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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis, molecular modeling, and ADMET studies of some pyrazoline derivatives as shikimate kinase inhibitors

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Abstract A series of pyrazoline derivatives were synthesized and their structures have been characterized by IR, ^{1}H NMR, ^{13}C NMR, mass spectral and elemental analysis. The novel compounds were designed as *Mycobacterium tuberculosis* shikimate kinase (*Mt*SK) inhibitors based on docking studies using Sybyl-X 2.0 software. In silico ADMET predictions revealed that all compounds had minimal toxic effects and had good absorption as well as solubility characteristics. Thus these compounds may serve as potential lead compound for developing new anti-tubercular drug. Among the tested compounds 4c, 5b, and 6a exhibited promising antitubercular activity. Additional, some compounds were also evaluated for their cytotoxic activity against EAC cell lines using the tryphan blue exclusion method.

Keywords Pyrazolines · Antitubercular activity · *Mycobacterium tuberculosis* Shikimate Kinase

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Introduction

Mycobacterium tuberculosis (*Mt*) is the causative bacterial pathogen of Tuberculosis (TB), which is one of the main causes of death (Swaminathan 2002). In the past 40 years, no new classes of drugs for TB have been developed. Novel active drugs are essential for the effective treatment of dormant tuberculosis infection.

The genomic information about the targets is greatly needed in the development of new classes of antitubercular drugs. The enzymes in the shikimate pathway are one of the prominent targets for the rational design of new antitubercular agents (Gu et al. 2002). Shikimate kinase (SK) is been identified as a very significant enzyme in various pathogenic bacteria. Moreover, it does not have any counterpart in human cells, which marks the striking property for the extension of new antibiotics. Phosphorylation of the 3-hydroxyl group of shikimic acid is carried out by the enzyme SK using ATP as a co-substrate. It belongs to the family nucleoside monophosphate kinases. The latest studies in the field of drug design has highlighted the basic interactions between *Mycobacterium tuberculosis* shikimate kinase enzyme (*Mt*-SK) and their substrates, which is very essential in the development of new generation of drugs against tuberculosis (Pereira et al. 2007; Blanco et al. 2013).

Good drug absorption and suitable drug delivery are very important in the development of drugs intended for oral use. Due to poor pharmacokinetics, about 30% of drugs fail in the initial stage of drug discovery and development. So in order to minimize these failures, proper understanding of molecular properties is very essential.

Medicinal chemists working in the area of tuberculosis have a great attraction towards the chemistry of nitrogen containing heterocyclic compounds. Pyrazole and their derivatives (Bandodkar and Schmitt 2010) containing the

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N–N bond are active against many mycobacteria (Khunt et al. 2012). Certain pyrazoline skeletals with anti-tubercular action reported are shown in Fig. 1 (Monga et al. 2014; Joshi et al. 2016). Pyrazolines have varied biological activities, like anti-inflammatory (Khalil 2012), antidepressant (Parmar et al. 2006; Soni et al. 1987), anticancer (Ghorab et al. 2006), antitumor (Insuasty et al. 2011) antimicrobial (Manna and Agrawal 2009), anti-tubercular (Taj et al. 2011; Manna and Agrawal 2011), and antioxidant activities (Kumar et al. 2013). It comprises the vast section of chemical entities, which motivated us to examine the biological activity by some structural changes.

The prevalent method for the synthesis of pyrazoline is of Fischer and Knoevenagel i.e. the reaction of α, β-unsaturated ketones with phenyl hydrazine in acetic acid under refluxing condition (Azarifar and Maleki 2005). Nevertheless, depending on the chemical nature of the molecules, they have been synthesized consuming various solvents, catalysts and different conditions, such as, Amberlyst-15 (Holla et al. 2006), tetrabutyl ammonium iodide Shandala and Hamdy 2008, triethanolamine (Nitin and Soni PA 2007).

Here in, we report a series of new chalcones synthesis by base catalyzed Claisen Schmidt condensation and were treated with substituted hydrazines according to the Michael addition reaction to obtain new pyrazoline derivatives. And molecular modeling of pyrazoline derivatives as potential *Mt*-SK inhibitors was performed. The interaction between 24 pyrazolines and *Mt*-SK has been evaluated by applying Surflex-Docking. Other therapeutically active groups were added to the target compounds to impart synergism. These newly designed compounds can support in finding the SAR of the pyrazoline derivatives and can aid in understanding the structural characteristics affecting the binding with shikimate kinase (Fig. 2) by molecular docking.

Experimental

Materials and methods

All used materials were obtained commercially, mostly from Sigma Aldrich, and were used without further purification. Melting points were found by capillary method and were uncorrected. Shimadzu Perkin Ekmer 8201 Pc IR Spectrometer was used to record IR spectra (KBr pellets) and frequencies were expressed in cm^{-1}. BrukerAvance II 400 NMR spectrometer recorded NMR spectra. All spectra were obtained in $CDCl_3$ and dimethyl sulfoxide (DMSO). Chemical shift values are reported as values in ppm relative to TMS ($\delta = 0$) as internal standard. JEOL SX-102/DA-6000 Mass spectrometer recorded FAB mass spectra using

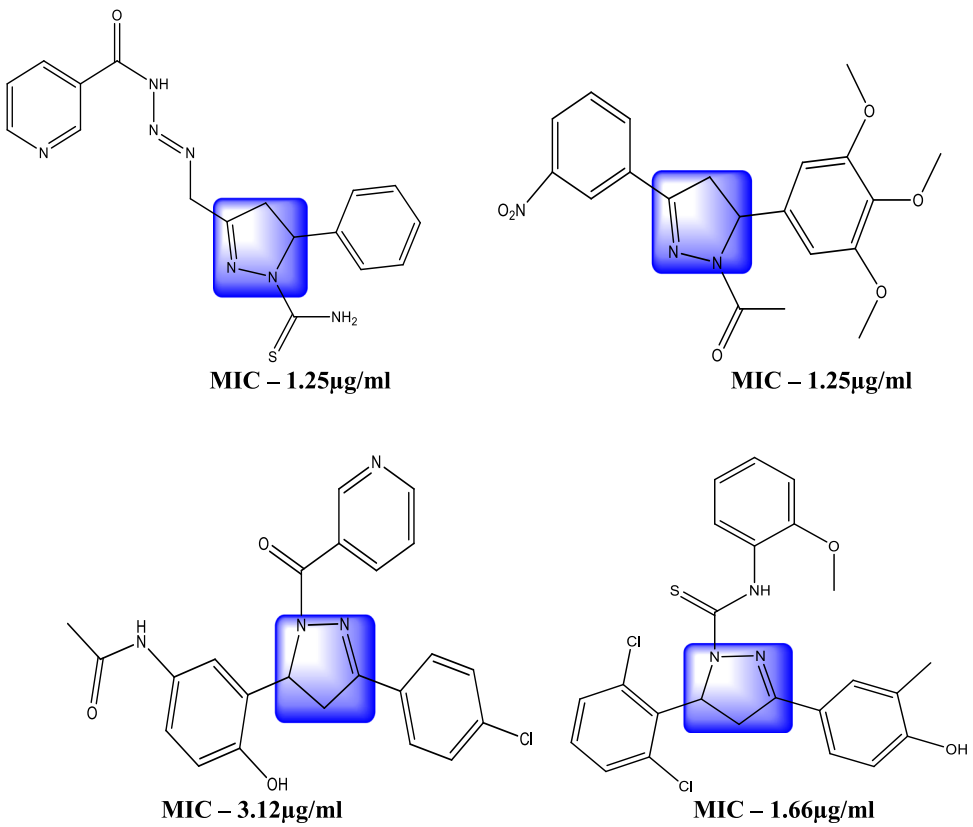


Fig. 1 Reported pyrazoline-based antitubercular agents

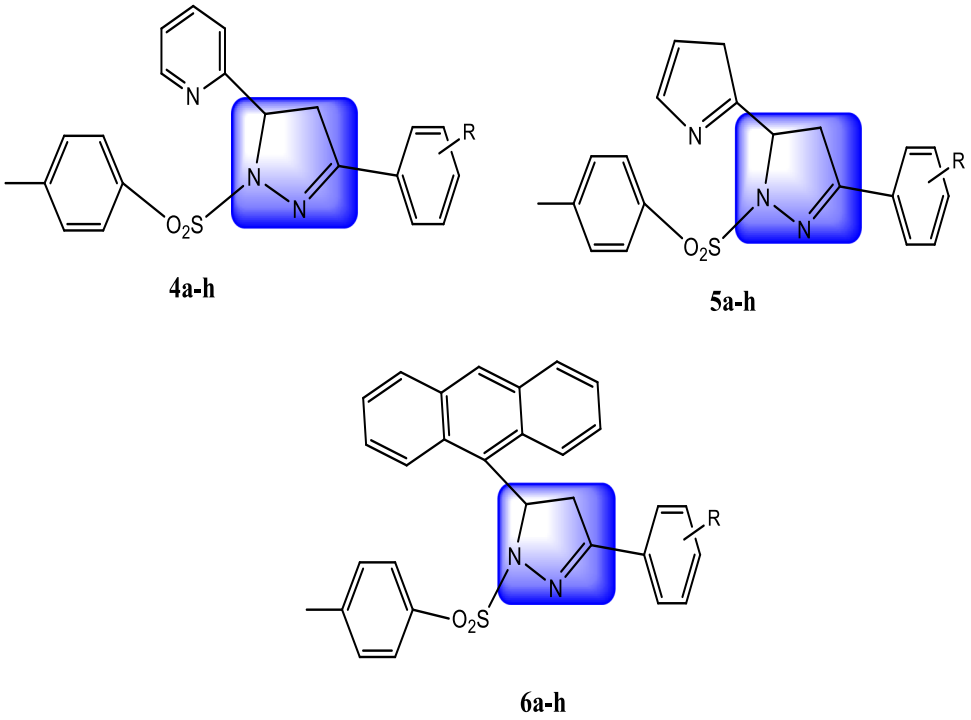


Fig. 2 Proposed antituberculostatic agents

Argon/Xenon (6Kv, 10 Ma) as the FAB gas. The elemental analysis has been obtained using VairoElementar Model, CHN analyser and the results were found to be within ±0.4%. The microwave reactor used was Cata-R, Catalyst Systems 140–700 W.

General methods of synthesis of chalcones (1a–h, 2a–h, 3a–h)

A mixture of substituted aldehydes (0.01 mol) and substituted acetophenones(0.01 mol) in ethanol (20 ml) were stirred together for 24 h. 20% NaOH (4 ml) were used as catalyst. After the completion of the reaction, it was poured into crushed ice and acidified with HCl. The solvent ethanol was used for recrystallization.

General methods of synthesis of pyrazolines (4a–h, 5a–h, 6a–h)

Chalcones (0.01 mol) and p-toluenesulfonylhydrazide (0.01 mol) were dissolved in DMF (30 ml) and was subjected to microwave irradiation for 6–8 min. After completion of the reaction, refluxing condenser was removed and the reaction solution was concentrated to 10–15 ml. It was left at room temperature to give crystalline compound. It was filtered and recrystallized from ethanol. Chloroform:Methanol (8:2) is the solvent system for thin layer chromatography. The same was carried under conventional heating by refluxing the reaction mixture for 12–14 h.

3-(Anthracen-9-yl)-1-(4-chlorophenyl)prop-2-en-1-one ***(3a)***

Red Crystals (EtOH). This compound was prepared by condensing 9-anthraldehyde with substituted acetophenones; m.p.88–90 °C; yield 80%; IR (KBr) cm^{-1}: 3048, (CH str), 1675 (α,β unsaturated keto group), 1510 (C=C str), 748 (C–Cl). ^{1}H NMR ($CDCl_3$, 400 MHz) δ / ppm: 7.43–7.44 (d, 1H, $J = 15.2$ Hz, H-2), 7.55–7.57 (d, 1H, J = 15.7 Hz, H-3), 7.20 (d, 1H, J = 8.1 Hz, Ar–H), 7.39 (d, 1H, $J = 8.3$ Hz, Ar–H), 7.86–7.89 (m, 4H, Ar–H), 8.54–8.58 (m, 6H, Ar–H), 8.2 (s, 1H, Ar–H). ^{13}C NMR ($CDCl_3$, 300 MHz) δ / ppm: 187.7 (C=O, C-1), 121.2, 145.1 (CH=CH, C-2, C-3), 168.7 (C-F), 125.3, 126.5, 126.4, 122.1, 121.5, 126.1, 123.1, 125.2, 124.2 (13 CH aromatic, C-1′, C-2′, C-3′, C-4′, C-5′, C-6′, C-7′, C-8′, C-10′, C-2″, C-3″, C-5″, C-6″), 133.5, 137.8 (2C aromatic, C-9, C-1″); Mass (*m/z*) (M^+) 342; Anal. Calcd for C, 80.58; H, 4.41, Found: C, 80.59; H, 4.43.

3-(Anthracen-9-yl)-1-(4-bromophenyl)prop-2-en-1-one ***(3b)***

Yellow Crystals (EtOH). This compound was prepared by condensing 9-anthraldehyde with substituted acetophenones; m.p.117–119 °C; yield 78%; IR (KBr) cm^{-1}:3040 (CH str), 1694 (α,β unsaturated keto group), 1509 (C=C str), 668 (C-Br). ^{1}H NMR ($CDCl_3$, 400 MHz) δ / ppm: 7.26–7.28 (d, 1H, $J = 15.2$ Hz, H-2), 7.4-7.43 (d, 1H, J = 15.4 Hz, H-3), 7.27–7.29 (d, 2H, J = 9.0 Hz, Ar–H), 7.44–7.45 (d, 1H, $J = 8.5$ Hz, Ar–H), 7.48–7.56 (m, 4H, Ar–H), 7.30–7.37 (m, 6H, Ar–H). ^{13}C NMR ($CDCl_3$,

300 MHz) δ / ppm: 187.7 (C=O, C-1), 121.2, 145.1 (CH=CH, C-2, C-3), 128.9 (C-Br), 125.3, 128.3, 125.4, 125.3, 125.6, 121.6, 128.3, 125.2, 125.9, 123.5 (13 CH aromatic, C-1′, C-2′, C-3′, C-4′, C-5′, C-6′, C-7′, C-8′, C-10′, C-2″, C-3″, C-5″, C-6″), 133.1, 136.0 (2C aromatic, C-9, C-1″); Mass (*m/z*) (M^+) 387, (M^+ 2) 389; Anal. Calcd for C, 71.33; H, 3.90, Found: C, 71.36; H, 3.87.

3-(Anthracen-9-yl)-1-(4-fluorophenyl)prop-2-en-1-one ***(3c)***

Dark Red Crystals (EtOH). This compound was prepared by condensing 9-anthraldehyde with substituted acetophenones; m.p.122–124 °C; yield 74%; IR (KBr) cm^{-1}: 3041, 3007 (CH str), 1696 (α,β unsaturated keto group), 1504 (C=C str), 1338(C–F). ^{1}H NMR ($CDCl_3$, 400 MHz) δ / ppm: 7.41–7.43 (d, 1H, $J = 15.0$ Hz, H-2), 7.53–7.54 (d, 1H, $J = 15.3$ Hz, H-3), 7.22–7.25 (d, 1H, J = 8.6 Hz, Ar–H), 7.42–7.45 (d, 1H, J = 8.5 Hz, Ar–H), 7.92–8.16 (m, 4H, Ar–H), 8.25–8.29 (m, 6H, Ar–H), 8.29 (s, 1H, Ar–H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ / ppm: 187.7 (C=O, C-1), 121.2, 145.1 (CH=CH, C-2, C-3), 168.7 (C-F), 125.4, 128.5, 126.4, 123.3, 121.4, 127.2, 124.2, 126.6, 124.2 (13 CH aromatic, C-1′, C-2′, C-3′, C-4′, C-5′, C-6′, C-7′, C-8′, C-10′, C-2″, C-3″, C-5″, C-6″), 133.4, 136.8 (2C aromatic, C-9, C-1″); Mass (*m/z*) (M^+ 1) 327; Anal. Calcd for C, 84.64; H, 4.63, Found: C, 84.60; H, 4.65.

3-(Anthracen-9-yl)-1-(4-aminophenyl)prop-2-en-1-one ***(3d)***

Light Yellow Crystals (EtOH). This compound was prepared by condensing 9-anthraldehyde with substituted acetophenones; m.p.132–135 °C; yield 76%; IR (KBr) cm^{-1}: 3012 (CH str), 1693(α,βunsaturated keto group), 1514 (C=C str), 3512, 3215 (NH_2). ^{1}H NMR ($CDCl_3$, 400 MHz) δ / ppm: 7.45–7.47 (d, 1H, $J = 15.2$ Hz, H-2), 7.48-7.49 (d, 1H, $J = 15.0$ Hz, H-3), 7.12-7.14 (d, 1H, $J = 8.1$ Hz, Ar–H), 7.41–7.44 (d, 1H, $J = 8.5$ Hz, Ar–H), 7.88–7.92 (m, 4H, Ar–H), 8.13–8.17 (m, 6H, Ar–H), 8.1 (s, 1H, Ar–H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ / ppm: 187.7 (C=O, C-1), 121.2, 145.1 (CH=CH, C-2, C-3), 154.2 (C-NH_2), 122.4, 128.3, 126.2, 125.3, 126.4, 127.5, 124.2, 126.2, 126.5, 124.5 (13 CH aromatic, C-1′, C-2′, C-3′, C-4′, C-5′, C-6′, C-7′, C-8′, C-10′, C-2″, C-3″, C-5″, C-6″), 135.4, 135.4 (2C aromatic, C-9, C-1″). Mass (*m/z*) (M^+) 228; Anal. Calcd for C, 85.42; H, 5.30, Found: C, 85.43; H, 5.46.

2-(3-(4-Chlorophenyl)-1-tosyl-4,5-dihydro-1H-pyrazol-5-yl)pyridine ***(4a)***

Yellow Crystals (EtOH). This compound was obtained by condensing substituted 4-chloro chalcones with *p*-toluenesulfonylhydrazide; m.p.167–169 °C; yield 81%; IR (KBr, cm^{-1}): 3114.56 (aromatic CH str), 1650.45 (C=N str), 1541.31 (C=C str), 724.34 (C-Cl); ^{1}H NMR (400 MHz, DMSO, δ/ppm): 2.34 (s, 3H, CH_3), 3.94 (dd, 1H, HA, JAB 18.1, JAx 9.5), 3.69 (dd, 1H, HB, JAB 18, JBx 9.51), 3.9 (dd, 1H, Hx, JAx 11.0, JBx 9.52), 6.83 to 7.98 (m, 11H, Ar–H); ^{13}C NMR (300 MHz, $CDCl_3$) δ /ppm: 144.7, 151.7 (C=N), 136.6 (C-Cl), 137.6 (C–CH_3), 128.7, 127.6, 128.5, 127.6, 126.4, 128.4, 127.8, 129.6, 127.5, 124.6, 126.3, 124.2 (12 CH aromatic, C-2″, C-3″, C-5″, C-6″, C-3‴, C-4‴, C-5‴, C-1⁗, C-2⁗, C-4⁗, C-5⁗), 134.3, 127.6, 141.1 (3C aromatic, C-1″, C-2‴, C-6⁗); Mass (FAB), *m/z*: (M^+) 411; Anal. Calcd for C, 61.23; H, 4.40; Found: C, 61.24; H, 4.42.

2-(3-(4-Bromophenyl)-1-tosyl-4,5-dihydro-1H-pyrazol-5-yl) pyridine ***(4b)***

Yellow Crystals (EtOH). This compound was obtained by condensing substituted 4-bromo chalcones with *p*-toluenesulfonylhydrazide; m.p.167–169 °C; yield 81 %; IR (KBr, cm^{-1}): 3114.56 (aromatic CH str), 1650.45 (C=N str), 1541.31 (C=C str), 724.34 (C-Cl); ^{1}H NMR (400 MHz, DMSO, δ/ppm): 2.34 (s, 3H, CH_3), 3.94 (dd, 1H, HA, JAB 18.1, JAx9.5), 3.69 (dd, 1H, HB, JAB 18, JBx 9.51), 3.9 (dd, 1H, Hx, JAx 11.0, JBx 9.52), 6.83 to 7.98 (m, 12H, Ar–H); ^{13}C NMR (300 MHz, $CDCl_3$) δ /ppm: 151.7, 144.7 (C=N),125.6 (C–Br), 137.6 (C–CH_3), 128.7, 127.6, 128.5, 127.6, 126.4, 128.4, 127.8, 129.6, 127.5, 124.6, 126.3, 124.2 (11 CH aromatic, C-2″, C-3″, C-5″, C-6″, C-3‴, C-4‴, C-5‴, C-1⁗, C-2⁗, C-4⁗, C-5⁗), 134.3, 127.6, 141.1 (3C aromatic, C-1″, C-2‴, C-6⁗); Mass (FAB), *m/z*: (M^+) 456; Anal. Calcd for C, 55.27; H, 3.98; Found: C, 55.28; H, 3.99.

2-(3-(4-Fluorophenyl)-1-tosyl-4,5-dihydro-1H-pyrazol-5-yl)pyridine ***(4c)***

Light Red Crystals (EtOH). This compound was obtained by condensing substituted 4-fluoro chalcones with *p*-toluenesulfonylhydrazide; m.p.154–156 °C; yield 78%; IR (KBr, cm^{-1}): 3136.78 (aromatic CH str), 1643.23 (C=N str), 1534.35 (C=C str), 1372.14 (C–F); ^{1}H NMR (400 MHz, DMSO, δ/ppm): 2.34 (s, 3H, CH_3), 4.02 (dd, 1H, HA, JAB 18.11, JAx 9.1), 3.43 (dd, 1H, HB, JAB 18.3, JBx 9.71), 3.24 (dd, 1H, Hx, JAx 11.5, JBx 9.21), 7.05 to 7.79 (m, 12H, Ar–H); ^{13}C NMR (300 MHz, $CDCl_3$, δ/ ppm): 144.4, 151.3 (C=N), 165.8 (C–F), 137.3 (C–CH_3), 128.2, 127.8, 128.4, 126.2, 126.3, 127.6, 128.4, 128.5, 128.7, 128.6, 126.9 (11 CH aromatic, C-2″, C-3″, C-5″, C-6″, C-3‴, C-4‴, C-5‴, C-1⁗, C-2⁗, C-4⁗, C-5⁗), 133.5, 127.5, 142.2 (3C aromatic, C-1″, C-2‴, C-6⁗); Mass (FAB), *m/z*: (M^+) 395; Anal. Calcd for C, 63.78; H, 4.59; Found: C, 63.77; H, 4.60.

*2-(3-(4-Aminophenyl)-1-tosyl-4,5-dihydro-1H-pyrazol-5-yl) pyridine **(4d)***

Brown Crystals (EtOH). This compound was obtained by condensing substituted 4-amino chalcones with *p*-toluenesulfonylhydrazide; m.p.178–180 °C; yield 77%; IR (KBr, cm^{-1}): 3128.52 (aromatic CH str), 1662.34 (C=N str), 1541.32 (C=C str), 3152.64 (C-NH_2); ^{1}H NMR (400 MHz, DMSO, *δ*/ppm): 2.48 (s, 3H, CH_3), 3.29 (dd, 1H, HA, JAB 18.11, JAx9.1), 3.38 (dd, 1H, HB, JAB 18.3, JBx 9.71), 3.67 (dd, 1H, Hx, JAx 11.5, JBx 9.21), 6.98 to 7.73 (m, 12H, Ar–H); ^{13}C NMR (300 MHz, $CDCl_3$, *δ* /ppm): 144.5, 151.3 (C=N), 150.7 (C–NH_2), 137.3 (C–CH_3), 127.3, 128.2, 127.8, 128.8, 127.4, 128.4, 127.5, 128.7, 128.6, 127.3 (11 CH aromatic, C-2″, C-3″, C-5″, C-6″, C-3‴, C-4‴, C-5‴, C-1⁗, C-2⁗, C-4⁗, C-5⁗), 133.5, 127.5, 142.2 (3C aromatic, C-1″, C-2‴, C-6⁗); Mass (FAB), *m/z*: (M^+) 392; Anal. Calcd for C, 64.27; H, 5.14; Found: C, 64.28; H, 5.15.

*2-(3-(4-Hydroxyphenyl)-1-tosyl-4,5-dihydro-1H-pyrazol-5-yl)pyridine **(4e)***

Bright Red Crystals (EtOH). This compound was obtained by condensing substituted 4-hydroxy chalcones with *p*-toluenesulfonylhydrazide; m.p.166–168 °C; yield 73%; IR (KBr, cm^{-1}): 3148.66 (aromatic CH str), 1667.54 (C=N str), 1556.43 (C=C str), 3461.53 (C–OH); ^{1}H NMR (400 MHz, DMSO, *δ*/ppm): 2.44 (s, 3H, CH_3), 3.76 (dd, 1H, HA, JAB 18.11, JAx 9.1), 3.45 (dd, 1H, HB, JAB 18.3, JBx 9.71), 3.57 (dd, 1H, Hx, JAx 11.5, JBx 9.21), 7.57 to 8.75 (m, 12H, Ar–H); ^{13}C NMR (300 MHz, $CDCl_3$, *δ* /ppm): 144.5, 151.3 (C=N), 160.8 (C–OH), 137.3 (C–CH_3), 128.2, 127.8, 128.4, 126.2, 126.3, 127.6, 128.4, 128.5, 128.7, 128.6, 126.9 (11 CH aromatic, C-2″, C-3″, C-5″, C-6″, C-3‴, C-4‴, C-5‴, C-1⁗, C-2⁗, C-4⁗, C-5⁗), 133.5, 127.5, 142.2 (3C aromatic, C-1″, C-2‴, C-6⁗); Mass (FAB), *m/z*: (M^+) 393; Anal. Calcd for C, 64.10; H, 4.87; Found: C, 64.11; H, 4.88.

*2-(3-(4-Nitrophenyl)-1-tosyl-4,5-dihydro-1H-pyrazol-5-yl) pyridine **(4f)***

Light Brown Crystals (EtOH). This compound was obtained by condensing substituted 4-nitro chalcones with *p*-toluenesulfonylhydrazide; m.p.196–199 °C; yield 68%; IR (KBr, cm^{-1}): 3154.08 (aromatic CH str), 1633.30 (C=N str), 1556.51 (C=C str), 1547.94 (C–NO_2); ^{1}H NMR (400 MHz, DMSO, *δ*/ppm): 2.67 (s, 3H, CH_3), 3.62 (dd, 1H, HA, JAB 18.11, JAx 9.1), 3.83 (dd, 1H, HB, JAB 18.3, JBx 9.71), 4.16 (dd, 1H, Hx, JAx 11.5, JBx 9.21), 8.01 to 8.99 (m, 11H, Ar–H); ^{13}C NMR (300 MHz, $CDCl_3$, *δ* /ppm): 144.4, 151.3 (C=N), 150.2 (C–NO_2), 137.3 (C–CH_3), 126.3, 126.7, 128.5, 127.4, 128.4, 128.4, 128.4, 128.3, 128.5, 126.2 (11 CH aromatic, C-2″, C-3″, C-5″, C-6″, C-3‴, C-4‴, C-5‴, C-1⁗, C-2⁗, C-4⁗, C-5⁗), 133.5, 127.5, 142.2 (3C aromatic, C-1″, C-2‴, C-6⁗); Mass (FAB), *m/z*: (M^+) 422; Anal. Calcd for C, 59.70; H, 4.29; Found: C, 59.71; H, 4.28.

*2-(3-p-Tolyl-1-tosyl-4,5-dihydro-1H-pyrazol-5-yl)pyridine **(4g)***

Red Crystals (EtOH). This compound was obtained by condensing substituted 4-methyl chalcones with *p*-toluenesulfonylhydrazide; m.p.89–91 °C; yield 66%; IR (KBr, cm^{-1}): 3142.43 (aromatic CH str), 1638.26 (C=N str), 1556.48 (C=C str); ^{1}H NMR (400 MHz, DMSO, *δ*/ppm): 2.38 (s, 3H, CH_3), 4.06 (dd, 1H, HA, JAB 18.11, JAx 9.1), 3.54 (dd, 1H, HB, JAB 18.3, JBx 9.71), 3.42 (dd, 1H, Hx, JAx 11.5, JBx 9.21), 6.87 to 7.89 (m, 12H, Ar–H); ^{13}C NMR (300 MHz, $CDCl_3$, *δ* /ppm): 144.4, 151.3 (C=N), 140.8 (C–CH_3), 137.3 (C–CH_3), 127.4, 127.5, 127.4, 128.4, 126.7, 127.3, 127.4, 126.5, 128.7, 128.8, 126.9 (11 CH aromatic, C-2″, C-3″, C-5″, C-6″, C-3‴, C-4‴, C-5‴, C-1⁗, C-2⁗, C-4⁗, C-5⁗), 133.5, 127.5, 142.2 (3C aromatic, C-1″, C-2‴, C-6⁗); Mass (FAB), *m/z*: (M^+) 391; Anal. Calcd for C, 67.50; H, 5.41; Found: C, 67.52; H, 5.40.

*3-(4-Chlorophenyl)-5-(1H-pyrrol-2-yl)-1-tosyl-4,5-dihydro-1H-pyrazole **(5a)***

Brown Crystals (EtOH). This compound was obtained by condensing substituted 4-chloro chalcones with *p*-toluenesulfonylhydrazide; m.p.191–193 °C; yield 82%; IR (KBr, cm-1): 3064.67 (aromatic CH str), 1665.52 (C=N str), 1569.32 (C=C str), 756.67 (C-Cl); ^{1}H NMR (400 MHz, DMSO, *δ*/ppm): 2.07 (s, 3H, CH_3), 3.21 (dd, 1H, HA, JAB 18.10, JAx 9.3), 3.45 (dd, 1H, HB, JAB 18.2, JBx 9.78), 3.89 (dd, 1H, Hx, JAx 11.2, JBx 9.1), 6.89 to 7.68 (m, 11H, Ar–H); ^{13}C NMR (300 MHz, $CDCl_3$, *δ* /ppm): 151.3 (C=N), 135.8 (C–Cl), 137.3 (C–CH_3), 128.4, 126.3, 127.4, 128.5, 128.7, 128.3, 128.3, 126.9, 128.7 (11 CH aromatic, C-2″, C-3″, C-5″, C-6″, C-3‴, C-4‴, C-5‴, C-1⁗, C-2⁗, C-4⁗, C-5⁗), 133.7, 127.4, 142.2 (3C aromatic, C-1″, C-2‴, C-6⁗); Mass (FAB), *m/z*: (M^+) 399; Anal. Calcd for C, 60.07; H, 4.54; Found: C, 60.05; H, 4.56.

*3-(4-Bromophenyl)-5-(1H-pyrrol-2-yl)-1-tosyl-4,5-dihydro-1H-pyrazole **(5b)***

Brown Crystals (EtOH). This compound was obtained by condensing substituted 4-bromo chalcones with *p*-toluenesulfonylhydrazide; m.p.204–206 °C; yield 85%; IR (KBr, cm^{-1}): 3072.46 (aromatic CH str), 1659.29 (C=N str), 1551.19 (C=C str), 634.12 (C-Br); ^{1}H NMR (400 MHz, DMSO, δ/ppm): 2.16 (s, 3H, CH_3), 3.18 (dd, 1H, HA, JAB

18.10, JAx 9.3), 3.35 (dd, 1H, HB, JAB 18.2, JBx 9.78), 3.78 (dd, 1H, Hx, JAx 11.2, JBx 9.1), 7.01 to 7.98 (m, 11H, Ar–H); ^{13}C NMR (300 MHz, $CDCl_3$, δ /ppm): 151.3 (C=N), 135.8 (C–Cl), 137.3 (C–CH_3), 128.4, 128.7, 129.4, 128.1, 128.9, 128.5, 128.2, 126.9, 128.4 (11 CH aromatic, C-2″, C-3″, C-5″, C-6″, C-3‴, C-4‴, C-5‴, C-1⁗, C-2⁗, C-4⁗, C-5‴), 133.7, 127.4, 142.2 (3C aromatic, C-1″, C-2‴, C-6⁗); Mass (FAB), *m/z*: (M^+) 444; Anal. Calcd for C, 54.06; H, 4.08; Found: C, 54.07; H, 4.07.

3-(4-Fluorophenyl)-5-(1H-pyrrol-2-yl)-1-tosyl-4,5-dihydro-1H-pyrazole ***(5c)***

Green Crystals (EtOH). This compound was obtained by condensing substituted 4-fluoro chalcones with *p*-toluenesulfonylhydrazide; m.p.174–176 °C; yield 80%; IR (KBr, cm^{-1}): 3078.54 (aromatic CH str), 1682.43 (C=N str), 1576.51 (C=C str), 1406.11 (C-F); ^{1}H NMR (400 MHz, DMSO, δ/ppm): 2.83 (s, 3H, CH_3), 3.67 (dd, 1H, HA, JAB 18.10, JAx 9.3), 3.88 (dd, 1H, HB, JAB 18.2, JBx 9.78), 4.12 (dd, 1H, Hx, JAx 11.2, JBx 9.1), 7.39 to 8.28 (m, 11H, Ar–H); ^{13}C NMR (300 MHz, $CDCl_3$, δ /ppm): 151.4 (C=N), 165.2 (C–F), 137.3 (C–CH_3), 128.2, 127.1, 127.6, 128.2, 126.7, 128.3, 128.5, 126.9, 128.5 (11 CH aromatic, C-2″, C-3″, C-5″, C-6″, C-3‴, C-4‴, C-5‴, C-1⁗, C-2⁗, C-4⁗, C-5‴), 133.7, 127.4, 142.2 (3C aromatic, C-1″, C-2‴, C-6⁗); Mass (FAB), *m/z*: (M^+) 383; Anal. Calcd for C, 62.65; H, 4.73; Found: C, 62.63; H, 4.74.

4-(5-(1H-Pyrrol-2-yl)-1-tosyl-4,5-dihydro-1H-pyrazol-3-yl) aniline ***(5d)***

Green Crystals (EtOH). This compound was obtained by condensing substituted 4-amino chalcones with *p*-toluenesulfonylhydrazide; m.p.182–184 °C; yield 78%; IR (KBr, cm^{-1}): 3081.31 (aromatic CH str), 1674.74 (C=N str), 1551.76 (C=C str), 3356.15 (C–NH_2); ^{1}H NMR (400 MHz, DMSO, δ/ppm): 2.33 (s, 3H, CH_3), 3.23 (dd, 1H, HA, JAB 18.10, JAx 9.3), 3.45 (dd, 1H, HB, JAB 18.2, JBx 9.78), 3.94 (dd, 1H, Hx, JAx 11.2, JBx 9.1), 7.15 to 8.17 (m, 11H, Ar–H); ^{13}C NMR (300 MHz, $CDCl_3$, δ /ppm): 151.4 (C=N), 150.7 (C–NH_2), 137.3 (C–CH_3), 128.1, 128.5, 127.7, 128.2, 126.7, 128.3, 128.6, 126.9, 126.5 (11 CH aromatic, C-2″, C-3″, C-5″, C-6″, C-3‴, C-4‴, C-5‴, C-1⁗, C-2⁗, C-4⁗, C-5‴), 133.7, 127.4, 142.2 (3C aromatic, C-1″, C-2‴, C-6⁗); Mass (FAB), *m/z*: (M^+) 380; Anal. Calcd for C, 63.14; H, 5.30; Found: C, 63.16; H, 5.32.

4-(5-(1H-Pyrrol-2-yl)-1-tosyl-4,5-dihydro-1H-pyrazol-3-yl) phenol ***(5e)***

Red Crystals (EtOH). This compound was obtained by condensing substituted 4-hydroxy chalcones with *p*-toluenesulfonylhydrazide; m.p.216–218 °C; yield 77%; IR (KBr, cm^{-1}): 3069.42 (aromatic CH str), 1691.33 (C=N str), 1568.39 (C=C str), 3526.71 (C-OH); ^{1}H NMR (400 MHz, DMSO, δ/ppm): 2.41 (s, 3H, CH3), 3.12 (dd, 1H, HA, JAB 18.2, JAx 9.0), 3.76 (dd, 1H, HB, JAB 18.3, JBx 9.1), 4.04 (dd, 1H, Hx, JAx 11.1, JBx 9.1), 7.15 to 8.14 (m, 11H, Ar–H); 13C NMR (300 MHz, CDCl3, δ /ppm): 151.4 (C=N), 160.8 (C–OH), 137.3 (C–CH_3), 127.1, 128.5, 127.6, 128.2, 126.7, 128.2, 128.6, 127.9, 126.5 (11 CH aromatic, C-2″, C-3″, C-5″, C-6″, C-3‴, C-4‴, C-5‴, C-1⁗, C-2⁗, C-4⁗, C-5‴), 133.7, 127.3, 142.1 (3C aromatic, C-1″, C-2‴, C-6⁗); Mass (FAB), *m/z*: (M^+) 381; Anal. Calcd for C, 62.97; H, 5.02; Found: C, 62.96; H, 5.01.

3-(4-Nitrophenyl)-5-(1H-pyrrol-2-yl)-1-tosyl-4,5-dihydro-1H-pyrazole ***(5f)***

Brown Crystals (EtOH). This compound was obtained by condensing substituted 4-nitro chalcones with *p*-toluenesulfonylhydrazide; m.p.123–125 °C; yield 64%; IR (KBr, cm^{-1}): 3072.34 (aromatic CH str), 1656.21 (C=N str), 1557.21 (C=C str), 1426.31 (C-NO_2); ^{1}H NMR (400 MHz, DMSO, δ/ppm): 2.67 (s, 3H, CH_3), 3.67 (dd, 1H, HA, JAB 18.2, JAx 9.0), 3.79 (dd, 1H, HB, JAB 18.3, JBx 9.1), 4.01 (dd, 1H, Hx, JAx 11.1, JBx 9.1), 6.89 to 7.82 (m, 11H, Ar–H); ^{13}C NMR (300 MHz, $CDCl_3$, δ/ppm): 151.4 (C=N), 150.2 (C-NO_2), 137.3 (C–CH_3), 127.1, 128.5, 126.6, 128.2, 127.7, 128.3, 128.2, 127.9, 126.1 (11 CH aromatic, C-2″, C-3″, C-5″, C-6″, C-3‴, C-4‴, C-5‴, C-1⁗, C-2⁗, C-4⁗, C-5‴), 133.7, 127.3, 142.1 (3C aromatic, C-1″, C-2‴, C-6⁗); Mass (FAB), *m/z*: (M^+) 410; Anal. Calcd for C, 58.53; H, 4.42; Found: C, 58.52; H, 4.43.

5-(1H-Pyrrol-2-yl)-3-p-tolyl-1-tosyl-4,5-dihydro-1H-pyrazole ***(5g)***

Brown Crystals (EtOH). This compound was obtained by condensing substituted 4-methyl chalcones with *p*-toluenesulfonylhydrazide; m.p.173–175 °C; yield 64%; IR (KBr, cm^{-1}): 3087.87 (aromatic CH str), 1659.78 (C=N str), 1551.62 (C=C str); ^{1}H NMR (400 MHz, DMSO, δ/ppm): 2.11 (s, 3H, CH_3), 3.21 (dd, 1H, HA, JAB 18.2, JAx 9.0), 3.43 (dd, 1H, HB, JAB 18.3, JBx 9.1), 3.93 (dd, 1H, Hx, JAx 11.1, JBx 9.1), 6.94 to 7.74 (m, 11H, Ar–H); ^{13}C NMR (300 MHz, $CDCl_3$, δ /ppm): 151.4 (C=N), 140.8 (C–CH_3), 137.3 (C–CH_3), 126.1, 127.5, 126.6, 128.6, 127.7, 128.6, 128.2, 126.9, 126.1 (11 CH aromatic, C-2″, C-3″, C-5″, C-6″, C-3‴, C-4‴, C-5‴, C-1⁗, C-2⁗, C-4⁗, C-5‴), 133.7, 127.3, 142.1 (3C aromatic, C-1″, C-2‴, C-6⁗); Mass (FAB), *m/z*: (M^+) 379; Anal. Calcd for C, 66.47; H, 5.58; Found: C, 66.46; H, 5.55.

*5-(Anthracen-9-yl)-3-(4-chlorophenyl)-1-tosyl-4,5-dihydro-1H-pyrazole**(6a)***

Yellow Crystals (EtOH). This compound was obtained by condensing substituted 4-chloro chalcones with *p*-toluenesulfonylhydrazide; m.p.167–169 °C; yield 81%; IR (KBr, cm^{-1}): 3144.48 (aromatic CH str), 2922.16 (CH_3 str), 1668.43 (C=N str), 1591.27 (C=C str), 789.09 (C-Cl). ^{1}H NMR (400 MHz, DMSO, *δ*/ppm): 2.48 (s, 3H, CH_3), 3.55 (dd, 1H, HA, JAB 18.1, JAx 9.5), 3.73 (dd, 1H, HB, JAB 18, JBx 9.51), 4.56 (dd, 1H, Hx, JAx 11.0, JBx 9.52), 7.27 to 8.58 (m, 17H, Ar-H); ^{13}C NMR ($CDCl_3$, 300 MHz) *δ* /ppm: 151.2 (C=N), 136.6 (C–Cl), 137.6 (C–CH_3), 123.7, 124.6, 122.5, 128.6, 121.4, 124.4, 126.8, 123.6, 124.5, 124.6, 126.3, 124.2 (17 CH aromatic, C-1′, C-2′, C-3′, C-4′, C-5′, C-6′, C-7′, C-8′, C-10′, C-2″, C-3″, C-5″, C-6″, C-2‴, C-3‴, C-5‴, C-6‴), 133.3, 135.6 (2C aromatic, C-9, C-1″); Mass (FAB), *m/z*: (M^+) 511; Anal. Calcd for C, 70.51; H, 4.54; Found: C, 70.53; H, 4.53.

*5-(Anthracen-9-yl)-3-(4-bromophenyl)-1-tosyl-4,5-dihydro-1H-pyrazole **(6b)***

Red Crystals (EtOH). This compound was obtained by condensing substituted 4-bromo chalcones with *p*-toluenesulfonylhydrazide; m.p.97–99 °C; yield 76%; IR (KBr, cm^{-1}): 3136.53 (aromatic CH str), 2945.69 (CH_3 str), 1642.67 (C=N str), 1598.52 (C=C str), 636.67 (C–Br). ^{1}H NMR (400 MHz, DMSO, *δ*/ppm): 2.67 (s, 3H, CH_3), 3.56 (dd, 1H, HA, JAB 18.3, JAx 9.4), 3.65 (dd, 1H, HB, JAB 18, JBx 9.51), 4.43 (dd, 1H, Hx, JAx 11.0, JBx 9.52), 7.27 to 8.58 (m, 17H, Ar–H); ^{13}C NMR ($CDCl_3$, 300 MHz) *δ* /ppm: 151.2 (C=N), 128.8 (C–Br), 137.6 (C–CH_3), 123.7, 124.6, 122.5, 128.6, 121.4, 124.4, 126.8, 123.6, 124.5, 124.6, 126.3, 124.2 (17 CH aromatic, C-1′, C-2′, C-3′, C-4′, C-5′, C-6′, C-7′, C-8′, C-10′, C-2″, C-3″, C-5″, C-6″, C-2‴, C-3‴, C-5‴, C-6‴), 133.3, 135.6 (2C aromatic, C-9, C-1″); Mass (FAB), *m/z*: (M^+) 554 Anal. Calcd for C, 70.51; H, 4.54; Found: C, 70.53; H, 4.52.

*5-(Anthracen-9-yl)-3-(4-fluorophenyl)-1-tosyl-4,5-dihydro-1H-pyrazole **(6c)***

Brown Crystals (EtOH). This compound was obtained by condensing substituted 4-fluoro chalcones with *p*-toluenesulfonylhydrazide; m.p.174–175 °C; yield 72%; IR (KBr, cm^{-1}): 3138.36 (aromatic CH str), 2953.47 (CH_3 str), 1674.64 (C=N str), 1585.53 (C=C str), 1354.43 (C–F). ^{1}H NMR (400 MHz, DMSO, *δ*/ppm): 2.39 (s, 3H, CH_3), 3.24 (dd, 1H, HA, JAB 18.1, JAx 9.4), 3.58 (dd, 1H, HB, JAB 18.4, JBx 9.34), 4.21 (dd, 1H, Hx, JAx 18.3, JBx 9.53), 7.46 to 8.68 (m, 17H, Ar–H); ^{13}C NMR ($CDCl_3$, 300 MHz) *δ* /ppm: 151.2 (C=N), 168.7 (C–F), 137.6 (C–CH_3), 122.4, 125.2, 128.2, 129.2, 124.1, 126.3, 128.2, 125.8, 122.3, 127.2, 122.9 (17 CH aromatic, C-1′, C-2′, C-3′, C-4′, C-5′, C-6′, C-7′, C-8′, C-10′, C-2″, C-3″, C-5″, C-6″, C-2‴, C-3‴, C-5‴, C-6‴), 133.3, 135.6 (2C aromatic, C-9, C-1″); Mass (FAB), *m/z*: (M^+) 494; Anal. Calcd for C, 72.85; H, 4.69; Found: C, 72.83; H, 4.68.

*5-(Anthracen-9-yl)-3-(4-aminophenyl)-1-tosyl-4,5-dihydro-1H-pyrazole **(6d)***

Brownish red Crystals (EtOH). This compound was obtained by condensing substituted 4-amino chalcones with *p*-toluenesulfonylhydrazide; m.p.143–145 °C; yield 76%; IR (KBr, cm^{-1}): 3157.56 (aromatic CH str), 2968.78 (CH_3 str), 1686.83 (C=N str), 1586.54 (C=C str), 3259.65 (C–NH_2). ^{1}H NMR (400 MHz, DMSO, *δ*/ppm): 2.53 (s, 3H, CH_3), 2.93 (dd, 1H, HA, JAB 11.4, JAx 9.2), 3.32 (dd, 1H, HB, JAB 18.4, JBx 9.43), 3.76 (dd, 1H, Hx, JAx 11.0, JBx 9.52), 7.11 to 7.90 (m, 17H, Ar–H); ^{13}C NMR ($CDCl_3$, 300 MHz) *δ* /ppm: 151.2 (C=N), 154.2 (C–NH_2), 137.6 (C–CH_3), 122.4, 122.7, 123.6, 127.7, 123.6, 122.6, 127.7, 125.6, 127.6, 126.3, 123.2 (17 CH aromatic, C-1′, C-2′, C-3′, C-4′, C-5′, C-6′, C-7′, C-8′, C-10′, C-2″, C-3″, C-5″, C-6″, C-2‴, C-3‴, C-5‴, C-6‴), 133.3, 135.6 (2C aromatic, C-9, C-1″); Mass (FAB), *m/z*: (M^+) 491; Anal. Calcd for C, 73.30; H, 5.13; Found: C, 73.28; H, 5.15.

*5-(Anthracen-9-yl)-3-(4-hydroxyphenyl)-1-tosyl-4,5-dihydro-1H-pyrazole **(6e)***

Red Crystals (EtOH). This compound was obtained by condensing substituted 4-hyroxy chalcones with p-toluenesulfonylhydrazide; m.p.168–169 °C; yield 80%; IR (KBr, cm^{-1}): 3146.75 (aromatic CH str), 2929.43 (CH_3 str), 1674.52 (C=N str), 1584.36 (C=C str), 1342.54 (C-OH). ^{1}H NMR (400 MHz, DMSO, *δ*/ppm): 2.43 (s, 3H, CH_3), 3.24 (dd, 1H, HA, JAB 18.1, JAx 9.5), 3.86 (dd, 1H, HB, JAB 18, JBx 9.51), 4.23 (dd, 1H, Hx, JAx 11.5, JBx 9.3), 7.78 to 8.64 (m, 17H, Ar–H); ^{13}C NMR ($CDCl_3$, 300 MHz) *δ* /ppm: 151.2 (C=N), 164.7 (C–OH), 137.6 (C–CH_3), 124.2, 124.6, 122.6, 121.6, 128.2, 122.5, 122.6, 126.3, 125.3, 128.6 (17 CH aromatic, C-1′, C-2′, C-3′, C-4′, C-5′, C-6′, C-7′, C-8′, C-10′, C-2″, C-3″, C-5″, C-6″, C-2‴, C-3‴, C-5‴, C-6‴), 133.3, 135.6 (2C aromatic, C-9, C-1″); Mass (FAB), *m/z*: (M^+) 492; Anal. Calcd for C, 73.15; H, 4.91; Found: C, 73.17; H, 4.93.

*5-(Anthracen-9-yl)-3-(4-nitrophenyl)-1-tosyl-4,5-dihydro-1H-pyrazole **(6f)***

Cream Crystals (EtOH). This compound was obtained by condensing substituted 4-nitro chalcones with p-toluenesulfonylhydrazide; m.p.184–186 °C; yield 79%; IR (KBr,

cm^{-1}): 3157.53 (aromatic CH str), 2943.54 (CH_3 str), 1653.65 (C=N str), 1564.52 (C=C str), 1495.45 (C-NO_2). ^{1}H NMR (400 MHz, DMSO, δ/ppm): 2.31 (s, 3H, CH_3), 2.68 (dd, 1H, HA, JAB 18.4, JAx 9.3), 3.03 (dd, 1H, HB, JAB 18.6, JBx 9.3), 3.74 (dd, 1H, Hx, JAx 11.3, JBx 9.4), 7.20 to 7.86 (m, 17H, Ar-H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ/ppm: 151.2 (C=N), 153.7 (C–NO_2), 137.6 (C–CH_3), 124.3, 128.2, 127.2, 124.3, 122.4, 124.6, 122.7, 127.4, 125.3, 127.2, 128.5 (17 CH aromatic, C-1′, C-2′, C-3′, C-4′, C-5′, C-6′, C-7′, C-8′, C-10′, C-2″, C-3″, C-5″, C-6″, C-2‴, C-3‴, C-5‴, C-6‴), 133.3, 135.6 (2C aromatic, C-9, C-1″); Mass (FAB), *m/z*: (M^+) 521; Anal. Calcd for C, 69.08; H, 4.44; Found: C, 69.08; H, 4.44.

5-(Anthracen-9-yl)-3-(4-methylphenyl)-1-tosyl-4,5-dihydro-1H-pyrazole ***(6g)***

Red Crystals (EtOH). This compound was obtained by condensing substituted 4-methyl chalcones with p-toluene-sulfonylhydrazide; m.p.197–199 °C; yield 71%; IR (KBr, cm^{-1}): 3163.48 (aromatic CH str), 2928.53 (CH_3 str), 1647.74 (C=N Str), 1545.45 (C=C Str), 2849.54 (C–CH_3). ^{1}H NMR (400 MHz, DMSO, δ/ppm): 2.35 (s, 3H, CH_3), 2.99 (dd, 1H, HA, JAB 18.1, JAx 9.5), 3.13 (dd, 1H, HB, JAB 18, JBx 9.51), 3.76 (dd, 1H, Hx, JAx 11.0, JBx 9.52), 7.79 to 66 (m, 17H, Ar–H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ /ppm: 151.2 (C=N), 140.6 (C–CH_3), 124.2, 125.2, 124.2, 127.2, 125.3, 127.3, 128.3, 122.7, 125.3, 125.8, 123.5, 124.6 (17 CH aromatic, C-1′, C-2′, C-3′, C-4′, C-5′, C-6′, C-7′, C-8′, C-10′, C-2″, C-3″, C-5″, C-6″, C-2‴, C-3‴, C-5‴, C-6‴), 133.3, 135.6 (2C aromatic, C-9, C-1″); Mass (FAB), *m/z*: (M^+) 490; Anal. Calcd for C, 75.89; H, 5.34; Found: C, 75.89; H, 5.34.

Biological activity

Antitubercular activity using microplate Alamar blue assay

The antimycobacterial activity of compounds was assessed against *Mt* using microplate alamar blue assay (Collins and Franzblau 1997, Franzblau et al. 1998). 100 µl of the Middlebrook 7H9 broth containing *Mt* and serial dilution of compounds was poured to 96 well plates. The different concentrations of compounds tested were 0.2 to 100.0 µM and standards used are Streptomycin and Isoniazid. And the plates were sealed and incubated for 7 days at 37 °C. After the incubation, 25 µl of freshly prepared 1:1 mixture of alamar blue reagent and 10 % tween 80 was added and incubated for 24 h. After the color change was noted, blue color indicated no bacterial growth and pink as growth. The least concentration at which drug prevents the color change from blue to pink is the MIC (minimum drug concentration).

Evaluation of anticancer activity

The in vitro cytotoxicity of the compounds was studied against Ehrlich Ascites Carcinoma (EAC)cells by tryphan blue exclusion method (Manojkumar et al. 2009; Ghorab et al. 2006). This method checks the viability of the tumor cells. The cell suspension was added to the tubes of various test concentrations. Phosphate buffered saline was used to make up the volume to 1 ml. Then incubated for 3 h at 37 ° C and the percent of dead cells were calculated.

Docking studies

Docking is one of the approaches of computer-aided drug design, which is involved in placing a ligand into the binding site of a receptor and finding the appropriate binding position within the receptor (Wermuth 1996; Cohen 1996). It determines the binding affinity between the molecules (drug and the receptor), and also reveals the possible conformations and orientations. From the different binding site conformations, the stable ligand-receptor complex can be determined. 3D structures of all compounds were constructed using the ChemDraw3D. Sybyl-X 2.0 software package (Tripos International 2012) was used to understand the binding mode between pyrazoline derivatives and *Mt*SK. Structural energy minimization was performed using the standard Tripos molecular mechanics force field and Gasteiger–Huckel charge (Gasteiger and Marsili 1980), the max interations for the minimization was set to 2000. The minimization was terminated when the energy gradient convergence criterion of 0.05 kcal/mol Å was reached. Crystal structure of shikimate kinase from *Mt* in complex with MGADP at 2.0 angstrom resolution was retrieved from the Brookhaven Protein Data Bank (PDB http://www.rcsb.org/pdb) (PDB entry code 1L4Y) (Gu et al. 2002) the structure of the biopolymer was analyzed and prepared for the docking experiment. Hydrogen atoms with essential H-bond orientation were added. All water molecules were removed and the relevant side-chains were repaired, also the bumps were fixed and finally the biopolymer was charged using AMBER7FF9902 method, then the Surflex-Dock program used to compute the binding mode of the pyrazoline derivatives and *Mt*SK that adopted an empirical scoring function anda patented searching engine (Jain 1996, 2003). In order to visualize secondary structure elements and the cavities and channels of the *Mt*SK pocket, the MOLCAD Robbin and Multi-Channel surfaces program were applied. AdmetSAR prediction tool (Cheng et al. 2012)was used for ADMET properties of the compounds.

Results and discussion

Chemistry

Substituted chalcones were prepared by base catalyzed Claisen Schmidt condensation of various ketones with aldehydes as reported in the literature (Kumar et al. 2010). The cyclization of chalcone with p-toluenesulfonylhydrazide yielded pyrazolines respectively (Mistry et al. 2012; Bano et al. 2011). Comparison of conventional and microwave techniques, in terms of time and yields have been described in Table 1. Spectral and elemental analysis data confirmed the structure of the compounds.

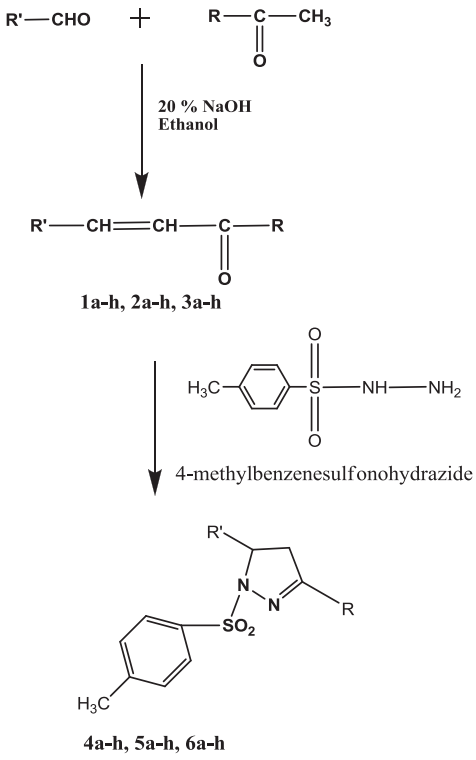
Table 1 Reaction time and yield of conventionally and microwave assisted synthesis of pyrazolines

Compounds	Conventional synthesis		Microwave assisted synthesis	
	Time (h)	Yield (%)	Time (min)	Yield (%)
Pyrazolines	6	52–75	5–6	73–84

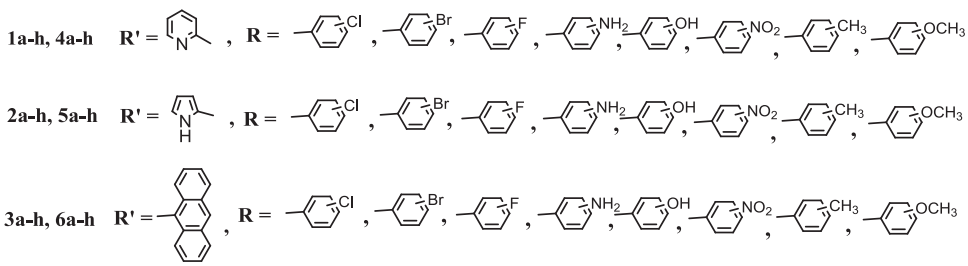
The plausible mechanism for the formation of pyrazoline is depicted in Schemes 1 and 2. The reaction proceed by Michael addition of (nucleophile) $R–N^{-}–NH_2$ to the chalcones (1,4-addition) followed by proton transfer, cyclization, hydrolysis, and spontaneous dehydration.

In IR spectra an absorption band in 4a afforded pyrazoline C–N stretching (1650.45 cm^{-1}) and 1541.31 (C=C str), bands. In ^1H NMR spectra, the CH_2 protons of the pyrazoline ring resonated as a pair of doublets of doublets at 3.94 ppm (HA), 3.69 ppm (HB). The CH (Hx) proton appeared as a doublet of doublets at 3.9 ppm.

In compound 5a, IR spectra afforded an absorption band of pyrazoline C-N stretching (1665.52 cm^{-1}) and 1569.32 (C=C str). In ^1H NMR spectra of Sp1, the CH_2 protons of the pyrazoline ring resonated as a pair of doublets of doublets at 3.21 ppm (HA), 3.45 ppm (HB). The CH (Hx) proton appeared as a doublet of doublets at 3.89 ppm. This confirmed the cyclization of chalcones to 2-pyrazolines.



Scheme 1 Synthesis of pyrazolines.



Scheme 2 Plausible mechanism for pyrazolines.

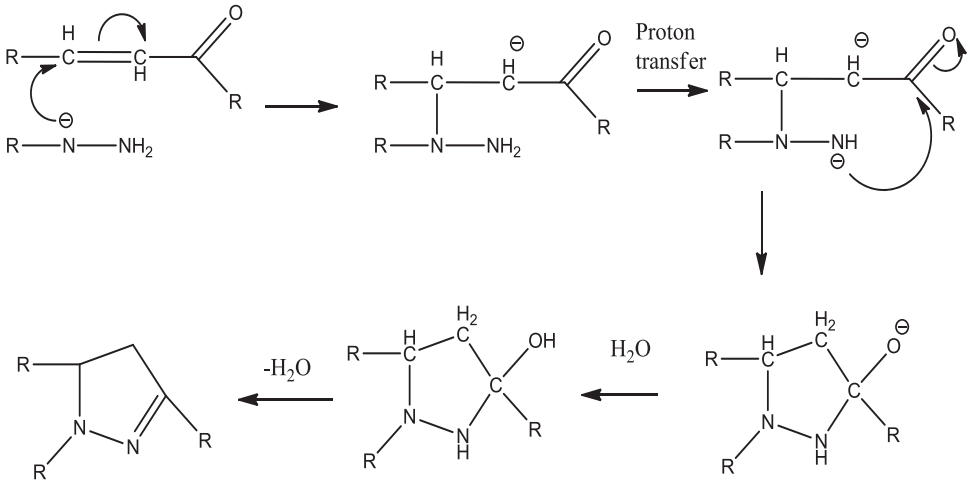


Table 2 Antitubercular activity and cytotoxicity activity of pyrazoline derivatives

Compound	R′	R	MIC / μM	Dead cells / (%) at 200 μM
4a	Pyridine	4-Cl -C_6H_4	0.8	87
4b	Pyridine	4-Br -C_6H_4	0.8	82
4c	Pyridine	4-F -C_6H_4	0.2	65
4d	Pyridine	4-NH_2 -C_6H_4	0.8	70
4e	Pyridine	4-OH -C_6H_4	0.8	71
4f	Pyridine	4-NO_2 -C_6H_4	0.8	83
4g	Pyridine	4-CH_3 -C_6H_4	0.8	68
5a	Pyrolle	4-Cl -C_6H_4	0.8	68
5b	Pyrolle	4-Br -C_6H_4	0.2	80
5c	Pyrolle	4-F -C_6H_4	0.8	88
5d	Pyrolle	4-NH_2 -C_6H_4	0.8	79
5e	Pyrolle	4-OH -C_6H_4	0.8	67
5f	Pyrolle	4-NO_2 -C_6H_4	0.8	85
5g	Pyrolle	4-CH_3 -C_6H_4	6.25	65
6a	Anthraldehyde	4-Cl -C_6H_4	0.2	78
6b	Anthraldehyde	4-Br -C_6H_4	0.8	86
6c	Anthraldehyde	4-F -C_6H_4	0.8	78
6d	Anthraldehyde	4-NH_2 -C_6H_4	6.25	59
6e	Anthraldehyde	4-OH -C_6H_4	6.25	65
6f	Anthraldehyde	4-NO_2 -C_6H_4	0.8	71
6g	Anthraldehyde	4-CH_3 -C_6H_4	12.5	61
Standard	Isoniazid		0.2	–
Standard	Streptomycin		0.8	–
Standard	5-Fluorouracil		–	98

The active compounds when compared with the standard are given in bold

In IR spectra an absorption band in 6a afforded pyrazoline C-N stretching (1650.45 cm^{-1}) and 1541.31 (C=C str), bands. In ^1H NMR spectra, the CH_2 protons of the pyrazoline ring resonated as a pair of doublets of doublets at 3.94 ppm (HA), 3.69 ppm (HB). The CH (Hx) proton appeared as a doublet of doublets at 3.9 ppm.

Mass spectra of compounds were collected and the molecular ion peak was found correct according to the molecular weight.

Biological activity studies

All the pyrazolines tested for antitubercular and anticancer studies showed promising activity when compared with the standard. The antitubercular screening studies showed that all the compounds exhibit moderate to good inhibitory activity against *Mt*. The results are tabulated in Table 2 with isoniazid (INH), streptomycin as reference drugs. Compounds 4c, 5b, and 6a were potent as standard isoniazid at

MIC 0.2 µM, while most of the compounds were active as streptomycin with inhibition of mycobacterium at MIC 0.8 µM. The presence of active hetero-aryl groups like pyridine, pyrolle, anthracene, which is attached to the pyrazoline ring may be responsible for the good antitubercular activity. Compounds 4a, 5c and 6b induced the greatest effect on EAC cells and found to be active anticancer agents (Table 2) when compared with standard 5-fluorouracil.

Molecular docking studies

The application of molecular modeling was employed to further understand the possible conformation of pyrazoline-*Mt*SK complex. The shikimate kinases are composed of three domains: Core domain, LID domain and shikimate-binding domain (SB domain). The LID and SB domains are responsible for large conformational changes during catalysis. LID domain residue Arg117 is involved in all pyrazoline derivatives binding.

Table 3 shows the energy scores of the inhibitors, which indicates that all the studied inhibitors were well docked into the binding site of *Mt*SK. The binding interactions between the receptor and the inhibitors 6eand 6awhich are potent as standard streptomycin and isoniazid respectively were selected for performing deeper docking studies and is shown in the Fig. 3a, b.

Table 3 Surflex-Dock scores (kcal/mol) of pyrazoline derivatives

Compound	C Score[a]	Crash Score[b]	Polar Score[c]	D score[d]	PMF score[e]	G score[f]	Chem score[g]
6e	8.92	−2.20	1.62	−187.11	−69.47	−354.25	−41.89
4c	8.69	−0.51	1.11	−189.89	−69.77	−368.82	−41.46
5b	8.53	−2.12	0.17	−183.90	−65.51	−389.25	−39.36
6a	8.43	−3.27	0.019	−171.89	−70.28	−350.14	−40.34
5a	7.96	−2.82	0.006	−178.70	−75.12	−349.13	−38.72
DHK	7.64	−0.80	5.56	−12.61	−55.35	−69.33	−18.18
6b	7.56	−0.55	0.04	−162.71	−60.41	−339.05	−38.60
6f	7.12	−2.90	0.03	−165.34	−58.17	−312.43	−38.57
Pyrazolone	7.06	−1.24	1.97	−128.03	−52.12	−231.40	−32.65
5c	6.98	−2.80	0.05	−162.63	−56.97	−310.67	−38.72
4a	6.88	−3.50	0.14	−174.82	−57.92	−321.86	−38.24
4b	6.75	−2.22	0.18	−168.66	−71.83	−300.00	−37.90
6g	6.71	−2.26	0.18	−168.66	−71.83	−300.00	−37.90
5d	6.65	−1.45	0.002	−168.34	−68.66	−318.87	−39.65
5e	6.60	−3.48	0.12	−170.44	−74.77	−329.99	−30.33
6c	6.56	−1.30	0.01	−161.00	−69.55	−347.77	−38.88
4f	6.48	−3.47	0.20	−175.28	−71.11	−310.02	−39.11
6h	6.41	−2.27	0.09	−169.99	−73.09	−333.76	−38.54
4e	6.39	−3.05	1.32	−170.87	−68.99	−329.14	−34.99
5f	6.28	−2.11	0.012	−164.64	−62.12	−308.22	−37.11
4d	6.23	−3.33	1.55	−160.60	−70.27	−311.22	−31.52
4h	6.18	−2.68	0.07	−172.47	−71.35	−335.44	−38.23
5g	6.17	−2.55	1.32	−175.73	−64.44	−328.56	−33.76
6d	6.11	−2.11	0.062	−172.62	−72.32	−327.87	−33.89
5h	6.08	−3.67	0.63	−168.89	−68.27	−326.87	−35.67
4g	6.01	−1.68	0.09	−175.28	−70.65	−316.75	−32.67

DHK 3-Dehydroshikimate; Pyrazolone 3,5-dichloro-N-(6-(3-methyl-5-oxo-2,5-dihydro-1H-pyrazol-1-yl)pyridin-3-yl)benzenesulfonamide

The compunds with high scores are in bold

[a] CScore (Consensus Score) integrates a number of popular scoring functions for ranking the affinity of ligands bound to the active site of a receptor and reports the output of total score

[b] Crash-score revealing the inappropriate penetration into the binding site. Crash scores close to 0 are favorable. Negative numbers indicate penetration

[c] Polar indicating the contribution of the polar interactions to the total score. The polar score may be useful for excluding docking results that make no hydrogen bonds

[d] D-score for charge and van der Waals interactions between the protein and the ligand

[e] PMF-score indicating the Helmholtz free energies of interactions for protein-ligand atom pairs (Potential of Mean Force, PMF)

[f] G-score showing hydrogen bonding, complex (ligand-protein), and internal (ligand-ligand) energies

[g] Chem-score points for hydrogen bonding, lipophilic contact, and rotational entropy, along with an intercept term

Compound 6e showed hydrogen bonding interaction, the hydrogen of the hydroxyl group present at the 4th position of the phenyl ring made hydrogen bond with oxygen of the residue Glu38. This compound also formed van der waals interactions with the residues Leu119, Arg136, Arg58, Arg117, Ala46, Asp34, Glu38, Asp32.

The binding interactions in the other compounds revealed that anthraldehyde/pyridine/pyrolle and bridge phenyl moiety was surrounded by the residues Arg117, Ala46, Leu119, Asp34, and Asp32.

The conserved residue Arg117 on the LID domain is involved in pyrazoline derivatives binding by forming one hydrogen bond or two hydrogen bonds with the sulfonamide oxygen atom, additional interaction observed at shikimate-binding domain (SB domain) the Glu38 of α2 helix make two hydrogen bonds with OH and NH_2 and the NO_2 make two H-bonds with Ala46 of α3 helix.

The carboxylic acid group of 3-dehydroshikimate (DHK) make three H-bonds with Arg136 of α8 helix and one H-bond with Arg58 of α4 helix and that of OH at 3rd position make one H-bond with Asp34 of α2 helix (Fig. 3c). Bandodkar and Schmitt (2007) reported pyrazolone derivative 3,5-dichloro-*N*-(6-(3-methyl-5-oxo-2,5-dihydro-1H-pyrazol-1-yl) pyridin-3-yl) benzenesulfonamide used to further understand the binding pattern. It showed four H-bonds, one H-bond with Arg136 of α8 helix, two H-bonds with Arg58 of α4 helix and one H-bond with Arg117 of LID domain (Fig. 3d).

It was important to note that pyrazoline derivatives was surrounded by residues Leu10, Pro11, Ser13, Lys15, Arg20, Asp32, Asp34, Glu38,Ile45, Ala46, Phe47, Phe57, Arg58, Gly79, Gly80, Gly81, Thr111, Thr115, Val116, Arg117, Pro118, Leu119, and Arg136 of *Mt*SK; which indicated that the binding location was LID domain (Fig. 3e).

Consensus score (C score) ranging 8.92-6.01 integrates a number of popular scoring functions for ranking the affinity of ligands bound to the active site of a *Mt*SK enzyme and reports the output of total score. Crash score showed that inappropriate penetration into the binding site was in favor of compounds 4c, 6b followed by DHK and other compounds. D score which shows the charge and van der waals interactions between the protein and the ligand suggested that compounds 4c, 6e, 5b, 5a, and 6a were the superior ligands than DHK and pyrazolone. PMF score gives the helmholtz free energies of interactions for protein-ligand atom pairs prefer that all the compounds over those of DHK and pyrazolone. Compounds 5b, 4c, 6e, and 6a showed better G score, which indicates better hydrogen bonding, complex (ligand-protein), and internal (ligand-ligand) energies than DHK, pyrazolone and other compounds in the series. Chem score corresponds to hydrogen bonding, lipophilic contact and rotational entropy, along with

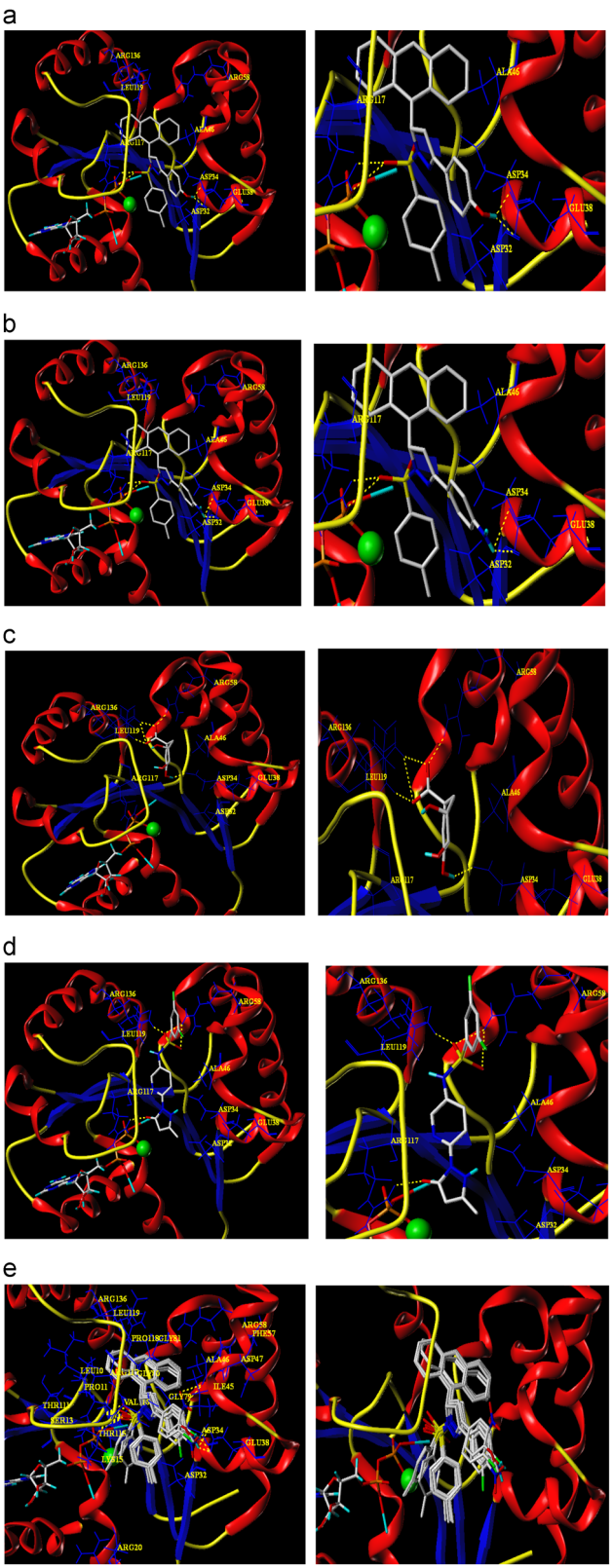


Fig. 3 **a**. Docking conformation of the compound **6e** at the MtSK active site. **b**. Docking conformation of the compound **6a** at the MtSK active site. **c**. Docking conformation of the DHK at the MtSK active site. **d**. Docking conformation of the pyrazolone at the MtSK active site. **e**. All pyrazoline derivatives at MtSK active site with surrounded amino acids

Table 4 ADMET properties of pyrazoline derivatives

Comp. Code:	BBB	HIA	Caco-2 permeability	CYP450 2D6 Substrate/ Inhibitor	AMES toxicity	Carcino - genicity	Acute oral toxicity
4a	0.7796	1.0000	0.5094	Non-substrate/ Non-inhibitor	Non AMES toxic	Non carcinogens	0.6209
4b	0.7941	1.0000	0.5169	Non-substrate/ Non-inhibitor	Non AMES toxic	Non carcinogens	0.6180
4c	0.8292	1.0000	0.5069	Non-substrate/ Non-inhibitor	Non AMES toxic	Non carcinogens	0.6075
4d	0.8501	1.0000	0.5292	Non-substrate/ Non-inhibitor	Non AMES toxic	Non carcinogens	0.6502
4e	0.5211	0.9930	0.5439	Non-substrate/ Non-inhibitor	Non AMES toxic	Non carcinogens	0.6160
4f	0.6784	0.9864	0.5713	Non-substrate/ Non-inhibitor	Non AMES toxic	Non carcinogens	0.6000
4g	0.8162	1.0000	0.5539	Non-substrate/ Non-inhibitor	Non AMES toxic	Non carcinogens	0.6371
5a	0.8384	1.0000	0.5149	Non-substrate/ Non-inhibitor	Non AMES toxic	Non carcinogens	0.6132
5b	0.8478	1.0000	0.5204	Non-substrate/ Non-inhibitor	Non AMES toxic	Non carcinogens	0.6112
5c	0.8774	1.0000	0.5062	Non-substrate/ Non-inhibitor	Non AMES toxic	Non carcinogens	0.5962
5d	0.8842	1.0000	0.5688	Non-substrate/ Non-inhibitor	Non AMES toxic	Non carcinogens	0.6405
5e	0.5231	1.0000	0.5711	Non-substrate/ Non-inhibitor	Non AMES toxic	Non carcinogens	0.6047
5f	0.7706	0.9970	0.5722	Non-substrate/ Non-inhibitor	Non AMES toxic	Non carcinogens	0.5983
5g	0.8625	1.0000	0.5605	Non-substrate/ Non-inhibitor	Non AMES toxic	Non carcinogens	0.6327
6a	0.8608	1.0000	0.5214	Non-substrate/ Non-inhibitor	Non AMES toxic	Non carcinogens	0.6348
6b	0.8723	1.0000	0.5262	Non-substrate/ Inhibitor	Non AMES toxic	Non carcinogens	0.6272
6c	0.8955	1.0000	0.5000	Non-substrate/ Inhibitor	Non AMES toxic	Non carcinogens	0.6149
6d	0.9051	1.0000	0.5291	Non-substrate/ Inhibitor	Non AMES toxic	Non carcinogens	0.6507
6e	0.7032	1.0000	0.5541	Non-substrate/ Inhibitor	Non AMES toxic	Non carcinogens	0.6174
6f	0.7803	0.9973	0.5689	Non-substrate/ Inhibitor	AMES toxic	Non carcinogens	0.6057
6g	0.8919	1.0000	0.5590	Non-substrate/ Inhibitor	Non AMES toxic	Non carcinogens	0.6364

intercept terms revealed that compounds 6e, 4c, and 6a showed increased interactions with the protein than pyrazoline.

ADMET properties

ADMET properties like blood brain barrier penetration (BBB), human intestinal absorption (HIA), Caco-2 permeability, CYP2D6 inhibition (CYP), AMES toxicity and acute oral toxicity have been calculated and the results are tabulated in Table 4.

Conclusions

In this present investigation, synthesis of new series of pyrazolines, along with spectral data as well as antitubercular, anticancer, and antioxidant activities has been reported. Microwave irradiated synthesis of pyrazolines resulted in higher yield with less reaction time period when compared to conventional method. In the development of new anti-TB drugs, these classes of pyrazolines might act as potential inhibitors and can be thought of as lead compounds due to their chemical flexibility, activity, and selectivity. Molecular docking studies proved that compounds 6e, 4c, 5b, and 6a interacted with *Mt*SK enzyme very competently and so, these can be further processed to improve their antitubercular activity. ADMET studies revealed that the pyrazolines showed considerable values with minimal toxic effects, good absorption as well as solubility characteristics. And also some of these compounds were found to be active as anticancer agents, which can be further studied for their respective enzyme interactions.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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