-3-methyl-4-[[2-(4-nitrophenyl)hydrazono)methyl]-1-phenyl-1H-pyrazole (B₄): Brick red solid 1.35 g, yield: 76 %. APCI-MS (m/z) calcd. (M^+) 355.7, found: 356.2. ¹H NMR spectrum (400 MHz, DMSO- d_6), δ , ppm (J , Hz): 2.50 (3H, s, CH₃), 7.08 (2H, d, $J = 7.3$, Ar-H), 7.50 (1H, s, N=C-H), 7.54-7.61 (5H, m, Ar-H), 8.04 (1H, s, N-H), 8.13 (2H, d, $J = 8.8$, Ar-H). IR spectrum (thin layer), ν , cm^{-1} : 3237 (N-H), 1601 (C=N), 1554, 1382 (NO₂), 1501, 1476 (C=C), 1241 (C-N). Anal. calcd.: C, 57.39; H, 3.97; N, 19.68. Found: C, 57.30; H, 4.01; N, 19.65 %.

3,5-Dimethyl-1-phenyl-4-[[2-(phenylhydrazono)methyl]-1H-pyrazole (C₁): Deep yellow solid 0.50 g, yield: 34 %. APCI-MS (m/z) calcd. (M^+) 290.3, found: 291.2. ¹H NMR spectrum (400 MHz, DMSO- d_6), δ , ppm (J , Hz): 2.38 (3H, s, CH₃), 2.43 (3H, s, CH₃), 6.68 (1H, t, $J = 7.3$, Ar-H), 6.96 (2H, d, $J = 7.7$, Ar-H), 7.18 (2H, t, $J = 7.8$, Ar-H), 7.41 (1H, s, N=C-H), 7.51-7.54 (5H, m, Ar-H), 7.90 (1H, s, N-H). IR spectrum (thin layer), ν , cm^{-1} : 3236 (N-H), 1600 (C=N), 1504, 1428 (C=C), 1263 (C-N). Anal. calcd.: C, 74.45; H, 6.25; N, 19.30. Found: C, 74.39; H, 6.18; N, 19.27 %.

4-[[2-(4-Fluorophenyl)hydrazono)methyl]-3,5-dimethyl-1-phenyl-1H-pyrazole (C₂): Deep yellow solid 0.75 g, yield: 49 %. APCI-MS (m/z) calcd. (M^+) 308.3, found: 309.1. ¹H NMR spectrum (400 MHz, DMSO- d_6), δ , ppm (J , Hz): 2.37 (3H, s, CH₃), 2.42 (3H, s, CH₃), 6.93 (2H, dd, $J = 9, 4.8$, Ar-H), 7.03 (2H, t, $J = 8.9$, Ar-H), 7.40 (1H, s, N=C-H), 7.46-7.56 (5H, m, Ar-H), 7.89 (1H, s, N-H). IR spectrum (thin layer), ν , cm^{-1} : 3222 (N-H), 1596 (C=N), 1502, 1425 (C=C), 1272 (C-N). Anal. calcd.: C, 70.11; H, 5.56; N, 18.17. Found: C, 70.05; H, 5.63; N, 18.01 %.

4-[[2-(3-Chlorophenyl)hydrazono)methyl]-3,5-dimethyl-1-phenyl-1H-pyrazole (C₃): Deep yellow solid 0.55 g, yield: 34 %. APCI-MS (m/z) calcd. (M^+) 324.8, found: 325.1. ¹H NMR spectrum (400 MHz, DMSO- d_6), δ , ppm (J , Hz): 2.37 (3H, s, CH₃), 2.43 (3H, s, CH₃), 6.69 (1H, s, Ar-H), 6.86 (1H, s, Ar-H), 6.95 (1H, s, Ar-H), 7.19 (1H, s, Ar-H), 7.42 (1H, s, N=C-H), 7.48-7.52 (5H, m, Ar-H), 7.91 (1H, s, N-H). IR spectrum (thin layer), ν , cm^{-1} : 3224 (N-H), 1597 (C=N), 1504, 1430 (C=C), 1265 (C-N). Anal. calcd.: C, 66.56; H, 5.28; N, 17.25. Found: C, 66.50; H, 5.29; N, 17.21 %.

4-[[2-(4-Bromophenyl)hydrazono]methyl]-3,5-dimethyl-1-phenyl-1H-pyrazole (C₄): Deep yellow solid 1.05 g, yield: 57 %. APCI-MS (*m/z*) calcd. (M⁺) 369.2, found: 370.1. ¹H NMR spectrum (400 MHz, DMSO-*d*₆), δ , ppm (*J*, Hz): 2.37 (3H, s, CH₃), 2.42 (3H, s, CH₃), 6.90 (2H, t, *J* = 5.9, Ar-H), 7.32 (2H, t, *J* = 5.9, Ar-H), 7.41 (1H, s, N=C-H), 7.48-7.54 (5H, m, Ar-H), 7.90 (1H, s, N-H). IR spectrum (thin layer), ν , cm⁻¹: 3236 (N-H), 1596 (C=N), 1500, 1427 (C=C), 1266 (C-N). Anal. calcd.: C, 58.55; H, 4.64; N, 15.17. Found: C, 58.50; H, 4.71; N, 15.12 %.

3,5-Dimethyl-4-[[2-(4-nitrophenyl)hydrazono]methyl]-1-phenyl-1H-pyrazole (C₅): Brick red solid 1.40 g, yield: 83 %. APCI-MS (*m/z*) calcd. (M⁺) 335.3, found: 336.1. ¹H NMR spectrum (400 MHz, DMSO-*d*₆), δ , ppm (*J*, Hz): 2.40 (3H, s, CH₃), 2.45 (3H, s, CH₃), 7.04 (2H, s, Ar-H), 7.42 (1H, s, C=N-H), 7.48-7.59 (5H, m, Ar-H), 8.10 (2H, s, Ar-H), 8.12 (1H, s, N-H). IR spectrum (thin layer), ν , cm⁻¹: 3231 (N-H), 1595 (C=N), 1553, 1378 (NO₂), 1503, 1432 (C=C), 1262 (C-N). Anal. calcd.: C, 64.47; H, 5.11; N, 20.88. Found: C, 64.45; H, 5.10; N, 20.78 %.

1-(4-Bromophenyl)-3,5-dimethyl-4-[(2-phenyl hydrazono)methyl]-1H-pyrazole (D₁): Yellow solid 1.55 g, yield: 84 %. APCI-MS (*m/z*) calcd. (M⁺) 369.3, found: 370.1. ¹H NMR spectrum (400 MHz, DMSO-*d*₆), δ , ppm (*J*, Hz): 2.48 (3H, s, CH₃), 2.49 (3H, s, CH₃), 6.85 (1H, s, Ar-H), 7.04 (3H, s, Ar-H), 7.31-7.35 (3H, m, Ar-H), 7.58-7.60 (2H, m, Ar-H), 7.61 (1H, s, N=C-H), 7.75 (1H, s, N-H). IR spectrum (thin layer), ν , cm⁻¹: 3231 (N-H), 1600 (C=N), 1496, 1425 (C=C), 1255 (C-N). Anal. calcd.: C, 58.55; H, 4.64; N, 15.17. Found: C, 58.51; H, 4.69; N, 15.07 %.

1-(4-Bromophenyl)-3,5-dimethyl-4-[[2-(*p*-tolyl)hydrazono]methyl]-1H-pyrazole (D₂): Yellow solid 1.45 g, yield: 76 %. APCI-MS (*m/z*) calcd. (M⁺) 383.3, found: 384.0. ¹H NMR spectrum (400 MHz, DMSO-*d*₆), δ , ppm (*J*, Hz): 2.18 (3H, s, CH₃), 2.36 (3H, s, CH₃), 2.43 (3H, s, CH₃), 6.85 (2H, d, *J* = 8.4, Ar-H), 6.98 (2H, t, *J* = 9.1, Ar-H), 7.47-7.50 (2H, m, Ar-H), 7.69 (1H, s, N=C-H), 7.70-7.77 (2H, m, Ar-H), 7.84 (1H, s, N-H). IR spectrum (thin layer), ν , cm⁻¹: 3235 (N-H), 1607 (C=N), 1498, 1426 (C=C), 1254 (C-N). Anal. calcd.: C, 49.54; H, 5; N, 14.62. Found: C, 49.46; H, 5.07; N, 14.67 %.

1-(4-Bromophenyl)-4-[[2-(4-fluorophenyl)hydrazono]methyl]-3,5-dimethyl-1H-pyrazole (D₃): Yellow solid 1.30 g, yield: 67 %. APCI-MS (*m/z*) calcd. (M⁺) 387.3, found: 388.1. ¹H NMR spectrum (400 MHz, DMSO-*d*₆), δ , ppm (*J*, Hz): 2.36 (3H, s, CH₃), 2.43 (3H, s, CH₃), 6.88-6.98 (2H, m, Ar-H), 7.02 (2H, m, Ar-H), 7.48 (2H, t, *J* = 7.9, Ar-H), 7.69 (1H, s, N=C-H), 7.70-7.77 (2H, m, Ar-H), 7.88 (1H, s, N-H). IR spectrum (thin layer), ν , cm⁻¹: 3233 (N-H), 1606 (C=N), 1500, 1426 (C=C), 1253 (C-N). Anal. calcd.: C, 55.83; H, 4.16; N, 14.47. Found: C, 55.79; H, 4.19; N, 14.39 %.

1-(4-Bromophenyl)-4-[[2-(4-bromophenyl)hydrazono]methyl]-3,5-dimethyl-1H-pyrazole (D₄): Yellow solid 1.50 g, yield: 67 %. APCI-MS (*m/z*) calcd. (M + Na)⁺ 448.2, found: 471.8. ¹H NMR spectrum (400 MHz, DMSO-*d*₆), δ , ppm (*J*, Hz): 2.36 (3H, s, CH₃), 2.42 (3H, s, CH₃), 6.84-6.98 (2H, m, Ar-H), 7.26-7.41 (2H, m, Ar-H), 7.45-7.57 (2H, m, Ar-H), 7.69 (1H, s, N=C-H), 7.71-7.75 (2H, m, Ar-H), 7.88 (1H, s, N-H). IR spectrum (thin layer), ν , cm⁻¹: 3236 (N-H), 1594

(C=N), 1496, 1426 (C=C), 1252 (C-N). Anal. calcd.: C, 48.24; H, 3.60; N, 12.50. Found: C, 48.20; H, 3.69; N, 12.47 %.

1-(4-Bromophenyl)-3,5-dimethyl-4-[[2-(4-nitrophenyl)hydrazono]methyl]-1H-pyrazole (D₅): Brick red solid 1.90 g, yield: 92 %. APCI-MS (*m/z*) calcd. (M + Na)⁺ 437.3, found: 437.1. ¹H NMR spectrum (400 MHz, DMSO-*d*₆), δ , ppm (*J*, Hz): 2.49 (3H, s, CH₃), 2.51 (3H, s, CH₃), 7.01 (2H, t, *J* = 7.2, Ar-H), 7.31-7.35 (2H, m, Ar-H), 7.60-7.64 (2H, m, Ar-H), 7.84 (1H, s, N=C-H), 7.91 (1H, s, N-H), 8.81 (2H, d, *J* = 9.3, Ar-H). IR spectrum (thin layer), ν , cm⁻¹: 3231 (N-H), 1606 (C=N), 1553, 1378 (NO₂), 1499, 1427 (C=C), 1254 (C-N). Anal. calcd.: C, 52.19; H, 3.89; N, 16.91. Found: C, 52.11; H, 3.99; N, 16.84 %.

3,5-Dimethyl-1-(4-nitrophenyl)-4-[(2-phenyl hydrazono)methyl]-1H-pyrazole (E₁): Deep red solid 1.10 g, yield: 66 %. APCI-MS (*m/z*) calcd. (M⁺) 335.4, found: 336.7. ¹H NMR spectrum (400 MHz, DMSO-*d*₆), δ , ppm (*J*, Hz): 2.42 (3H, s, CH₃), 2.55 (3H, s, CH₃), 6.69 (1H, t, *J* = 7.3, Ar-H), 6.96 (2H, d, *J* = 7.6, Ar-H), 7.12-7.29 (2H, m, Ar-H), 7.83-7.87 (2H, m, Ar-H), 7.89 (1H, s, N=C-H), 8.30-8.41 (2H, m, Ar-H), 10.11 (1H, s, N-H). IR spectrum (thin layer), ν , cm⁻¹: 3235 (N-H), 1600 (C=N), 1551, 1376 (NO₂), 1503, 1427 (C=C), 1262 (C-N). Anal. calcd.: C, 64.47; H, 5.11; N, 20.88. Found: C, 64.38; H, 5.18; N, 20.82 %.

3,5-Dimethyl-1-(4-nitrophenyl)-4-[[2-(*p*-tolyl)hydrazono]methyl]-1H-pyrazole (E₂): Deep red solid 1.30 g, yield: 74 %. APCI-MS (*m/z*) calcd. (M⁺) 349.4, found: 350.2. ¹H NMR spectrum (400 MHz, DMSO-*d*₆), δ , ppm (*J*, Hz): 2.19 (3H, s, CH₃), 2.40 (3H, s, CH₃), 2.55 (3H, s, CH₃), 6.87 (2H, d, *J* = 8.4, Ar-H), 7.00 (2H, d, *J* = 8.2, Ar-H), 7.83 (1H, s, N=C-H), 7.85-7.89 (2H, m, Ar-H), 8.31-8.39 (2H, m, Ar-H), 9.98 (1H, s, N-H). IR spectrum (thin layer), ν , cm⁻¹: 3236 (N-H), 1595 (C=N), 1550, 1380 (NO₂), 1506, 1428 (C=C), 1260 (C-N). Anal. calcd.: C, 65.32; H, 5.48; N, 20.04. Found: C, 65.25; H, 5.51; N, 20.07 %.

4-[[2-(4-Fluorophenyl)hydrazono]methyl]-3,5-dimethyl-1-(4-nitrophenyl)-1H-pyrazole (E₃): Deep red solid 1.35 g, yield: 76 %. APCI-MS (*m/z*) calcd. (M⁺) 353.4, found: 354.2. ¹H NMR spectrum (400 MHz, DMSO-*d*₆), δ , ppm (*J*, Hz): 2.40 (3H, s, CH₃), 2.54 (3H, s, CH₃), 6.92-6.97 (2H, m, Ar-H), 7.00-7.09 (2H, m, Ar-H), 7.81-7.86 (2H, m, Ar-H), 7.87 (1H, s, N=C-H), 8.30-8.40 (2H, m, Ar-H), 10.09 (1H, s, N-H). IR spectrum (thin layer), ν , cm⁻¹: 3235 (N-H), 1594 (C=N), 1553, 1370 (NO₂), 1504, 1430 (C=C), 1267 (C-N). Anal. calcd.: C, 61.18; H, 4.56; N, 19.82. Found: C, 61.11; H, 4.59; N, 19.77 %.

4-[[2-(3-Chlorophenyl)hydrazono]methyl]-3,5-dimethyl-1-(4-nitrophenyl)-1H-pyrazole (E₄): Deep red solid 1.25 g, yield: 68 %. APCI-MS (*m/z*) calcd. (M⁺) 369.8, found: 370.1. ¹H NMR spectrum (400 MHz, DMSO-*d*₆), δ , ppm (*J*, Hz): 2.40 (3H, s, CH₃), 2.54 (3H, s, CH₃), 6.88-6.98 (2H, m, Ar-H), 7.00-7.10 (2H, m, Ar-H), 7.81-7.86 (2H, m, Ar-H), 7.87 (1H, s, N=C-H), 8.30-8.39 (2H, m, Ar-H), 10.09 (1H, s, N-H). IR spectrum (thin layer), ν , cm⁻¹: 3234 (N-H), 1594 (C=N), 1556, 1386 (NO₂), 1506, 1429 (C=C), 1268 (C-N). Anal. calcd.: C, 58.46; H, 4.36; N, 18.94. Found: C, 58.45; H, 4.39; N, 18.91 %.

4-[[2-(4-Bromophenyl)hydrazono]methyl]-3,5-dimethyl-1-(4-nitrophenyl)-1H-pyrazole (E₅): Deep red solid 1.25 g, yield: 60 %. APCI-MS (*m/z*) calcd. (M⁺) 414.5, found: 415.3.

^1H NMR spectrum (400 MHz, DMSO- d_6), δ , ppm (J , Hz): 2.50 (3H, s, CH_3), 2.60 (3H, s, CH_3), 6.93-7.00 (2H, m, Ar-H), 7.35-7.37 (2H, m, Ar-H), 7.67-7.70 (2H, m, Ar-H), 7.73 (1H, s, $\text{N}=\text{C}-\text{H}$), 8.35-8.38 (2H, m, Ar-H), 10.05 (1H, s, $\text{N}-\text{H}$). IR spectrum (thin layer), ν , cm^{-1} : 3231 ($\text{N}-\text{H}$), 1601 ($\text{C}=\text{N}$), 1553, 1378 (NO_2), 1503, 1427 ($\text{C}=\text{C}$), 1263 ($\text{C}-\text{N}$). Anal. calcd.: C, 52.19; H, 3.89; N, 16.91. Found: C, 52.13; H, 3.97; N, 16.88 %.

3,5-Dimethyl-1-(4-nitrophenyl)-4-[[2-(4-nitrophenyl)-hydrazono]methyl]-1H-pyrazole (E_6): Brick red solid 1.40 g, yield: 74 %. APCI-MS (m/z) calcd. (M^+) 380.4, found: 381.5. ^1H NMR spectrum (400 MHz, DMSO- d_6), δ , ppm (J , Hz): 2.44 (3H, s, CH_3), 2.57 (3H, s, CH_3), 7.05 (2H, d, $J = 8$, Ar-H), 7.82-7.93 (2H, m, Ar-H), 8.10-8.11 (2H, m, Ar-H), 8.13 (1H, s, $\text{N}=\text{C}-\text{H}$), 8.30-8.45 (2H, m, Ar-H), 11.26 (1H, s, $\text{N}-\text{H}$). IR spectrum (thin layer), ν , cm^{-1} : 3236 ($\text{N}-\text{H}$), 1596 ($\text{C}=\text{N}$), 1556, 1387 (NO_2), 1502, 1427 ($\text{C}=\text{C}$), 1266 ($\text{C}-\text{N}$). Anal. calcd.: C, 56.84; H, 4.24; N, 22.10. Found: C, 56.80; H, 4.29; N, 22.02 %.

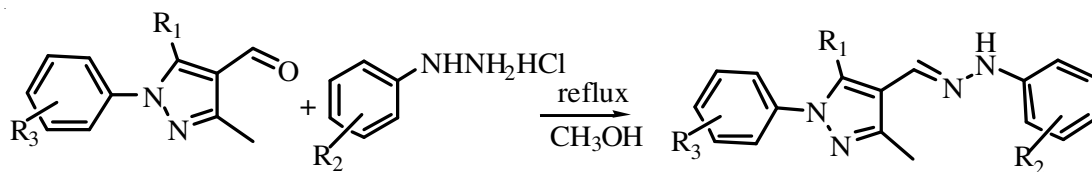
RESULTS AND DISCUSSION

These Schiff base compounds were synthesized by condensation reaction with 1-arylpyrazol-4-carbaldehyde and benzene hydrazine or phenylhydrazine hydrochloride as the starting materials (Fig.1). The phenylhydrazine hydrochloride can dissociate by weak base (e.g., CH_3COONa), but it was found that the post processing of the reaction would become very complex after adding CH_3COONa and the products were difficult to separate out from the reaction system. Generally, weak acid can be used as a catalyst in this reaction, however, it was found that it is disadvantageous to the separation of products as adding acid and phenylhydrazine hydrochloride as the substrate maybe enhance the acidity of reaction system, so there is no other acid has been added as catalyst. In the meantime, the results showed that we can get good yields and pure products in the presence of methanol as the solvent and refluxing 2 h under 80 °C. It is relatively complex to the purification of Schiff base compounds, for the Schiff base can decompose in the silica gel column and these compounds can't be separated by column layer chromatography. At last, the crystallization method has been used to separate these Schiff base compounds while EtOH and CH_2Cl_2 ($V_{\text{EtOH}}:V_{\text{CH}_2\text{Cl}_2} = 1:3$) as solvent.

The existence of electron-withdrawing substituent on the pyrazole ring is beneficial to the reaction and the experimental results show that the reaction is easier and the yield of the target compounds is higher when $\text{R}_1 = \text{Cl}$ and $\text{R}_3 = \text{F}$, Cl , Br and $-\text{NO}_2$ as compared with the electron-donating substituent on the pyrazole ring. For R_2 the existence of electron-donating substituent is disadvantageous to the reaction, but it's easier for product to separate out from the reaction system when $\text{R}_2 = -\text{NO}_2$.

Structural characterization: The structure of target compounds have been characterized by means of ^1H NMR, FT-IR, MS and elemental analysis. In the ^1H NMR spectrum, it was found that the $\text{N}=\text{C}-\text{H}$ chemical shift was at 7.40-8.13 (7.45-7.49 for A-series; 7.40-7.50 for B-series; 7.40-7.42 for C-series; 7.61-7.84 for D-series; 7.73-8.13 for E-series), is a singlet; it can be seen that the data clearly are divided into two groups and have small difference for it, the results illustrate that R_3 has certain influence to the chemical shift of $\text{N}=\text{C}-\text{H}$ but these influence is very less. For A-series the chemical shift of $\text{O}-\text{H}$ is located in the scope of 10.22 to 10.93 with a strong singlet. The $\text{N}-\text{H}$ appeared in the range of 7.69 to 8.12 (7.79-8.11 for A-series; 7.69-8.04 for B-series; 7.87-8.12 for C-series; 7.75-7.91 for D-series) and 9.98-11.26 for E-series. The abnormal of E-series can be interpreted as the influence of the intermolecular hydrogen bond. In the IR spectrum, the absorption of $\text{N}-\text{H}$ peak was within the scope of 3237-3222 cm^{-1} ; The stretching vibration absorption of $\text{C}=\text{N}$ appeared in the range of 1607-1587 cm^{-1} ; 1506-1425 cm^{-1} was the scope of absorption peaks of aromatic ring skeleton; 1278-1241 cm^{-1} belonged to the vibration absorption peak of $\text{C}-\text{N}$. And it appeared two obviously characteristic absorption peaks, in 1556-1550 and 1382-1370 cm^{-1} , respectively for the compounds that containing the substituent of $-\text{NO}_2$.

in vitro Antitumor activity: We predict that the as-synthesis Schiff base compounds containing pyrazole group has biological activity for the Schiff base and pyrazole derivatives have been widely used in biomedical fields. For this reason, we choose compounds B_2 and B_4 as examples to explore their *in vitro* antitumor activity. Their antiproliferative activity against K562 (human leukemia cell line) was evaluated by *in vitro* MTT assays. Amonafide was tested as a reference compound. The biological results were shown in Table-1. It can be seen



$\text{R}_1 = \text{OH}$; $\text{R}_3 = \text{H}$; $\text{R}_2 = p\text{-CH}_3(\text{A}_1)$; $p\text{-F}(\text{A}_2)$; $m\text{-Cl}(\text{A}_3)$; $p\text{-Br}(\text{A}_4)$; $p\text{-NO}_2(\text{A}_5)$

$\text{R}_1 = \text{Cl}$; $\text{R}_3 = \text{H}$; $\text{R}_2 = p\text{-F}(\text{B}_1)$; $m\text{-Cl}(\text{B}_2)$; $p\text{-Br}(\text{B}_3)$; $p\text{-NO}_2(\text{B}_4)$

$\text{R}_1 = \text{CH}_3$; $\text{R}_3 = \text{H}$; $\text{R}_2 = \text{H}(\text{C}_1)$; $p\text{-F}(\text{C}_2)$; $m\text{-Cl}(\text{C}_3)$; $p\text{-Br}(\text{C}_4)$; $p\text{-NO}_2(\text{C}_5)$

$\text{R}_1 = \text{CH}_3$; $\text{R}_3 = p\text{-Br}$; $\text{R}_2 = \text{H}(\text{D}_1)$; $p\text{-CH}_3(\text{D}_2)$; $p\text{-F}(\text{D}_3)$; $p\text{-Br}(\text{D}_4)$; $p\text{-NO}_2(\text{D}_5)$

$\text{R}_1 = \text{CH}_3$; $\text{R}_3 = p\text{-NO}_2$; $\text{R}_2 = \text{H}(\text{E}_1)$; $p\text{-CH}_3(\text{E}_2)$; $p\text{-F}(\text{E}_3)$; $m\text{-Cl}(\text{E}_4)$; $p\text{-Br}(\text{E}_5)$; $p\text{-NO}_2(\text{E}_6)$

Fig. 1. Synthesis of Schiff base compounds

from Table-1 that the compounds **B**₂ and **B**₄ have showed anti-proliferative activity against K562 and can inhibit the growth of K562 cells.

TABLE-1
INHIBITION RATES OF COMPOUNDS
B₂ AND **B**₄ TO K562 CELL LINES

Compound	K562 (%)	
	10 $\mu\text{mol L}^{-1}$	30 $\mu\text{mol L}^{-1}$
Amonafide	18.5	39.0
B ₂	10.6	11.8
B ₄	0.31	9.1

Conclusion

We have synthesized 5 series novel Schiff bases containing pyrazole group and characterized by means of ¹H NMR, FT-IR, MS and elemental analysis. The test shows that the compounds **B**₂ and **B**₄ have showed antiproliferative activity against K562 and can inhibit the growth of K562 cells.

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